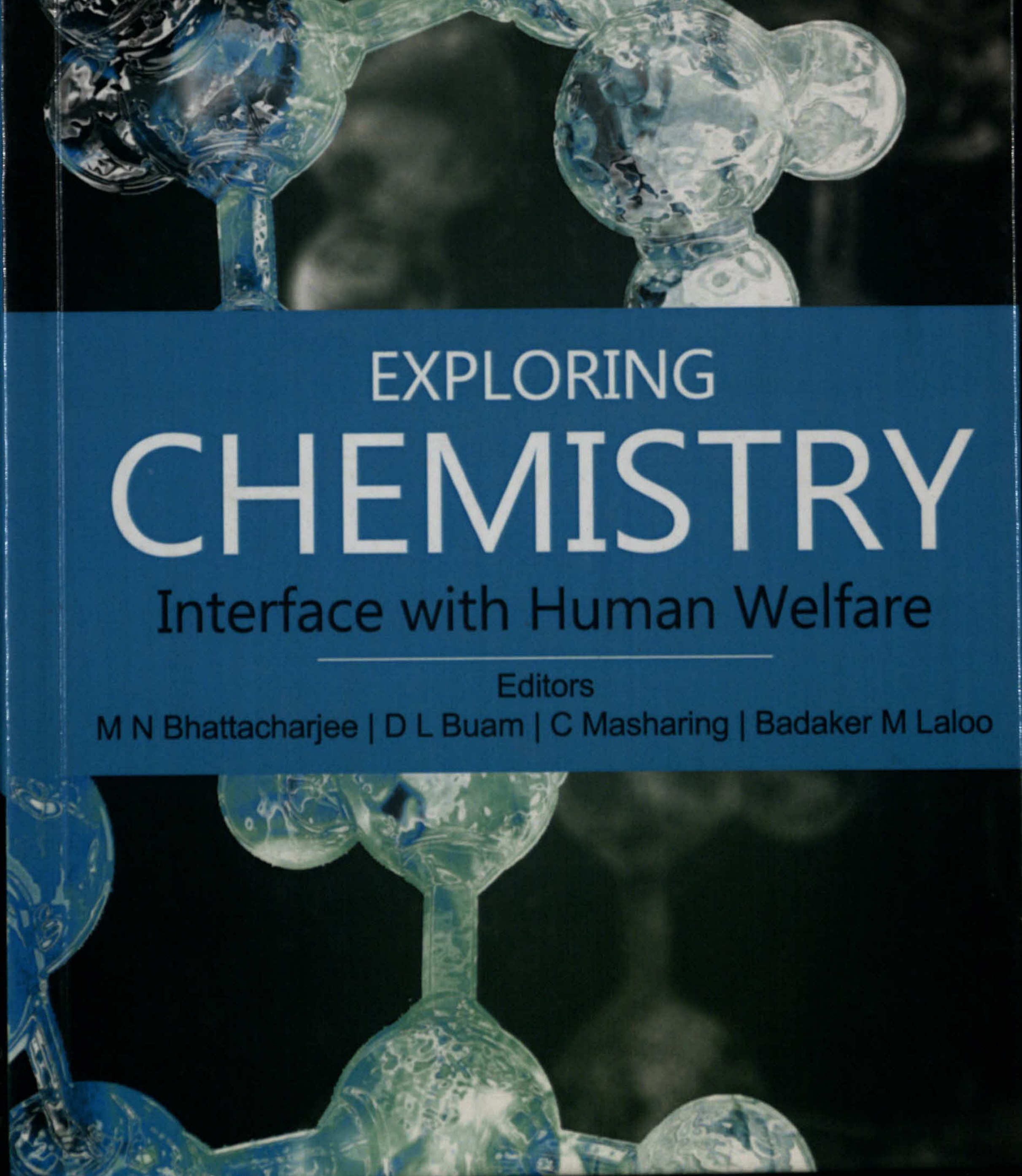


EXPLORING

CHEMISTRY

Interface with Human Welfare

Editors

M N Bhattacharjee | D L Buam | C Masharing | Badaker M Laloo

Exploring Chemistry

Interface with Human Welfare

Exploring Chemistry

Interface with Human Welfare

Editors

Dr. M. N. Bhattacharjee
Dr. (Smt.) D. L. Buam
Dr. C. Masharing
Dr. (Smt.) Badaker M. Laloo

EBH Publishers (India)
Guwahati-1

UNIVERSITY OF SCIENCE & TECHNOLOGY, MEGHALAYA
(Established under act 6 of 2008 enacted by the State Legislative Assembly of Meghalaya and
incorporated under section 22 & 2(f) of the UGC Act 1956)

Dr PG Rao, Ph D, FNASc, FIE, FIIChE
Vice Chancellor

File No: USTM/VC/Preface/2016/
Date 30.4.2016

Preface

I am happy to learn that, Department of Chemistry, Shillong College, Shillong is bringing out a book on the proceedings of the seminar held on "Recent Advances : Exploring Chemistry for humanwelfare", held at Shillong College, during 30-31 july 2015. My compliments to the organizing team and the team bringing out these proceedings.

The first chapter covers the theme of the seminar by Prof O K Medhi, which forms the road map for other chapters. A good number of topics are focused on water quality, drinking water, removal of contaminants of water etc. As water is the basis for the survival and welfare of human beings, the topics has been dealt by many authors, with focus to north east.

Few topics are covered on Inorganic materials and nano science, which is now helping to develop products, processes, which could give tailor made solutions to the problems of life.

Green chemistry has revolutionalized, to look back all the solutions of the past in a more environmental friendly and also extend the same to future solutions also, by reducing quantum of hazardous chemicals usage, safer and simpler reactions etc. Some of the experts have covered some examples on the subject.

The book of the proceedings have highlighted many other live examples of application chemistry in different context. I am sure the book will be reference guide of latest developments to Post Graduate students and others.

I would like to thank the editors for giving the opportunity to write preface to this book on proceedings of the seminar

With best wishes

PG Rao

Contents

List of Figure

List of Tables

List of Contributors

1. **Ambikesh Mahapatra**, Department of Chemistry, Jadavpur University, Kolkata 700 032, e-mail: ambikeshju@gmail.com & amahapatra@chemistry.jdvu.ac.in.
2. **Apurba K. Bhattacharjee**, Adjunct Faculty Professor, Department of Microbiology and Immunology, School of Medicine, Georgetown University, Washington, DC 20057 (U.S.A.), Email: ab3094@georgetown.edu.
3. **Badaker M. Laloo**, Department of Chemistry, Shillong College, Shillong 793003, Meghalaya.
4. **Banteiskhem Kharwanlang**, Department of Biochemistry, St. Edmund's College, Shillong-793003, email: banteiskhemkharwanlang@yahoo.in
5. **Bekington Myrboh**, Centre for Advanced Studies in Chemistry, Department of Chemistry, North-Eastern Hill University, Mawlai, Shillong -793022, India
6. **Bharati Deka**, Research scholar, Department of Chemistry, Gauhati University, Guwahati 781014, Assam deka.bharati@ rediffmail.com
7. **Chira R Bhattacharjee**, Department of Chemistry, Assam University, Silchar 788011, Assam India, crbhattacharjee@rediffmail.com
8. **Cornelia Mary Lyngdoh**, Department of Chemistry, Union Christian College, Barapani
9. **Deboshikha Bhattacharjee**, Centre for Advanced Studies in Chemistry, Department of Chemistry, North-Eastern Hill University, Mawlai, Shillong -793022, India
10. **Devolakshi Handique**, Department of Chemistry, Gauhati University, Guwahati 781014, Assam, handiquedevolakshi@gmail.com
11. **E. K. Mawkhiew**, Department of Geology, Kiang Nangbah Government College, Jowai, 793 150.
12. **F. Rumnong**, Department of Chemistry, Kiang Nangbah Government College, Jowai, 793 150
13. **Ghanashyam Bez**, Department of Chemistry, North Eastern Hill University, Shillong-793022, bez@nehu.ac.in
14. **Gitimoni Deka**, Associate Professor, Department Of Chemistry, Rangia College, Rangia - 781354, Assam.
15. **Iasylloklang Rynjah**, Department of Environmental Science, St. Edmund's College - 793003, Shillong, Meghalaya, email - evs07.sec@gmail.com
16. **Jasmine T. Sawian,** Department of Environmental Science, St. Edmund's College - 793003, Shillong, Meghalaya, email - evs07.sec@gmail.com

17. **Kali Prasad Sarma**, Department of Environmental Science, Tezpur University, Napaam, Sonitpur, Assam-784-028, India, sarmakp@tezu.ernet.in
18. **K. Belho**, Department of Biochemistry, North-Eastern Hill University, Shillong 793 022 Meghalaya, India
19. **Krishna G. Bhattacharyya**, Department of Chemistry, Gauhati University, Guwahati 781014, Assam, kgbhattacharyya@gmail.com
20. **Larihun Jeengaph**, Department of Environmental Science, St. Edmund's College - 793003, Shillong, Meghalaya, email - evs07.sec@gmail.com
21. **Lashngain R. Kharkongor**, Department of Biochemistry, School of Life Sciences, North-Eastern Hill University, Shillong 793 022, Meghalaya, India
22. **Leena Deka**, Department of Chemistry, Gauhati University, Guwahati - 781014, Assam. leenadeka_gu@rediffmail.com.
23. **Manalisha Deka**, Assistant professor, Department of Zoology, BBK Collge, Nagaon, Barpeta Assam 781311, e-mail: manalishad@gmail.com
24. **Michelle Khongwir**, Department of Environmental Science, St. Edmund's College - 793003, Shillong, Meghalaya, email - evs07.sec@gmail.com
25. **Moonmoon Choudhary**, Research Scholar, Department of Chemistry, Gauhati University, Pin-781014, Assam, India, moonschem@gmail.com
26. **Moutusi Das**, Research scholar, Department of Chemistry, Gauhati University, Guwahati, Assam 781014, E-mail: das.moutusi@gmail.com
27. **O. K. Medhi**, former Vice Chancellor, Gauhati University, Guwahati, Assam.
28. **O. Risuklang Shangpliang**, Department of Chemistry, North-Eastern Hill University, Shillong-793022, Meghalaya, India, Email: o.risuklang@gmail.com
29. **P. K. Ambasht**, Department of Biochemistry, North-Eastern Hill University, Shillong 793 022 Meghalaya, India, pravin.ambasht@gmail.com
30. **Porag Bora**, Department of Chemistry, North Eastern Hill University, Shillong-793022
31. **Pranabjyoti Das**, Research Scholar, Department of Chemistry, Gauhati University, Guwahati, Assam 781014. pranabjyoti021@gmail.com
32. **R.A. Lal**, Centre for Advance Studies, Department of Chemistry, North-Eastern Hill University, Shillong-793022, Meghalaya, India.
33. **R.N. Dutta Purkayastha**, Department of Chemistry, Tripura University, Suryamaninagar - 799022, West Tripura.
34. **Rajat Shubro Bose**, Department of Environmental Science, Tezpur University, Napaam, Sonitpur, Assam-784028, India.
35. **Rajib Panchadhayee**, Department of Chemistry, Assam University, Silchar-788011, Assam, India, rajibcdri@gmail.com
36. **Rekibuddin Ahmed**, Department of Chemistry, Rangia College, Rangia - 781354, Assam

37. **S. Choudhury**, Department of Chemistry, Kiang Nangbah Government College, Jowai, 793 150.
38. **Sujit Kumar Ghosh**, Department of Chemistry, Assam University, Silchar-788011, India, E-mail: sujit.kumar.ghosh@aus.ac.in
39. **Sunshine D. Kurbah**, Centre for Advance Studies, Department of Chemistry, North-Eastern Hill University, Shillong-793022, Meghalaya, India.
40. **Valentina Basumatary**, Department of Chemistry, Gauhati University, Guwahati 781014, Assam, basumataryvalen@gmail.com

Introduction

Role of Chemistry on human life and progress of civilisation is always intrinsic. Every sphere of human activities is influenced by developments in various fields of chemistry since the recorded history of mankind, and it will continue to be so. Moreover, with the passage of time, chemical work extended to the realms of physical sciences on one side and biological sciences on the other side. Some of the celebrated achievements in the fields of Physics and Biology are also perceived as landmark achievements in Chemical Sciences. Consequently, Chemistry is now increasingly referred as the 'Central Science'.

The role played by Chemistry as a 'Central Science' also influences its future developments. Chemistry is the connecting link between physical sciences and life sciences (e. g., earth science, nano science, study of brain, environment, medical science, agro science etc.) as well as with social sciences (e. g., health, economics, law) with the ultimate aim of dealing with the welfare of humanity.

The famous play-write George Bernard Shaw once quipped: 'Science is always wrong. It never solves a problem without creating ten more'. Being a branch of science, chemistry may also have to bear the brunt of this criticism. In essence, taking the positive aspect of this comment, it only implies the ever expanding field of research. The history of chemistry shows that there are instances of great successes leading to more number of successes. As a result, new branches of chemistry are progressively emerging that has significant effect on Human Welfare.

One such example is Green chemistry. In general, when we talk about green chemistry it includes the invention, design and application of chemicals and chemical processes to reduce the use of hazardous substances that have potential to eliminate the generation. Risk for life in the living world depends both on the hazard and exposure to hazard. Green chemistry is part of the future chemistry, because of its importance as a tool in reducing risk and preventing pollution. As a chemical philosophy, it applies to all classical branches of chemistry such as organic, inorganic, analytical and biochemistry. Green chemistry is also sometimes described as 'sustainable chemistry'. With the increasing demands of society for safer and more convenient products, the chemical industry is facing the possible depletion of natural resources and the damage that may be caused to environment. But the future chemistry will consider the demands of sustainability along with the conversion processes.

Future chemistry may see greater strides in instrumentation yielding sharper observations. It also creates hopes for alternative sources of energy that is safe and renewable. In brief, 'future chemistry' beckons humanity with hopes of greater understanding of nature and greater welfare of the biosphere.

The main learning objectives emphasized in the chemistry curriculum not only stress the need for students to learn and master primary concepts of chemistry but also undergo intellectual development as scientifically literate citizen. These objectives can only be achieved if students gain conceptual understanding in science knowledge. It is more challenging to motivate a student in lower classes towards a particular discipline and therefore nature of teaching-learning activity plays a very important role. In a way, therefore the teaching in degree colleges is more demanding as the students are to be motivated in the subject concerned at this stage so that they may opt for post graduate course in a particular discipline. It is well established that what an individual learns is as a result of the learner's actively relating what is already known and what is being taught or experienced. The high demands of conceptual learning require chemistry teachers to letting go of transmission-oriented practices; they need to carefully prepare lesson plans, student worksheets, assignments, and assessment tasks to be able to think about and convey the subject matter in different ways.

There is phrase often used in English, i.e. 'in the limelight". Limelight is an intense white light which is produced by heating a piece of lime in a flame of burning oxygen and hydrogen. The effect was discovered in the 1820s by Goldsworthy Gurney and the application of the process to create a bright light was developed by Thomas Drummond around 1825, though credit for which is normally given to Robert Hare. In 1825, a Scottish Engineer, Thomas Drummond (1797-1840), saw a demonstration of the effect by Michael Faraday and realized that the light would be useful for surveying. Drummond built a working version in 1826, and the device is sometimes called the Drummond Light. The earliest known use of limelight at a public performance was outdoors, over Heme Bay Pier, Kent, on the night of 3 October 1836 to illuminate a juggling performance by magician Ching Lau Lauro. This performance was part of the celebrations following the ceremony of laying of the Foundation Stone of the Clock Tower. The advertising leaflet called it koniaphostic light, and announced that: "the whole pier is overwhelmed with a flood of beautiful white light. It was widely used in 19th century theatres to illuminate the stage and was first used in a public theatre at Covent Garden in London in 1837 and then enjoyed widespread use in theatres around the world during later part of 19th century. Limelights were employed to highlight solo performers in the same manner as modern spotlights. Clearly, actors who were the centre of attention on stage being said to be in the limelight. The figurative use, to people or things that were the centre of attention outside the world of theatre, came into use around the turn of the 20th century and is now very common all around.

Developments in chemical research and the related areas should always be deliberated and discussed with attention in various scientific forums, seminars etc. This seminar organised by the Department of Chemistry, Shillong College in collaboration with IQAC of the college was an activity in this direction and, therefore, has contributed to throw more information on how chemistry is continuing to influence the mankind and also glimpses of what more are expected. The response of the academicians and researchers was overwhelming and thus the seminar became a 'repository of knowledge' and this will definitely encourage our students to opt for a career in Science, specifically in Chemistry. To make the deliberations reach a larger audience of teachers and students of chemistry, the proceedings are published in the form of this book with an aim to share the knowledge with all. For obvious reasons, the contents are not extensive as no seminar can cover all aspects of chemistry that influences human welfare. Some of the papers presented and included in this volume may not look having any direct consequence in our welfare but let us remember that every scientific discovery, when accumulated and applied systematically, lead to some effect in the life and function of mankind.

It was a daunting task for the Editorial Board to compile the papers, study the conformity of the contents of various articles with scientific findings and concepts and finally to put in proper format. Notwithstanding this job, the credit and responsibility of the various scientific facts, experiments and their veracity lie entirely on the authors of each article, and the members of the Editorial Board are not responsible for any claim of the scientific facts, data and conclusion.

Dr. M. N. Bhattacharjee
Dr. (Smt.) D. L. Buam
Dr. C. Masharing
Dr. (Smt.) B. M. Laloo

1

Chemistry for Human Welfare

O. K. Medhi

Chemistry has emerged as systematic science sometimes around the last quarter of the 18th century and ever since it contributed to many developments in human society. Contribution to human knowledge and civilization is brought about with increasing understanding of fundamental and applied aspects of chemistry. It has contributed directly or indirectly to the silent technological revolutions in agriculture, communications, medicine, metallurgy, public health, environment, textiles, petrochemicals; and countless other fields.

Chemistry is a molecular science and deals with structure and properties of molecules. Combined knowledge in other fields such as Physics, Biology, Material science , Environmental Science, Earth Science and Computational aspects played an important role in applications of chemistry. Chemistry is an Inter disciplinary Science and it has smooth transition of knowledge from one field to another. The great chemist Linus Pauling made a smooth transition of knowledge from theoretical chemistry to molecular biology. Most of the current research in chemistry is in the interface of chemistry with other disciplines.

The objective of applications of chemistry is to take threats to present day civilizations e.g. population explosion, hunger, disease, environmental pollution, depletion of source of natural resources among other things. The world population would be double in 35 years and food is going to be short very soon if the population is not controlled. There would be a major energy deficit when the world population reaches about 60 billions. Since the 19th century the chemists are helping food production by manufacturing fertilizers, pesticides, insecticides and producing genetically modified seeds. This has brought about a green revolution and many countries including India has become self sufficient in food. Animal farming using the help of chemicals for protecting animals from disease lead to increase in the production of milk and meat. Simultaneously chemists have produced birth control chemicals in an attempt to keep population explosion under control. Also attempts are made to find newer sources of food and nutrients.

Availability of pure and fresh drinking water is becoming increasingly rare. It is believed that the next world war would be on controlling the sources of water. It is not just the scarcity of water which is a problem but the contamination of the available sources of water with toxic substances such lead, arsenic, flouride, and iron is the problem. It is a challenge to chemists to find ways and means to remove such pollutants to provide pure water. Reverse osmosis is a way to obtain pure water by desalination of sea water in coastal areas.

Synthesis or isolation of drugs from medicinal plants is a major contribution of chemists to human society. Aspirin, sulfa drugs, antibiotics (penicillin, streptomycin), anti malarial drugs (quinine, artemycin), anaesthetics cortisone (for Rh fever, Rh arthritis), anti cancer drugs, and drugs for HIV patients are examples of such efforts by chemists. Design of drugs by structure-activity correlations and molecular modeling is one of the recent areas of research in chemistry.

Chemistry techniques are used to purify crude oil and then separate hydrocarbons. Heavier hydrocarbons are cracked into lighter ones forming gasoline used in automobiles. Petrochemicals have extensive use for human society. Chemists have used synthetic fibres, synthetic rubber, resins, plastics, detergents, cement, metals alloys, glass and ceramics which have extensive applications.

In the later half of the 20th century and in the early 21st century chemistry has become an interdisciplinary science. Several other areas in science such as physics, biology, mathematics, computer science, environmental science, earth science, medical science and material science are now integral part of chemistry. It has been found that new knowledge is found at the interface of two or more disciplines.

Recently chemistry has made important discoveries in the areas of material science. Chemists has produced electronic sensor materials, compounds which can be used as magnetic memory devices, optoelectronic materials, semiconductors, high temperature super conducting materials, and soft materials (surfactants, liquid crystals, biological materials), and catalysts for various applications. Very recently chemists have made very important contributions to the synthesis and applications of nanomaterials, carbon nanotubes, graphemes etc. Nanomaterials have a great deal of applications and may control the development of science and technology in the next few decades.

The interface of chemistry and biology in the latest areas of research, emerging subjects such genetics, drug design, synthesis of metal containing anti cancer drugs, microbiology and bioengineering depends heavily on knowledge and techniques developed by chemists.

Chemists have found environmental solutions for sustainable developments, solving problems of climate change. Green chemistry has become increasingly important in reducing the environmental pollutions that arise due to chemical processes and chemical industry.

The major areas of research in chemistry for future lie in the interface of chemistry with biology, materials, environmental science and computational chemistry and molecular modelling.

2

Inorganic Materials: Beneficial Aspect, Applications–An overview

R. N. Dutta Purkayastha

Abstract

Inorganic chemistry has always been playing a major role in the progress of human civilisation since time immemorial. Development in science and technology has remained largely dependent on the advances in inorganic chemistry that also includes catalysis, coordination chemistry, electronics, material chemistry and many other emerging fields in addition to classical chemistry. Nevertheless, some of the developments posses problems as well, particularly with reference to environmental pollution. This paper presents an overview of the recent developments in these areas, more relating to solid fuels cells, super-conductors, molecular materials and fullerides, hydrogen as alternative fuel, molecular magnets, inorganic-organic hybrid materials and similar recent advances in the use of inorganic materials and chemistry in the welfare of mankind in the present rapidly advancing society.

Key words: *Mixed ionic-electronic conductors, Fuel cells, Solid Oxide Fuel Cells, Superconductors, Zeolites, Fullerenes, Hydrogen Storage Materials, Hydrides, Molecular Magnets, Graphene, Inorganic -Organic Hybrids, Nanomaaterials.*

Inorganic chemistry is advancing rapidly at its frontiers, especially when research impinges on other disciplines such as life sciences, condensed matter physics, material science, environmental chemistry and such related fields. These rapidly developing fields also represent many areas of inorganic chemistry where novel types of compounds are used in catalysis, electronics, pharmaceuticals, solid state material chemistry, metal-organic hybrids, hydrogen storage materials, molecular materials and nano materials etc.

The area of material chemistry is developing rapidly and there is a great interest in synthesis and properties of novel inorganic solids. The chemistry of solid state is an exciting area partly on account of technological applications and because of their properties quite which are challenging to understand.

Much of current research interest in solid state is motivated by the search for commercially useful materials such as components of batteries, fuel cells, catalyst for hydrocarbon conversion, molecular separations and improved electronic, photonic devices for information processing and storage. Few selective examples related to the development and useful applications of intercalation compounds, complex electronic oxides such as high temperature superconductors, magnetic compounds, materials exhibiting giant magneto resistance, metal-organic frame work compounds (MOFs), zeolites and molecular materials etc. are presented in the following sections.

Some solid materials exhibit both ionic and electronic conductivity and one such material is Mixed ionic-electronic conductors. Mixed ionic-electronic conductors (MIECs) are materials that conduct both ions (σ_i) and electronic (σel) charge carriers (electrons and/or holes). This definition can also be extended to say that a MIEC is a material that conducts matter (ions and/or atoms) and electronic charge carriers. Some examples of such class of compounds are, $CeO_{2-\delta}$, which conducts both oxygen ions and electrons, $YBa_2Cu_3O_{6+\delta}$ diffusion of oxygen and Pd, diffusion of hydrogen. However, it is not clear whether the matter propagates as ions with a close electron cloud or as atoms. Another important application of solid state materials are FUEL CELLS, an electrochemical devices, which can directly convert the chemical energy of gas fuel into electrical energy and heat. One of the promising applications of fuel cells is MIECs composites for Solid Oxide Fuel Cells (SOFCs). The SOLID OXIDE FUEL CELLS, is a source of Portable power applications, use an oxide ion conductor as electrolyte. The advantages of the cell are high efficiency, use of relatively inexpensive materials, fuel flexibility and low sensitivity to impurities in the fuel. The major disadvantages of cells are thermal stresses of materials and possibility of undesired reactions.

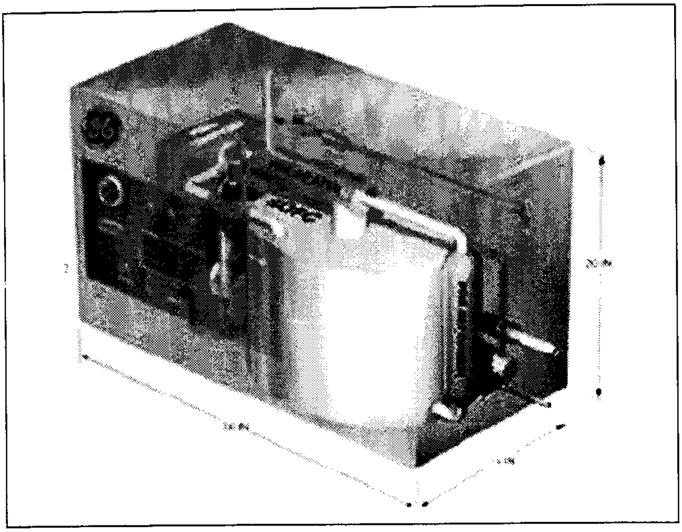


Fig. 2.1: Solid Oxide Fuel Cell

Most of the ionic conductors have low electronic conductivity. In some cases a combination of electronic and ionic conductivity is desirable, and this type of behaviour can be found in some d -metal compounds where defects allow O_2-conduction and the metal d-orbitals provide an electronic conduction band. Many of such materials are having perovskite based structures e.g. $La_{1-x}Sr_xCo_{3-y}$ and $La_{1-x}Sr_xFeO_{3-y}$. These oxide systems are good electron conductors. The versatility of solids with perovskities structure extends to superconductivity because most of the high temperature superconductivity, reported first time in 1986, can be viewed as variants of perovskite structure. High temperature superconductors have two striking features, below a critical temperature Tc (critical temperature), they enter superconducting state and DC electrical resistivity goes to zero. In this superconducting state they exhibit Meissner effect i.e. Magnetic flux present in superconductor is ejected when superconductor cooled through Tc. There are two types of Superconductors, Type I Superconductors (soft superconductors), many types of pure materials have this behavior such as Zn, Cd, Hg, etc. Type II Superconductors tend to be alloys, has a region that is a mixture of superconducting and non-superconducting states (vortex state). The Meissner effect is the basis of the superconductivity in which a pellet of superconductors levitates above a magnet .It also the basis for a number of applications that include magnetic levitation as in MagLev Trains, MRI (non-invasive health imaging) and other Possible applications could be in power generation and transmission.

Materials for Superconductors

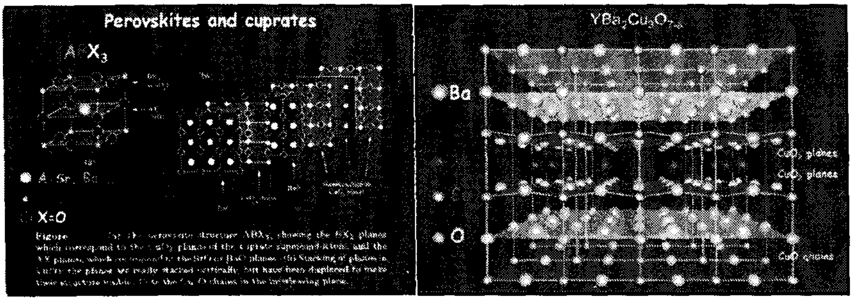


Fig. 2.2: Super-conductors

Frame Work Structures

Zeolites are framework silicates, with a completely linked framework of tetrahedra, each consisting of 4 O^{2-} surrounding a cation usually Si^{4+} or Al^{3+}.The framework contains open cavities in the form of channels and cages. Channels are large enough to allow passage of guest species. Channels and cages are occupied by H_2O molecules and extra-framework cations K^+, Na^+, Ca^+ and others that are commonly exchangeable. Common properties of Zeolites' are high degree of hydration, low density and large void volume when hydrated, stability of the crystal structure when dehydrated, cation exchange properties, uniform molecular-sized channels in dehydrated crystals, ability to absorb gases and vapours and catalytic properties.

Following the discovery of large number of new microporous structures from 1950 onwards many new zeolite framework structures are synthesized by using complex template molecules. Industrial applications of zeolites for separation and purification include petroleum refining processes, where they are used to remove water, CO_2, chloride, mercury, and the desulfurization of natural gases, removal of water and carbon dioxide from air before liquefaction and separation by cryogenic distillation and drying and removal of odor from pharmaceuticals products. The other excellent ion-exchange properties of zeolites results from the open structures and ability to trap significant quantities of cation, selectively, within pores. The important area where ion exchange properties of zeolites are exploited is the trapping and removal of radio nuclides from nuclear waste. Zeolites also find useful applications in removal of ammonium ion from waste stream treatment, sewage treatment, pet litter and aquaculture etc. They find application in heavy metal ion removal from nuclear, mine, and industrial wastes.

Agricultural applications include use as soil conditioner and animal feed supplements. The major utilities of Zeolites are however in adsorption and catalysis.

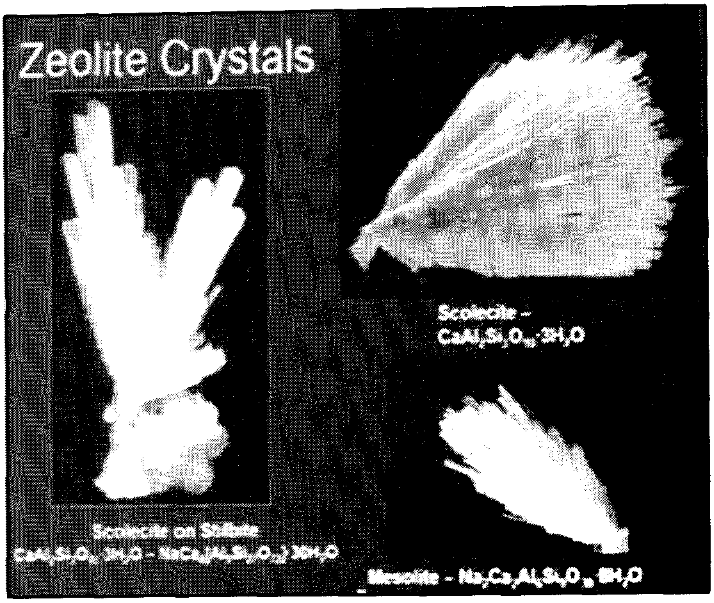


Fig. 2.3 : Sample Zeolite Crystal

Table 2.1: Sample Zeolite Formulas

Mineral Name	**General Formula**
Analcime	$Na_{16}[Al_{16}Si_{32}O_{96}].16H_2O$
Chabazite	$(Ca_{0.5}Na,K)_4[Al_4Si_8O_{24}].12H_2O$
Chnoptilolite	$(Na,K)_6[Al_6Si_{30}O_{72}].20H_2O$
Erionite	$K_2(Na,Ca_{0.5})_8[Al_{10}Si_{26}O_{72}].28H_2O$
Heulandites	$(Na,K)Ca_4[Al_9Si_{27}O_{72}].24H_2O$
Laumontite	$Ca_4[Al_8Si_{16}O_{48}].18H_2O$
Masolite	$Na_{16}Ca_{16}[Al_{48}Si_{72}O_{240}].64H_2O$
Scolecite	$Ca_8[Al_{16}Si_{24}O_{80}].24H_2O$
Stilbite	$NaCa_4[Al_9Si_{27}O_{72}].30H_2O$

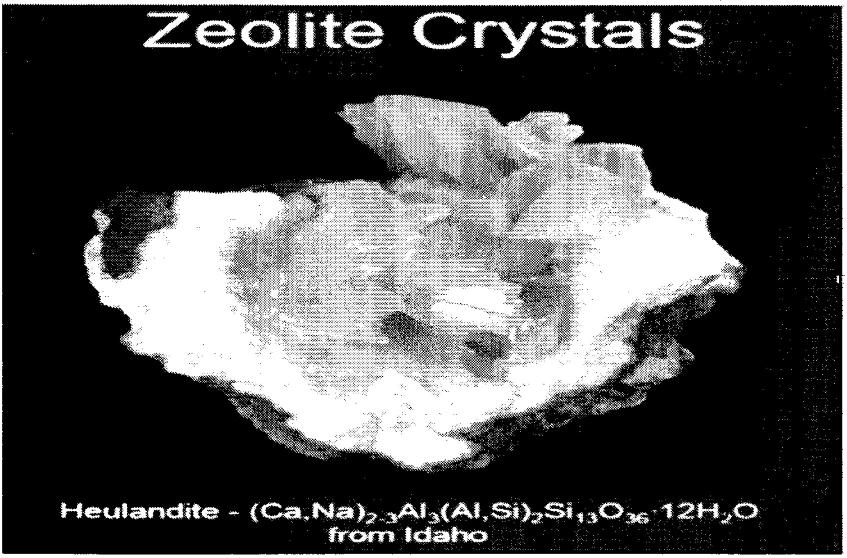
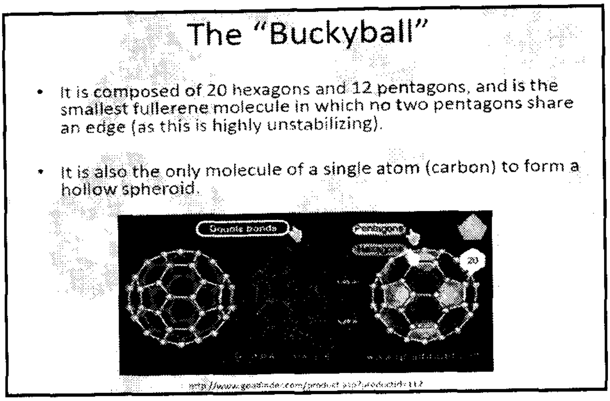


Fig. 2.4: Zeolite structures

The majority of the compounds discussed so far have materials with extended structures, which are widely used in heterogeneous catalysis, rechargeable batteries and electronic devices due to their chemical and thermal stability. The desire to combine the synthetic and chemical flexibility of molecular chemistry with the properties of classical solid-state materials has led to the rapid emergence of the area of molecular material chemistry.

Molecular Materials and Fullerides

Fullerenes and "Buckyballs" - Fullerene are any molecule composed entirely of carbon in the form of an ellipsoid, tube, or hollow sphere. Fullerenes were discovered in 1985 by Richard Smalley from Rice University. The "buckyball", the first fullerene discovered has the molecular formula of C_{60} and is a true hollow sphere. The solid fullerene $C_{60}(s)$, the MnC_{60} fullerides derivatives contain discrete $C_{60}n-$ molecular anions. Exposure of solid C_{60} to alkali metal; vapour results in the formation of a series of compounds of the type MxC_{60}, however, the precise stoichiometry depends on properties of reactants . Compounds with stiochiometry M_3C_{60} are the most interesting, which become super conducting in the temperature range of 10-40K depending on the type of metal. K_3C_{60} is superconducting on cooling to 18K.



The "Buckyball"

- It is composed of 20 hexagons and 12 pentagons, and is the smallest fullerene molecule in which no two pentagons share an edge (as this is highly unstabilizing).
- It is also the only molecule of a single atom (carbon) to form a hollow spheroid.

http://www.goalfinder.com/product.asp?productid=112

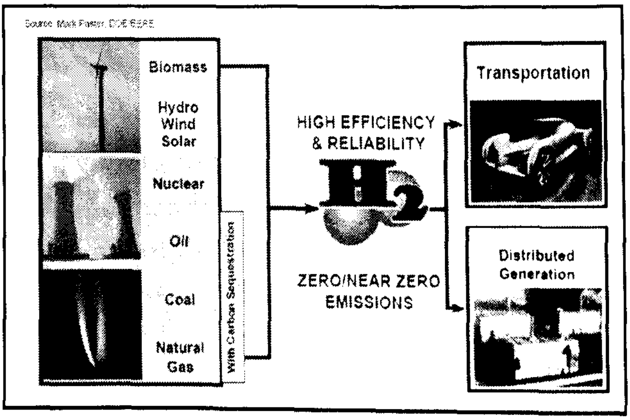
Fig. 2.5: The Wonder Buckyball

Hydrogen as Alternative Fuel

Hydrogen Storage Materials

All the fossil fuels we are using at present are depleting rapidly and use of them increases carbon dioxide proportions in atmosphere, results in greenhouse effects and causing Global Warming. Some fossil fuels contain sulphur and on burning produces sulphur dioxide, a poisonous gas which reacts with water in the atmosphere to form sulphuric acid, causes Acid Rain. In view of such problems the alternating fuels are looked for. Hydrogen gas is very powerful; it has the highest energy per unit of weight of any chemical fuel. Hydrogen is highly abundant element, one of the most common substances on earth, and environmentally friendly as its oxidation product is only water.

Hydrogen Production



Source: Mark Paster, DOE EERE

Fig. 2.6: Production, Transport and Distribution Hydrogen as Fuel

Challenges of Using Hydrogen

Even though, hydrogen is an attractive alternative to hydrocarbon fuels such as gasoline, however, the storage of hydrogen still remains a major problem and scientists look for light, affordable, and safe method for storing of hydrogen. Problems associated with hydrogen storage are its low energy density and hydrogen has a tendency to leak because of its small size. Hydrogen that has leaked out could accumulate and increase the risk of fire or explosion.The development of materials for use in a future hydrogen based energy economy is a key challenge faced by Inorganic chemists. One such area is the new materials that needed for hydrogen storage. High pressure gas cylinders and liquefaction routes are unlikely to meet the requirements of hydrogen storage applications, e.g. transport or distribution of power supplies due to their weight and safety issue. New materials for storing hydrogen will be required to have high capacities both volumetrically and gravimetrically also being relatively cheap.Two main approaches to the new materials currently pursued are: chemically bound hydrogen viz. metal hydrides and new porous or high surface area compounds that physiosorb hydrogen. Many metal hydrides and complex metal hydrides releases H_2 when heated and are potential materials for hydrogen storage. These metal hydrides have an important safety advantage over pressurized or liquefied H_2.

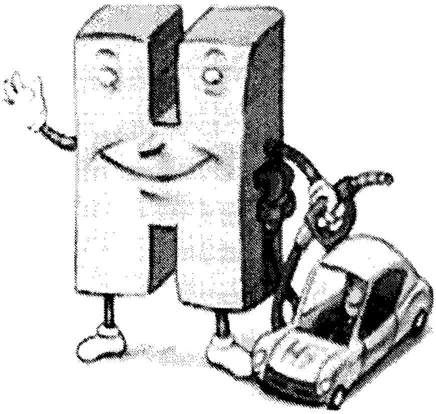


Fig. 2.7: Representative Hydrogen Fuel Car

Since heat is required to release the hydrogen, this method avoids safety concerns surrounding leakage that can be a problem with compressed hydrogen and liquefied H_2. In fact, metal hydrides are one of the safest methods for storing hydrogen.

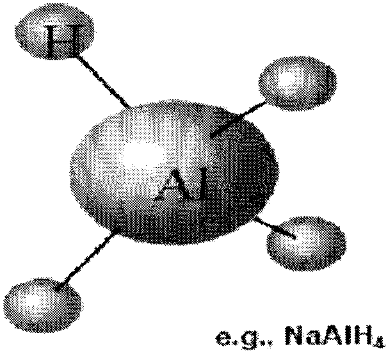


Fig. 2.8: $[AlH_4]^-$ **ion**

The recent discovery of hydrogen sorption by inter-metallic compounds created great hopes and stimulated research and development worldwide of using the metal hydrides as a new alternative for storing and delivering pure hydrogen that can be very useful for fuel cell technology. The conventional metal hydride alloy families are the AB_5, AB_2, and AB type inter-metallic compounds.

Table 2.2: Hydrides: New Materials for Storing Hydrogen

Hydrides	Material	H_2[mass%]	T_{dec} [pC] 1 bar
	$LaNi_5H_6$	1.49	15
Metal Hydrides	$TiMn_{1.5}H_{2.5}$	1.76	
	$FeTiH_2$	1.86	-10
MgH_2, AlH_3	ZrH_2	2.16	
	$TiCr_{1.8}H_{3.5}$	2.43	
$XAlH_4$, XBH_4	Mg_2NiH_4	3.62	300
	VH_2	3.81	-10
$-NH_2$, -NH	TiH_2	3.98	780
	NaH	4.20	430
$H^{\delta+}$ and H^{δ}	CaH_2	4.79	1000
	Li_2NH + LiH	5.50	600
	$LiNH_2$ + LiH	6.50	300
	$NaAlH_4$	7.46	30, 120
	MgH_2	7.66	320
	AlH_3	10.07	< RT

Hydrides	Material	H_2[mass%]	T_{dec} [pC] 1 bar
	$LiAlH_4$ $NaBH_4$ LiH $Al(BH_4)_3$	10.62 10.66 12.86 16.90	-93 620 900 < 100

Inter-metallic Compounds

Table 2.3: Inter-metallic Compounds and their Hydrogen-storage Properties

Type	Metal	Hydride	Structure	Mass%	P_{eq}, T
Elemental	Pd	$PdH_{0.6}$	Fm3m	0.56	0.020 bar, 298 K
AB_5	$LaNi_5$	$LaNi_5H_6$	P6/mmm	1.37	2 bar, 298 K
AB_2	ZrV_2	$ZrV_2H_{5.5}$	Fd3m	3.01	10^{-8} bar, 323 K
AB	FeTi	$FeTiH_2$	Pm3m	1.89	5 bar, 303 K
A_2B	Mg_2Ni	Mg_2NiH_4	P6222	3.59	1 bar, 555 K
Body-centred cubic	TiV_2	TiV_2H_4	b.c.c.	2.6	10 bar, 313 K

Molecular Magnets

Molecular solids containing individual molecules, clusters or linked chains of molecules can show bulk magnetic effects. Molecular inorganic magnetic materials, in which individual molecules, or units constructed from such

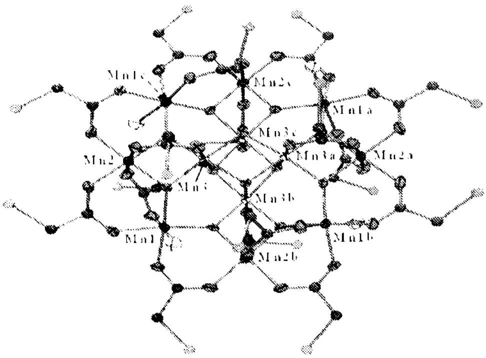


Fig. 2.9: Structure of $[Mn_{12}O_{12}(O_2CR)_{16}(H_2O)_4]$

molecules, contain d-metal atoms with unpaired electrons, is a class compound of growing interest. The Criteria for Molecular Magnets are large molecules, little intermolecular interaction; high total spin (S), High magnetic anisotropy (directional dependence).The incorporation of several d-metal ions into a single complex provides an opportunity to produce a molecule that acts as a tiny magnet. Such compounds have been termed as Single -molecule magnets (SMMs). One such example is the complex manganese acetate, $[Mn_{12}O_{12}(O_2CR)_{16}(H_2O)_4]$ which contains a cluster of 12 Mn (Mn_{12}) atoms linked through O of acetate . The features of this Mn_{12} family are S = 10, D = -0.40 to -0.50 cm-1 (-0.58 to -0.72 K), magnets below volume of the $Mn_{12}O_{12}$ magnetic core ~ 0.1 nm3

A New Generation of Mn Clusters, containing Mn84 atoms

$[Mn_{84}O_{72}(O_2CR)_{78}(H_2O)_{42}(MeOH)_{12}(H_2O)(OH)_6].xH_2O$ has been synthesized .The structure contains 84 Mn(III) ions in a large doughnut-shaped molecule 4nm in diameter. The ability to magnetise such individual SMMs potentially provides a route to storing information at extremely high densities.

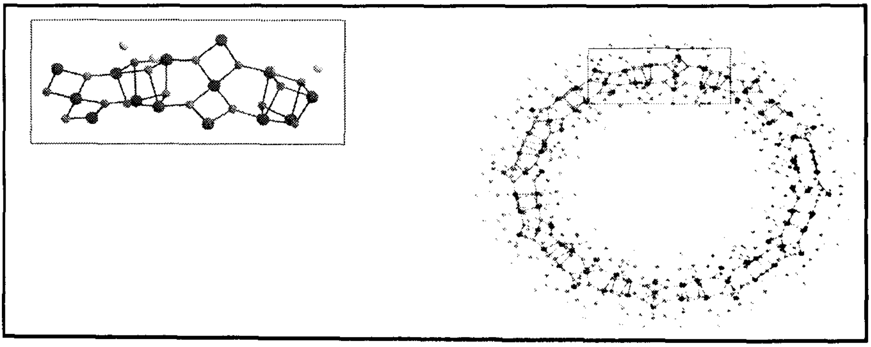


Fig. 2.10: Structure of New Generation Mn Cluster with 84 Mn atoms

Graphene

Graphene is the latest wonder material, a new type of carbon based material. Graphene is fundamentally one single layer of graphite; a layer of sp^2 bonded carbon atoms arranged in a hexagonal honeycomb lattice. The name "graphene" was first coined in 1987 by S. Mouras and co-workers to describe the graphite layers that had various compounds inserted between them forming the so-called graphite intercallation Compounds or GIC's. Graphene, is the strongest material ever recorded. In 2004: Andre Geim and Kostya Novoselov at Manchester University managed to extract single-atom-thick crystallites (graphene) from bulk graphite.

Andre and Kostya were awarded the 2010 Nobel prize in physics for their pioneering work in this field. Graphene has recently attracted considerable attention owing to its remarkable properties and potential applications in many emerging technologies.

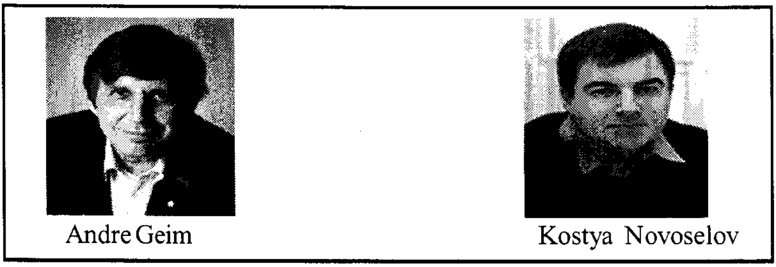


Fig.2.11: Pioneers of Graphene Materials

An important derivative of graphene is graphene oxide. It finds useful application in touch screen devices, electrode, flexible solar cell, large screen display systems, catalyst for organic reactions and many more.

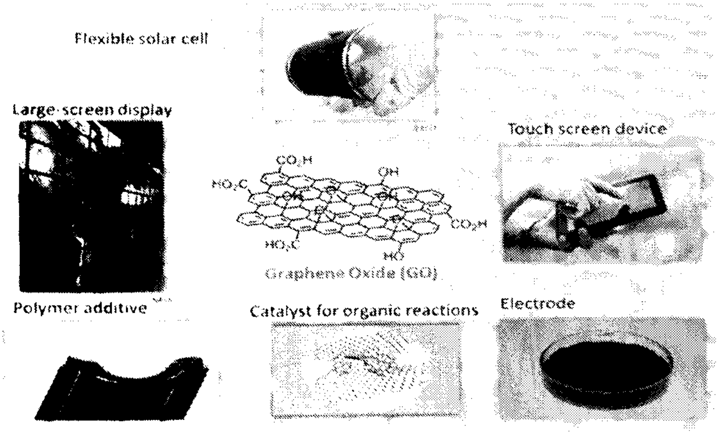
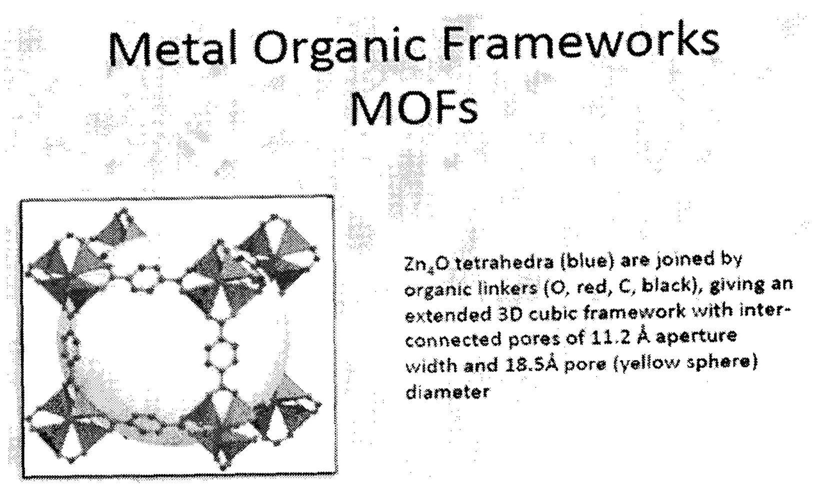


Fig. 2.12: Graphene and Graphene Oxide in Application in various Fields

Inorganic - Organic Hybrids: Ordered Structures

The novel inorganic-organic hybrid framework compounds has attracted great attention in recent years , not only owing to their intriguing variety of architectures and topologies , but also due to their potential applications in different spheres. Frame work complexes composed of metal centers linked by organic spacers are important in many field of chemistry and material science due to their potential applications as functional materials in nonlinear optics , medicines, gas-host chemistry, supramolecular chemistry , sensors, luminescence materials , magnetic materials and in catalysis.

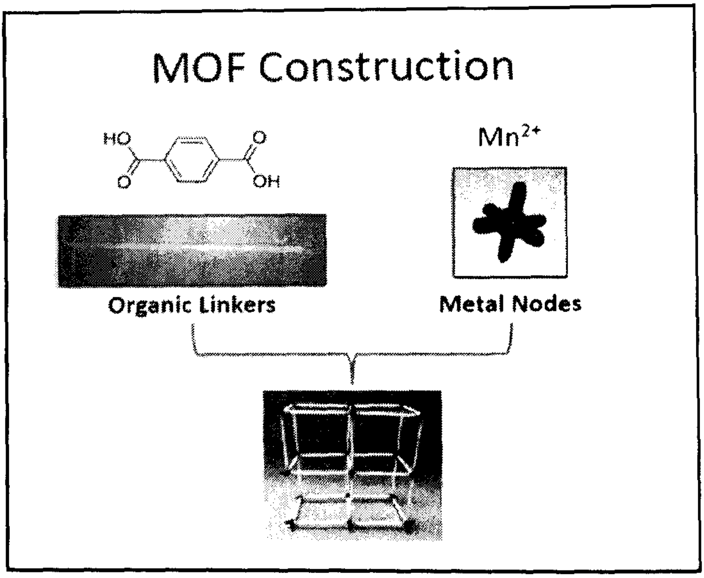


Metal Organic Frameworks
MOFs

Zn_4O tetrahedra (blue) are joined by organic linkers (O, red, C, black), giving an extended 3D cubic framework with inter-connected pores of 11.2 Å aperture width and 18.5Å pore (yellow sphere) diameter

Fig. 2.13: Metal Organic Framework

A general approach for construction of MOF is shown below:



MOF Construction

Mn^{2+}

Organic Linkers

Metal Nodes

Fig. 2.14: Construction of Metal Organic Framework

MOFs are used as hydrogen storage materials, in CO_2 sequestration, as CO_2 storage materials, as well as optical, magnetic and bio-medical materials and catalyst.

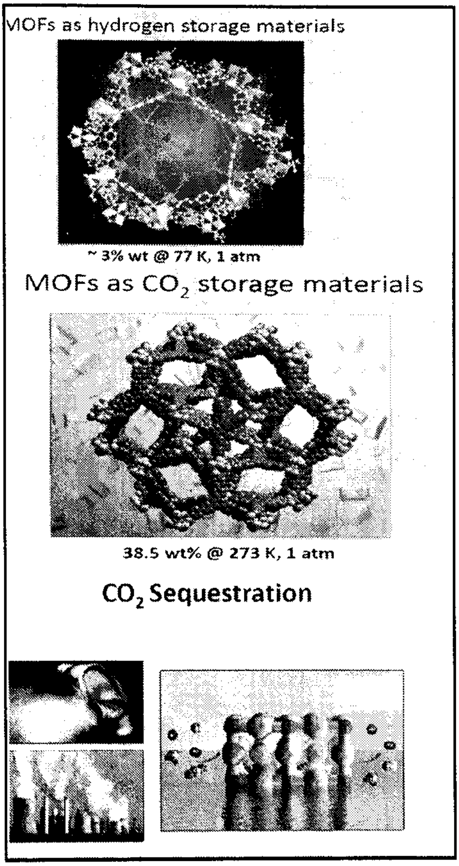


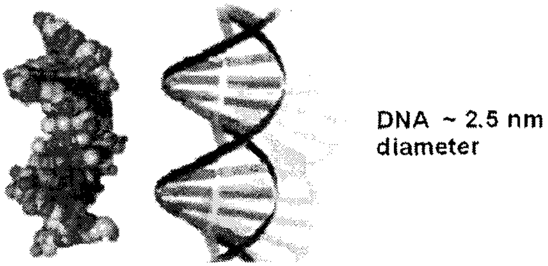
Fig. 2.15: Application of MOF in Some Important Areas Human Welfare

Nanomaterials: Developments and Promises

"To understand the large, we must understand the very small" - Democritus (400 BC).

"Nano technology will be the future with nanoscience developing nanomaterial and devices .This will lead to further convergence of technology with wide applications" - Dr. A.P.J. Abdul Kalam

A nano material is any material that has a critical dimension on the scale of 1to 100nm and exhibit properties absent in both molecular and bulk solids. The original version of nano technology occurred in nature, where organisms developed an ability to manipulate light and matter on atomic scale to build devices that perform specific functions, such storage of information, reproducing themselves etc. and in this sense DNA is the ultimate nano materials, as it stores information as a sequence of base pairs that are spaced about 0.3nm apart. Folded DNA molecules have information density of more than ~ 1Tb cm-2. Human beings have practiced nanotechnology for centuries.



(Courtsey : Nanoscale Science, Engineering, and Technology in the Office of Basic Energy Sciences (BES) of the U.S. Department of Energy (DOE))

Fig. 2.16: Diameter of DNA - Comparison with Nano-particles

Fabrication of nano materials are generally carried out by two general approaches, i.e., Top-down (Break down)and Bottom-up (Build up)approach.

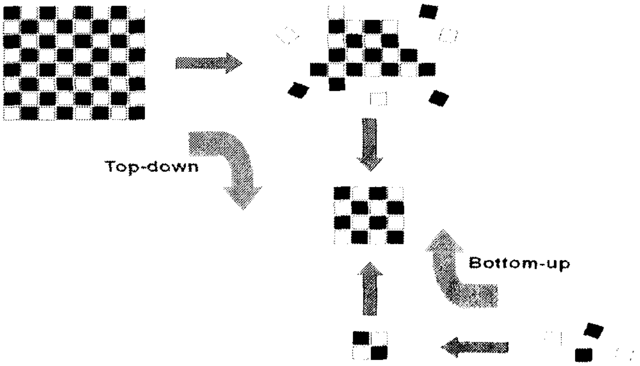


Fig. 2.17: Top-down and Bottom-up Fabrication of Nano-particles

Nano particles exhibit very different properties as compared to their bulk counterparts in respect of Colour, solubility, surface area/volume ratio, mechanical, electronic and magnetic properties.

Nanotechnology find applications in diversified fields such as computing, data storage and communication, materials, manufacturing industry, health and medicine, energy, environment, transportation and space exploration. Nano science and nano technology has spread its wings in almost every sphere of human activity and development. Herein, presented a few selective examples based on inorganic components to highlight the importance of nano materials.

Nano particles are very efficient catalyst. Increased surface area means more efficient chemical reactions when used as a catalyst. One such example is "Micro-carnation". This 'micro-carnation' is actually a catalyst containing phosphorus and vanadium ($V(OH)PO_4.½H_2O$). It is a selective catalyst used in a natural gas purifying process (changing normal butane into maleic acid anhydride). Another selective example is molecular filters. Large molecules are generally a few nanometers in size. They can act as very efficient filters selectively allowing the passage of some substances while blocking others. A network of these can act as a molecular filter and catalyst.

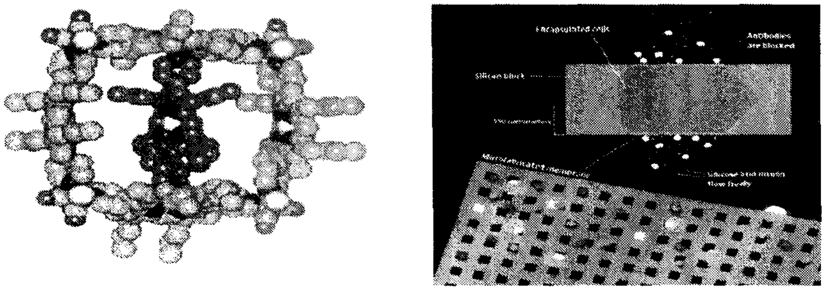


Fig. 2.18: Nano-particles as they act in Cells

Even nano-sized pores are useful! For example, transplanted pancreatic cells shielded in a silicon block having pores so small that they allow glucose and insulin to flow through freely allowing normal metabolism, but are too small to allow antibodies to enter and attack the cells. The example of use of metal oxide nano particle is antibacterial Magnesium Oxide Nanoparticles.

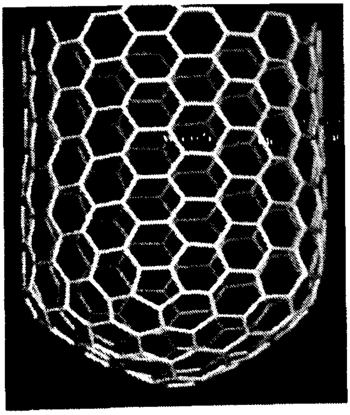


Fig. 2.19: A Nano Tube

A very promising and versatile nano material is carbon nanotubes discovered in 1990. Carbon nanotubes are the best example of novel nanostructures fabricated through bottom up chemical synthesis approaches. They have simple chemical composition and atomic bonding but exhibit remarkable diverse structure and unparalleled physical properties. These novel materials find application as sensor, fuel cells, field effect transitors, electrical interconnects and host of others. Carbon nanotubes are the strongest and most flexible molecular material, has high thermal conductivity, very good electrical conductivity, can be metallic or semiconducting depending on structure and can be chemically modified.

One representative example of use of carbon nanotubes in bio sensor is carbon nano tube based DNA sensor.

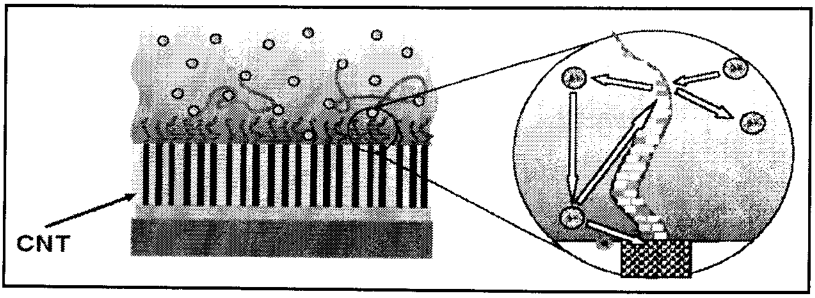


Fig. 2.20: Sample of Carbon Nano Tube

Carbon nanotube (CNT) array with attached DNA probe acts as an ultrasensitive sensor for detecting the hybridisation of target DNA from the sample using signals from the redox bases in the excess DNA single strands. The signal is amplified using metal ion mediators- oxidation of $[Ru(bpy)_3]^{2+}$ by guanine.

Now a day, one can even write nano letters: The technique is called dip pen nanolithography. One molecule thick letters are written on the surface of a piece of gold using a molecule called octadecanethiol as the ink. It is then visualized using an atomic force microscope.

The science and engineering of nanotechnology began to take shape in latter part of the twentieth century. The areas of nanoscience and technology are a multidisciplinary and well diverse in scope. The foregoing discussion highlighted the importance of chemistry in multidisciplinary research directed towards understanding nanomaterials.

In this presentation, an effort has been made to highlight certain aspects of development and applications based on inorganic materials in various domain of human welfare.

The discussions is non exhaustive, only certain selective aspects have been highlighted to provide an idea of contribution of inorganic chemistry in development of science and technology for the benefit and welfare of mankind.

References

1. Shriver & Atkins', Inorganic Chemistry, - Atkins, P., Overton, T., Rourke, J., Weller, M. and Armstrong, F., 5th Edn., Oxford University Press, 2012.
2. Hazen, R. M., "The Breakthrough: The Race for the Super Conductors.", Sumit Books ,New York ,1988.
3. Hampton, M. D., Schur, D.V., Zaginaichenko, S. Yu and Trefilov, V. I., "Hydrogen Materials, Science and Chemistry of Metal Hydrides", Kluwer Academic Publishers, Dordrecht, 2002.
4. Zuttel, A., Borgschulte, A. and Schlapbach, L., "Hydrogen as Future Energy Carrier", Wiley, VCH, Weinheim, 2008.
5. Day, P., "Molecules in Materials: Case Studies in Materials Chemistry" , World Scientific Publishing, Singapore, 2007.
6. P. Ball, "Made to Measure: New Materials for 21st century", Princeton University Press, Princeton,1997.
7. West, A. R., "Basic Solid State Chemistry", Wiley, Newyork, 1998.
8. Yao, H.B., Gao, M. R. and Yu , S. H., Nano Scale, 2010, 2, 323.
9. Rao, C. N. R. Chetham, A. K. and Thirumirugan, A., J. Phys : Condensed Matter ,20, 2008, 083202.
10. Chethm, A. K. Rao, C. N. R. and Feller, R. K., Chem. Commun.,2006, 4780.
11. D. N. Bruce and D. O'Hare, Inorganic materials, Wiley, New York, 1997.
12. Lee, C., Wei, X. D., Kysar, J.W. and Hone, J., Science 2008, 321, 385-388.
13. Stoller, M. D. S., Park, J. Zhu, Y. W. An, J. H and Ruoff, R. S., Nano Lett. , 2008, 8, 3498-3502.
14. Geim, A. K. and Novosselov, K.S., "The Rise of Graphene", Nature Materials, 6(3),183-91,2007.
15. Sakamoto, J. et. al., Angew. Chem. Intl. .Ed., 48(16), 1030-69, 2009.
16. Geim, A., "Graphene : Status and Prospects", Science, 324, (5934) ,1530, 2009.
17. "Carbon Wonderland", Scientific American, April, 2008.
18. Gao, Y., Karasawa, N. and Goddard, N. A., Nature, 351, 264,1991.

3

Quantum Mechanical Treatment of Non-classical Spinning Motion: NMR Spectroscopy

Ambikesh Mahapatra

Abstract

MRI or Magnetic Resonance Imaging is a useful diagnostic tool for in medical sciences. The commercial MRI machine is principled on Magnetic resonance spectroscopy (Electron Spin Resonance or ESR and Nuclear Magnetic Resonance or NMR) and is a blessing to our society, and to mankind.

Spinning motion, observed in visible objects of the macroscopic/ classical world, is purely a non-classical concept. In classical mechanics there are mathematical tools to handle angular motion with associated characteristic properties like angular displacement (θ), angular speed (ω), moment of inertia (I), rotational energy (Erot) and angular momentum (L) and its direction. But we have no such tools in hand to handle spinning motion in classical mechanics. Quantum mechanics provides us a tool to handle the spinning motion of electron, nucleon (proton & neutron) particles of microscopic/ quantum world. Ceaseless spinning motion of quantum particles (electron, proton, and neutron) in atoms produces Spin Angular Momentum with associated current strength and finally magnetic dipole moment with magnetic field. Applied external magnetic field interacts with the magnetic field created by spinning motion of electron/nucleon particles in atom/ molecule. Degeneracy of spin energy levels of the nuclei/electrons is thus lost. This sets the stage for magnetic resonance spectrum.

Keywords: Quantum Mechanics, NMR, Angular Momentum, Magnetic Field, Larmor Precision, Resonance, Diagnostics.

Introduction

Down the Memory Lane of Development of Quantum Mechanics

Angular motion: In the development of Newtonian Mechanics/ Classical Mechanics scientific community knew the characteristic properties of angular/ circulatory motion with its mathematical tools like angular displacement (θ), angular speed ($\omega \equiv \theta/t$), moment of inertia ($I \equiv \Sigma m_i r_i^2$), angular momentum ($L \equiv I\omega$) with its direction and rotational energy ($E \equiv I\omega^2/2$ or $L^2/2I$). However even though people observed and knew spinning motion, they did not know any mathematical tools to quantify its characteristic properties in Classical Mechanics.

Failure of Classical Mechanics to Explain the Experimental Findings is a Dawn of Quantum Mechanics:

1. In 1885, Secondary School teacher Johann J Balmer published emission spectrum of H-atom which was discontinuous line spectrum in opposition to the expected continuous spectrum. Later Lyman, Paschen and Bracket independently published the same i.e., emission spectrum of H-atom which was discontinuous line spectrum in opposition to the expected continuous spectrum.
2. In 1897, Joseph J Thomson discovered sub-atomic negatively charged particle electron and bagged a Nobel Prize in Physics in 1906.
3. In 1900, Max K E L Planck explained successfully the energy distribution curves of Black Body radiation at different temperatures assuming a quantum hypothesis that energy of an oscillator in solid state is an integral multiple of small amount of energy which is equivalent to ($h\nu$) where h is fundamental constant having dimension of angular momentum and very small value of 6.626×10^{-34} J s, named as Planck constant and ν is frequency of oscillator. For this, Planck was honored with Nobel Prize in Physics in 1918. Before 1900, many scientists namely, Lord Rayleigh, Sir James Jeans, W Wien, Stefan, Boltzmann tried independently or jointly to explain the same using classical concepts but repeatedly failed all the times.
4. In 1905, Albert Einstein successfully explained all the observations of photoelectric experiment using particle or photon concept of light beside the well established wave property of it. Thus light energy is also quantized or discrete and should be an integral multiple of photon energy ($\equiv h\nu$) where ν is frequency of light or electromagnetic radiation. Einstein also bagged the Nobel Prize in Physics in 1921.
5. In 1913, Neils H D Bohr proposed postulates for electronic energy of H-atom, assuming one quantum postulate {angular momentum of rotating electron around the nucleus is an integral multiple of ($h/2\pi$)} and successfully

explained the Balmer, Lyman, Paschen and Bracket emission spectra of H-atom. Bohr was also honored with Nobel Prize in Physics in 1922.

6. Louis de Broglie proposed wave-particle duality with a relation, $\lambda = h/p$, from different experimental findings.
7. Warner Heisenberg projected the uncertainty principle to determine simultaneous values of some properties associated with each other for moving particles.
8. In 1927, George P Thomson discovered wave property of particle electron with Davisson and was honored with a Nobel Prize in Physics in 1937.

With these quantum theories, especially applicable for subatomic/ molecule/ atom/ microscopic particles, quantum mechanics developed. By this time, we came to know about the orbital motion of electron around the nucleus and the associated quantized electronic energies, quantized orbital angular momentum and its quantized direction in space. Then Theoretical Physicist **Erwin Schrödinger** predicted one spectral line at around **590** nm for atomic spectrum of Sodium but observed two closely spaced lines (doublet) at **589.59** & **588.99** nm. In 1925, to explain this doublet spectral lines young Dutch Physicists **George Uhlenbeck** and **Samuel Goudsmit** suggested an electron behaves like a spinning top having quantized z-components of spin angular momentum or S_z is $(+½)\hbar$ or $(-½)\hbar$.

In quantum mechanics, for orbital motion, orbital angular momentum (**L**) has an operator and corresponding quantum mechanical expression of **L**. Similarly **L** has its z-component operator and corresponding quantum mechanical expression of $\mathbf{L}_z$. *For spinning motion of electron, in early 1930s, the English Physicist **Paul Dirac** simply extended the quantum theory of orbital motion of electron to its spinning motion.* Like orbital motion, spinning motion of electron has an operator $\hat{S}$ and corresponding quantum mechanical expression of **S**. Similarly **S** has its z-component operator and corresponding quantum mechanical expression of $\mathbf{S}_z$. However, we do not know the exact form of operators $\hat{S}$ & $\hat{S}_z$ and exact expressions $\alpha(\tau)$ & $\beta(\tau)$ along with their co-ordinates. But $\hat{S}$ & $\hat{S}_z$ are linear & Hermitian operators and $\alpha(\tau)$ & $\beta(\tau)$ are well behaved, orthonormal wave functions for $\boldsymbol{\alpha}$ & $\boldsymbol{\beta}$ spin states of electron.

An electron is a charged particle, so its intrinsic spin confers to it, the property of magnetic dipole moment. Like electron, nuclei have also an intrinsic spinning motion, spin angular momentum (**I**) and associated magnetic dipole moment ($\boldsymbol{\mu}$). Where spin angular momentum,

$$\boldsymbol{I} = \sqrt{\boldsymbol{S}(\boldsymbol{S}+1)}\hbar$$

(S = total spin no. of nucleus and has a single value for a particular nucleus) and z-component spin angular momentum, $I_z = m_I \hbar$

m_I = -S, (-S+1), ... ,-1, 0, +1, ... , (S-1), S: for S = integer

m_I= -S, (-S+1), ... ,-½, +½, ... , (S-1), S: for S = half of an integer

Like orbital motion, there is a relationship between **μ** & **I** for the spinning motion of nucleus. This is

$$\mu = g\left(\frac{q}{2m_N}\right)I = g\left(\frac{q}{2m_N}\right)\sqrt{S(S+1)}\hbar$$

For ^{1}H nucleus (a" proton)

$$\mu = g\left(\frac{e\hbar}{2m_p}\right)\sqrt{S(S+1)} \equiv g\mu_N\sqrt{S(S+1)}$$

On application of external magnetic field........

External magnetic field interacts with the magnetic field produced by spinning motion of nucleus, thus lifting the degeneracy of different spin states. As a result, an energy gap ("V) is produced, the value of which depends on the external magnetic field strength (**B**). "V = $g\mu_N$**B** and frequency of electromagnetic radiation equivalent to energy gap, is $\nu = (g\mu_N/h)\mathbf{B}$.

Energy difference between two energy levels can also be expressed in units of angular frequency, ω. $\omega\ (rad\ s^{-1}) = 2\pi = 2\pi(g\mu_N/h)\mathbf{B} = \gamma\mathbf{B}$.

In presence of applied external magnetic field, the magnetic moment (**μ**) associated with spinning motion of nucleus experiences a coupled force or torque which tends to align **μ**-vector along the direction of applied magnetic field i.e., z-direction (say). However this is ***not possible at all because of quantum mechanical restriction***. The only other effect of this force (or torque) that remains is to rotate the **μ**-vector around the field direction (z-axis) all the while keeping its orientation angle θ, as constant for quantum mechanical restriction. This type of rotation is known as ***Larmor Precession***. According to classical mechanics, the frequency of ***Larmor Precession*** or ***Larmor frequency*** (in rad s^{-1}) is

$$\omega_{\text{Larmor}} = \frac{\boldsymbol{magnetic\_moment}}{\boldsymbol{angular\_momentum}} \times \boldsymbol{B}$$

$$= \frac{\mu}{I}\boldsymbol{B} = \frac{g\mu_N\sqrt{S(S+1)}}{\sqrt{S(S+1)}\hbar} \times B$$

$$= \frac{g\mu_N}{\hbar} B = \gamma B$$

Hence,

ωLarmor = ωRadiation or νLarmor = νRadiation

i.e., Resonance which leads to Nuclear Magnetic Resonance Spectroscopy or NMR Spectroscopy.

Advancement of NMR Spectroscopy using quantum mechanics and other related advancement provides the following information about the molecule-

1. .No. of spectral lines in NMR spectrum depend on how many different environments of ^{1}H nuclei are present in the molecule.
2. Intensity of spectral lines depends on how many ^{1}H nuclei are present in each environment.
3. Multiplicity of spectral lines depends on how many neighboring ^{1}H nuclei are present for each H in the molecule.

Development of NMR Spectroscopy and its application

- The NMR spectroscopy utilizes EM radiation in the radio frequency region (lowest frequency region) of electromagnetic spectrum.
- In 1936, Gorter first predicted NMR spectrum.
- In 1946, Block *et al* and Purcell *et al* first detected the NMR spectral line.
- In 1949, it was first applied to solving a chemical problem.
- In 1953, first commercial NMR spectrometer was developed.
- Since then several revolutionary developments have happened.

In early 1970s high resolution technique (in the detecting device) based on FT principle developed that helped:

- To study multiple nuclei by one spectrometer
- To obtain high resolution spectra of solid samples
- For 2-D NMR
- For MRI

The above developments became possible rapidly because of:

- ✓ Use of high magnetic field strength (using super conducting magnet)
- ✓ Improved electronics & designing
- ✓ Advancement of computer & spin physics
- ✓ Urge to solve biological problems including large molecules
- ✓ Use of pulse radiation

Development of Quantum Mechanics gave tools for studying spinning motions, with their related properties, especially spin angular momentum and magnetic dipole moment—application of external magnetic field—interaction of it with the magnetic field of spinning nuclei—splitting of degenerate energy states—stage is set for NMR spectrum—advanced to Magnetic Resonance Imaging (MRI)—helps in diagnostics of various ailments.

4

Computer Assisted Chemistry - A New Application in Service for Mankind

Apurba K. Bhattacharjee

Abstract

The role of Chemistry in service to mankind cannot be described in nutshell. It plays such an important and integral part of our life that no numbers can short list its functions. The present article discusses an application of Computers in Chemistry known as Computer Assisted Molecular Modeling (CAMM) specifically focusing on the discovery and development of chemotherapeutics as drugs for human use and welfare. Although a relatively new technology, application of computers in Chemistry has been playing a remarkable major role in the service and welfare of mankind. The role of virtual screening of virtual libraries (compound databases), structure based drug discovery and development including the role of pharmacophore will be discussed.

Key words: *Computer-Assisted Molecular Modeling(CAMM), drug-target docking, drug discovery, Pharmacophore, Norepinephrine Transporter (NETa).*

Introduction

Chemistry, the science of matter has served mankind since time immemorial. Over ages, human beings have skilfully used a variety of techniques and methods to extract potential applications from matter. This way, from the earliest primitive tools like knives, arrows, spears to modern day chemicals including computer chips, nano-materials, soaps, cosmetics, detergents, exotic new chemical entities, fertilizers, pesticides, medicines as well as chemical weapons and nukes are all contributions of skilful application of Chemistry for human use. However, the present discussion will be on the application of Chemistry for human welfare, particularly focusing on chemotherapeutics.

Use of computers in Chemistry, commonly known as the *in silico* approach is relatively a new technique to extract information about matter for a multitude of applications. Although *in silico* techniques are recent applications in Chemistry, the methods have made enormous progress to impact human welfare by serving remarkably well in past few decades. Specifically, the branch of Computer-Assisted Molecular Modeling (CAMM) has made significant contribution in mechanistic drug design and discovery of novel bioactive chemicals [1-3]. In late 1990s, combinatorial chemistry coupled with high-throughput screening shifted most of the drug discovery attention from age old chemical structure based synthesis and *in vitro* tests. However, in recent years, due to genome mapping and large scale determination of protein structures, new chapters have opened up for chemotherapeutic drug discovery processes. The new paradigm starts from active site analysis of structure-based crystallographic protein structure to design and identify new compounds as potential therapeutics through virtual (computer based) screening of compound databases. The procedure has revolutionized the drug discovery programs in pharmaceutical industries. Computational approaches commonly used in CAMM include ligand (compound)-based molecular design (specifically, 3D pharmacophore modeling to identify the arrangement of chemical features, such as hydrogen bonds, hydrophobic sites, and ionizable sites that are essential for a specific biological activity of a molecule), structure-based molecular design for known X-ray crystallographic structure of a target protein (drug-target docking), and QSAR/QSPR (quantitative structure-activity relationship and quantitative structure-property relationship) studies. CAMM techniques provide five major types of molecular information that are useful for mechanistic design of new bioactive chemical entities. The information can be summarized as: (1) three-dimensional structure of a molecule, (2) physio-chemical properties and arrangement of chemical features of a molecule, (3) comparison of molecular structures and properties, (4) graphical visualization of stereo-electronic (three dimensional arrangement of atoms along with electron distribution in molecules) properties and complexes formed between the bioactive molecule and the target protein, and (5) predictions of how a related molecule matches with the new ones and an estimate or prediction of activity for each of them.

Developments and Discussion

In this article as an example for application of CAMM, I have discussed only the *in silico* applications to chemotherapeutic drug discovery and development process. Discovery and development of a new chemical entity (NCE) as a drug are expensive and complex processes with ever changing technologies. On an average, it takes about 10 years and approximately five hundred million to a billion U.S. dollar to bring a drug from the bench of discovery to the market for human use [2, 4] (Figure 4.1).

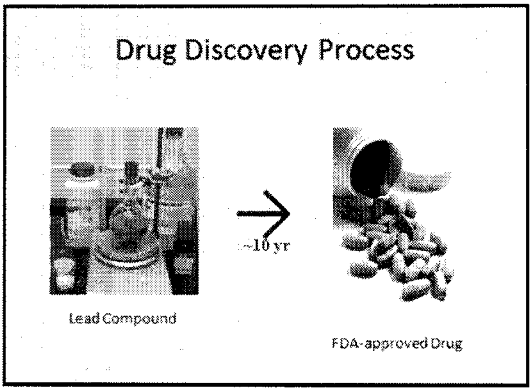


Fig. 4.1: Drug Discovery Process

Drug discovery involves multiple steps of laborious and stringent regulatory efforts that may be summarized in Fig. 4.2. Toxicity evaluation of a new potential drug candidate is considered the key component for development of a drug before human trial. In this context, computer aided toxicity evaluations are also useful. These methods are rapidly becoming more advanced, reliable and far less expensive than experimental toxicity evaluations. On an average, discovery process of a drug costs one-third of the total cost whereas the developmental phase takes away two-third of the total cost before marketing. When a drug is marketed, only three in ten achieve revenue greater than the development costs [5].

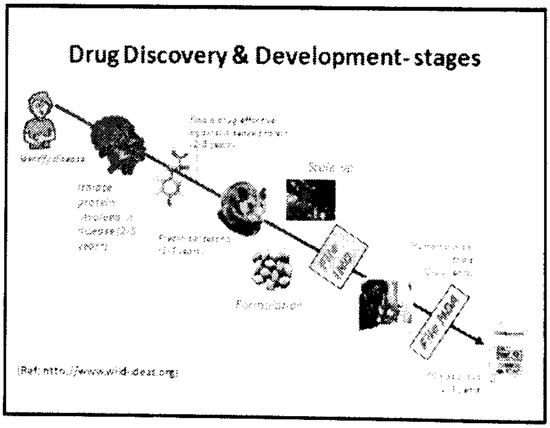


Fig. 4.2: Drug Discovery and Development Stages

Therefore, any technology that can improve the efficiency of the process is considered highly valuable to the pharmaceutical industry.

With the advent of high speed modern computers, astronomical memory and graphic tools, accomplishing computations and visualization of small to large bio-molecules including proteins have become more efficient with greater precision. The graphic tools in modern computers have not only made visualization of three-dimensional structures of large protein molecules possible, but allowed interactive virtual docking experiments between a potential drug molecule and the binding sites of a protein. Applications involving molecular modeling or in silico techniques have now become an integral part of basic science research and studies that require an understanding of molecular level information about environmental, biochemical, and biological processes. The current advances in these methodologies have direct applications ranging from accurate ab initio quantum chemical calculations of stereo-electronic properties [6], generation of three-dimensional pharmacophores, and performance of database searches to identify bioactive agents [7].

Increasing costs for pharmaceutical development have resulted in the emergence of these computational approaches for improving the efficiency of the process. Among them, in silico screening or virtual screening of databases to identify potential new compounds has been remarkably successful [7, 8,]. Virtual screening is a process of intelligent use of computing to analyze large databases of chemical compounds to identify potential drug candidates. The process can serve as a complimentary tool to high-throughput screening (HTS) for rapid and effective experimental assay for a large pool of compounds. Screening compounds by this method is essentially a knowledge-based approach and thus implicitly requires certain information about the nature of the receptor binding site or the nature of ligand that is expected to bind effectively at the active site. However, the type of procedure followed in virtual screening for compound databases depends upon availability of information as input and requirement for the output [7, 8].

Although active site analysis of crystallographic protein structure to design and identify new ligands as potential therapeutics can be very useful for drug discovery but when the target structure of a protein is not known (i.e., crystallographic structure not yet determined), pharmacophore based methods are found to be quite effective. Pharmacophore is defined as "an ensemble of steric and electronic features that are necessary for optimal interaction with a specific receptor target structure (a protein or an enzyme) to trigger or inhibit its biological response" [9, 10]. It is an abstract concept but can be geometrically represented as a distribution of chemical features in a three dimensional (3D) space. The common chemical features are hydrogen bond acceptors & donors, aliphatic & aromatic hydrophobic sites, ring aromaticity, and ionizable sites that interact with complementary sites of a target structure. Pharmacophore based methods are not only useful for virtual screening and identification of new compounds from databases but also provide useful insight for designing novel compounds as well as complementarity for binding at the active sites of unknown target structures [11-15]. It is both an intelligent knowledge based as well as efficient approach for discovery of potential bioactive compounds. Since pharmacophore transcends chemical structure and captures only the features, pharmacophore based method has an additional advantage for identification of new chemical compounds of new classes or chemo-types.

A few pharmaceutical success stories involving computational chemistry technologies include, ARICEPT for Alzheimer's disease (Eisai), TRUSOPT for glaucoma treatment (Merck), CRIXIVAN for AIDS (Merck), VIRACEPT for AIDS (Lilly and Agouron), and ZOMIG for migraine treatment (Wellcome, Zeneca). The list is ever growing. Although in silico technologies started developing from the mid-1980s, rapid growths started to emerge only with the advent of powerful modern computers. Since a drug takes approximately 10 to 12 years from the discovery stage to the market, we are just beginning to see the success stories [4].

Pharmacophore models have led to the discovery of XEN2174 compounds as Norepinephrine Transporter (NETa) and are in phase II clinical trials as pain killer [16]. Pharmacophores have been developed for screening growth factor (ALK5) receptor inhibitors [17], Checkpoint1 kinase inhibitors by retrieving potent molecules from databases, such as Specs, NCI, Maybridge, and CNPD [18]. For prediction of juvenile hormone antagonists, pharmacophore models have been generated from 3D QSAR studies [19].

Although a CAMM model can be built solely from structure-activity relationship, the model should be consistent with the observations of X-ray crystal structure if the target structure is known. For an unknown receptor structure, pharmacophore based methods could be helpful to predict the complimentary interaction capabilities in the active site of the target. However, it is important to note that despite perfect mapping onto the model, there may be many short listed compounds that may show poor experimental activity. Thus, although the pharmacophore directed 3D database search is an efficient approach for extracting potential bioactive compounds, it is necessary to experimentally test the identified compounds and iteratively refine the model. Perfect mapping of any molecule to the pharmacophore model does not guarantee its experimental activity despite reflecting receptor complementarity. There may be several factors lacking, such as the perfect fit to the active site due to steric hindrance, electrostatics, lipophilicity, and other unforeseen parameters. Nonetheless, pharmacophore models are useful tools for identification of new compounds. Undoubtedly, computers in Chemistry can maximize the efficiency of chemotherapeutic drug discovery.

Conclusion

Finally, without rating any of the above mentioned computational methods, it may be concluded that an integration of structure-based methods, pharmacophore modeling, virtual screening, and combinatorial chemistry could all together play a better basis for more efficient drug discovery and development by reducing both costs and time.

References

1. Kapetanovic, I. M., Computer-aided Drug Discovery and Development *(CADDD): in silico-chemico-biological approach., Chem. Biol. Interact.* , 2008, 171(2), 165-176.
2. Janseen, D. The Power of Prediction, Drug Discov. 2002, pp. 38.
3. Podlogar, B. L., Muegge, I., and Brice, L. J., Computational Methods to Estimate Drug Development Parameters, *Curr. Opinion Drug Disc.*, 2001, 12, 102-109.
4. Kubinyi, H., *Success Stories of Computer-aided Design, in Computer Applications in Pharmaceutical Research and Development*, S. Ekins and B. Wang, eds., Wiley-InterScience, New York 2006, pp. 377-424.
5. Bleicher, K. H., Bohm, H. J., Muller, K., and Alanine, A. I., Hit and Lead Generation Beyond High-throughput Screening, *Nat. Rev. Drug. Discov.*, 2003, *2*(5), 369-378.
6. Bhattacharjee, A. K. and Karle, J. M., Molecular Electronic Properties of a Series of 4-quinolinecarbinolamines define Antimalarial Activity Profile, *J. Med. Chem.*, 1996, 39, 4622.
7. Walters, W. P., Stahl, M. T. and Murko, M. A., Virtual Screening - An Overview, *Drug Discov. Today*, 1998, 3, pp. 160-178.
8. Lyne, P. D., Structure-based Virtual Screening - A Review, *Drug Discov. Today, 2002*, 7, pp. 1047-1055.
9. Leach, A. R., Gillet, V. J., Lewis, R. A and Taylor, R., Three-Dimensional Pharmacophore Methods in Drug Discovery, *J. Med. Chem.*, 2010, 53, pp. 539-558.
10. Güner, O. F. (ed.), *Pharmacophore, Perception, Development, and Use in Drug Design*, University International Line (IUL Biotechnology Series), San Diego, 2000.
11. Bhattacharjee, A. K., Hartell, M .G, Nichols, D. A., Hicks, R. P., Stanton, B., van Hamont, J. E. and Milhous, W. K., Structure-activity Relationship Study of Antimalarial Indolo [2,1-b]quinazoline-6,12-diones (tryptanthrins). Three Dimensional Pharmacophore Modeling and Identification of New Antimalarial Candidates, *Eur. J. Med. Chem.*, 2004, 39, pp. 59-67.
12. Bhattacharjee, A.K., Kuèa, K., Musilek,K. and Gordon, R.K., In Silico Pharmacophore Model for Tabun-inhibited Acetylcholinesterase (AChE) Reactivators: a Study of their Stereoelectronic Properties, *Chem. Res. Toxicol.* , 2010, 23, pp. 26-36.

13. Bhattacharjee, A. K., Gordon, J. A., Marek, E., Campbell, A. and Gordon, R. K., 3D-QSAR studies of 2,2-diphenylpropionates to Aid Discovery of Novel Potent Muscarinic Antagonists, *Bioorg. & Med. Chem.*, 2009, 17, pp. 3999–4012.
14. Bhattacharjee, A. K., Marek, E. Le, H. T. and Gordon, R. K., Discovery of Non-oxime Reactivators using an in silico Pharmacophore Model of oxime reactivators of OP-inhibited Acetylcholinesterase, *Eur. J. Med. Chem.*, 2012, 49, pp. 229-238.
15. Bhattacharjee, A. K., Marek, Le, E. H. T. Ratcliffe, R., DeMar, J. C., Pervitsky, D. and Gordon, R. K.., Discovery of Non-oxime Reactivators using an in silico Pharmacophore Model of Oxime Reactivators of OP-inhibited Acetylcholinesterase., *Eur. J. Med. Chem.*, 2015, 90, pp. 209-220.
16. Brust, A., Palant, E., Croker, D. E., Colless, B., Drinkwater, R., Patterson, B., Schroeder, C. I., Wilson, D., Nielson, C. K., Smith, M. T., Alewood, D., Alewood, P. F. and Lewis, R. J., ã-Conopeptide pharmacophore Development: toward A Novel Class of Norepinephrin Transporter Inhibitor (Xen2174) for Pain., *J. Med. Chem.*, 2009, 52, pp. 6991–7002.
17. Ren, J. X., Li, L. L., Zou, J., Yang, L. Yang, J. L. and Yang, S. Y., Pharmacophore Modeling and Virtual Screening for the Discovery of New Transforming growth factor b type I Receptor (ALK5) Inhibitors, *Eur. J. Med. Chem.*, 2009, 44, pp. 4259–4265.
18. Chen, J. J., Liu, T. L., Yang, L. J., Li, L. L., Wei, Y. Q. and Yang, S. Y., Pharmacophore Modeling and Virtual Screening Studies of Checkpoint kinase 1 Inhibitors., *Chem. Pharm. Bull.*, 2009, 57, pp. 704–709.
19. Liszekova, D., Palacovicova, M., Beno, M. and Farkas, R., Molecular Determinants of Juvenile Hormone Action as Revealed by 3D QSAR Analysis in Drossophila, *Plos One*, 2009, 4, pp. 1–15

5

Liquid Crystal - Nanoparticle (LCNP) Hybrid Soft Material - A Novel Supramolecular Self Assembly

Chira R Bhattacharjee

Particles of sizes in the range of 1-100nm are called nanoparticles irrespective of whether they are dispersed in gaseous, liquid or solid medium. The properties of nanometer dimension material are significantly different from those of atoms and bulk materials in terms of colour, conductivity, melting points, strength etc. It would be quite educative to glance at the Fig.5.1 for a practical idea of sizes of different material *vis-à-vis* nanomaterial.

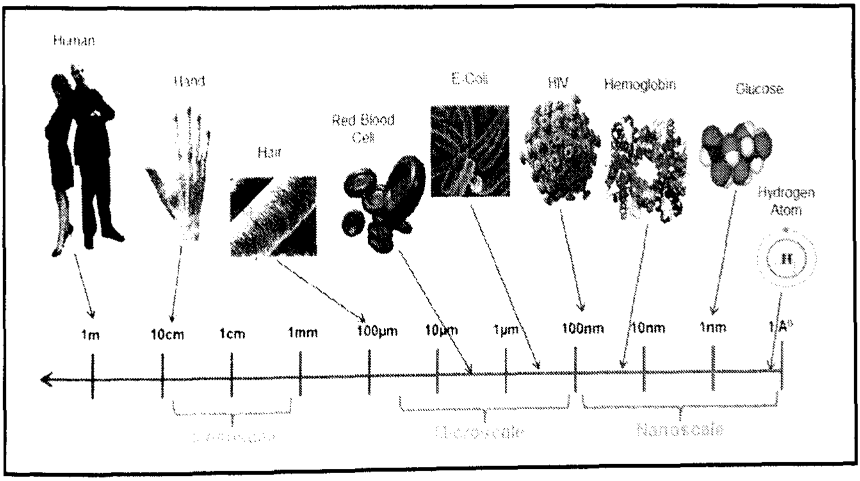


Fig.5.1: Size Dimension of Some Materials

Some nanomaterials occur naturally, but engineered nanomaterials (EN) are of particular interest, which find applications in many commercial products and processes. These materials have been applied for the preparation of sunscreens, cosmetics, sporting goods, stain-resistant and wrinkle free clothing, tyres, electronics, and many other items of everyday use. These materials are also used in medicine for diagnosis, imaging and drug delivery. Properties at the molecular (nanometre) level due to their small size are generally not encountered in their conventional, bulk counterparts. The two main reasons why materials at the nano scale differ in properties are increased relative surface area and new quantum effects. The field of nanoscience is rapidly growing providing access to number of structural and functional materials, both inorganic and organic, permitting manipulation of mechanical, electric, magnetic, catalytic, optical and electronic functions.

Quite apart from nanomaterials, another group of material called liquid crystal, a partially ordered state intermediate between ordinary liquid and solid is a well established field of research currently enjoying tremendous attention due to their potential in multifaceted application in various fields. Liquid crystal may flow like a liquid, but its molecules may be oriented in a crystal-like way (Fig. 5.2). The liquid crystal state is a distinct phase of matter observed between the crystalline (solid) and isotropic (liquid) states. There are many types of liquid crystal states, depending upon the amount of order in the material. A large number of chemical compounds are known to exhibit one or several liquid crystalline phases. Despite significant differences in chemical composition, these molecules have some common features in chemical and physical properties. There are three types of thermotropic liquid crystals: discotics, bowlics and rod-shaped molecules.

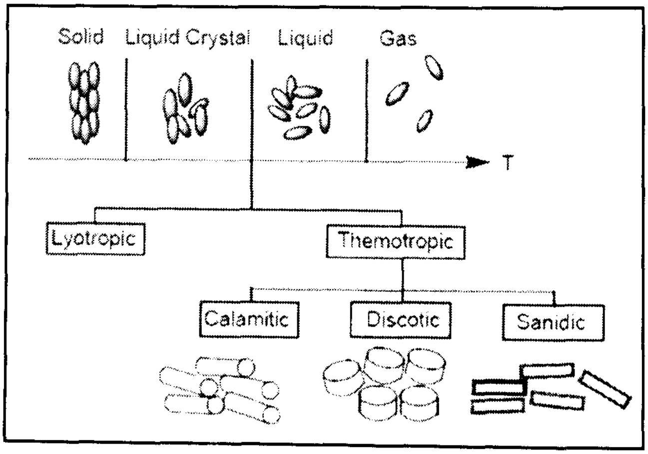


Fig. 5.2: Orientational Order of Molecules in a Liquid Crystal

Discotics are flat disc-like molecules consisting of a core of adjacent aromatic rings; the core in a bowlic is not flat but like a rice bowl (a three-dimensional object). This allows for two dimensional columnar ordering, for both discotics and bowlics. Rod-shaped molecules have an elongated, anisotropic geometry which allows for preferential alignment along one spatial direction. Perhaps the most ambitious goal of this field of endeavour is the creation of molecular machines and devices, with dimensions on the order of atoms and molecules, capable of performing specific predetermined tasks [1].

Interests in nanoparticle science are ever expanding which originate from the desire to understand the synthesis and fundamental properties [2-4] and the myriad of potential applications of these unique species [5]. Fields as diverse as biology and medicine [6,7], optics and electro-optics [8,9], catalysis [10], and environmental remediation [11] are expected to benefit from the unique properties and promises offered by nanoparticles. In order to exploit the exciting properties of NPs, it is deemed necessary to incorporate them into a more complex structure, often through the use of a suitable organic coating, that imparts stability against aggregation and also generates other important features such as solubility, modularity, and optical and self-organisation properties.

Both nanoparticles and liquid crystalline material exhibit unique property of self assembly depending on different conditions and template. Liquid crystal-nanoparticle (LCNP) hybrids, a new emerging class of soft materials have drawn significant attention with enormous scope. The LC-nanoparticle hybrid systems allows for investigating a mutually beneficial combination of the unique properties emanating from the individual entities of LCs and nanoparticles. Liquid crystal-nanoparticle composites have significant importance for modulating nanoparticle properties using LCs as well as tuning the LC properties using NPs. Hybridisation of liquid crystals and nanoparticles provide novel materials that may be useful in many device applications such as photoconductors, light emitting diodes, photo-voltaic solar cells, thin film transistors etc. The present paper will dwell on the supramolecular self assembly of hybrid LCNP materials highlighting related work on synthesis of some novel hybrid materials with gold nanomaterials and graphitic nanomaterials from our laboratory.

Noble metal nanoparticles are of great interest because of their intense tunable absorption and scattering resonances caused by collective oscillations of the conduction band electrons, which are known as surface plasmons. The surface plasmon resonance maximum is very sensitive to the dielectric constant of the surrounding media. Thin metal films, gold nanoparticle arrays, metallic hole arrays, gold nanorods, and single gold nanosphereshave been dispersed in LC. The shift in the surface plasmon resonance has been exploited to measure the local orientation of the liquid crystal molecules. To afford such a composite material, metal nanoparticles is needed to be dispersed within a nematic liquid crystal. Composite materials consisting of liquid crystals doped with nanoparticles have attracted much scientific and technological interest, as the incorporation of nanomaterials enhances the electro-optical properties of the liquid crystal itself [12]. Ferromagnetic $Sn_2P_2S_6$ nanoparticles when suspended in a nematic liquid crystal host were found to enhance the dielectric anisotropy of the liquid crystal leading to a lower threshold voltage. In the following section we qualitatively discuss some of the cases of synthesis of novel LCNP hybrid material accomplished in our research group. One such case is the room temperature synthesis of 1-dodecane thiol stabilised Schiff base decorated gold nanoparticle. The alkane thiol performed two-fold role - to reduce the gold(III) to gold(0) while preventing the agglomeration of the NP formed. The thiol decorated GNP on treatment with the LC Schiff base ligand afforded the LCNP material (Fig. 5.3). Both Schiff base LC as well as Composite show blue light fluorescence at wavelength 442 and 470 nm when excited at 334nm and 394 nm, respectively. The emission intensity for the composite was found to be higher than that of bare LC and which may be due to inclusion of gold nanoparticles in the large matrix of Schiff base.

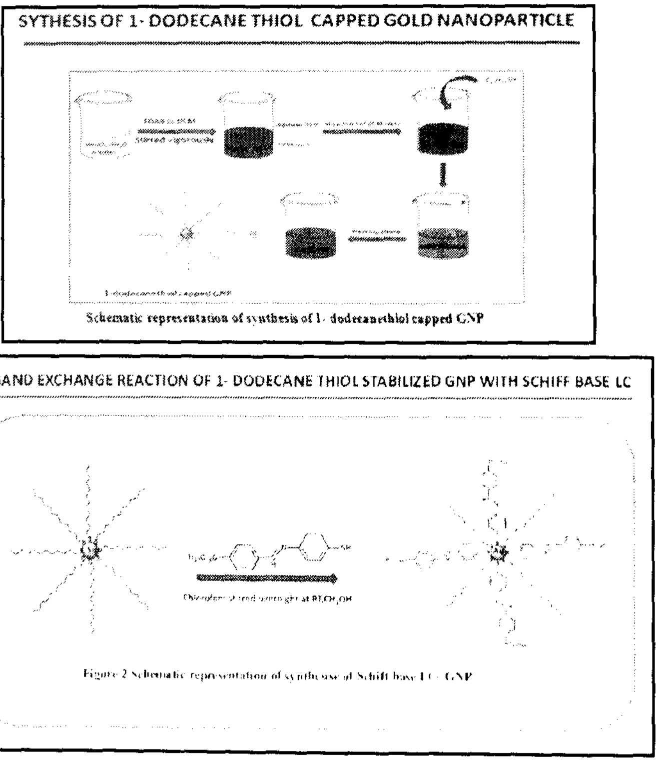


Fig. 5.3: GNP-LC Synthesis

Another interesting example is the ZnO quantum dot decorated with Schiff base liquid crystal (Fig. 5.4). The effect of variation in relative concentration of the ZnO QD and LC has also been studied. The white ZnO quantum dot material were prepared using a simple and convenient strategy wherein zinc acetate was treated with oleic acid at 140^{0}C followed by triphenylphosphine at 240^{0}C. The LCNP hybrid material was obtained as a yellow material simply by stirring the ZnO with preformed LC Schiff base ligand at room temperature.

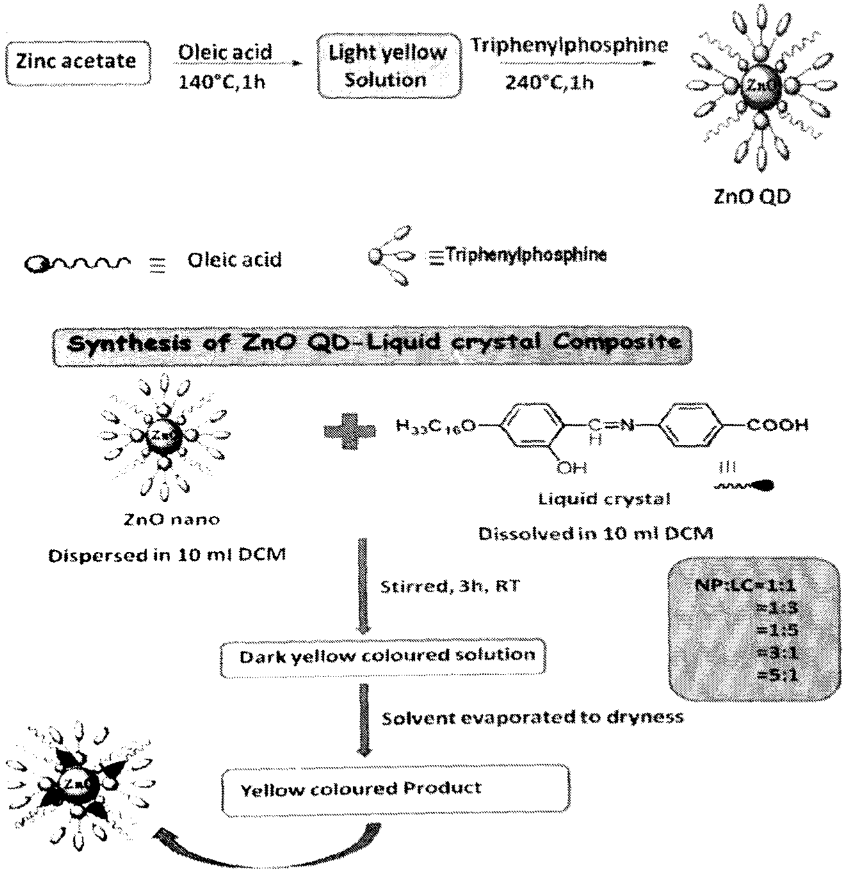


Fig. 5.4: ZnO QD Synthesis

Yet another example is of tagging graphene nanosheet with amino terminated LC molecules achieved simply by stirring at ambient temperature (Fig. 5.5 and Fig. 5.6). The phase transition temperature drops to by half in the composite from that in the bare LC molecule. The graphene oxide sheets are prepared by oxidising graphite.

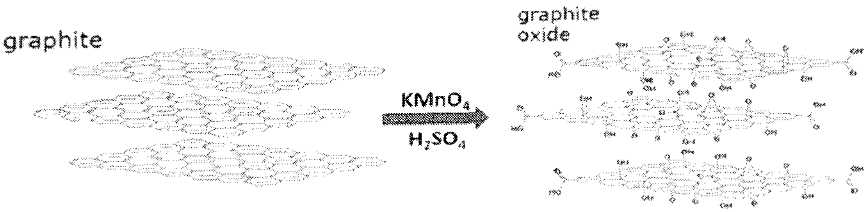


Fig. 5.5: Graphene Oxide Synthesis

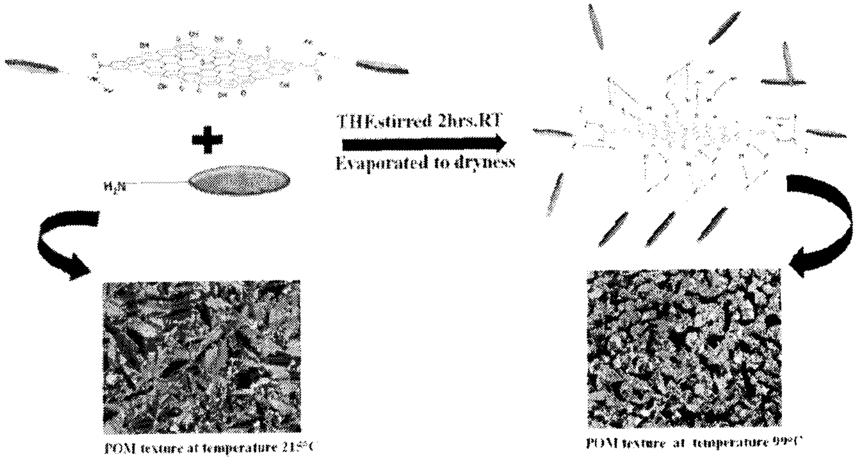


Fig. 5.6: Graphene Oxide - LC Synthesis

The field of LCNPs is wide open for chemists to design and synthesise new hybrids- wider variety of phases and new applications for these novel materials are anticipated. By controlling the spatial assembly of NPs into ordered structures and combining the processability and defect tolerance of the LC state, the structural and functional versatility of the organic coating, and the unique magnetic, optical and electrical properties of NPs, liquid-crystalline nanoparticles are poised to address some of the exciting challenges of this century. Preparation of addressable assemblies of multifunctional hybrids, including high-density recording media, single-electron microelectronic and charge transport devices, nanoscale plasmon waveguides and metamaterials throw many research challenges leading to newer high-end applications.

References

1. Balzani, V., Credi, A., and Venturi, M., Molecular Devices and Machines - A Journey into the Nano World; Wiley-VCH: Weinheim, Germany, 2003. doi:10.1002/3527601600.

2. Zabet-Khosousi, A., and Dhirani, A.-A., Chem. Rev., 2008, 108, 4072-4124. doi:10.1021/cr0680134.
3. Jun, Y.-w., Choi, J.-s., and Cheon, J., Angew. Chem., Int. Ed., 2006, 45, 3414-3439. doi:10.1002/anie.200503821.
4. Lu, A.-H., Salabas, E. L., and Schüth, F., Angew. Chem., Int. Ed., 2007, 46, 1222-1244. doi:10.1002/anie.200602866.
5. Goesmann, H., and Feldmann, C., Angew. Chem., Int. Ed., 2010, 49, 1362-1395. doi:10.1002/anie.200903053.
6. Kennedy, L. C., Bickford, L. R., Lewinski, N. A., Coughlin, A. J., Hu, Y., Day, E. S., West, J. L. and Drezek, R. A., Small 2011, 7, 169-183. doi:10.1002/smll.201000134.
7. Daniel, M.-C., and Astruc, D., Chem. Rev., 2004, 104, 293-346.doi:10.1021/cr030698+.
8. Haase, M., and Schäfer, H., Angew. Chem., Int. Ed., 2011, 50, 5808-5829. doi:10.1002/anie.201005159.
9. Talapin, D. V., Lee, J.-S., Kovalenko, M. V., and Shevchenko, E. V., Chem. Rev., 2010, 110, 389-458. doi:10.1021/cr900137k.
10. Schlögl, R., and Hamid, S. B. A., Angew. Chem., Int. Ed., 2004, 43, 1628-1637. doi:10.1002/anie.200301684.
11. Kaur, A. and Gupta, U. J., Mater. Chem., 2009, 19, 8279-8289. doi:10.1039/b901933b.
12. Khatua, S., Manna, P., Chang, Wei-Shun., Tcherniak, A., Friedlander, E., Zubarev, E R., and Link, S., J. Phys. Chem. C, 2010, 114, 7251-7257.

6

Arsenic Contamination in Groundwater: A Global Perspective with Special Emphasis on the Brahmaputra and Barak Valley Regions of Assam, India

Kali Prasad Sarma
Rajat Shubro Bose

Abstract

During the past two decennia, occurrence of high concentrations of arsenic (As) in drinking-water has been recognized as a major public-health concern in several parts of the world. Some of the well-documented and most severe cases of arsenic contaminated groundwater have been found in aquifers in Asia and South America. The problem is particularly severe in backwards and developing countries like India and Bangladesh where a large part of the population depends on groundwater extraction for their livelihood but do not have basic sanitation and purification facilities. *Besides*, a number of other States, namely; Jharkhand, Bihar, Uttar Pradesh and several states of NE India comprises the vast plains of the *Ganges* and *Brahmaputra* rivers and are underlain by thick piles of alluvial sediments of Tertiary and Quaternary age where groundwater is contaminated by As. The problem of groundwater As contamination from the fluvial plain of the Brahmaputra and the Barak is known to have affected 7 lakhs of population in 21 of the 27 districts of Assam which highlight the extent of the problem in the state. Newly deposited riverine Holocene sediments provide a very favourable environment for As enrichment in groundwater. In Assam, the Brahmaputra Flood Plains (BFP) and the Barak Valley Plains (BVP) are the two most important river systems which have given rise to extensive alluvial plains on which majority of the population of the state is dependent. In Assam, similar to West Bengal the source of arsenic is geogenic and released from soil under conditions conducive to dissolution of Arsenic from solid phase. In most of the high groundwater As areas in the two river plains elevated iron levels were detected and positive correlation was observed between the Fe and As. The reductive dissolution of iron hydroxides is considered as the most widely accepted mechanism for high concentrations of As in the groundwater of these two river plains.

Keywords: *Groundwater arsenic, Brahmaputra Flood Plain (BFP), Barak Valley Plain (BVP), Arsenic mobilation mechanism, Arsenic speciation, Source of Arsenic.*

Introduction

The problem of naturally occurring As pollution in groundwater is now recognised to be one of the world's greatest environmental hazards,, threatening the lives of the million across the globe [1-15]. Geogenic arsenic pollution occurs in diverse geological and climatic conditions ranging from the hot and humid tropics, to Arctic Alaska and hyperarid deserts.

Natural arsenic pollution in excess of the permissible limit of 10μg/L in potable groundwater resources of north-east India was first reported in the year 2004 [16,17]. However, the scale of the problem is potentially large in the state of Assam where 21 of the total 27 districts are severely affected by groundwater As contamination [17] and have affected about 7 lakh people. The elevated level of As was found in the flood plain of Jorhat, Lakhimpur, Nalbari, and Nagaon dis-tricts [17-23]. When scientific studies highlighting a potentially severe As problem in the state was published, a number of state and central government organisations came forward to make their own assessment of groundwater As in the state. The PHED (Public Health Engineering Department) Assam decided to conduct an assessment of "Presence of Arsenic in Groundwater of Assam" in collaboration with UNICEF. The objective of the study was to identify areas at risk of contamination and to develop a comprehensive database on As status of the state, covering an area of 192 blocks spread across 22 districts. The results of the Assessment program revealed that 76 blocks in 18 districts were indeed at risk, with As level greater than 50 μg/L [24]. When the studies showed high As concentrations (> 50 μg/L) are widespread in groundwater aquifers in the state, the As screening and surveillance programme was implemented jointly by the PHED, UNICEF and IIT Guwahati. To suggest the government on the issue and to demarcate groundwater sources measuring less than or more than 50 μg/L as safe, and unsafe respectively a blanket screening of an estimated 56,180 public water sources was carried out in the identified high-risk blocks of the state. This study also showed that the concentrations were localized at certain pockets of the floodplain and is narrowly spread compared to the Ganga Brahmaputra Meghna

(GBM) floodplain in Bangladesh and West Bengal. The result of the analysis was used for preparation of district level As concentration maps. To understand the nature of As release into the groundwater from borehole, sediments were investigated to find out the role of Fe in leaching As to groundwater. This study showed that reductive dissolution of ferric hydroxides is believed to be the release mechanism for controlling the mobility of As in Assam [25]. The reducing condition by microbial activities controls the dissociation of Fe from its compounds (iron hydr(oxides), ferromanganese compounds), which is responsible for the leaching of Fe into the groundwater from the aquifer sediments [26, 27].

Almost all the As affected ground waters in Assam and Manipur are located in the floodplains of Brahmaputra and Barak respectively [16, 17, 28]. The As affected districts of Assam are overlain by successions of alluvial Quaternary sediments. The source of the As enriched sediments is widely believed to be the mountainous rocks, Himalayas towards the north appear to be the source of As in the BFP; and the Barail in the south appear to the source of the As in the BVP [12, 16, 22, 29]. However, the probable As release mechanism in these two flood plains appear to be reductive dissolution of iron hydroxides [14,30-36]. Under such an anoxic condition reduction of iron hydr(oxides) coatings on sand, silt and clay particle is a common phenomenon, which ultimately lead to the release of the adsorbed As [5, 31, 34, 37-39].

In terms of the percentage of tube wells with high As concentration, Assam is the worst affected states in the North-east India. There are several important rivers in the state, the entire northern and middle half of the state is drained by the river Brahmaputra, while towards the south the Barak river is a very important river system. It has been established that almost all the known As hotspots of the state are situated in these riverine plains. The people here depend on groundwater for a variety of utilities, most importantly drinking.

Sources of Arsenic in Drinking Water

Arsenic is ubiquitous element and twentieth most abundant element in the earth's crust. Its concentration in earth crust is 2-5 mg/kg and occurs in 200 diff minerals [40]. Arsenic is introduced into the environment through weathering of rocks and dissolution of minerals. Besides, it can also be introduced by anthropogenic activities. Although, arsenic occurs widely in rocks and soils, but high concentrations of arsenic in water are uncommon. Generally, As concentration in groundwater is below 10 μg/l. But very high concentration of As in groundwater are found in regions with active volcanism, sedimentary rocks, geothermal system, and in soil with a high concentration of sulphide. Arsenic occurs in rocks, soil, water, air, and in biota. Naturally As has been mobilized such as weathering of rocks, microbial activities, besides anthropogenic activities viz, mining, burning of fossil fuel.

Arsenic Chemistry: Speciation and Toxicity

Arsenic is colourless, tasteless and odourless element. High concentration of As in groundwater is not associated with any change of colour, taste, and odour of water. Therefore, detection of arsenic in groundwater require sophisticated analytical technique.

Arsenic is perhaps unique among the heavy metalloids and oxyanion-forming elements. Its distribution, mobility, and source in the water system depends on several geochemical factors, such as pH, ORP, presence of other ionic species in aquatic system and microbial activity [41].

Arsenic toxicity strongly depends on the form in which arsenic is present. The toxicity of As(III) is greater than that of As(V) since it coagulates proteins and forms complexes with coenzymes, and inhibits ATP production [42]. Arsenic in water occurs in two primary forms: inorganic and organic. The most common organic forms of arsenic are mono-methylated acids (MMA) and dimethylated acids (DMA) present abundantly in seafood and are relatively less toxic to human health. Inorganic arsenic can occur in the environment in several forms. In drinking-water, it is mostly found as trivalent arsenite (As (III)) or pentavalent arsenate (As (V)) which exhibit different grades of toxicity. The valency and species of inorganic arsenic are dependent on the redox conditions and the pH of the water. In general, the reduced trivalent form [As(III)], is normally found in anoxic groundwater and arsenate, the oxidised pentavalent form [As(V)], is found in oxic surface water. Some ground waters have been found to have only As(III), others only As(V), while in some others both forms have been found in the same water source [43-46].

Under oxic condition As(V) exists in four forms in aqueous solution based on pH: H_3AsO_4, H_2AsO^{4-}·$HAsO_4{}^{2-}$, and $AsO_4{}^{3-}$ depending on the Eh and pH conditions [43]. Similarly, Under reducing condition and at thermodynamic equilibrium over a wide range of pH As(III) exists in five forms: $H_4AsO_3{}^-$, H_3AsO_3, H_2AsO_3 , $HAsO_3{}^2$, and $AsO_3{}^{3-}$. The ionic forms of As(V) dominate at pH >3, and As(III) is neutral at pH <9 and ionic at pH >9.

The toxicity and mobility of arsenic varies with its valence state and chemical form. As(III) is generally more toxic to humans and four to ten times more soluble in water than As(V) [47, 48].

Mechanism of As Mobilization

There are four different mechanisms for mobilization of arsenic in groundwater namely *Reductive dissolution, Alkali desorption, Sulphide oxidation and Geothermal water.*

Reductive Dissolution

Although, there are four basic mechanisms that can mobilise arsenic to groundwater but reductive dissolution is the most important one among the four. In this hypothesis iron oxide which is present in the sediments of aquifer act as a scavengers of AS. Arsenic is a natural component of rocks, which can result in release of arsenic into water. Under reducing conditions, the iron oxides minerals to which arsenic is adsorbed break down under the influence of decaying organic matter and dissolve, causing arsenic to be released into water. A strong statistical correlation between the arsenic and iron concentrations in groundwater of Bangladesh, West Bengal lend support to this hypothesis. In this process degradation of organic matter generates high bicarbonate concentrations, with high concentrations of manganese, ferrous iron, and ammonium, while oxidized species such as dissolved oxygen, nitrate and sulphate are usually very low or absent.

Alluvial sediments probably acquire most arsenic by adsorbing As(V) in oxic environments, where As(III) is not stable. Organisms such as *Shewanella alga* can increase the rate of Fe(III) reduction, accelerating the release of As(V) into solution. *Sulfurospirillum barnesii* can reduce arsenate to arsenite, both in solution and when adsorbed on ferrihydrite. The redox reactions controlling iron in natural waters have been studied in great detail by Langmuir [49], and the process of iron reduction by organic matter may be represented by the following equation:

$$8FeO(OH) + CH_3COOH + 14H_2CO_3 => 8Fe^{2+} + 16HCO_3^- + 12H_2O$$

This reaction requires sedimentary organic matter, which consumes all available sources of oxygen. [33].

Alkali Desorption

The alkali desorption is used to depict a set of processes that involve an increase in pH that promote desorption of arsenic from iron oxides. Alkali desorption occurs at high pH (≥8.0) and in the presence of dissolved oxygen, nitrate or sulphate which can be termed 'alkali-oxic' and which have low concentrations of iron and manganese. The occurrence of arsenic in alkali-oxic ground waters in Argentina, the southwestern and central USA, Spain and other locations are some of the examples.

Sulphide Oxidation

The oxidation and break-down of sulphide minerals containing arsenic is another important mechanism of mobilization of arsenic. Sulphide oxidation occurs where pyrite or arsenopyrite are exposed to oxygen, to produce waters that are typically both acid and sulphate-rich, but not necessarily high in iron [49]. The pyrite oxidation hypothesis support the release of As to the aquifers in the shallowest ground waters close to the water table where sulfate concentrations is usually high. The sulphide oxidation can proceed under both biotic and abiotic conditions. The arsenopyrite (FeAsS) is one of the most important stores of arsenic and their oxidation can be important sources of arsenic pollution. The iron sulphide minerals (FeS_2) and arsenopyrite (FeAsS) are the commonest sulphide mineral, and is most abundant in marine sediments, igneous rocks and areas of hydrothermal alteration. Oxidation of arsenic-bearing sulfides is commonly associated with mining activities in many regions.

Pyrite may be oxidised by oxygen, nitrate or ferric iron, and the processes have been studied by many authors [49, 50].

The dissolve oxygen in oxygen-rich water is the most significant oxidant of pyrites:

$$2FeS_2 + 7O_2 + 2H_2O => 2Fe^{2+} + 4SO_4^{2-} + 4H^+$$

Pyrite can also be oxidized by Fe(III) and in some wells exhibit low pH, and in these wells Fe(III) may be an important oxidant.

$$FeS_2 + 14Fe^{3+} + 8H_2O =>15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

The reaction consumes seven molecules of O_2 for every two of iron sulphide and it produces acidity, accounting for the low pH values. Where additional oxygen is present, the dissolved ferrous iron is then oxidised to ferric iron, consuming some of the acidity, as follows [50]:

$$Fe^{2+} + 1/4\ O_2 + H^+ => Fe^{3+} + ½\ H_2O$$

The ferric iron will be precipitated as ferrihydrite, which will adsorb at least some of the released arsenic.

Pyrite may also be oxidised by nitrate, a process that could be important in areas of intensive farming that overlie sulphide-rich subsoils. The equation for the reaction with nitrate is:

$$5FeS_2 + 14NO_3^- +4H^+ => 5Fe^{2+} + 10SO_4^{2-} + 7N_2 + 2H_2O$$

In the absence of free oxygen, nitrate may oxidise ferrous to ferric iron, which is precipitated as ferrihydrite, while the other reaction products are nitrogen gas and acidity [50]:

$$10Fe^{2+} + 2NO_3^- + 14H_2O =>10FeOOH + N_2 + 18H^+$$

Geothermal Water

The geothermal mechanism involves mixing with geothermal waters, where arsenic was leached from rocks by hot water, either at great depth or in areas of volcanic activity. Geothermal waters reach the surface as natural springs and then mix with surface water flows or shallow groundwater bodies. Since As contained in the geothermal fluid, it causes As pollution in the surface and shallow groundwater [51]. The temperature of geothermal fluids may be elevated and usually also characterized by a correlation of arsenic with chloride [52]. Geothermal arsenic is common in active and former continental-volcanic settings such as in New Zealand, the Andes, southern Italy and the Rockies, and to a lesser extent in oceanic-volcanic terrains.

Health Problems with Arsenic

Arsenic is now recognised to be one of the world's greatest environmental hazards and millions of individuals across the globe are chronically exposed through drinking water. It has been reported that [53] over 137 million people in more than 70 countries are probably affected by arsenic poisoning from drinking water. The disease symptoms caused by chronic arsenic ingestion are called *arsenicosis* and develop when arsenic contaminated water is consumed for several years. Long-term ingestion of drinking water having As concentration beyond the permissible limit leads to harmful effects on human health. There is a strong body of evidence that inorganic As is a serious toxicant and can cause a variety of adverse health effects, such as dermal changes, respiratory, pulmonary, cardiovascular, gastrointestinal, haematological, hepatic, renal, neurological, developmental, reproductive, immunologic lead to cancer and other degenerative effects of the circulatory and nervous system [11, 54-56]. Epidemiological studies have shown that arsenic is also contributed to various other cardiovascular, pulmonary, immunological, neurological, peripheral vascular and endocrine diseases.

Human exposure to arsenic can take place through ingestion, inhalation or skin adsorption; however, ingestion is the predominant form of arsenic intake. The relationship between arsenic and skin cancer has been well documented over the past several decades [57, 58]. Exposure to low level of arsenic in drinking water also causes skin pigment changes which is black brown in colour described as *melanosis*. These lesions are relatively early manifestations of arsenic toxicity and hardening of palms and soles known as *keratosis*. The most visible symptoms of long term exposure to arsenic concentrations in drinking water are development of skin de-pigmentation resulting in white spots that looks like raindrops known as raindrop *melanosis* [59]. Palms and soles further thicken and painful cracks emerge, mainly at palms and the sole. These lesions are commonly found in chronically exposed populations called *hyperkeratosis* and can lead on to skin cancer [60]. Arsenical keratoses are the most characteristic skin feature of long-term arsenic exposure. Elevated concentration of arsenic in drinking water may attack internal organs without causing any visible external symptoms, making arsenic poisoning difficult to recognize. Hair, nails, urine and blood can be an indicator of human exposure to arsenic before visible external symptoms. The disruption of the peripheral vascular system leading to gangrene in the legs, known in some areas as black foot disease in China (province of Taiwan) in the first half of twentieth century was observed due to exposure of arsenic for several years.

Guidelines and Standards

Because of the proven negative health effects on humans and widespread distribution of As in groundwater, WHO in 1993 has lowered its earlier permissible limit of 50 µg/L in drinking water to 10 µg/L. The guideline value for arsenic is provisional because there is clear evidence of hazard but uncertainty about the actual risk from long-term exposure to very low arsenic concentrations [61,62]. The value of 10 µg/ was set as realistic limit taking into account practical problems associated with arsenic removal to lower levels. The WHO provisional guideline of 10 µg/L has been adopted as a national standard by most countries, including Japan, Jordan, Laos, Mongolia, Namibia, Syria and the USA, and by the European Union (EU).

Global Arsenic Problem

The presence of elevated concentration of As in groundwater have been documented from various Countries across the world in a large geological and climatic settings. Generally, surface water contain low level of AS and most of the major rivers of the world contain dissolved concentrations of 1–3 ppb As. Some smaller rivers that contain very high level of arsenic include the Loa (1400 ppb) and Toconce (800 ppb) in Chile, the Waikato (150 ppb) in New Zealand and the Madison (72 ppb) in the USA, and all receive discharges from geothermal springs.

In the 1960s, groundwater arsenic poisoning became well known in Taiwan, which has a special place in the history of epidemiological studies of arsenic. It caused a range of severe illnesses, including Blackfoot Disease, which is almost unique to southwest Taiwan.However, Until the 1980s, groundwater arsenic problem did not attract international attention.

Arsenic poisoning in West Bengal was first clinically diagnosed since early 1980s although it was not appreciated internationally until mid 1990s. Now, high arsenic concentrations beyond permissible limit for drinking water have been confirmed in many countries on all continents and this should therefore be regarded as a global issue. With the discovery of newer sites in the recent past, the arsenic-con-tamination scenario around the world, especially in Asian countries, has changed considerably. Occurrence of high concentrations of arsenic in drinking-water has been recognized as a major public-health concern in several parts of the world. In the Americas the incidence of high concentrations of arsenic in drinking-water has been reported from Mexico, United States, Chile and Argentina. In fact, in North America, the USA is one of the most widely affected countries in the world, although the health impacts are quite small. An extremely widespread and diverse case of arsenic contamination in well was first reported from Argentina in 1917 [63]. The Pampean Plains of Argentina and the Pacific Plains of Chile are the areas in South America have severe arsenic pollution. As per a report at least four million people are exposed to arsenic level > 50 μg/L in Latin America alone [64]. In northern Chile (region I and II) both surface water and groundwater in the region II, consisting of Antofagasta, Calama, and Tocopilla, have shown high concentrations of ar-senic (*Caceres et al 1992*). The rivers in northern Chile (region I and II) have high natural concentra-tions of arsenic, particularly those from the region of Antofagasta. Hungary, Serbia and Croatia are most affected country in Europe where groundwaters are contaminated with arsenic. An account of groundwater quality conducted in Hungary demonstrated that drinking water for almost 400 towns and villages in the Great Hungarian Plain contain arsenic concentrations several times higher the WHO and EC guidelines. The arsenic level ranging from 60 to 4,000 μg/L was reported in well-water during 1941-1983 [65, 66]. A few thousand people showed symptoms of arsenic poisoning, such as melanosis, hyperkeratosis, skin cancer, internal cancer, bronchitis, gastroenteritis, or haematologic abnormalities [67]. Later, arsenic exceeding 50 μg/L was identified in groundwater from alluvial sediments associated with the River Danube in the southern part of the Great Hungarian Plain [68].

In Africa, arsenic pollution is well documented only from southwest Ghana, which is partly anthropogenic. However, it appears from literature that large areas of Africa, groundwater has simply not been tested.

In Australasia, New Zealand arsenic has been reported from geothermal sources on Hawaii and Iceland., An elevated levels of arsenic in surface water and groundwater of Victoria, mostly around the gold-mining regions has been reported in 1991 . The survey data revealed that the concen-trations of arsenic in groundwater varied from <1 to 300,000 μg/L (n=109), while in surface water these were in the range of <1-28 300 μg/L (n=590) [69] . At present prevalence of arsenic in drinking water has been identified in the most widespread As enrichment in groundwater has been documented from parts of Asia, including Bangladesh, India, Nepal, Cambodia, Thailand, Vietnam, China, and Taiwan, this region is known as south and southeast Asian As belt (SSAAB). In terms of the clinical manifestation due to As induced diseases and percentage of tube wells with high As concentration, Bangladesh is the worst affected nation in the world [2, 3, 8, 9, 74, 75]. In the recent decades, arsenic contamination in groundwater in the have been reported in Gangetic plains of West Bengal, Jharkhand, Bihar, Uttar Pradesh and the flood plains of the Brahmaputra and Barack in addition to Bihar, UP, Jharkhand), lower Ganga (West Bengal) [14, 36, 76-79] as well as several states of northeast India [16, 17, 29, 81-84]. However, in India the worst cases of As related health problem have been reported from the state of West Bengal where As problem is endemic and the first case of chronic As poisoning due to drinking water was diagnosed from the state way back in 1983 [5,6, 35, 36, 42, 70, 80, 85-88].

Study Area, Hydrology, Climatic and Geology

The BFP forms a part of the extended Ganga-Brahmaputra flood plain system and has a unique pattern of morphology characterised by extremely variable flow, enormous rates of sediment discharge, rapid channel, aggradations, and accelerated rates of basin denudation [89].

The vast alluvial plains of Brahmaputra valley occupy most of the North Assam and it extends from Sadiya in the east to Dhubri in the west. The greater part of Assam lies within the Brahmaputra valley and its length is nearly 640 Km and has the width of about 100 km. The Brahmaputra plain is formed of the sediments carried by the Brahmaputra and its tributaries from the Himalaya and surrounding hills. It is bounded by Arunachal Himalaya in the north and northeast, Patkai -Naga - Lushai range of Nagaland and the Shillong Plateau in the south and southeast. The southern part of Assam (Barak Valley) consists of three districts viz. Cachar, Karimganj and Hailakandi (24°8′ and 25°8′ N latitudes and 92°15′ and 93°15′ E longitudes. The valley is bounded by the North Cachar Hills District of Assam and the state of Meghalaya to the north; Manipur to the east; Mizoram to the south; and Tripura and Bangladesh to the west. The climate of the region is described as 'Tropical Monsoon Climate', which is characteristically warm and humid, and experiences a high rainfall under the influence of the south-east monsoon from June to September. Mean annual rainfall is 320 cm with a variability of 15-10%. Rainfall varies along the stretch of the plains, from as low as 175 cm in the Kapili basin in central Assam to as high as 410cm in Jidhal basin in the north-eastern part [89]. Towards the south the rains are scantier in the rain shadow zones of Nagaon and Karbi Anglong. Minimum temperature during winter in the plains and the valley areas of the basin varies from 9°C in the western part to 4°C in the north-eastern part, summer temperatures are around an average of 35°C, maximum temperature can go higher than 40°C, especially in regions like Silchar in the extreme south [89].

The Brahmaputra and the Barak valleys of Assam were formed by the deposition of older and newer alluvium and more than 75% of the state is underlain by unconsolidated formation comprising of clay, silt, sand, gravel, pebble where ground water is available at low to moderate depth. The state of Assam has a unique geology, the northern plains (BFP) are dominated by deposits of new Quaternary Holocene alluvium, but tertiary sandstones along the fractures and solution cavities in limestone also occur [89]. The BFP which is underlain by recent alluvial deposits are about 200-300 m thick and consist of clay silt, sand and gravels.

The Surma basin of South Assam (Barak Valley) forms a part of the greater Bengal basin [90]. Barak Valley consists of hilly terrain surrounded by bowl shaped synclinal valley elongated towards east to west. In Assam, Surma group is exposed in Surma valley and north Cachar hills. Geologically the Surma (Lower to Middle Miocene) are the oldest rock and has been subdivided into a lower arenaceous facies (Bhuban) formation and an upper argillaceous facies (Bokabil Formations). A major part of the Barak valley consist of Tertiary upland surfaces with the presence of shale, sandstone, ferruginous sandstone, mottled clay, pebble and boulder beds or lowland valley areas with thin cover of Holocene Newer Alluvium sediments on top. Both newer and older alluvium are found in the area. The semi-consolidated older rocks consists of shale, ferruginous sandstone, mottled clay, pebble bed and boulder bed etc., belonging to Bhuban and Barail groups of rocks formed under marine condition. Newer Alluvium Holocene deposits along Barak Valley in the central parts mainly consist of sand, silt, carbonaceous shale, clay with gravel [91].

Distribution of As in the State of Assam

In a study conducted on the groundwater arsenic status of Assam by NERIWALM [17] during post-monsoon in the year 2003, showed that 21 out of 24 districts have elevated groundwater As content exceeding 50 μg/L. It was found that arsenic concentration was found to be higher adjacent to Himalayan foothills. The concentration of As was relatively high in shallow tube well (15-40 m deep) as compared to deep tube well and rings well. No report of *Arsenocosis* from any area of the region has been known till date. Chakraborty *et al* [16] also reported high groundwater As in the plains of upper Brahmaputra

The groundwater As problem of Assam has been investigated by various researchers [19, 23, 84, 92, 93, 94,83]. The groundwater of Assam valleys is highly ferruginous [16, 95] and a number of scientific studies [18, 23, 83, 84 96, 97] focus mainly on the physicochemical analyses of As contaminated groundwater.

Distribution in the Brahmaputra Flood Plain

The Majuli is the largest inhabited riverine island in the world [98]. A preliminary survey of As content in ground water from shallow tube wells/hand pumps within 50 m depth of Majuli Island, was first conducted by *Saha et al.* [99]. Results of the chemical analysis of the 24 groundwater samples collected from shallow groundwater abstraction structures mainly tubewells have shown that the As ranged from 6 to 90 μg/L in the area.

Arsenic in groundwater of Majuli [28] revealed that As in excess of 50 μg/L occur in the groundwater of many villages. Arsenic concentration in groundwater of Majuli was found to decrease gradually beyond 25 m depth of tube-wells and no correlation was observed between the concentration of As in water and depth. It was observed that As levels in tubewells deeper than 45 m are found to be less than 10 μg/L and is considered as safe. Similar results are also reported by Kumar *et al.* [14] from the middle Gangetic plains where elevated As levels were mostly observed in the tube-wells with a depth range of 10-20 m and As level decreased beyond a depth of 40 m. The Majuli research on As in groundwater has revealed that levels of As in the aquifer sediment is not rich in As and concentration is lower than background levels (5-10 mg/kg) suggesting that the mobilization of As in groundwater could be derived locally or proximally [28]. Besides, the As/Fe (identical values) in sediments from consecutive layers of a single borehole or different boreholes indicate that both Fe and As are coming from a definite source. Similar result was also observed in the earlier study in the Bengal delta region[100]. Statically a poor correlation was found between As and Fe levels in sediment and the possible explanation of such poor relationship could be other processes by which Fe may be removed from groundwater and that some of these do not involve As [34]. Similarly low correlation (r=0.23) between As and Fe which was observed in the groundwater of Majuli. Researchers have already reported such poor relation between As level and Fe level in both water and sediments from GMB delta plain [8, 9, 32, 35]. One of the possible reasons of this behaviour could be difference in the conservation status of the two the species (Fe and As) in groundwater; another reason could be spatial difference of the ratio of As and iron hydr(oxides) based on the chemical characteristics, abundance and concentrations of the mineral phases [8,9,28]. Several other processes like mineral weathering, oxygen consumption and nitrate reduction may influence As release in groundwater, which can explain the poor relation observed between As and Fe in the groundwaters of the middle Gangetic plains of Ghazipur Uttar Pradesh, India [14].

Arsenic concentration in biological samples (hair, nail and urine) of the inhabitant residing in the high As affected areas of the Majuli river island was investigated by Goswami *et al.*,[28] to evaluate the As body burden. The result of analysis of biological samples revealed that about 90% urine samples, 100% hair samples and 97% nail samples contained As level above the maximum normal level. The maximum normal level of As in urine, hair and nail are 50 μg/kg, 200 μg/kg and 500 μg/kg respectively. It is noteworthy that the As in the hairs of the residents of Majuli was found out to be higher than those recorded in previous studies from Vietnam and Cambodia [101,102,103].

Mahanta et al.[25] had established that reductive dissolution of iron hydr(oxides) was the cause of As release in the flood plains of Assam. To investigate the possible release and mobilization mechanism of As in the aquifer. Baviskar and Mahanta, [22] studied the borehole sediments from As enriched areas of Jorhat, Assam. It was observed that the groundwater in the study area was under a reducing condition and Fe (II) and As (III) were the dominant species.

The nature of As release into the groundwater from borehole sediments were investigated by [22]. The objective of the study was to gain an understanding about the process of Fe leaching and As mobilization in Assam by investigating the sediments and groundwater samples collected from As enriched areas of Jorhat, Assam. Combined Eh-pH stability diagram of Fe and As indicated the presence of Fe (II) and As (III) species in the groundwater, indicating the presence of a reducing condition. Under reducing conditions arsenic generally exists as a trivalent form such as $H_2ASO_3^-$ and $HASO_3^{2-}$ and the qualitative relationships between arsenic species can be understood with the help of Eh-pH diagram. However in oxidising environment As is present as the arsenate form [9]. Concentrations of elevated As in the sediment samples collected at variable depths of the three boreholes ranged between 1 mg/kg to 18.6 mg/kg, with maximum concentrations found in the fine sand fractions. The silt and the fine sand sediment fractions seem to be the key repositories of high Fe and As concentrations in the study area, which is supported by the SEM/EDX and XRD analysis. Similar results are also reported from the fluvio deltaic sediments of West Bengal and Bangladesh [31, 35,104,105, 106].

Groundwater As exceeding 10 μg/L were detected in Dhubri and Bongaigaon districts of Assam by Reddy, 2012 especially three places Charkola, Paglahat and Tamarhat have very high level of As. The two districts are located on the north bank of the river Brahmaputra and are part of the BFP. The groundwater and sediment chemistry of Bongaigaon and Darrang districts of Assam were investigated to find out the distribution, origin and release mechanisms of arsenic [12]. Fifty groundwater samples from domestic wells and public water supply schemes, and 5 sediment samples were collected. Fifteen groundwater samples had As concentration above the Indian national drinking standard of 50 μg/L, and

33 of them had As concentration above the WHO guideline of 10 μg/L, besides DOC levels were moderately high ranging from 0.3-4.2 mg/L. The study could not identify distinct zones or depths with specific sedimentological features producing arsenic free water were. However, the sediments with a green-olive colour are probably more likely to bear As contaminated water than white sediments and proposed that reductive dissolution of ferric hydroxides is thought to be the release mechanism controlling the mobility of As. As no relation was found between dissolved As and SO_4^{2-}; oxidation of arsenopyrite was ruled out in this study. According to Mc Arthur *et al.* 2004, aquifers having grey coloured sediments has elevated concentration of As as compared to brownish due to reduction of iron hydr(oxides). In the extreme north-eastern part of the state the district of Dhemaji which lies in the north bank of the river Brahmaputra experience annual episodes of mass flooding and deposition. Arsenic level as high as 100 to 200 μg/L has been detected in Dhemaji district, the locations where high As levels in groundwater has been detected are Sissiborgaon, Dhemaji town and Jonai [94]. Spatial distribution maps for arsenic in different seasons were prepared using curve fitting method in Arc View GIS software. Chetia *et al.* [23] investigated the As content in the 222 groundwater samples collected from shallow and deep tubewells of six blocks of Golaghat district (Assam). The results of the analysis showed that 67%, 76.4%, and 28.5% were found contaminated with higher metal contents (for total As, Fe, and Mn, respectively). A strong significant correlation was observed between As and Fe (0.697 at $pH<0.01$), suggesting a possible reductive dissolution of As-Fe-bearing minerals for the mobilization of As in the groundwater of the region. Borah *et al.* 2009 studied the As, F^-, and Fe contamination in the tea gardens of Darrang district. Bhuyan, 2010 found high levels of As in 3 developmental blocks of Lakhimpur district, Assam.

Arsenic Distribution in the Barak Valley Plain

In Cachar and Karimganj districts of Barak Valley, Assam the presence of naturally occurring As in shallow tube wells (14-40 m) have been reported where 66% and 26% tubewells have As concentration above the 10 μg/L and 50 μg/L respectively. The earlier study carried by Shah, 2007 also detected elevated As in groundwater from parts of Barak Valley, Cachar and Karimganj districts. The As affected villages of these two districts are situated in entrenched channels and flood plains of Barak-Surma-Langai Rivers system. In contrast, Plio-Pleistocene older alluvium aquifers, which are located at higher altitude or have thin cover of Newer Alluvium sediments, are safe from As [29].

The results from the quite recent studies performed by Thambidurai et al. [83,84] have shown that As enriched groundwater exists in Barak valley. The presence of high level of As (ranging from 12 to 97μg/L) was detected in 17 of the 50 groundwater samples from Barak Valley area, As is being leached into the groundwater from soil and rocks [84]. The elevated levels of As in the bedrock and soil of the area and suggest that the source of As contamination is geogenic. It was observed that As contaminated aquifers are generally situated in the Holocene alluvial terrain.

Conclusion

In Assam, similar to Middle Gangetic Plain (Bihar, Jharkhand and UP) and lower Gangetic Plain of West Bengal plain in India and Padma-Meghna-Brahmaputra of Bangladesh, the source of As is geogenic. The process of reductive hydrolysis of iron hydr(oxides) appears to be the principal process of As release in the flood plains of the Brahmaputra and Barak, bacterial oxidation of organic matter in the newer sediments creates a reducing environment which is conducive for As release. Although reductive dissolution is the process of As mobilization in BFP and BVP, but the actual source of As appears to be the sediments which are deposited by the rivers. Both the Brahmaputra as well as the Barak are mountainous rivers, the former originating from the Himalayas while the latter originating from the Barail hills in the border areas of Assam, Nagaland and Manipur. Arsenic is inherently present in the mountain sediments. Arsenic bearing minerals like arsenopyrite which was detected in the sediments of two of our studies could originate in the mountains. Thus the ultimate source of As in BFP could be the Himalayan mountains while the Barail hill range could be the source of As in the BVP. No case of arsenicosis has been reported in the state of Assam due to the widespread use of sand filters among the common people.

References

1. Nickson, R.T., McArthur, J.M., Sengupta, B., Kyaw-Myint, T.O., and Lowry, D., 2005, Arsenic and other Drinking water quality issues, Muzaffargarh District, Pakistan., Appl. Geochem., 20, pp.55-68.
2. Nickson,R., McArthur, J.M., Burgess, W., Ahmed, K.M., Ravenscroft, P., Rahman, M., 1998, Arsenic Poisoning of Bangladesh Groundwater, *Nature* 395, 338.
3. Smith, A.H., E.O. Lingas and M. Rahman., 2000, Contamination of Drinking-water by Arsenic in Bangladesh: A Public Health Emergency, *Bulletin of the World Health Organization*, 78(9), 1093–1103.
4. Berg, M., Tran, H. C., Nguyen, T. C., Pham, H. V., Schertenlrib, R., and Giger, W., 2001, Arsenic Contamination of Groundwater and Drinking Water in Vietnam: A human Health Threat, *Environ. Sci. Technol*, **35**, 2621–2626.
5. Smedley, P. L. and Kinniburgh, D. G., 2002, A Review of the Source, Behavior and Distribution of Arsenic in Natural Waters", *Appl. Geochem.*, 17(5), 517–568.
6. Smedley, P.L., Zhang, M., Zhang, G. and Luo, Z., 2003, Mobilisation of Arsenic and

other Trace Elements in Fluvio-lacustrine Aquifers of the Huhhot Basin, Inner Mongolia, *Appl. Geochem.*, 18(9), 1453–1477.

7. Guo, H. M., Wang, Y. X., Shpeizer, G. M., and Yan, S. L., 2003, Natural Occurrence of Arsenic in Shallow Groundwater, Shanyin, Datong basin, China, *J. Toxicol. Environ. Health,* Part A Environmental Science and Engineering & Toxic and Hazardous Substance Control, **38**, 2565–2580.
8. Ravenscroft, P., Burgess, W.G, Ahmed, K.M., Burren, M., Perrin, J., 2005, Arsenic in Groundwater of the Bengal Basin, Bangladesh: Distribution, Field Relations, and Hydrological Setting, *Hydrogeol. J.* , 13, 727–751.
9. Ravenscroft, Peter., Brammer., Hugh and Richards., Keith., 2009, Arsenic Pollution: A Global Synthesis, John Wiley & Sons, Ltd., Publication ISBN: 978-1-405-18602-5.
10. Polya, D. A., Gault, A. G, Diebe, N., Feldman, P., Rosenboom, J. W., Gilligan, E., et al., 2005, Arsenic Hazard in Shallow Cambodian Groundwaters, *Mineralogical Magazine*, 69, 807–823. doi:10.1180/0026461056950290.
11. (a) Anawar, H. M., Akai. J., Yoshioka. T., Konohira. E., Lee. J. Y., Fukuhara. H., Tari Kul Alam. M., and Garcia Sanchez A., 2006. Mobilization of Arsenic in Groundwater of Bangladesh: Evidence from an Incubation Study, *Environ Geochem Hlth.*, **28**, 553–565.

    (b) Anawar, H.M., Akai, J., Mostofa, K.M.G., Safiullah, S., and Tareq, S.M., 2002b. Arsenic Poisoning in Groundwater: Health Risk and Geochemical Sources in Bangladesh, *Environ. Int.*, **27**, 597– 604.
12. Enmark, Gustav., and Nordborg, Daniel., 2007, Arsenic in the Groundwater of the Brahmaputra Floodplains, Assam, India – Source, Distribution and Release Mechanisms Committee of Tropical Ecology, ISSN 1653-5634, Minor Field Study 131, Uppsala University, Sweden, Uppsala.
13. Nriagu, J. O., Bhattacharya, P., Mukherjee, A. B., Bundschuh, J., Zevenhoven, R., Loeppert, R. H., 2007, Arsenic in Soil and Groundwater: An Overview. In: Bhattacharya, P.,Mukherjee, Perrin, J., 1998, Arsenic in Groundwater at Meherpur, Bangladesh: A Vertical Porewater Profile and Rock/water interactions. M.Sc. Thesis (unpub), University College London.
14. Kumar, P., Kumar, M., Ramanathan, A.L., and Tsujimura, M., 2010, Tracing the Factors Responsible for Arsenic Enrichment in Groundwater of the Middle Gangetic Plain, India: A Source Identification Perspective, *Environ Geochem. Hlth.*, **32**, 129–146.
15. Bundschuh, J., Litter , M. I., Parvez, F., Román-Ross Gabriela., Nicolli, Hugo B., Jean, Jiin-Shuh., Liu, Chen-Wuing., María Dina López., Armienta, A., Guilherme Luiz R.G, Cuevas A G, Cornejo L, Cumbal L., and Toujaguez Regla., 2012, *One Century of Arsenic Exposure in Latin America: A review of History and Occurrence from 14 Countries*, **429**, 2–35.
16. Chakraborti, D., Sengupta, M. K., Rahman, M. M., Ahamed, S., Chowdhury U. K., and Hossain, M. A. et al., 2004, Groundwater Arsenic Contamination and its Health effects in the Ganga– Meghna–Brahmaputra Plain, *J. Environ Monitor*, **6**, 75N–83N.

17. Singh, A.K., 2004., Arsenic Contamination in Groundwater of North Eastern India Published in Proceedings of National seminar on Hydrology with focal theme on "Water Quality" held at National Institute of Hydrology, Roorkee during Nov 22-23.
18. Hazarika, Sikharani., Baruah, M. K., Kotoky, P., Baruah, J., Borah, G C., and Bora, P. K., 2003, Arsenic Association and Distribution in Carbonaceous Materials in Northeastern India, *Curr. Sci.*, **85**, pp. 204-208.
19. Borah, K., Bhuyan, B., Sarma, H. P., 2009, Lead, Arsenic, Fluoride, and Iron Contamination of Drinking Water in the Tea Garden Belt of Darrang District, Assam, India, *Environ Monit Assess.*, **169**, pp. 347-352.
20. CGWB, 2009. Central Ground Water Board, Ministry of Water Resources, Government of India. Retrieved from url: http://cgwb.gov.in/ .
21. Devi, N. Linthoingambi., Yadav, Ishwar Chandra., Shihua,Q.I. 2009. Recent Status of Arsenic Contamination in Groundwater of Northeastern India – A Review Report and Opinion, **1(3)**, http://www.sciencepub.net, sciencepub@gmail.com.
22. Baviskar, S. M., Mahanta, C., and Choudhary, R., 2011, Leaching of Iron, Controlling Factors and Implication to Arsenic Mobilization in an Aquifer of the Brahmaputra Floodplain, *Annual International Conference 2011 Programme RGS-IBM*, London, UK.
23. Chetia, M., Chatterjee, S., Banerjee, S., Nath, M.J., Singh, L., Srivastava, R.B., and Sarma, H.P., 2011, Groundwater Arsenic Contamination in Brahmaputra River Basin: A Water Quality Assessment in Golaghat (Assam), India, *Environ Monit Assess*, **Feb;173(1-4)**, 371-85. doi: 10.1007/s10661-010-1393-8.
24. JOPA, 2005, Joint plan of Action for Arsenic and Fluoride Mitigation in Assam, Public health Engineering Department, Assam. Manak Bhavan, 9 Bahadur Shah Zafar Marg New Delhi.
25. Mahanta, C., Pathak, N., Bhattacharya, P., Enmark, G, and Nordborg, D., 2008, Source, Distribution and Release Mechanisms of Arsenic in the Groundwater of Assam Floodplains of Northeast India, *Proceedings of the World Environmental and Water Resources Congress Sponsored by Environmental and Water Resources Institute (EWRI) of the American Society of Civil Engineers*, pp. 1-19.
26. Bhattacharya, P., Jacks, G, Ahmed, K. M., Khan, A. A., and Routh, J., 2002b, Arsenic in Groundwater of the Bengal Delta Plain Aquifers in Bangladesh, *Bull. Environ. Cont.Toxicol.*, **69 (4)**, 538–545.
27. Roden, E. E., 2006, Geochemical and Microbiological Controls on Dissimilatory Iron Reduction, *C.R. Geosciences*, 338, 456–467.
28. Goswami, R., Rahman, M. M., Murril, M., Sarma, K. P., Thakur, R., and Chakraborti, D., 2013, Arsenic in the Groundwater of Majuli - The Largest River Island of the Brahmaputra: Magnitude of Occurrence and Human Exposure, *J. Hydrol.*, (article in press).
29. Shah, Babar., 2012, Role of Quaternary Stratigraphy on Arsenic-contaminated Groundwater from parts of Barak Valley, Assam, North-East India, *Environ. Earth Sci.*, 66(8), 2491-2501.

30. Bhattacharya, P., Chatterjee, D., and Jacks, G., 1997, Occurrence of Arsenic Contamination of Groundwater in Alluvial Aquifers from Delta Plain, Eastern India: Option for Safe Drinking Supply. *Intl. J. Water Resour.*, **D 13**, 79–92.
31. Nickson, R.T., 1997, Arsenic in Groundwater, Central Bangladesh. Unpublished M.Sc. thesis, Univ. College London, London.
32. Nickson, R.T., Mcarthur, J.M., Ravenscroft, P., Burgess, W.G., and Ahmed, K.M., 2000, Mechanism of Arsenic Release to Groundwater, Bangladesh and West Bengal., *Appl. Geochem.* ,15, 403-413.
33. McArthur, J. M., Ravenscroft, P., Safiullah, S., and Thirlwall, M. F., 2001, Arsenic in Groundwater: Testing Pollution Mechanisms for Sedimentary Aquifers in Bangladesh, *Water Resources Research*, **37**, 109-117.
34. Harvey, C.F., Swartz C.H., and Badruzzaman A.B.M., 2002. Arsenic mobility and groundwater extraction in Bangladesh. Science, 298, 1602–1606.
35. Ahmed, K.M., Bhattacharya, P., Hasan, M.A., Akhter, S.H., Alam, S.M.M., Bhuyan, M.A.H., Imam, M.B., Khan, A.A., and Sracek, O., 2004., Arsenic enrichment in groundwater of the alluvial aquifers in Bangladesh: An Overview. *Appl. Geochem.*, **19**, 181–200.
36. Kumar, Manish., Kumar, Pankaj., Ramanathan, A. L., Bhattacharya, Prosun., Thunvik, Roger., Singh, Umesh K., Tsujimura,M., and Sracek, Ondra., 2010, Arsenic Enrichment in Groundwater in the Middle Gangetic Plain of Ghazipur District in Uttar Pradesh, *India J. Geochem Explor.*, **105**, 83 –94.
37. Meharg, A. A. and Rahman, M., 2003, Arsenic Contamination of Bangladesh Paddy Fields Soils: Implications for Rice Contribution to Arsenic Consumption, *Environ. Sci. Technol.*, **37**, 229–234.
38. McArthur, J. M., Banerjee, D. M., Hudson-Edwards, K. A., et al., 2004, Natural Organic Matter in Sedimentary Basins and its Relation to Arsenic in Anoxic Groundwater: the Example of West Bengal and its Worldwide Implications, *Appl. Geochem.*, **19**, 1255–1293.
39. Zheng, Y., Stute, M., Van Geen, A., Gavrieli, I., Dhar, R., Simpson, H.J., Schlosser, P. and Ahmed, K.M., 2004, Redox Control of Arsenic Mobilization in Bangladesh Groundwater, *Appl. Geochem.*, 19(2), 201–214.
40. Appelo, Tony, 2006. As in Groundwater - A World Problem, *Proceedings Seminar Utrecht.*
41. Shih, M.-C., 2005, 'An overview of Arsenic Removal by Pressure-driven Membrane Processes'. In: Desalination, vol. 172, no. 1, p. 85-97.
42. Bhattacharya, P., Frisbie, S.H., Smith, E., Naidu, R., Jacks, G, and Sarkar, B., 2002A, Arsenic in the Environment: A Global Perspective. In: Sarkar, B. (Ed.). *Handbook of Heavy Metals in the Environment.*, Marcell Dekker Inc., New York, pp. 147–215.
43. Ferguson, J. F. and Gavis, J. 1972, A Review of the Arsenic Cycle in Natural Waters'. In: Water *research*, **vol. 6, no. 11**, p. 1259-1274.
44. Korte, N. E. and Fernando, Q. 1991, 'A Review of Arsenic(III) in Groundwater'. In: *Critical Reviews in Environmental Control*, **vol. 21, no. 1**, p. 1-39.

45. Cheng, R. C., Liang, S., Wang, H. C. and Beuhler, M. D., 1994, 'Enhanced Coagulation for Arsenic Removal', In: Journal AWWA, **vol. 86, no. 9**, p. 79-90.

46. Hering, J. G. and Chiu, V. Q., 2000, 'Arsenic Occurrence and Speciation in Municipal Groundwater based Supply System', *Journal of Environmental Engineering*, **vol. 126, no.5** p. 471-474. *Geochimica Cosmochimica Acta*, **41**, 527–538.

47. USEPA., 1997, Treatment Technology Performance and Cost Data for Remediation of Wood Preserving Sites. Washington, DC, USA, US EPA Office of Research and Development, Available at. http://www.epa.gov/nrmrl/pubs/625r97009/625r97009.pdf

48. US Occupational Safety and Health Administration, Available at USOSHA, 2001. Occupational safety and health guidelines for arsenic, organic compounds (as As). Washington, DC,USA, http://www.osha.gov/SLTC/healthguidelines/arsenic/recognition.html

49. Langmuir, D., 1997, Aqueous Environmental Geochemistry. New York: Prentice-Hall.

50. Appelo, C.A.J. and Postma, D., 1996, Geochemistry, Groundwater and Pollution., Rotterdam: Balkema.

51. Ballantyne, J. M. and Moore, J. N., 1988. Arsenic Geochemistry in Geothermal Systems., *Geochimica Cosmochimica Acta*, **52(2)**, 475–483.

52. Ellis, A.J., Mahon, and W. A. J., 1967, Natural Hydrothermal Systems and Experimental Hot-water/rock interactions (Part II), *Geochimica et Cosmochimica Acta*, **31**, 519-538.

53. International Agency for Cancer Research (IARC), "Some Drinking-water Disinfectants and Contaminants including Arsenic," in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **vol.** *84, International Agency for Cancer Research (IARC),* Lyon, France, 2004.

54. Golub, M. S., Macintosh, M. S., and Baumrind, N., 1998, Developmental and Reproductive Toxicity of Inorganic Arsenic: Animal Studies and Human Concerns, *J. Toxicol. Environ. Health*, **Part B 1**, 199–241.

55. Lin, T.-H., Y.-L. Huang, and M.-Y. Wang, 1998, Arsenic Species in Drinking Water, Hair, Fingernails and Urine of Patients with Blackfoot Disease, *J. Toxicol. Environ. Health*, **53**, 85–93.

56. National Research Council. 2001. Arsenic in Drinking Water 2001 update. Washington DC: National Academy Press.

57. Melkonian, S., Argos, M., B. Pierce, L. et al., 2011, "A Prospective Study of the Synergistic Effects of Arsenic Exposure and Smoking, Sun Exposure, Fertilizer use, and Pesticide use on Risk of Premalignant Skin Lesions in Bangladeshi Men," *American Journal of Epidemiology*, 173(2), pp. 183–191.

58. Saha K. C, 2003, "Diagnosis of Arsenicosis," *Journal of Environmental Science and Health*, **Part A, vol. 38, no. 1**, pp. 255–272.

59. Guha Mazumder, D. N., et al., 1998, 'Arsenic Levels in Drinking Water and the Prevalent of Skin Lesions in West Bengal, India', *International Journal of Epidemiology*, **vol. 27, no. 5**, p. 871-877.

60. WHO., 2001. Arsenic in Drinking Water., Fact sheet No. 210.

61. WHO.,1993. Guidelines for Drinking-water Quality. Vol. 1. Recommendations. 2nd ed. Geneva, Switzerland, World Health Organization.

62. WHO., 2004. Guidelines for Drinking-water Quality. Vol. 1. Recommendations. 3rd ed., Geneva, Switzerland, World Health Organization.

63. Arguello, R. A., Cenget, D. D., and Tello, E. E., 1938 .[Cancer and endemic arsenism in the Cordoba region], *Rev Argent Dermatosifiorg*, **22**, 461-87 (Spanish).

64. Bundschuh J., Garcia, M. E. and Birkle, P., 2006, 'Rural Latin America : A Forgotten part of the Global Groundwater Arsenic Problem, In: *Proceedings of the As 2006 International Congress: "Natural Arsenic in Groundwaters.*

65. Egyedi, K., and Pataky, E., 1978, Dermatologic Aspects of Ar-senic Poisoning caused by Drinking Water, Derm Beruf Umwelt, **26**, 54-6.

66. Nagy, G., and Korom L., 1983, Late Skin Symptoms of Ar-senic Poisoning in the Arsenic Endemy in Bugac-Alsomonostor, *Z. Hautkr*, **58**, 961-4.

67. Pataky J, and Lusztig, G., 1958, Arsenic Liver Cirrhosis in several members of a Family, *Z Gesamte Inn Med*, 17, 668-71.

68. Varsanyi I., 1989, Arsenic in Deep Groundwater. In: Miles DL, editor. Proceedings of the 6th Inter-national Symposium on Water-rock interaction (WR1-6). Malvern: AA Balkema Rotterdam, 715-8.

69. Smith, E., Smith, J., Smith, L., Biswas, T., Correll, R., and Naidu, R., 2003, Arsenic in Australian Environment: An Overview, *J. Environ. Sci. Health*, 38, 223-39.

70. Bhattacharyya, R., Jana, J., Nath, B., Sahu, S., Chatterjee, D., and Jacks, G., 2003, Groundwater Arsenic Mobilization in the Bengal Delta Plain, the Use of Ferralite as a Possible Remedial Measure — A Case Study, *Appl. Geochem.*, **18**, 1435–1451.

71. Bhattacharya, P., Ahmed, K. M., Hasan, M. A., Broms, S., Fogelström, J., Jacks, G., Sracek, O., von Brömssen, M., and Routh, J., 2006, Mobility of Arsenic in Groundwater in a part of Brahmanbaria District, NE Bangladesh.

72. Bhattacharya, P., Hasan, M.A., Sracek, O., Smith, E., Ahmed, K.M., von Brömssen, M., Huq, S. M. I., and Naidu, R., 2009, Groundwater Chemistry and Arsenic Mobilization in the Holocene Flood Plains in South-central Bangladesh. *Environ Geochem. Hlth.*, **31**, 23–44.

73. Acharya, S.K., Chakraborty, P., Lahiri, S., Raymahasay, B.C., Guha, S., and Bhowmik, A., 1999, Arsenic Poisoning in Ganges Delta, *Nature,* **401**,545.

74. Kinniburgh, D. G., and Smedley, P. L., 2001, Arsenic Contamination of Groundwater in Bangladesh, *Hydrochemical Atlas, British Geological Survey Technical Report,* **Vol. 3**, p. 124.

75. Shamsudduha, M., Uddin, A., Saunders, J.A., and Lee, M.K., 2008, Quaternary Stratigraphy, Sediment Characteristics and Geochemistry of Arsenic-contaminated Alluvial Aquifers in the Ganges– Brahmaputra Floodplain in Central Bangladesh, *J. Contam. Hydrol.*, 99,112–136.

76. Chakraborti, D., Mukherjee, S. C., Pati, S., Sengupta, M. K., Rahman, M. M., Chowdhury, U. K., Lodh, D., Chanda, C. R., Chakraborti, A. K., and Basu, G. K.,

2003, Arsenic Groundwater Contamination in Middle Ganga Plain, Bihar, India: A Future Danger, *Environ Health Persp.*, **111**, 1194–1201.

77. Ahamed, S., Kumar Sengupta, M., Mukherjee, A., Amir, H. M., Das, B., Nayak, B., Pal, A., Mukherjee, C. S., Pati, S., Nath, D. R., Chatterjee, G., Mukherjee, A., Srivastava, R., and Chakraborti, D.,2006, Arsenic Groundwater Contamination and its Health Effects in the State of Uttar Pradesh (UP) in Upper and Middle Ganga Plain, India: a Severe Danger, *Sci. Total Environ.*, **1;370(2-3):**310-22.

78. Nayaka, Bishwajit., Dasa, Bhaskar., Mukherjee, Subhash Chandra., Pala, Arup., Ahamed, Sad., Hossain, M. Amir., Maitya, Priyanka., Dutta, Rathindra Nath., Dutta, Subir., and Chakraborti, Dipankar, 2008, Groundwater Arsenic Contamination in the Sahibganj district of Jharkhand State, India in the Middle Ganga plain and Adverse Health Effects, *Toxicol Environ Chem.*, 90:4, 673-694.

79. Shah, B. A., 2008, Role of Quaternary Stratigraphy on Arsenic contaminated Groundwater from parts of Middle Ganga Plain, *Environ. Geol.*, 53, 1553-1561.

80. Shah, B. A., 2010, Arsenic contaminated Groundwater in Holocene Sediments from parts of Middle Ganga Plain, Uttar Pradesh, *Curr. Sci.*, 98, 10.

81. Chakraborti, D., Singh, E., Das, B., Shah, B. A., Hossain, M. A., Nayak, B., Ahamed, S., and Singh, N. R., 2008, Groundwater Arsenic Contamination in Manipur, One of the Seven North-Eastern Hill States of India: A Future Danger. *Environ Geol.*, **56**, 381–390.

82. Shah, B. A., 2007, Arsenic in Groundwater from parts of Barak Valley, Cachar and Karimganj Districts, Assam, *Ind. J. Geol.*, 79, 59–62.

83. Thambidurai, P., Chandrasekharam, D., Chandrashekhar, A.K., and Farooq, S.H., 2012, Arsenic Contamination in Groundwater of Surma Basin of Assam and Mizoram, North Eastern India: 4th International Congress on Arsenic in the Environment; Cains, Australia, p.47-49.

84. Thambidurai, P., Chandrashekhar, A. K., Chandrasekharam, D., 2013, Geochemical Signature of Arsenic-Contaminated Groundwater in Barak Valley (Assam) and Surrounding Areas, Northeastern India, Procedia Earth and Planetary Science, Volume 7, 834–837 Proceedings of the Fourteenth International Symposium on Water-Rock Interaction, WRI.

85. Bhattacharya, P., Jacks, G., Jana, J., Sracek, A., Gustafsson, J. P., and Chatterjee, D., 2001, Geochemistry of the Holocene Alluvial Sediments of Bengal Delta Plain from West Bengal, India: Implications on Arsenic Contamination in Groundwater, In: Jacks, G., Bhattacharya, P., Khan, A.A. (Eds.), *Groundwater Arsenic Contamination in the Bengal Delta Plain of Bangladesh*, KTH Special Publication, TRITA-AMI Report 3084, pp. 21–40.

86. Ben, D. S., Berner, Z., Chandrasekharam, D., and Karmakar, J., 2003. Arsenic Enrichment in Groundwater of West Bengal, India: Geochemical Evidence for Mobilization of As under Reducing Conditions, *Appl. Geochem.*, **18**, 1417–1434.

87. Rahman Mohammad Mahmudur, Sengupta Mrinal Kumar, Ahamed Sad, Chowdhury Uttam Kumar, Das Bhaskar, Hossain Md. Amir, Lodh Dilip, Saha Kshitish Chandra, Palit Shyamal Kanti, Chakraborti Dipankar, 2005, A Detailed Study of the Arsenic

Contamination of Groundwater and its Impact on Residents in Rajapur Village of the Domkal block, District Murshidabad, West Bengal, India, *Bulletin of the World Health Organization*, 83(1), 49-57.

88. Samanta Gautam, Sharma Ramesh, Roychowdhury Tarit, Chakraborti Dipankar, 2004, Arsenic and other Elements in Hair, Nails and Skin-scales of Arsenic Victims in West Bengal, India, *Sci Total Environ.*, 326 (1-3), 33-47.

89. Jain, K.S., Agarwal, P. K., and Singh. V. P., 2007, Hydrology and Water Resources of India, *Water Sci and Technology Library*, **57**, 419-472. Springer.

90. Sarkar, K., and Nandy, D. R., 1977, Structures and Tectonics of Tripura-Mizoram Area, India, *Geol Surv. India Misc Publ*, 34 (1), 141-148.

91. Dasgupta, A. B., Biswas, A. K., 2000, Geology of Assam. Geological Society of India, Bangalore, p 169., Devi, N. Linthoingambi., Yadav, Ishwar Chandra., Shihua, Q.I. 2009. Recent Status of Arsenic Contamination in Groundwater of Northeastern India – A Review Report and Opinion, **1(3)**, http://www.sciencepub.net, sciencepub@gmail.com.

92. Baruah, M.K., Kotoky, P., Baruah, J., Borah, G.C., and Bora, P. K., 2003, Arsenic Association and Distribution in Carbonaceous Materials in Northeastern India, *Curr Sci.*, **85**, 204-208.

93. Bhuyan, Bhabajit., 2010, A Study on Arsenic and Iron Contamination of Groundwater in Three Development Blocks of Lakhimpur District, Assam, India, *Report and Opinion*; **2(6)**, 82-87, http://www.sciencepub.net/report.

94. Buragohain, Mridul and Sarma, H. P., 2012, A Study on Spatial Distribution of Arsenic in Groundwater Samples of Dhemaji District of Assam, India by using Arc View GIS Software, *Sci. Revs. Chem. Commun.*, **2(1)**, 7-11, ISSN 2277-2669.

95. Aowal, A.F.S.A., 1981, Design of an Iron eliminator for hand tube wells, *Journal of I.W.W.A.*, **Vol XIII, No-1**, 65.

96. Mukherjee, A. B., Bhattacharya, P., Jacks, G., Banerjee, D. M., Ramanathan, A. L., Mahanta, C., Chandrashekharam, D. and Naidu, R., 2006, Groundwater Arsenic Contamination in India: Extent and Severity (R. Naidu, E. Smith, G. Owens, P. Nadebaum, & P. Bhattacharya (Eds.), Managing Arsenic in the Environment: From Soil to Human Health.(pp 553-594). Melbourne: CSIRO Publishing).

97. Singh, A.K., 2006. Chemistry of Arsenic in Groundwater of Ganges-Brahmputra River Basin, India, *Curr. Sci.*, 91(5), 1-7.

98. Sarma, J. N., and Phukan, M. K., 2004, Origin and Some Geomorphological Changes of Majuli Island of Brahmaputra River in Assam, India, *Geomorphology*, 60, 1-19.

99. Saha, G.C., Samanta, S.K., & Kumar, S., 2009, Arsenic Contamination in Ground Water in Majuli Island, Jorhat District, Assam, *Bhujal News, Quarterly Journal of Central Ground Water Board.*

100. Chakraborti, D., Basu, G. K., Biswas, B. K., et al., 2001, Characterization of Arsenic bearing Sediments of the Gangetic Delta of West Bengal, India. In: *Chappell.*

101. Agusa, T., Kunito, T., Fujihara, J., et al. 2006, Contamination by Arsenic and other Trace Elements in Tube-well Water and its Risk Assessment to Humans in Hanoi, Vietnam, *Environ Pollut*, **139(1)**, 95–106.

102. Berg, M., Stengel, C., Trang, P. T. K., Viet, P. H., Sampson, M. L., Leng, M., and Samreth, S., and Fredericks, D., 2007, Magnitude of Arsenic Pollution in the Mekong and Red 581 River deltas – Cambodia and Vietnam, *Sci. Total Environ.*, **372**, 413–425.
103. Nguyen, V.A., Bang, S., Viet, P.H., Kim, K.W., 2009, Contamination of Groundwater 703 and Risk Assessment for Arsenic Exposure in Ha Nam Province, Vietnam. 704, *Environ. Intl.*, 35, 466–472.
104. AAN, 1999. Arsenic Contamination of Groundwater in Bangladesh: Interim Report of the Research at Samta Village. Asian Arsenic Network, Research Group for Applied Geology and the National Institute for Preventive and Social Medicine, Bangladesh.
105. DPHE, 1999. Groundwater Studies for Arsenic Contamination in Bangladesh, Rapid Investigation Phase, Final Report, Mott MacDonald Ltd and British Geological Survey, Report for the Department for Public Health Engineering and the Department for International Development.
106. Hasan, M. A., von Brömssen, M., Bhattacharya, P., Ahmed, K. M., Sikder, A. M., Jacks, G., and Sracek, O., 2009, Geochemistry and Mineralogy of Shallow Alluvial Aquifers in 499–511.

7

As (III) Removal from Drinking Water using Cellulose-based Biosorbents : Characterization and Study of Various Parameters

Moonmoon Choudhary
Krishna G. Bhattacharyya

Abstract

This study investigates the removal of trivalent arsenic from drinking water using biosorbents derived from leaves of *Caesalpinia bonducella* (Radhachura). The biosorbent was characterised by specific surface area, anion exchange capacity and Fourier transform infrared spectroscopy. Uptake was rapid and equilibrium was achieved within 90 minutes for biosorbent of particle size 63-75 μm. Effects of As (III) concentration (0.5-5.0 mg L^{-1}) and biosorbent amount (1.0-5.0 gL^{-1}) was studied. The optimum pH values for adsorption of As (III) was found to be 8.0. The biosorbent was found to be efficient in removing As (III) from aqueous solutions as compared to other biosorbents already used for As (III) removal. Also the rapid As (III) uptake and low cost of Radhachura leaf powder makes them a promising material for arsenite removal.

Keywords: *As (III) Removal, Biosorbent, Radhachura Leaf Powder, Drinking Water, pH.*

Introduction

Arsenic is widely distributed in the nature and is 20th most abundant element in the earth's crust [1]. Arsenic is found in nature by volcanic eruptions, weathering reactions, biological activity and anthropogenic activities like mining, use of arsenic in pesticides, industrial waste discharges etc [2,3,4]. Long term ingestion of arsenic containing water and food leads to some serious health problems like keratosis, hyperkeratosis in hands and foot, cancer, respiratory and nervous disorders [5]. In humans, it can also cross the placental membranes and cause disorders to the unborn baby [6]. Arsenic contamination in groundwater is a worldwide problem including countries like USA, Mexico, Hungary, Poland and many of the asian countries which is affected most and includes largest arsenic-affected population. Among asian countries, the largest arsenic-affected population resides in Bangladesh, followed by west Bengal in india [7,8]. The deltaic region of GBM River system (Bengal Basin) is the most affected region in the world to a threatened level [9,10]. In natural water arsenic exist in two inorganic forms – As(III) or arsenite and As(V) or arsenate, among which As(III) is more toxic and mobile compared to As(V) [11]. The adverse effects of arsenic on human health have led WHO to set a maximum limit of arsenic in drinking water, i.e. 10μg/L [12].

The necessity of arsenic removal from water includes several methods like solvent extraction, chemical precipitation, iron coprecipitation, ion exchange and reverse osmosis [13, 14, 15]. Among the technologies to decontaminate arsenic from water, adsorption is most widely used due to its easy operation, cost effectiveness and eco-friendly nature [16, 17]. Various adsorbents like agricultural waste and plant biomass has been used for arsenic decontamination.

The present work uses *Caesalpinia bonducella* (Radhachura) leaf powder (RLP) for removal of As(III) from arsenite spiked water.

Materials and Methods

Biosorbent Preparation

Mature leaves of the Radhachura plants were collected from Botanical garden of Gauhati University. The leaves were washed with clean water, first sun dried followed by drying in an oven at around 343K for several hours till they became crisp. Leaves were then powdered in a grinder followed by several washings till the washings were colourless. The washed leaf powder was again dried in an oven for several hours up to 343K till water is removed completely. Lumps were ground in a mortar and the powder was sieved through a 75 μm and then by 63 μm sieve to obtain particles of uniform size, which were preserved in clean plastic containers. RLP of size between 75-63 μm were used for carrying out adsorption experiments.

Characterization of Biosorbents

The adsorbents were characterized with FTIR using KBr pallet (IR Affinity-1 Fourier Transform Infrared Spectrophotometer, Shimadzu, range 4000 – 400 cm^{-1}).

The surface area of the biosorbents was determined by the methylene blue adsorption method by using the following equation [18,19,20],

$$S = (q_m \cdot N_A \cdot A) / M \qquad (1)$$

where, q_m ($mg\ g^{-1}$) is the maximum adsorption capacity which can be obtained from Langmuir model, N_A is the Avogadro's number ($6.023 \times 10^{23} mol^{-1}$), A is the surface occupied by a molecule of Methylene Blue (taken as 130 $Å^2$) and M is the molecular weight of Methylene Blue (319.86 g mol^{-1}) [21,22].

Anion exchange capacity (AEC) of the biosorbents were determined by conductometric method and was calculated using the formula [23],

$$AEC = NV/W \qquad (2)$$

where N is the normality of $AgNO_3$ solution and V is its volume required by W g of anion exchanger.

Preparation of As (III) Solution

A stock of 1000 ppm sodium arsenite solution was prepared by dissolving the required amount of $NaAsO_2$ in double distilled water. Solutions of desired concentrations were prepared from the stock solution in its natural pH. Sodium arsenite AR (Loba Chemie, Mumbai) was used without further purification and obtained as sealed from the chemical store.

Adsorption Experiments

The adsorption experiments were carried out in 100 ml Erlenmeyer flasks by mixing a fixed amount of bioadsorbents with 50 ml of aqueous As (III) solution. The mixture was agitated in a thermostatic water bath (NSW, Mumbai, India) for a pre-determined time interval. The mixture was centrifuged for 15 min (Eltec centrifuge, TC4100 ~ 8000 rpm) and As (III) remaining unadsorbed in the supernatant liquid was determined with the help of an atomic absorption spectrophotometer (Varian SpectrAA 220). Before the actual experiments, sets of blank experiments were done to find if there was any adsorption on the walls of the container. The batch adsorption experiments were done under different conditions (Table 6.1).

Table 7.1: Various experimental conditions for adsorption of As (III) on RLP

pH	: 6.0 - 8.5
Interaction time (min)	: 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360
Amount of bioadsorbent (g L^{-1})	: 1.0, 2.0, 3.0, 4.0, 5.0
As (III) (mg L^{-1})	: 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0.
Temperature (K)	: 303
Adsorbent particle size (μm)	: 63 - 75

Results and Discussion

Characterization of Biosorbents

The specific surface area and anion exchange capacity was found to be 367 m^2/g and 8.87 meq $100g^{-1}$ respectively.

Peaks at 663 cm^{-1}, ~2850 cm^{-1} and 2920 cm^{-1} correspond to C-Cl, $-CH_2-$ (sym. stretch) and $-CH_2-$ (asym. stretch) groups respectively with %T values almost similar for RLP and ARLP. Peaks between 1000-1200 cm^{-1} indicate the presence of -C-C-, -C-N-, -C-O- groups. A strong peak for RLP at 1624 cm^{-1} can be assigned to N-H(bend) functional group (Fig. 6.1) [24,25].

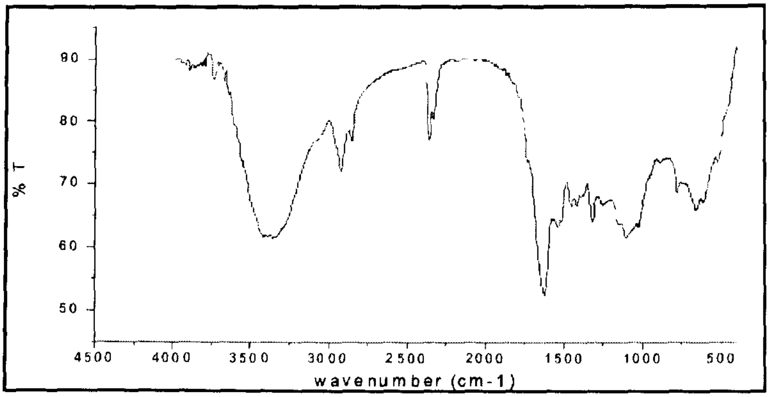


Fig. 7.1: FTIR spectra of RLP before adsorption

Adsorption Experiments

Effects of Agitation Time

Adsorption of As(III) on leaf powder attained rapid equilibrium within 90 min RLP. From Figure 2a, it is clear that adsorption occurred in two distinct phases – a very fast phase initially up to ~10 min and a comparatively slow phase resulting in equilibrium. Initially, there were large number of vacant active binding sites available at first phase of experiment and large amount of As(III) were bound rapidly on adsorbent surface at a faster rate. As binding sites became limited, the it became difficult for As(III) anions to attach themselves to the remaining vacant sites due to the repulsive forces developing between the arsenite ions and the solid surface. Equilibrium is reached when the adsorbent surface is saturated with As(III).

Effects of Biosorbent Amount

It was observed that percent removal of As(III) increases from 35.6 to 69.8% as shown in Fig. 2b) with increase in biosorbent amount which may be due to the increase in the number of active sites of the biosorbent available for adsorption.

Effects of pH

The pH is an important factor that often influences the mechanism of adsorption. The pH of the aqueous As (III) solution was maintained by adding a few drops of 0.1N NaOH or 0.1N HCl solution such that the volume of the solution had very little change.

From the Fig. 2c, it is clear that highest adsorption of 78.5 % was observed at pH 8.0 and the lowest adsorption of 62.1 % at pH 6.5. Similar results were also obtained for adsorption of As(III) using agricultural residue rice polish [26].

This suggests that the adsorbents were very effective in the natural pH range of drinking water. Arsenite exist as a stable neutral and non-ionic form H_3AsO_3 at $pH \leq 9$. In acidic environment, adsorbent surface becomes highly protonated, which is not favorable for adsorption of arsenite. So there occurs no electrostatic interaction between As(III) species and adsorbent surface at low pH values. As pH of the system increases, surface protonation decreases gradually. Maximum removal is observed at around pH 7.5 where only neutral species are available, and this may be attributed to the slow dissociation of the weak acid (H_3AsO_3) at pH (7.0 – 9.0) producing arsenite ion. This partially neutral and partially negatively charged arsenite ion is attracted to the positively charged (below pH 8.2) surface resulting in high As(III) removal to form H_2AsO_3. In alkaline medium, the negative charged $H_2AsO_3^-$ species starts dominating and the biosorbent surface also tends to acquire negative charges. This tendency of adsorbate species and adsorbent surface will continue to increase with increase of pH causing a gradual increase in the repulsive forces between the surface and adsorbate species resulting in a decrease of adsorption.

Effects of As(III) Concentration

Increase in the initial concentration of As(III) (0.5 – 5.0 mg/L) resulted in a decrease in the extent of adsorption from 73.00 to 35.28 % for RLP at 303 K.

When the As (III) concentration is low, the ratio of the number of As (III) anions to the number of available adsorption sites is small and consequently, adsorption is high and is independent of the concentration. As (III) anions are driven to the adsorbent surface by a force arising from the concentration gradient between the surface and the bulk solution. If a relatively higher concentration of As (III) is used, there will be many more As (III) anions for each active site on the biosorbent surface and the active sites are likely to be saturated with As (III).

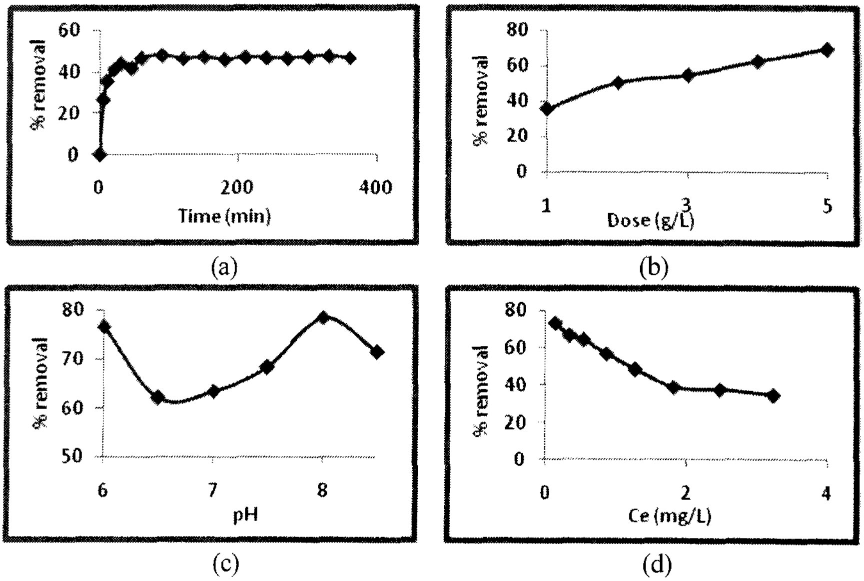


Fig. 7.2: (a) Effect of time, (b)Effect of dose, (c)Effect of pH and (d)Effect of concentration on biosorption of arsenite by RLP

Conclusion

Arsenite sorption was favoured around neutral pH, so RLP can be used to remove As(III) from natural water. Under optimized conditions the removal efficiency can be reached >90% in a relatively short operating time for batch process. This makes RLP an ideal candidate for As removal from drinking water or high volume low concentration complex waste waters. Easy-to-use system makes it suitable for homemade approaches for arsenic removal which do not require much technical knowhow. The selected plants are widespread species with the potential for application for the development of cheap arsenic filters with minimal capital investment.

References

1. Woolson, E.A., Arsenical Pesticides, ACS Symposium Series, 1975, 7, 108.

2. Smedley, P. L. and Kinniburgh, D. G,. A Review of the Source, Behaviour and Distribution of Arsenic in Natural Waters, Applied Geochemistry, 2002, 17, 517.
3. Khan, M. A. and Ho, Y. S., Arsenic in Drinking Water: A Review on Toxicological Effects, Mechanism of Accumulation and Remediation, Sian Journal of Chemistry, 2011, 23(5), 1889.
4. Jain, C. K. and Singh, R. D., Technological Options for the Removal of Arsenic with Special Reference to South East Asia, Journal of Environmental Management, 2012, 107, 1.
5. Saqib, A. N. S., Waseem, A., Khan, F. A., Mahmood, Q., Khan, A., Habib, A. and Khan, R.A., Arsenic Bioremediation by Low Cost Materials Derived from Blue Pine (Pinus Wallichiana) and Walnut (Juglans Regia), Ecological Engineering. 2013, 51, 88.
6. Squibb, K. S. and Fowler, B. A., The Toxicity of Arsenic and its Compounds in Biological and Environmental Effects of Arsenic, Edited by Fowler, B.A., Amsterdam, Elsevier, 1983, 233.
7. Mohan, D., Pittman Jr., C. U., Bricka, M., Smith, F., Yancey, B., Mohammad, J., Steele, P. H., Alexandre-Franco, M. F., Serrano, V. G and Cong, H., Sorption of Arsenic, Cadmium and Lead by Chars Produced from Fast Pyrolysis of Wood and Bark during Bio-oil Production, Journal of Colloid and Interface Science, 2007, 310, 57.
8. Guo, H.M., Wen, D.G., Liu, Z.Y., Jia, Y.F., Guo, Q. A Review of High Arsenic Groundwater in Mainland and Taiwan, China: Distribution, Characteristics and Geochemical Processes, Applied Geochemistry, 2014, 41, 196.
9. Nickson, R. T., McArthur, J., Burgess, W., Ahmed, K. M., Ravenscroft, P. and Rahman, M., Arsenic Poisoning of Groundwater in Bangladesh, Nature. 1998, 395.
10. Burgess, W. G., Hoque, M. A., Michael, H. A., Voss, C. I., Breit, G. N. and Ahmed, K. M., Vulnerability of Deep Groundwater in the Bengal Aquifer System to Vontamination by Arsenic, Nature Geoscience, 2010, 3, 83.
11. Malik, A. H., Khan, Z. M., Mahmood, Q., Nasreen, S. and Bhatti, Z.A., Perspectives of Low Cost Arsenic Remediation of Drinking Water in Pakistan and other Countries, Journal of Hazardous Materials, 2009, 168, 1.
12. World Health Organisation, Environmental Health Criteria, 224, 2nd ed., Geneva, 2001.
13. Kundu, S. and Gupta, A.K., Investigations on the Adsorption Efficiency of Iron Oxide Coated Cement (IOCC) towards As(V) - Kinetics, Equilibrium and Thermodynamic Studies, Chemical Engineering Journal, 2006, 273, 121.
14. Kumari, P., Sharma, P., Srivastava, S. and Srivastava, M. M., Biosorption Studies on Shelled Moringa Oleifera Lamarck Seed Powder: Removal and Recovery of Arsenic from Aqueous System, International Journal of Mineral Processing, 2006, 78, 131.
15. Choonga, T. S. Y., Chuaha, T. G., Robiaha, Y., Koaya, F. L. G. and Azni, I., Arsenic Toxicity, Health Hazards and Removal Techniques from Water: An Overview, Desalination, 2007, 217, 139.

16. Sun, X., Hu, C., Hu, X., Qu, J. and Yang, M., Characterization and Adsorption Performance of Zr-doped Akaganèite for Efficient Arsenic Removal, Journal of Chemical Technology and Biotechnology, 2013, 88, 629.
17. Márcia, C. S. F., Renedy, S. R., Cleide, A. B., Douglas, S. M., Fernando, B., Luiz, C. A. O., Mariandry, R., Márcio, C. P. and Jairo, L. R., Arsenic Removal from Contaminated Water by Ultrafine d-FeOOH Adsorbents, Chemical Engineering Journal, 2014, 237, 47.
18. Hang, P. T. And Brindley, G.W., Methylene Blue Adsorption by Clay Minerals. Determination of Surface Areas and Cation Exchange Capacities (clay-Organic Studies XVIII), Clays and Clay Minerals, 1970, 18, 203.
19. Lagaly, G., Characterization of Clays by Organic Compounds, Clay Minerals, 1981, 16, 1.
20. Schoonheydt, R. A. and Heughebaert, L., Clay Adsorbed Dyes: Methylene Blue on Laponite, Clay Minerals, 1992, 27, 91.
21. Farooq, U., Kozinski, J. A., Khan, M. A. and Athar, M., Biosorption of Heavy Metal Ions using Wheat-based Biosorbents - A Review of the Recent Literature, Bioresource Technology, 2010, 101, 5043.
22. Los, J. M. and Tompkins, C. K., Adsorption of Methylene Blue on a Positively Charged Mercury Surface, Journal of Chemical Physics, 1956, 24, 630.
23. Anirudhan, T. S. and Jalajamony, S., Cellulose-based Anion Exchanger with Tertiary Amine Functionality for the Extraction of Arsenic(V) from Aqueous Media, Journal of Environmental Management, 2010, 91, 2201.
24. Banwell, C. N. and McCash, E. M., Characteristic Stretching Frequencies of Some Molecular Groups in Fundamentals of Molecular Spectroscopy, Tata McGraw Hill, 2006, 86.
25. Pavia, D. L., Lampman, G. M. and Kriz, G. S., Infrared Spectroscopy in Spectroscopy, 2010, Fourth edition.
26. Ranjan, D., Talat, M. and Hasan S. H., Biosorption of Arsenic from Aqueous Solution using Agricultural Residue 'Rice Polish', Journal of Hazardous Materials, 2009, 166, 1050.

8

Förster Resonance Energy Transfer in Biology and Medicine

Sujit Kumar Ghosh

Abstract

While a donor molecule is placed in the vicinity of a metallic nanostructure, not only resonant energy transfer takes place but also the radiative lifetime of the donor molecule changes. The total decay rate of an excited molecule near a small sphere is governed by radiative loss through the modification of the boundary conditions of the electromagnetic field and non-radiative decay through which energy is dissipated. Resonant energy transfer systems, consisting of organic dye molecules and noble metal nanoparticles, have received considerable interest in designing synthetic light harvesting systems or as a spectroscopic ruler for tracking large-scale biomolecular conformational changes, drug discovery, and cellular imaging. In this article, theoretical perspectives and possible applications of resonant energy transfer systems have been reviewed.

Introduction

The elegant theory of transfer of energy, known as Förster Resonance Energy Transfer (FRET) was developed in 1948 by Professor Theodor Förster to explain the rate of fluorescence resonance energy transfer between a donor and an acceptor [1]. This theory assumes the dipole-dipole interaction between a fluorescent donor and an acceptor molecule of point dipoles and predicts a $1/R^6$ dependence of rate of energy transfer in center-to-center separation distance, R. Moreover, a pre-averaging over the orientations of the two dipoles is considered and has contribution in rate of energy transfer. The extent of FRET is readily predictable from the spectral overlap between the donor emission and absorption of the acceptors. FRET occurs between a donor fluorophore in the excited state and ground state acceptor molecule and not due to absorption of photon emitted from donor fluorophore but is the result of dipole–dipole interactions between the donor and acceptor. The energy transfer rate depends upon the extent of spectral overlap between donor emission and acceptor absorption, the quantum yield of the donor, extinction of the acceptor, the relative dipole orientation of the donor and acceptor, and the distance between the donor and acceptor molecules.

Discussion

The rate of Förster resonance energy transfer (non-radiative) for a donor and acceptor separated by a distance r is given by,

$$k_T(r) = \frac{Q_D K^2}{\tau_D r^6}\left(\frac{90000(In10)}{128\pi^5 N_A \eta^4}\right) J(\lambda) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 \quad (1)$$

where, Q_D is the quantum yield of the donor in the absence of acceptor, η the refractive index of the medium, N_A Avogadro's number, r the distance between the donor and acceptor and τ_D the excited-state radiative lifetime of the donor in absence of acceptor, and R_o is the Förster separation, $J(\lambda)$ is overlap integral between the emission spectrum of donor fluorophore and absorption spectrum of acceptor molecule. The term K^2 is describing the relative orientation in space of the transition dipoles of the donor and acceptor with respect to each other such that $K = \cos\theta_T - 3\cos\theta_D \cos\theta_A$ where, θ_T is the angle between the emission transition dipole of the donor and the absorption transition dipole of the acceptor, θ_D and θ_A are the angles between these dipoles and the vector joining the donor and the acceptor. The value of κ can vary from 0 to 4 depending on the relative orientation of the donor and the acceptor and usually assumed to be equal to 2/3, which is appropriate for dynamic random averaging of the donor and acceptor. The overlap integral $J(\lambda)$ expresses the extent of spectral overlap between the emission of donor fluorophore and absorption spectrum of the acceptor molecules could be written as [2],

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda = \frac{\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda)d\lambda} \quad (2)$$

where, $F_D(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta\lambda$ with the total intensity (area under the curve) normalized to unity, $\varepsilon_A(\lambda)$ the extinction co-efficient of the acceptor at λ, which is typically in units of M^{-1} cm^{-1} and is dimensionless. If is expressed in units of M^{-1} cm^{-1} and is in nanometers, then is in units of M^{-1} cm^{-1} nm^4. Förster separation distance,, corresponding to a rate of FRET, half the donor molecules decay by energy transfer and half decay by the usual radiative and non-radiative rates, is a function of the refractive index of the medium, and the expression can be written in terms of following equation,

$$R_0 = \left(\frac{9000(In10)k_p^2 Q_D}{128 N_A \pi^5 \eta_D^4} J(\lambda) \right)^{1/6} \tag{3}$$

The above expressions indicate that a quantum yield is necessary for determination of the Förster distance. The FRET efficiency, , is the fraction of photons absorbed by the donor which are transferred to the acceptor and could be expressed as in equation,

$$E_{ET} = \frac{k_T(r)}{\tau_D^{-1} + k_T(r)} = \frac{R_0^6}{R_0^6 + r^6} \tag{4}$$

accounts for the fraction of excitons transferred from donor to acceptor non-radiatively. The efficiency can be, experimentally, measured by monitoring changes in the donor or/and acceptor fluorescence intensities, or changes in the fluorescent lifetimes of the fluorophores using the following expression,

$$E = 1 - \frac{F_{DA}}{F_D} = 1 \frac{\tau_{DA}}{\tau_D} \tag{5}$$

where the fluorescence intensity of the donor in the absence and presence of acceptor are and and the lifetimes under these respective conditions are and respectively. Thus, FRET is a powerful photophysical technique and has been extensively used in the variety of investigations in the different branches of sciences.

When the dimensions of nanoparticles become smaller than the wavelength of the exciting light, energy can be confined in small spatial regions through the local excitation of surface plasmon resonances. The wavelength-dependent characteristics of surface plasmon modes on metal nanoparticles could be utilized for novel optical and electronic properties [3]. Utilizing these novel properties, nanoscience and nanotechnology are having an impact in biophotonics as well as in material science through radiative decay engineering of organic molecules in the close vicinity of conducting metal surfaces [2]. Förster resonant energy transfer systems, consisting of organic dye molecules and noble metal nanoparticles, have received considerable interest in designing synthetic light harvesting systems or as a spectroscopic ruler for tracking biomolecular conformational changes, drug discovery, and cellular imaging [4-6].

While Förster resonance energy transfer (FRET) is perhaps the best known of these optical probe methods, engineering fluorescent properties has led to significant advances in the fields of high-throughput DNA detection, bioimaging, drug delivery, and structural biology by utilizing the optical signature of the fluorescent molecule as a marker for imaging applications, or in energy-transfer studies [7,8]. Energy transfer can be radiative or nonradiative and is the basis of optical molecular rulers, where a donor and acceptor exhibit distance-dependent coupling [9,10]. These have the increased detectability and photostability of fluorophores and chemiluminescent species, improved DNA detection, the release of self-quenched fluorescence of the molecular probe over labeled proteins, enhanced wavelength-ratiometric sensing, and the application of metallic surfaces to amplified (ultrafast and sensitive) assay detection and diagnostic technology [11]. Gold nanoparticles can serve as ultra-efficient quenchers of molecular excitation energy in chromophore-Au NP composites, outranging the quenching efficiency of organic acceptor molecules. Moreover, many unique properties of gold nanoparticles, such as, colorimetric, conductivity, and nonlinear optical properties have been explored for potential applications in biomolecular detection [12]. The development of surface-based bioanalytical-sensor concepts has enabled selective detection of bio-recognition reactions. Conjugates of gold nanoparticles with oligonucleotides are of great current interest because of the potential use of the programmability of DNA base pairing to organize nanocrystals in space and the multiple ways of providing a signature for the detection of precise DNA sequences [13,14]. This biological strategy for fabrication of Au/DNA conjugates may be applied to molecular imaging, nanomedicine, and nanobiosensor technology [15]. Significantly extending the range of optical based methods in molecular rulers is an important step toward the biophysics [16]. Of particular interest in certain donor-acceptor systems whose luminescence properties have been thoroughly studied and exploited for purposes ranging over energy harvesting, trace analysis, and microscopy. The combination of conjugated polymers with gold nanoparticles can be used as donor-acceptor pairs in biophysical FRET experiments *in vitro* as well as *in vivo* [17].

Fluorophores have also been used as effective signal mediators for detecting biomarkers in bio-samples. The enhancement of the fluorescence can, therefore, improve the sensitivity of fluorophore-mediated biosensors which are expected to be effective for fluorophore-mediated bioimaging as well as biosensing [18]. Taking advantage of these beneficial roles of gold nanoparticles in accepting and shuttling the photogenerated electrons in Chl*a* to the collecting electrode, a superior photoelectrochemical cell based on gold nanoparticles and Chl*a* could be constructed that lead to an enhancement in charge separation efficiency [19]. It has been found that the gold nanoparticles play active role on the oxidation of NADH to NAD^- (an important coenzyme involved in the production of ATP) where the oxygen molecules are adsorbed on the surface of the particles due to the high surface-to-volume ratio of the gold nanoparticles [20]. The composites of dye aggregate and metal nanoclusters have been extensively used in color photography and light energy harvesting systems. Therefore, the convergence of nanoscale science with modern biology (Figure 1) is serving the multidisciplinary needs in developing organic-inorganic hybrid nanoassemblies for applications in material science, chemical sciences, biological science and medicinal applications.

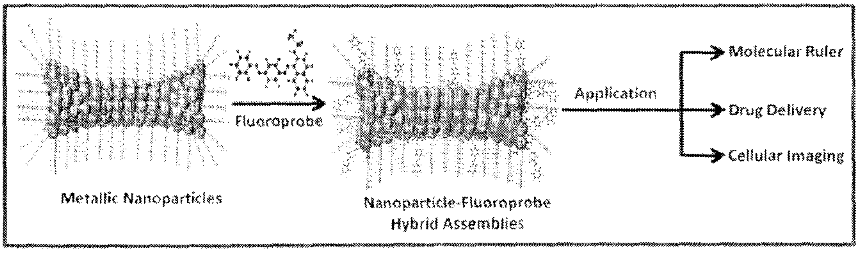


Fig. 8.1: Schematic presentation of the interdigitation into nanomaterial-fluoroprobe hybrid assembly for the generation of functional nanodevices.

References

1. Forster, T., *Ann. Phys.*, 1948, **437**, 55"75.
2. Geddes, C. D., Aslan, K.., Gryczynski, I., Malicka J. and Lakowicz, J. R., Radiative Decay Engineering, in: Topics in Fluorescence Spectroscopy, Kluwer Academic/ Plenum Publishers, New York, 2005.
3. Johansson, P., Xu, H. and Käll, M., *Phys. Rev. B*, 2005, **72**, 035427 1-17.
4. Jennings, T. L. and Strouse, G. F., *Adv. Exp. Med. Biol.* 2007, **620**, 34"47.
5. Medintz, I. L., Uyeda, H. T., Goldman, E. R. and Mattoussi, H, *Nat. Mater.*, 2005, **4**, 435"446.
6. Swierczewska, M., Lee, S. and Chen, X. Y., *Phys. Chem. Chem. Phys.*, 2011, **13**, 9929"9941.
7. Anker, J. N., Hall, W. P., Lyandres, O., Shah, N. C., Zhao, J. and Van Duyne, R. P., *Nat. Mater.*, 2008, **7**, 442"453.
8. Dubertret, B., Calame, M. and Libchaber, A. J., *Nat. Biotechnol.* 2001, **19**, 365"370.
9. Lakowicz, J. R., *Anal. Biochem.* 2001, **298**, 1"24.
10. Chen, N. T., Cheng, S. H., Liu, C. P., Souris J. S., Chen, C. T., Mou, C. Y. and Lo, L. W., *Int. J. Mol. Sci.*, 2012, **13**, 16598"16623.

11. Zhang, Y., Aslan, K., M. Previte, J. R. and Geddes, C. D., *Chem. Phys. Lett.*, 2006, **432**, 528-532.
12. Chowdhury, S., Wu, Z., Jaquins-Gerstl, K. A., Liu, S. P., Dembska, A., Armitage, B. A., Jin, R. C., and Peteanu, L. A., *J. Phys. Chem. C*, 2011, **115**, 20105"20112.
13. Basu, S., Jana, S., Pande, S. and Pal, T., *J. Colloid Interface Sci.*, 2008, **321**, 288-293.
14. Lytton-Jean, A. K. R. , Han, H. S., and Mirkin, C. A., *Anal. Chem.*, 2007, **79**, 6037-6041.
15. Tsai, C-Y., Shiau, A-L., Cheng, P-C., Shieh, D-B., Chen, D-H., Chou, C-H., Yeh, C-S., and Wu, C-L., *Nano Lett.*, 2004, **4**, 1209-1212.
16. Hong, B., and Kang, K. A., *Biosens. Bioelectr.*, 2006, **21**, 1333-1338.
17. Fan, C., Wang, S., Hong, J. W., Bazan, G C., Plaxco, K. W., and Heeger, A. J., *Proc. Nat. Acad. Sci.*, 2003, **100**, 6297-6301.
18. Ghosh, S. K., and Pal, T., *Chem. Rev.*, 2007, **107**, 4797-4862.
19. Barazzouk, S., Kamat, P. V., and Hotchandani, S., *J. Phys. Chem. B*, 2005, **109**, 716-723.
20. Huang, X., El-Sayed, I. H., Yi, X., and El-Sayed, M. A., *J. Photochem. Photobiol. B*, 2005, **81**, 76-83.

9

Green Chemistry: Brief Review of a Remedy for Sustainable Development

Cornelia Mary Lyngdoh

Abstract

Chemistry makes everything we do possible in our daily life: clothes, life saving drugs, food , homes, polymer products, all means of transportations etc. are all made possible by chemical industry. The sad part is that it releases hundreds of tonnes of hazardous waste every hour to land, water and air. It is associated with alarming health and environmental effects. As a result, the world faces serious threats and challenges to its sustainability. The tremendous pressure of technology based human activity has again given rise to the maximum uncertainty and insecurity in the concept of sustainability. However, with the advanced knowledge of Chemists, the sustainability dilemma has been devised. Green Chemistry plays a vital role for the growing concern about the environmental sustainability by introducing new chemical processes that do not pollute, reducing chemical waste and disposing them off in the appropriate way without causing much damage to the environment.

Keywords: *Green Chemistry, hazardous waste, environment, sustainable development*

Introduction

In 1991 Prof. Paul Anastas used the term "Green chemistry" [1] in a special program launched by the US Environment Protection Agency to implement sustainable development in Chemistry and chemical technology by industry, academia and government. In 1977 Green Chemistry Institute was formed to facilitate the communication between government agencies and Industrial corporations with Universities and research Institutes to design and implement new technologies that will be less harmful to human health and environment. In the same year, first conference highlighting Green chemistry was held in Washington. Thereafter, books and journals on the subject of green chemistry were introduced and also scientific conferences have soon held regularly. Green chemistry is defined[2-8] as the invention, design of various chemical products and application of chemical products and processes to reduce or to eliminate the use or generation of hazardous substances. It aims mainly to develop a sustainable approach to a non – polluted environment by reducing or eliminating the hazardous substances or wastes. It is a new philosophy of chemists "how to get good amount of products in the chemical industry by inventing a new chemical processes or route which lead to safer environment to achieve the goals to sustainable development. In other words, the main philosophy of green chemistry is to focus on the environment friendly chemicals in the new synthetic processes or routes. It also focuses mainly to increase the rate of the chemical reaction by using specific catalyst and lower the reaction temperatures to save energy. Chemistry has improved our life drastically. It has provided innumerable materials in our daily life in the form of food products, cosmetics, clothes, dyes, polymers, liquid crystals, nanoparticles, life saving drugs, new energy sources and many more[6]. The processes on the commercial scale require many chemical reactions using large quantities of reagents, solvents, acids, bases etc. These chemical processes produce the required products along with hazardous substances. We are proud of this successful achievement of chemists but it has negative effects on the environment. The principle of Green chemistry can be used to change all these negative impacts by inventing innovative chemical processes to restore the global sustainable development. The aim of today's chemical industry is to produce varieties of products that are so vital to the comfort and convenience of our daily life. But the big question is "How long will we continue this comfortable life at the expense of the environment?" To save the environment it is the right time to invent all these products through non- pollutive processes.

It must be made necessary to use the 12 principles [1-8] of Green chemistry to achieve the sustainable development. These principles are discussed briefly as follows:

1. **Prevention**: It is better to prevent waste than to treat or clean up waste after it has been formed. The generation of hazardous waste can be minimised by redesigning the processes of the chemical reaction.
2. **Atomic Economy**: Synthetic methods should be designed to maximize the incorporation of all the starting materials into the final product. It generates more require products and less waste.

$$\text{Percentage atomic economy} = \frac{\text{No. of atoms incorporated}}{\text{No. of atoms in the reactants}} \times 100$$

Example: Atomic Economy [5] of Diels - Alder reaction is 100%. The ideal E factor is zero; it indicates that positive environmental impact.

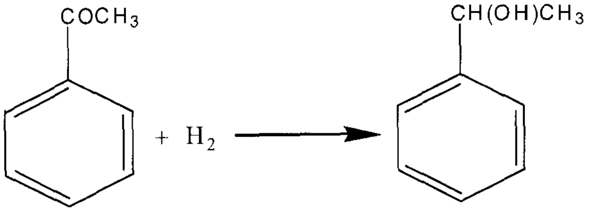
Table 9.1: The E-factor in the Chemical Industry

Industry segment	Product tonage	E factor (kg waste/kg product)
Oil refining	106 –108	– 0.1
Bulk Chemicals	104 –106	1 – 5
Fine chemicals	102 –104	5 – 50
Pharmaceutical	10 –103	25 – 100 +

Roger A. Sheldon, Chemtech, 1994

From the above table it is clear that the E factor increases from top to bottom. It is basically due to the fact that the production of fine chemicals generally involves multi-step syntheses and also due to stoichiometric reagents rather than catalytic methods. Further, the production of pharmaceutical product needs more steps and more purifying reagents to get pure form of the product.

Environmentally friendly catalytic reaction [9]:

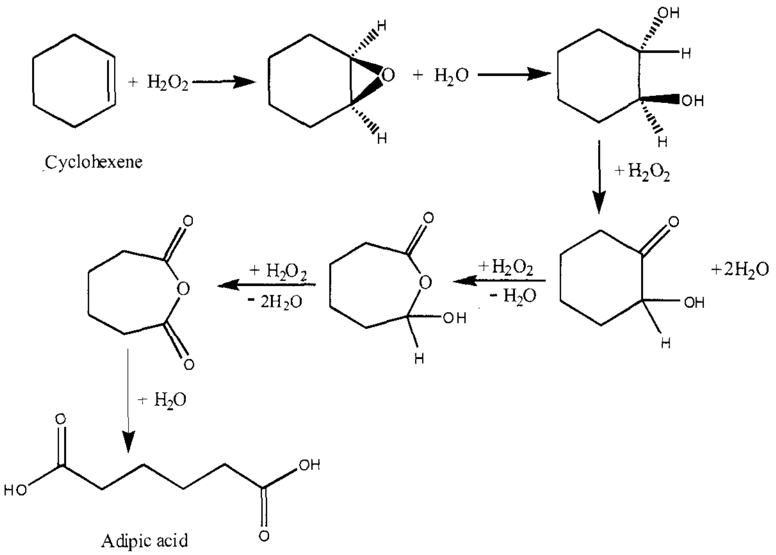


Atomic utilization 100%

Scheme I: Catalytic reduction reaction

3. Less Hazardous Chemical Synthesis: Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

   Example:



Scheme II: Synthesis of adipic acid by oxidation of cyclohexane using hydrogen peroxide [24, 25]

4. Designing Safer Chemicals: Chemical products should be designed to affect the desired function while minimising their toxicity.

   Example: Racemic thalidomide when administered during pregnancy leads to birth defects in many new borns. According to literature [5] one of the enantiomers has the curing effect while the other is the cause of defects.

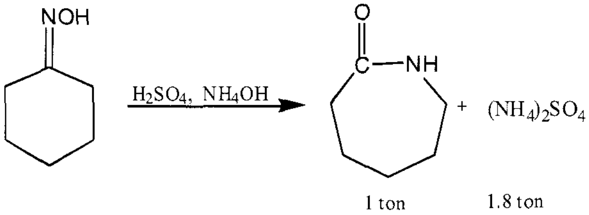
5. Safer Solvents and Auxiliaries: The use of solvents and auxiliary substances should be made unnecessary wherever possible and innocuous when used. Use of carcinogenic solvents like benzene, alcohol etc and CCl_4, $CHCl_3$, $CHCl_2$ etc. have now been replaced by supercritical CO_2 fluid and supercritical water [6, 10]. These are now becoming important of chemical separation solvent because of its low toxicity and non-inflammability.

6. Design Syntheses for Energy Efficiency: Energy requirements of a chemical processes should be kept to a minimum and reactions should be carried out at low temperature and pressure. Reactions using ultrasound energy are carried at room temperature with excellent yields.

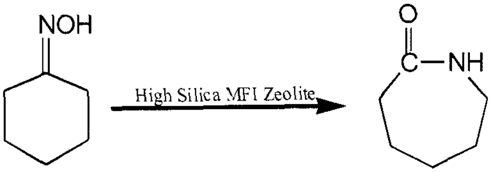
   Example: Ullmann's coupling which takes place at higher temperature giving low yields by conventional method gives more yields at low temperature and in short duration with ultrasound energy.

7. Use of Renewable Feedstocks: A raw materials and feedstocks should be renewable rather than depleting whenever technically and economically practicable. For green synthesis, the feedstocks should replace the petroleum sources.

   Example: Benzene used in the commercial synthesis of adipic acid has been replaced to some extent by the renewable and non-toxic glucose and the reaction is carried out in water.
8. Reduce Derivatives: Unnecessary derivatization (use of blocking groups, protection, deprotection) should be avoided whenever possible, because such steps require additional reagents and can generate more waste.
9. Catalysis: Catalytic reagents (as selective as possible) are superior to stoichiometric reagents. Catalysts are used in small quantity and can carry out a single reaction many times whereas stoichiometric reagents are used in large quantity and work only once. Example: Enzymes are the most efficient and commonest of the catalysts found in nature. Other examples [11-19] are

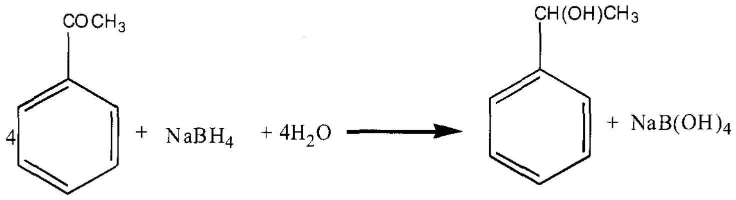


Scheme III: Current Catalytic Process for Bechmann Rearrangement discarding large amount of waste



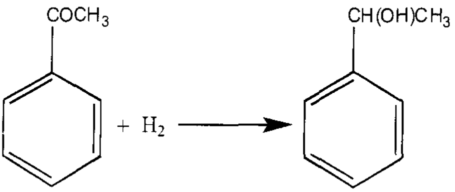
Scheme IV: New catalytic process for Bechmann Rearrangement without discharging waste, more greener process of synthesis

The scheme III produces large quantity of waste whereas the scheme IV does not. Therefore, scheme IV is more feasible for sustainable development. Similarly from the following Scheme V to IX [22], it is clear that scheme VI, VIII and IX are higher in atomic utilization, therefore they are more feasible for the sustainable development than others.



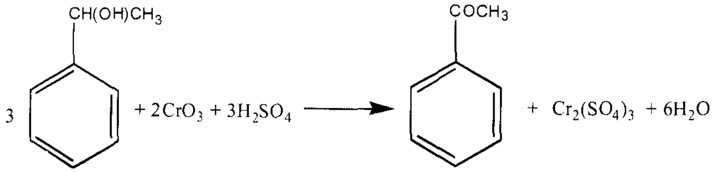
Atomic utilization 82%

Scheme V: Stoichiometric reduction reaction



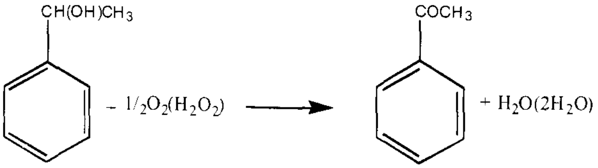
Atomic utilization 100%

Scheme VI: Catalytic reduction reaction



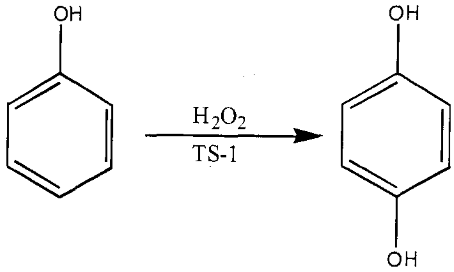
Atomic utilization 44%

Scheme VII: Stoichimetric oxidation reaction



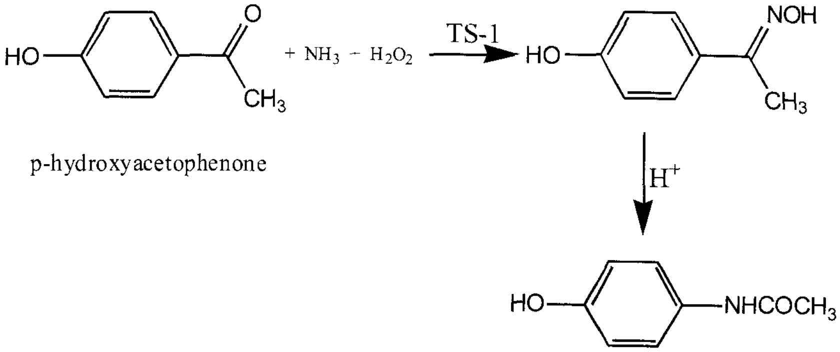
Atomic utilization 87%

Scheme VIII: Catalytic oxidation reaction



* TS-1 = Titanium silicate

Scheme IX: Synthesis of 4-hydroxyphenol



** TS-1 = Titanium (IV) silicate

Paracetamol

Scheme X: Paracetamol synthesis via ammoximation

10. Design for degradation: Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. Real - time analysis for pollution prevention: Analytical methodologies need to be further developed to allow for real time, in process monitoring and control prior to the formation of hazardous substances.

12. Inherently safer chemistry for accident prevention: Substances and the form of substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions and fires.

Example: Manufacture of gold atom nanoparticles used diborane (highly toxic and bursts into flame near room temperature) and benzene (cancer causing). Now diborane has been replaced by $NaBH_4$ and also eliminates the use of benzene.

Importance of these Principles

All these principles are not easy to apply immediately for many chemical processes. It needs a lot of positive hit and trial experiments to conclude percentage yield of the products without producing hazardous substances to the environment. It is very clear from the above that the principle of Green chemistry is to change the negative impacts through design innovation and to restore the planet's sustainable development [20,21]. As we all know that today's well developed industries depend upon petroleum. Petroleum is the building block to generate the chemicals. The chemicals produced like this require high energy, produce health hazard waste and pollute the environment. In view of deteriorating the environment, it is to prioritise the use of the principles of green chemistry by the chemical industries and researchers for the sustainable development. It should not be considered as a new philosophy within chemistry but rather the set of collections of the individual contribution in the various fields of sciences [7]. Basically it can be considered as set of principles eliminating out pollution and reducing the hazardous waste at the source for tackling the sustainable development. The waste produced in every reaction must be assessed for any hazard properties that they possess. Example: The oxidation reactions involving O_2 and H_2O_2 will be the outstanding achievement [23] as they give water as a by-product but specific experiments are required to prove it.

Economy and Environment

At present, millions of people have no proper health care due to many factors and the world population is increasing day by day. It is expected that the world population to increase by about 6 billions over the next hundred years. As the population increases, the poverty, education and healthcare should also be improved. To deal the situation effectively, the world economy needs to grow. However, economy growth is closely linked to environmental pollution [17]. Unless we save the environment from the pollution, it will delay our journey to a sustainable development. It will be more and more critical as the world population increases. There will be huge challenges faced by humanity in the sustainability of our lifestyles and systems [9]. Therefore it is necessary to uplift Green Chemistry for the sustainable development. Green chemistry may refer to as sustainable Technology. Sustainable development is our goal for future generation and green chemistry is the means to achieve it.

Advantage of Green Chemistry

In fact, Green chemistry promote more safer chemicals, atom economy in all the chemical processes, energy efficiency and the elimination of health hazard waste to protect the environment [1-9]. It is a highly effective approach to prevent pollution because it applies innovative scientific solutions to real world environmental situations. It is the right time for the chemical industries to rethink for the economic growth by using the principles of Green Chemistry. Some have reported that the supercritical CO_2 (use for dry cleaning) [20, 21] and supercritical H_2O can be used as solvent in place of volatile organic solvent, catalyst to bring down the activation energy and to get maximum yield at lower temperature. Therefore, the successful application of Green Chemistry depends upon the education and training to the young and future chemist, so that they can practice Green Chemistry for the sustainable development.

Green Chemistry Education

Teaching of green chemistry must be with practice. Different methodology should be used to educate the young minds particularly the promising young future chemists. The basic concept should be popularised in the schools. The difference between the stoichiometric and catalytic reactions should be taught at higher classes. The advantage of catalytic reaction over stoichiometric reactions should be emphasised to achieve the ideal E factor. Thereafter, the application of green chemistry in the research institutions and chemical industries should be made mandatory to achieve the sustainable development. The up to date educational materials, research papers should be readily available in the market, on the internet or any other form of electronic media.

Conclusion

Green Chemistry is not a new branch of chemistry rather it is the philosophical thoughts to achieve the sustainable development. For a sustainable development, first of all, the design and invention of new ideal process for every chemical reaction are still undertaken. In a sustainable society, green technologies must be used for production of required substances which are not harmful to the environment. To get non polluting products it is necessary to look specific starting materials [19]. If our generation were to consume natural resources to the extent that they were no longer a viable and usable option for future generations, this would violate the goals of sustainability. Therefore, renewable resources such as plant-based substances or solar energy can be used instead of fossil fuels that will eventually run out with respect to time. Re-cyclisation of the non-biodegradable [25] substances at the end of their use must be mandatory. Pharmaceutical industry has been trying to replace the traditional processes with greener. Many scholars have identified areas where improvement is needed. The concept of ideal E factors, atom economy is introducing to the organic synthesis both in the academia and industries. Consequently, green chemistry is leading to economic, environmental benefits particularly in the chemical and pharmaceutical industries.

References

1. Anastas, P.T. and Warner, J.C., Green Chem. Theory and Practice, Oxford Univ. Press. New York (1998).
2. Anastas, P.T. and Horvath, I.T., Chem. Rev., 2007, 107, 2169.
3. Trost, B. M., Angew. Chem. Int. Ed. Engl., 1995, 34, 259.
4. Sheldon, R.A., Green Chem., 2005, 7, 267.
5. Bharti, V.B., Resonance, 2008, 1041.
6. Ravichandaran, S., Int. J. Chem. Tech. Res., 2010,2(4) 2188.
7. Suresh D. Dhage, International Journal of Research in Pharmacy and Chemistry, 2013, 3(3) 518.
8. Sheldon, R.A., J. Chem. Tech. Biotechnol., 1997, 68, 381.
9. Cheng, M., Lobkovsky, E.B. and Coates, G.W., J. Am. Chem. Soc., 1998, 120(42) 11018.
10. Trost, B.M., Science, 1991, 254, 1471.
11. Sheldon, R.A., Chem. Commun., 2008, 3352.
12. Sheldon, R.A., Chem. Tech., 1994, March, 38.
13. Sheldon, R.A., J. Mol. Catal. A: Chemical, 1996, 107, 75.
14. Sheldon, R.A., Chem. Ind. (London), 1992, 903.
15. Sheldon, R.A., J. Chem. Tech. Biotechnol., 1997, 68, 381.
16. Sheldon, R.A., Pure Appl. Chem., 2000, 72(7) 1233.
17. Notari, B., Studies in Surface Science Catalysis, 1988, 37, 413.
18. le Bars. J, Dakka, J. and Sheldon, R.A., Appl. Catal. A: General, 1996, 69, 136.
19. Ahluwaliya, V.K. and Kidwai, M., New Trends in Green Chemistry, Anamaya Publisher, New Delhi 2004.
20. Micell Technology, Website: www.micell.com, accesses Dec. 1999).
21. US Patent, 4, 410,50(1983) and Maspero and U. Romano, J. Catal., 1994,146,476.
22. Gross, R.A. and Kelra, B., Science, 2002, 297, 803.
23. Saito, K., Aoki M. and Noyori, R., Science, 1998, 281, 1646.
24. Scott. G., Polym. Degrad. Stab., 2000, 68(1) 1.
25. Scott M.R. and James E.H., Journal of Chemical Education, 2000,77(12) 1627.

10

Synthesis, Characterisation and Application of Metal Nanoparticles

Deboshikha Bhattacharjee
Bekington Myrboh

Abstract

Nanomaterials are at the foremost edge of the rapidly developing field of nanotechnology. Their unique size-dependent properties make these materials superior and vital in many areas. Metal nanoparticles have been extensively studied because of their unique properties and potential applications in gas sensors, catalysis, drug carrier systems, solar cells and in photocatalytic treatment. Thus, development of facile procedures for the synthesis of metal nanoparticles and finding its application is an important area of research in recent era. Herein, we report a facile, efficient synthesis, characterisation and application of metal nanoparticles.

Key words: *SnO_2, Nano-particles, Catalyst, Anti-Bacterial Activity.*

Introduction

In the recent past enormous efforts are being directed towards the development of nanometer sized materials and finding applications of these materials. Metal nanoparticles are used for a wide range of applications in spectroscopy, biomedicine, and catalysis, which is the result of their unique catalytic, optical, electronic, and magnetic properties [1]. SnO_2 is one of the multifunctional materials having a variety of applications such as gas sensors, catalysis, optoelectronic devices, transparent conducting electrodes, and electrodes for Li-ion batteries [2]. The good stability, non-toxicity, and low cost of SnO_2

allow for its reliable commercial applications. The preparation of nano tin dioxide has attracted much attention recently, since it shows outstanding properties when used as a gas sensing material and also as a catalyst support [3]. In addition to the research on novel methods of synthesis, a lot of emphasis is also being paid to the antibacterial and antimicrobial studies of the synthesized nanoparticles. Antibacterial studies have become important primarily due to the bacterial resistance to conventional medical antibiotics. Moreover, the need for higher doses of the antibiotics has also been a matter of concern.

Materials and Methods

We purchased CTAB (99 %)) from Fluka, Sodium borohydride ($NaBH_4$) and tin chloride ($SnCl_2$) from Sigma-Aldrich, Millipore water was used for preparing all solutions.

Method

The experiments were performed in such a way as to keep the concentrations of all the components fixed except one whose effect is to be studied. In all the cases, the reducing agent was added at the end. The antibacterial activity of the synthesized SnO_2 NPs was evaluated using the agar well diffusion method.

Characterization of SnO2 NPs

SnO_2 NPs were characterized using UV-visible (VIS) spectroscopy. Dynamic Light Scattering (DLS) measurements were performed in a Malvern Nano ZS instrument for determining the size of SnO_2 NPs. Transmission electron microscope (TEM) images and selected area electron diffraction (SAED) data were acquired with a JOEL-JEM- 2100 instrument operating at 200 kV.

Result and Discussions

The SnO_2 nanoparticle has attracted much attention in the last decade because of its applications as gas sensors, but its use as a catalyst is not being explored yet to best of our knowledge. So, in spite of so many methods developed for the synthesis of Pyrazolo[4',3':5,6]pyrido[3,2-d]pyrimidine, an important biologically active compound, they suffer from certain drawbacks such as longer reaction times, unsatisfactory yields, high costs, harsh reaction conditions, and the use of a large quantity of volatile organic solvents. Thus, the possibility of performing multi-component reactions under room temperature using catalysts SnO_2 nanoparticles could enhance their efficiency from an economic as well as a green point of view.

It is worthwhile to test the antibacterial effect of the SnO_2 NPs prepared in the present study at nearly neutral medium, because often chemical methods of synthesis of SnO_2 NPs and the antibacterial studies were carried out in basic medium. The synthesized SnO_2 NPs were tested against five indicator bacteria for the antimicrobial efficacy, which resulted in varying zone of inhibitions indicating their antibacterial properties. The SnO_2 NPs have been found to show good inhibition of growth for all the five tested bacteria.

Conclusion

In the present study, we have successfully synthesized SnO_2. The synthesized NPs have been characterized by the visible spectra and also by DLS and TEM measurements. The nanoparticles proved to be a good catalyst for the synthesis of heterocyclic compound. The antibacterial effect of the synthesized NPs has been compared.

Acknowledgements

We would like to thank the Department of Chemistry and Sophisticated Analytical and Instrumentation Facility (SAIF) of North-Eastern Hill University and Ms Koel Biswas for antibacterial tests.

References

1. Wuithschick, M., Paul, B., Bienert, R., Adnan Sarfraz, A., Ulla Vainio, U. Sztucki,M.,Ralph Kraehnert, R. Strasser, P. Rademann,K. Emmerling, F. rg Polte, J. *Chem. Mater.* 2013, *25*, 4679.
2. Suematsu, K., Shin, Y., Hua, Z., Yoshida, K., Yuasa,M., Kida,T., Shimanoe, K., *Appl. Mater. Interfaces*, 2014, *6*, 5319.
3. Singh, A. K., Nakate, U.T. , *Advances in Nanoparticles*, 2013, *2*, 66

11

Assessment of Drinking Water Quality from Shallow Wells and Springs of Jowai, West Jaintia Hills

E. K. Mawkhiew
S. Choudhury
F. Rumnong

Abstract

Groundwater through shallow, open hand-dug wells, seepage wells and springs is a major source of water supply for domestic purposes for many households in and around Jowai. To determine the suitability of water from these sources for drinking purposes, samples were collected from 27 wells and 4 springs for analyses. The parameters considered include appearance, odour, hardness, pH, conductivity, temperature, turbidity, total dissolved solids (TDS), sulphate, phosphate, nitrate, bicarbonate, chloride, calcium, magnesium, sodium, potassium, iron, manganese, zinc, copper, lead, and total coliform.

The results show that the water samples are, in general, colourless, odourless, with low pH. The chemical constituents present in the groundwater are within permissible limit set by Bureau of India Standards (BIS) and World Health Organization (WHO). However, bacteria have been reported in almost 75% of the samples and 48% of the samples are found unsatisfactory for human consumption.

It is recommended that the water sources be tested annually for coliform bacteria and proper hygiene and sanitation of the areas surrounding the sources is to be maintained.

Keywords: *Groundwater Quality; Wells; Springs; pH; TDS; Hardness; Total Coliforms.*

Introduction

Water is the most essential natural resource for life next to air and water of adequate quantity and quality, is essential for healthy life. Besides piped water supply, groundwater is a major source of water supply for domestic purposes for many households in Jowai. The main source of groundwater is through shallow, open hand-dug wells, seepage wells, and springs even though bore holes are now being sunk in few households. Water from these wells and springs are used for domestic purposes. The area has a good number of private water wells and the depths of hand dug wells range from less than a meter to about 10 meters.

The interest in groundwater quality is mainly to ensure good quality drinking water as the quality of water of most of the wells and springs are not known, unlike public drinking water systems through piped water supply where the water is regularly checked and treated. Safe drinking water should be clear in appearance, tasteless and colourless (for aesthetic purposes), low in concentrations of toxic chemicals and free of pathogens.

Water, in general, is an excellent solvent and tends to dissolve minerals. Groundwater quality has been an issue of lower priority than groundwater availability and hence received relatively very little attention. However, not all groundwater is directly potable because it can contain lots of dissolved chemicals.

Since groundwater moves through rocks and subsurface soil, it has a lot of opportunity to dissolve substances as it moves. Therefore, groundwater will often have more dissolved substances than surface water. The farther, deeper, and longer that water migrates through an aquifer, the more dissolved minerals it will pick up. This process is influenced by several factors, including rock type, chemical weathering processes and anthropogenic factors. Locally, groundwater quality may be influenced by the atmosphere, soils, plants, and human activities, particularly in the areas of recharge near the land surface. Shallow groundwater is most susceptible to pollution and has significant potential for being contaminated by domestic as well as agricultural and industrial sources.

The primary objective of the study is to assess the drinking water quality of private/public drinking and domestic water supplies of shallow dug wells and springs in the area. A secondary goal is to create awareness to the local residents which could increase their knowledge of water management so they can use it as an opportunity to coordinate local efforts on water issues.

Study Area

Jowai, the district headquarters of the West Jaintia Hills is situated at 1310 m above mean sea level, atop a dissected plateau having a general NNE-SSW trend. It is located at latitude 25°26'32" N and longitude 92°12'00"E about 64 kms east of Shillong, the capital of Meghalaya along the NH- 44 connecting the other states of Mizoram, Tripura and Assam (Cachar).

The area is part of the Shillong plateau which represents a remnant of an ancient plateau of Pre-Cambrian Indian shield block. It consists of hard rock formation comprising of the Precambrian gneisses and Shillong Group quartzite along with younger intrusive and sedimentary rocks. Within Jowai town, the Precambrian rocks are concealed under a sequence of medium to coarse grained sandstone of Tertiary age. At some locations, conglomerate is also observed demarcating the base of the sedimentary cover.

The highest point is observed at Kiang Nangbah Government College in the northern part at 1372m above mean sea level, and the lowest point is near Syntu Ksiar, in the southern eastern part at 1199m above mean sea level. Under the influence of the various agents of weathering, mainly rain water, the area represents an extremely rugged topography with flat topped hillocks.

The drainage is carried mainly by the Myntdu River which surrounds Jowai on three sides, at the western, the southern and the eastern part and then flows south towards the Surma valley in Bangladesh. Most of the feeding streams are not perennial in nature and are dependent on the seasonal rainfall.

Materials and Methods

Sampling

Water sampling was carried out during the pre-monsoon months (March-April 2015) from 27 wells and 4 springs (Fig. 11.1); most of the wells are shallow hand-dug wells and seepage wells with depths ranging from 0.75 m to 10 m (Fig. 11.2). Sampling was also taken from one drilled well. The pH, conductivity, and temperature were measured in the field using portable meters. Samples for chemical analyses are collected in plastic containers (2000 ml) and for trace metals analyses, samples are collected in polyethylene bottles (1000 ml) acidified with concentrated nitric acid to a pH≤2. The bottles are washed with the water to be sampled several times. For bacteriological analyses, the samples are collected in (200-250 ml) glass bottles collected from the laboratory, washed and sterilized before hand with their caps wrapped with brown paper to prevent contamination while handling the bottle. All the samples collected are then transported to the laboratory within 24 hours of the sampling time.

Analysis

The standards for drinking water are specified by the World Health Organisation (WHO 2011) on the basis of several factors, viz., taste and odour, pH, TDS, hardness and chloride content, among others. According to Apello and Postma (2005), a standard groundwater chemical analysis will as a minimum comprise values for temperature, electrical conductivity (EC), pH, the four major cations (Na^+, K^+, Mg^+, Ca^+) and four major anions (Cl^-, HCO_3^-, SO_4^{2-}, NO_3^-).

Analyses of the samples were carried out at King Edward VII Memorial Pasteur Institute and Medical Research Institute, Shillong and Meghalaya State Pollution Control Board Central Laboratory, Shillong. The methods used for estimation of the various parameters are shown in Table 11.1.

Results and Discussion

Appearance

Generally groundwater has no colour. The colour of the water is due to the presence of substances in solution. All the samples have been reported as 'clear' in appearance.

Turbidity

Turbidity in water is because of suspended solids and colloidal matter. Turbidity values of the samples ranges from 1.2 to 2.7 NTU (Nephelometric Turbidity Units), which are within desirable limits.

pH

pH is a measure of the intensity of acidity or alkalinity and the concentration of hydrogen ion in water. No health-based guideline value is proposed for pH. Although pH usually has no direct impact on consumers, it is one of the most important operational water quality parameters.

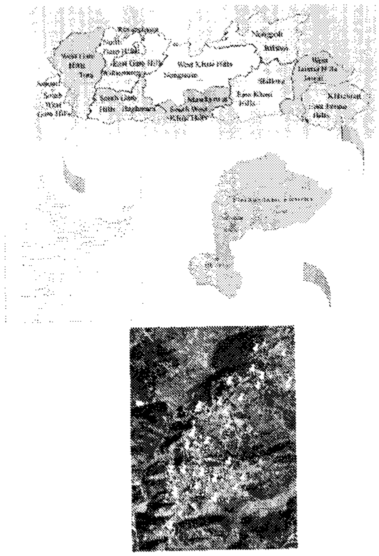


Fig. 11. 1: Location map of Study Area

The pH values of the water samples collected from springs and wells are on the acidic side varying from 4.7 to 6.7, which is lower than the maximum contaminant level for pH in drinking water as given by the WHO and BIS (6.5–8.5).

Fig. 11. 2(a) A spring.

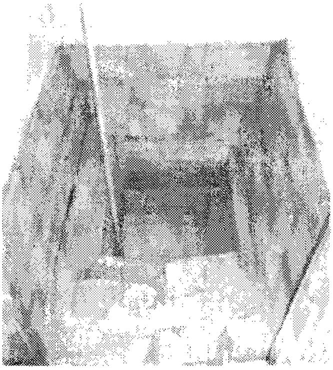


Fig. 11.2(b) Seepage well (lined with stone blocks).

Fig. 11. 2(c) Seepage well

Fig. 11.2(d) Dug well

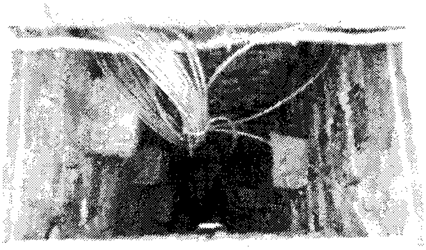


Fig. 11.2(e) Dug well (lined with stone blocks).

Fig. 11.2: Wells and Springs within the Area of Study.

Table 11.1: Parameters and Methods

Sl. No.	Parameter	Method
1	pH	pH Meter
2	Temperature	Thermometer
3	Electrical Conductivity	Conductivity Meter
4	Total Dissolved Solids (TDS)	Filtration Method
5	Turbidity	Nephelometric Method
6	Total Hardness	EDTA Titration Method
7	Calcium	EDTA Titration Method
8	Magnesium	Calculated from 6 and 7
9	Sodium	Flame Photometric Method
10	Potassium	Flame Photometric Method
11	Chlorides	Argentometric Method
12	Bicarbonates	Titration
13	Sulphate	Stannous Chloride Method
14	Nitrate	Selective Electrode Method
15	Phosphate	Stannous Chloride Method
16	Iron	Phenanthroline Method
17	Manganese	AAS Method
18	Zinc	AAS Method
19	Copper	AAS Method
20	Lead	AAS Method
21	Total Coliform	MPN Method

Determination of Various Parameters

Temperature

Determination of temperature is an important observation and measurements were taken in situ with the help of glass thermometers in shallow wells and springs and in case of deeper wells measurement is done with the help of an infra red thermometer. It is an important factor influencing the palatability and desirability of drinking water. The temperature of the wells and springs is found to be between 14.7°C and 20.1°C.

Electrical Conductivity (EC)

Electrical conductivity is a measure of water capacity to convey electric current. It signifies the amount of total dissolved salts. Electrical conductivity

(EC) is a parameter related to total dissolved solids (TDS). The conductivity values for the samples ranged from14 ìS/cm to 307ìS/cm.

Total Dissolved Solids (TDS)

High concentrations of total dissolved solids may cause adverse taste effects. Total dissolved solids are the residue of a filtered water sample after evaporation. The TDS of water samples show values between 22 mg/l to 450 mg/l indicating the fresh water class (< 1,000 mg/l) and satisfy the criteria of WHO (2011) for drinking water.

Hardness

Hardness in water is caused by dissolved calcium and, to a lesser extent, magnesium. The total hardness of the water samples varies between 16 mg/l to 150 mg/l. In general, except for two wells (samples no. 13 and 29) where the water is found to be moderately hard (76 mg/l and 150 mg/l), the hardness of the water samples is found to be soft and within the desirable limits (< 300 mg/l) specified by the ISI (1991) for drinking water.

Table 11.2: Classification of Water Hardness

Hardness (mg/L)	*Degree of hardness*
0-75	Soft
75-150	Moderately hard
150-300	Hard
>300	Very hard

(Source: Sawyer and McCarty, 1967)

Sulphate

Presence of sulphate is detected in 24 of the samples and range in values from 1.28 mg/l to 37.3 mg/l, which fall within the desirable limits (<200 mg/l) specified by the ISI (1991) for drinking water.

Phosphates

Presence of phosphate is detected in 27 of the samples and range in values from 0.002 mg/l to 0.019 mg/l.

Nitrate

Groundwater can also be contaminated by sewage and other wastes rich in nitrates. The presence of nitrate and nitrite in water has been associated with methaemoglobinaemia, especially in bottle-fed infants. Nitrate may arise from the excessive application of fertilizers or from leaching of wastewater or other organic wastes into surface water and groundwater (WHO, 2011). In all samples the nitrate content is found to be within the desirable limits (<45 mg/l) as specified by the ISI (1991) for drinking water except for one well (sample no. 29) where the nitrate content is reported to be higher at 66.3 mg/l but within permissible limits(<100 mg/l).

Calcium

Calcium (as $CaCO_3$) is reported to be between 8.5 mg/l to 40 mg/l (Desirable limit < 75 mg/l) in all the samples other than sample no. 29 which has a higher value of 120 mg/l (Permissible limit < 200 mg/l).

Magnesium

Magnesium (as $MgCO_3$) is between 5 mg/l to 31 mg/l (Desirable limit < 30 mg/l).

Bicarbonates

Bicarbonates in the samples vary from 12 mg/l to 40 mg/l which are within acceptable limits.

Chloride

The most important source of chlorides in the waters is the discharge of domestic sewage. The chloride content of the samples is between 6 mg/l to 50 mg/l which is within the admissible limits.

Sodium

The amount of sodium present in the samples is from 1 mg/l to 17 mg/l.

Potassium

Potassium concentration in the samples analyzed ranged from 2 mg/l to 11 mg/l.

Iron

Iron occurs in water either as ferrous iron or as ferric iron. It is dissolved practically from all types of rocks particularly from those containing minerals like pyroxene, magnetite, pyrite etc. It may also be added to the groundwater from contact with iron objects like well casing, piping etc. Levels as low as 0.2 mg/l to 0.3 mg/l will usually cause brown or reddish staining of laundry and plumbing fixtures. The presence of iron bacteria in water supplies will often cause these symptoms at even lower levels. The total iron content of the well and springs varies from 0.09 mg/l to 0.13 mg/l which is below the maximum contaminant level of 0.3 mg/l.

Zinc

Zinc is detected in 12 of the samples and is within the desirable limit (< 5 mg/l).

Manganese

Presence of manganese is reported in 5 samples in minor amount ranging from 0.03 mg/l to 0.1 mg/l (Permissible limit < 0.3 mg/l).

Copper and Lead

Copper and lead are not detectable in the samples.

Total Coliform

Testing for bacteria is the most common quality check for well water. Bacteria and microorganisms present in groundwater are too small to be seen by the naked eye; some are harmless while some are pathogenic. According to the U.S. Environmental Protection Agency's website on drinking water, "*...the presence of coliform bacteria indicates that the water is potentially dangerous and should not be consumed unless boiled.*"

In the present study tests for total coliform were carried out in the water samples collected from wells and springs. Results are reported as a most probable number (MPN). The MPN is only a statistical estimate of the number of bacteria that, more than any other number, would probably give the observed result; it is not an actual count of the bacteria present.

Of the 31 samples, in 8 samples no bacteria were reported, in 5 samples the MPN of coliform in 100 ml of water is found to be less than 2; 3 samples were reported as suspicious and 15 samples were reported as unsatisfactory for human consumption with MPN of coliform in 100 ml of water from 12 to 180+.

Table 11.3: Presumptive Total Coliform Count

Class	Grade	MPN of Coliform in 100ml of water
I	Excellent	0
II	Satisfactory	1-3
III	Suspicious	4-10
IV	Unsatisfactory	>10

(Source: Institute of Tropical Medicine, Antwerp)

The water quality analysis results of the samples collected during the study are given in Table 11.3. All Units are in mg/l except E.C., which is expressed in microsiemens/cm (ìS/cm), temperature, expressed in degree Celsius (°C) and pH.

The results of the analyses indicate that the groundwater around the study area fall within the acidic range with pH values ranging from 4.7 to 6.7 below the permissible limit (BIS: 6.5-8.5). The acidic nature of water may be attributed the geology of the area.The electrical conductivity values vary from 14 to 307ìS/cm. Except for two wells where the water is "moderately hard", the hardness in the other samples indicate that the water is soft in quality. Cl^-, HCO_3^-, SO_4^{2-}, NO_3^-, Na^+, $K^{+,}$ $Mg^{+,}$ Ca^+, Fe, Zn and Mn are present within permissible limit as per Bureau of Indian Standard (IS: 10500-91) and World Health Organization (2011) guidelines.

Of the 31 samples analyzed, presence of bacteria have been reported in 23 of the samples and 15 of the samples are found to be unsatisfactory for human consumption. It was observed that wells and springs which are located very near to residential areas and at a comparatively lower altitude than the surroundings show higher incidences of bacterial contamination. Due to time and other constraints, the actual sources of the contaminants could not be ascertained by this study and a thorough study is needed but it is recommended that the wells and springs be tested annually for coliform bacteria to detect contamination.

Table 11.4: Analytical Data of the Water Samples in the Study Area

Sample No.	Type	Temperature °C	pH	Conductivity (?S/cm.)	TDS (mg/l)	Turbidity (NTU)	Total Hardness(mg/l)	Sulphate(mg/l)	Phosphates(mg/l)	Nitrate (mg/l)	Bicarbonates(mg/l)	Chlorides(mg/l)	Sodium(mg/l)	Calcium as CaCO3(mg/l)	Magnesium as MgCO3(mg/l)	Potassium(mg/l)	Iron(mg/l)	Zinc(mg/l)	Manganese(mg/l)	Copper (mg/l)	Lead(mg/l)	MPN of Coliform
1	Dugwell	17	5.5	43	116	1.6	20	1.28	0.005	1.14	20	9	3	12	8	2	0.09	BDL	BDL	BDL	BDL	0
2	Dug well	20	5.6	36	70	1.7	16	1.36	0.005	2.29	12	8	3	8.5	7.5	4	0.1	0.19	BDL	BDL	BDL	0
3	Seepage well	16.8	5	36	144	1.4	26	1.47	0.003	6.99	12	19	8	16	10	4	0.09	BDL	0.07	BDL	BDL	5
4.	Dug well	17.2	6.7	76	92	1.8	30	1.28	BDL	1.37	32	17	7	20	10	5	0.09	BDL	BDL	BDL	BDL	18
5.	Spring	17.5	5.3	14	22	1.7	26	BDL	0.014	0.3	12	6	1	15	11	2	0.11	BDL	BDL	BDL	BDL	1
6.	Dug well	18.1	5.6	91	132	2	40	4.2	0.005	1	40	7	2	25	15	2	0.09	BDL	BDL	BDL	BDL	0
7.	Seepage well	19.4	5.8	85	202	1.6	34	BDL	0.006	2.6	32	15	5	20	14	2	0.1	0.06	0.04	BDL	BDL	1

Sample No.	Type	Temperature °C	pH	Conductivity (?S/cm.)	TDS (mg/l)	Turbidity (NTU)	Total Hardness(mg/l)	Sulphate(mg/l)	Phosphates(mg/l)	Nitrate (mg/l)	Bicarbonates(mg/l)	Chlorides(mg/l)	Sodium(mg/l)	Calcium as CaCO3(mg/l)	Magnesium as MgCO3(mg/l)	Potassium(mg/l)	Iron(mg/l)	Zinc(mg/l)	Manganese(mg/l)	Copper (mg/l)	Lead(mg/l)	MPN of Coliform
8.	Dug well	20.1	5.4	49	202	1.7	20	4.1	0.003	1.7	14	13	4	12	8	2	0.09	BDL	BDL	BDL	BDL	12
9	Dug well	18.7	5.3	156	140	1.5	34	4.1	BDL	6	22	32	16	19	15	10	0.12	0.01	0.1	BDL	BDL	0
10	Seepage well	19.3	5.9	198	170	1.6	62	12.3	0.002	15.3	24	26	13	45	17	8	0.12	0.35	BDL	BDL	BDL	20
11.	Spring	19.1	5.3	190	326	2.3	46	4.56	0.002	17.1	14	29	15	30	16	9	0.13	0.01	BDL	BDL	BDL	0
12.	Dug well	20	5.7	135	258	2.1	44	8.2	0.003	10.2	20	21	11	30	14	2	0.1	0.1	BDL	BDL	BDL	1
13.	Dug well	18.4	5.8	307	348	2	76	19.2	0.008	13.4	40	50	17	45	31	10	0.1	BDL	BDL	BDL	BDL	3
14.	Seepage well	18	5.2	67	164	1.5	20	4.6	0.019	2.78	16	16	4	15	5	4	0.11	0.05	0.02	BDL	BDL	180+
15.	Spring	19.4	6	120	192	2.2	38	BDL	0.014	5.88	24	16	6	25	13	7	0.1	0.1	BDL	BDL	BDL	160
16.	Seepage well	18.8	5.1	155	270	1.2	40	5.5	0.004	10.7	20	24	7	30	10	3	0.11	0.01	0.03	BDL	BDL	8
17.	Dug well	17.4	5.5	89	86	1.7	30	BDL	0.005	5.8	20	17	7	20	10	3	0.12	0.02	BDL	BDL	BDL	180+
18.	Dug well	17.8	6.4	156	102	1.7	40	BDL	0.01	7.9	32	28	13	30	10	10	0.1	0.1	BDL	BDL	BDL	90
19.	Seepage well	19.3	5.7	126	92	1.6	36	5.6	0.003	6	20	29	11	20	16	5	0.12	0.03	BDL	BDL	BDL	1
20.	Dug well	17.4	4.8	70	74	1.7	26	7.6	0.004	4	12	18	5	15	11	4	0.13	BDL	BDL	BDL	BDL	14
21.	Drilled well	18.4	5.4	83	130	1.8	50	37.3	0.002	1.7	18	19	9	35	15	10	0.1	BDL	BDL	BDL	BDL	0
22.	Seepage well	17.4	4.8	51	114	1.8	22	5.6	0.002	8	12	20	7	12.5	9.5	6	0.1	BDL	BDL	BDL	BDL	180+
23.	Seepage well	19.8	5.4	85	170	1.7	60	6.9	0.009	17.4	26	35	17	40	20	10	0.11	BDL	BDL	BDL	BDL	180+
24	Dug well	14.7	5	44	64	1.8	28	12.3	0.005	5.1	16	15	5	15	13	9	0.12	BDL	BDL	BDL	BDL	180+
25.	Seepage well	19.8	5	44	34	1.5	22	6	0.007	4.8	12	18	7	15	7	5	0.1	BDL	BDL	BDL	BDL	160
26.	Seepage well	18.9	6.7	65	74	1.7	32	7.3	0.008	8.5	18	25	11	20	12	10	0.1	BDL	BDL	BDL	BDL	160
27.	Seepage well	17.9	5.8	46	106	1.8	28	7.4	0.015	6.7	18	16	7	15	13	5	0.1	BDL	BDL	BDL	BDL	20
28.	Seepage well	17.4	4.7	196	450	2.7	150	6.1	0.002	66.3	14	47	17	120	30	11	0.11	BDL	BDL	BDL	BDL	0
29.	Dug well	19.5	5.2	184	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	160
30.	Dug well	19	5.9	74	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1
31.	Spring	21	5.8	48	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0

Conclusion

As per result of the groundwater analyses as far as the tested parameters are concerned, the water samples collected from the wells and springs within the study area are, in general, colourless, odourless, and the pH is low indicating that the waters are acidic in nature. While humans have a higher tolerance for pH levels there are still concerns. The implications of low pH may include corrosion, solubility of heavy metals and it imparts bitter and metallic taste to the water.

The chemical constituents present in the groundwater are within permissible limit set by BIS and WHO. However, bacterial contamination is a problem with most of the water sources. Bacteria have been reported in almost 75% (23 out of 31) of the samples and about 48% (15 out of 31) of the samples are found unsatisfactory for human consumption. Although coliforms are harmless, their presence in groundwater indicates the possibility of the presence of pathogenic bacteria. Water from these sources should not be consumed directly unless properly boiled.

While groundwater is an immensely important resource, it will become even more so as Jowai develops and the population continues to grow in the years to come. Groundwater, even when located at depths is still susceptible to pollution and excessive withdrawals. Therefore, it must be protected and managed. It is suggested that people become aware of the types of activities that are occurring near the drinking water sources that could potentially impact the water quality and quantity. Proper hygiene and sanitation of the areas surrounding the drinking water sources need to be maintained by the well owners and the local community as well. Proper well construction and continued maintenance are keys to the safety of the water supply. The wells should be located far from possible sources of contamination and ensure that rainwater flows away from the water sources as rainwater can pick up harmful bacteria and chemicals on the land's surface.

Acknowledgements

This study is part of a project carried out with financial assistance from the UGC in the form of a Minor Research Project. The authors are thankful to our students, Edify and Bobby; Manda, our office staff and Sowat, our friend and colleague for their help during the collection of water samples. Getting the samples analyzed would not have been possible without the kind help and cooperation of the staff of King Edward VII Memorial Pasteur Institute & Medical Research Institute, Shillong and Meghalaya State Pollution Control Board Central Laboratory, Shillong.

References

1. Apello, C. A. J. and Postma, D., 2005, Geochemistry, Groundwater and Pollution (2^{nd} Edition), A. A. Balkema Publishers, Leiden, Netherlands.

2. BIS 1991, Specifications for Drinking Water, IS: 10500:1991, Bureau of Indian Standards, New Delhi.
3. EPA (Environmental Protection Agency) 2001, Parameters of Water Quality: Interpretation and Standards, Environmental Protection Agency, Ireland.
4. Fetter, C.W., 2007, Applied Hydrogeology (2nd Edition Reprint), CBS Publishers & Distributors, New Delhi, India, ©1988 Merril Publishing House.
5. Patil, V.T. and Patil, P.R., 2011, Groundwater Quality of Open Wells and Tube wells around Amalner Town of Jalgaon District, Maharashtra, India. *E-Journal of Chemistry*, **8(1)**, 53-58. http://www.e-journals.net (accessed on 16.07.2015).
6. Todd, D.K., 1980, Groundwater Hydrology (Second Edition), Wiley India (P.) Ltd., New Delhi.
7. WHO (World Health Organization) 2011, Guidelines for Drinking Water, Fourth Edition, Geneva: WHO.
8. http://www.fondriest.com/environmental-measurements/parameters/water-quality/turbidity-total-suspended-solids-water-clarity (accessed on 25.07.2015).

12

Catalytic Wet Peroxide Oxidation Of Industrial Organic Pollutants From Water By Ni(II)-Impregnated Fly Ash As Catalyst: A Case Study With Phenol And 2-Chlorophenol

Bharati Deka
Krishna G. Bhattacharyya

Abstract

Phenol and its Chlorinated derivatives were persistent and toxic organic compounds which are harmful to organisms and humans even at very low concentrations. Biological oxidation used for removing these organics from industrial effluents is not suitable and catalytic wet oxidation is one of the major alternatives for their destruction. In the present work, phenol and its chlorinated derivatives (2-chlorophenol) were undergo wet peroxide oxidation catalyzed by Ni(II) impregnated fly ash at 323K in water. The catalyst was prepared by impregnating Ni(II) over water washed fly ash by refluxing for 6 h. Characterization of the catalyst was done by AAS, FT-IR and BET. The material was calcined at 773 K for 5 h before use. To optimize the reaction conditions, the effects of different parameters, such as reaction time, molar ratio of hydrogen peroxide, catalyst dose and the initial reactant concentration of the phenol and 2-chlorophenol were evaluated in batch reactor. It was confirmed that the catalyst had nearly no effect on the degradation of phenolic compounds without hydrogen peroxide by doing some blank experiments. The conversion with impregnated Ni(II)-fly ash for phenol and 2-chlorophenol is respectively 64.8 and 59.7 % in 300 min for the reactant: H_2O_2 mole ratio 1:1. The conversion was increased with time and with increasing molar ratio of hydrogen peroxide in all cases. The catalytic oxidation was found to follow second order kinetics having rate constant 1.17 and 2.21 g mol^1 min^{-1} respectively.

Keywords: *Phenol; 2-chlorophenol; Catalytic Wet Peroxide Oxidation; Ni(II)-fly ash, Second Order Kinetics.*

Introduction

Many industries like petroleum, petrochemical and pharmaceutical generate wastewater that contains lots of organic products which are hazardous to the environment especially to natural water resources. Phenol and 2-chlorophenol was found to be one of the most important water pollutants as it has extreme toxicity to the aquatic life, carcinogenic nature and resistance to biodegradation. In UESEPA list both the organic compounds are recognized to be the most important pollutants [1, 2]. The toxicity of phenol and substituted phenols towards aquatic environment and human life, the lack of biodegradability, generation of disagreeable odor in the receiving waters, are some of the reasons for classifying them as priority pollutants [3, 4, 5]. These toxic, carcinogenic and mutagenic pollutants enter into the environment mainly from the paper industries, domestic and agro industries, petrochemicals, plastic and insecticidal industries [6, 7, 8]. Therefore specific chemical processes are required to convert this carcinogenic, no biodegradable compound into industrially degradable products.

Different strategies such as "clean" technology, improvement of existing technologies, or development of new, innovative technologies (advanced oxidation) have been developed to reach to reasonably satisfactory level of the desired objectives. Among these, the catalytic wet air oxidation (CWAO process) appear to be very promising to achieve high conversion of organic pollutants, but unfortunately this process need high pressure and temperature implying increasing the investment costs [9, 10, 11, 12]. By contrast, the use of hydrogen peroxide (CWPO process) allows performing oxidation reaction at ambient conditions, limiting the investment costs, even if the low reaction rates compare to those of CWAO process needs high volume reactors. Hydrogen peroxide appears to be an appropriate and potential oxidant due to high oxygen content and yielding water as the only byproduct. Again heterogeneous catalysts are found to be more desirable because homogeneous catalysts require an additional separation step for the soluble species leading to high treatment costs. Among various catalysts, transition metal oxides and supported noble metals have been reported to be widely applied in CWAO [13, 14, 15]. But their use is limited by durability constraints and difficulties in recovery after use. This problem is usually overcome by dispersing the catalyst components on a porous support such as charcoal, polymers, zeolites and layered structures (pillared clays and hydrotalcites) by direct intercalation, ion exchange and encapsulation [16].

In the present work, Ni(II) impregnated fly ash was used as the catalyst for oxidation of phenol and 2-chlorophenol in water in presence of H_2O_2. The reaction time, molar ratio of hydrogen peroxide, catalyst dose and the initial reactant concentration were chosen as the variables.

Materials and Methods

Coal fly ash, obtained from Bongaigaon Thermal Power Station, Assam, India, has been utilized as the catalyst support material.

Chemicals

Nickel(II) nitrate hexahydrate from E. Merck, Mumbai, India was used for metal incorporation, Phenol and 2-chlorophenol from E. Merck Mumbai, India, H_2O_2 from E. Merck, Mumbai, India was used as oxidant.

Synthesis of Ni(II) impregnated fly ash

Fly ash is first washed with distilled water and dried. Each type of ash is then refluxed with 1.0 M Ni(II) solution (10 g in 40 ml solution) for 6 h. After refluxing, the material was washed with distilled water and dried. Calcinations of this material was done at 773 K for 5 h and then preserved.

Catalyst Characterization

The percentage of Ni(II) entering into fly ash was determined with Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 220, Australia). The catalysts were further characterized by FTIR measurements (Shimadzu IR Affinity-1 FTIR Model range 4400 to 440 cm^{-1}, KBr pellet technique). In the present work, Nitrogen adsorption–desorption measurements were performed at liquid nitrogen temperature of 77 K with a Surface Area and Porosity Analyzer (Tristar Micromeritics, Model 2220). Before the actual measurement, the catalyst was degassed at 250^0 C in an inert atmosphere of nitrogen gas for three hours. The isotherms were used to determine BET surface area.

Wet Oxidation of Phenol and 2-Chlorophenol

The catalytic wet oxidation was carried out in a batch process with stoppered 50 mL Erlenmeyer flasks kept in a thermostatic water-bath shaker (NSW, India) having equal volumes (25 mL each) of reactant (phenol or 2-chlorophenol) and H_2O_2 (concentration 5×10^{-3} M each) with catalyst load of 1.0 g/L at 323 K under an atmospheric pressure for 300 min. When evaluating the effects of a particular variable, appropriate changes were made in the values of the variable. After the reaction was complete, the mixture was filtered and the unconverted reactant was estimated spectrophotometrically using Hitachi UV-visible U3210.

The rate of the catalytic reaction with respect to phenol and 2-chlorophenol oxidation was tested for first order reaction mechanism, and since the experimental results did not conform to first-order kinetics, second order kinetics was applied using the following equations [17]:

Second order rate equation:

$$dC/dt = k_2 (C_0 - C_t)^2 \quad (1)$$

Or, the linear form

$$1/C_t = k_2 t + 1/C_0 \quad (2)$$

where C_0 and C_t represent the initial concentration and any concentration at time 't' of the reactant, k_2 is the second order rate coefficients. The linear plots obtained from the linear forms of the rate equations were used for obtaining the values of the rate coefficients.

Results and Discussion

Characterization of Catalyst

From AAS analysis, it is found that the Ni impregnated catalyst has 45.05 mg of Ni(II) per 1.0 kg of fly ash. The amount of Ni present in the calcined raw fly ash (O) was 12.52 mg/kg.

FT-IR spectra of raw fly ash (O) and Ni(II) impregnated fly ash catalyst (A) showed a characteristic FT-IR band at 1085, 1080 cm^{-1} respectively (Fig. 12.1). Strong mineral band observed around 1095 cm^{-1} which is normally assigned for Si-O stretching. The broad absorption band was found in the range of 3460 and 3300 cm^{-1} may be attributed to –OH group of water (hydrogen-bonded). The band between 2920 and 2850 cm^{-1} in the fly ash samples may be assigned to the

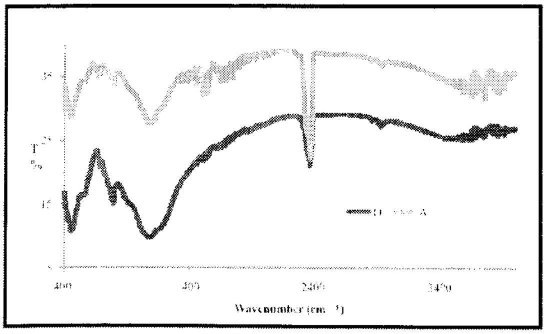


Fig. 12.1: FT-IR Spectra of Calcined Raw Fly Ash (O) and of Calcined Samples of Ni(II) inserted Fly Ash (A)

asymmetric and symmetric stretching of $-CH_2$ group. IR bands in the regions, 800–795 cm^{-1} correspond to aromatic C–H bending vibrations and some bands are observed in these regions [18, 19].

Both surface area and pore volume of fly ash, calculated from N_2 adsorption desorption isotherm data, increased after metal impregnation. The surface area, pore volume and average pore diameter data are given in Table 12.1. The BET surface areas of the raw fly ash and the catalyst were 1.87 and 2.09 m^2/g. Li *et al.* [20] have also found similar type of result having pore size of 21 to 505 Å. Reinik *et al.* [21] have reported that oil shale fly ash collected from a Power Plant had mesopores along with micro pores and have surface area around 6.3 m^2/g.

Table 12.1: BET Surface Area, Pore Volume and Pore Width of Raw and Ni(II)- impregnated Fly Ash

Parameters	Fly ash (O)	Ni(II)- Fly ash (A)
BET Surface Area (m^2/g)	0.64	2.87
BJH cumulative surface area (m^2/g) (pores between 1.7 and 300 nm)	1.12	6.06
Single point adsorption total pore volume of pores less than 146.5 nm diameter at P/Po = 0.99 (mm^3/g)	4.76	19.06
Dubinin-Astakhov micropore volume (mm^3/g)	0.55	2.89
BJH average pore diameter (nm) (4V/A)	16.8	12.6

Wet Oxidation of Phenol and 2-chlorophenol

Blank Experiments

A set of blank experiments were carried out for the following systems under the same conditions of temperature (323 K), atmospheric pressure and a time interval of 300 min:

(i) phenol or 2-chlorophenol alone without any catalyst and H_2O_2,

(ii) phenol or 2-chlorophenol and H_2O_2 (1:1 molar ratio) without any catalyst.

(iii) phenol or 2-chlorophenol with fly ash as the catalyst (1.0 g/L).

(iv) phenol or 2-chlorophenol and H_2O_2 (1:1 molar ratio) with fly ash as a catalyst.

No measurable conversion could be recorded in (i) and (iii) while the conversions observed in (ii) and in (iv) were about ~ 9.2 and 20.4% for phenol and 6.8 and 18.0 % for 2-chlorophenol respectively. From these blank experiments it can be concluded that the reactants (phenol or 2-chlorophenol) are normally very stable and show very little decomposition at 323 K as found in experiment (i), and again fly ash itself is a poor catalyst which itself could not give any significant decomposition of the pollutants as found in experiment (iii). While some amount of decomposition was observed in experiment (ii) and (iv) which may be due to the fact that some of the $\dot{O}H$ radicals were produced from hydrogen peroxide at the temperature of reaction (i.e., 323 K) and also fly ash itself had some adsorption property which provide an appreciable amount of oxidation. All these wet oxidation were carried out at the natural pH of the aqueous phenol or 2-chlorophenol solutions respectively.

Effects of Reaction Time

The phenol or 2-chlorophenol conversion increases with reaction time (5–300 min) till equilibrium conditions were reached (Fig. 11.2). The conversion of phenol or 2-chlorophenol in presence of H_2O_2 in 1:1 molar ratio was 47.2 and 45.9 % respectively after 15 min of reaction, which was increased to 64.8 and 59.7 % after 300 min. From Fig. 12.2 it is observed that amount of conversion of each reactant was very fast at the initial period of time (about 15-20 min) and thereafter the rate was found to decrease. The higher reaction rate at the initial period (first 20 min) may be due to the availability of an increased number of vacant sites on the catalyst surface at the initial stage [18].

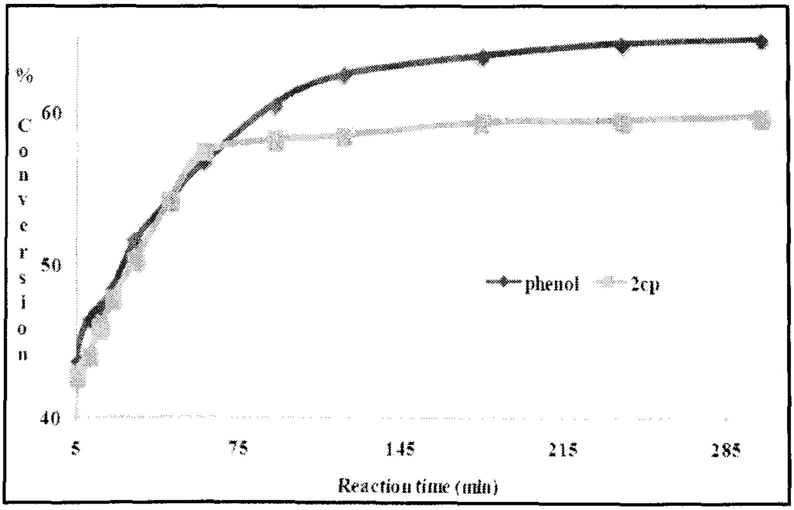


Fig. 12. 2: Effect of Reaction Time on Oxidation of Phenol and 2-Chlorophenol with Ni(II)-impregnated Catalyst at 323 K with Catalyst load 1.0 g/L, Reactant and H_2O_2 concentration of 5×10^{-3} M

The reaction rates of the oxidative degradation have been tested for both first and second order kinetics. For both the cases, the second order kinetics obtained by plotting $1/C_t$ versus time (Fig. 12.3) showed better fit with the experimental results (regression coefficient, R= 0.94 to 0.97). The second order rate coefficient was 1.76 and 2.07 L mol^{-1} min^{-1} respectively for phenol and 2-chlorophenol.

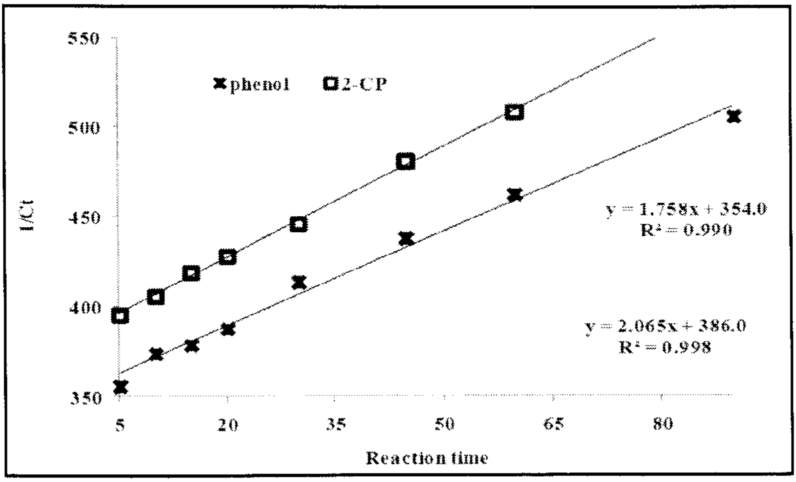


Fig. 12.3: Second Order Kinetic Plots for Wet Oxidation of Phenol and 2-Chlorophenol with H_2O_2 at 323 K over Ni(II)-fly ash (Catalyst 1.0 g/L, 2-CP: H_2O_2 mole ratio 1:1).

Effects of Mole Ratio of the Reactants

The oxidative destruction of phenol or 2-chlorophenol was influenced by the mole ratio of reaction mixture. With the mole ratio of H_2O_2: reactant (phenol or 2-chlorophenol) increasing from 1:1 to 20:1, the reaction was enhanced while keeping the reaction time, temperature and catalyst load (1.0 g/L) constant (Fig. 12.4). It is obvious considering the fact that increasing the relative amount of H_2O_2 in the reaction mixture, more and more $\dot{O}H$ radicals were come out which enhances the oxidation of the reactants. The stoichiometric equation for the complete oxidation of phenol and 2-chlorophenol with H_2O_2 are given in equation (3) and (4) respectively below:

$$C_6H_5(OH) + 14\ H_2O_2 \rightarrow 6\ CO_2 + 17\ H_2O \quad (3)$$

$$C_6H_4Cl(OH) + 13\ H_2O_2 \rightarrow 6\ CO_2 + 15\ H_2O + HCl \quad (4)$$

From these equations, it is clear that for bringing about the complete oxidation

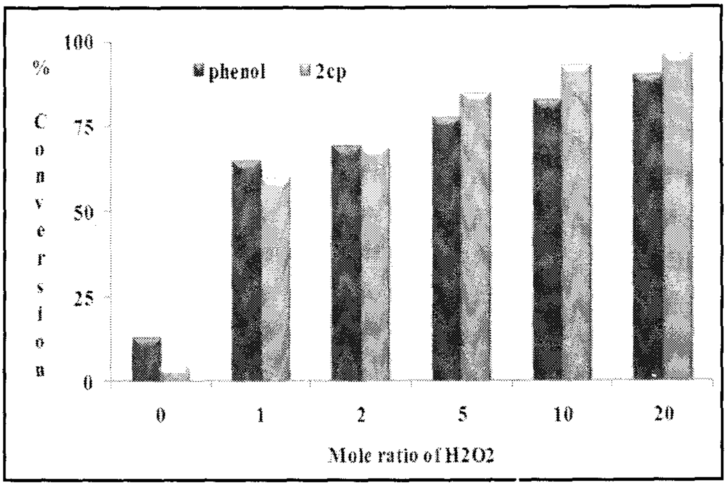


Fig. 12.4: Effect of Molar Ratio on Catalytic Wet Oxidation of Phenol and 2-Chlorophenol with Ni(II)-impregnated Fly Ash at 323 K with Reaction Time 300 min, catalyst load 1.0 g/L

of 1 mole phenol 14 mole H_2O_2 would be required whereas for 2-chlorophenol, 13 mole H_2O_2 was sufficient.

Effects of Reactant Concentration

When the concentration of phenol or 2-chlorophenol was increased from 4.0 x 10^{-4} to 5.0 x 10^{-3} M, the conversion showed a downward trend (Fig. 12.5) at a fixed reaction time of 300 min and catalyst load (1.0 g/L). The negative effect may be due to the fact that as the concentration of organic reactants viz. phenol and 2-chlorophenol increases, more and more reactant molecules were coming out which flocking together on the catalyst surface competing for the active site among themselves lowering the reaction rate.

Effect of Catalyst Load

With the increase on catalyst load from 0.4–8 g/L, keeping the other parameters constant, phenol or 2-chlorophenol conversion showed reasonable enhancement (Fig. 12.6). At the lowest catalyst load of 0.4 g/L, the catalyst could convert 55.7 % of phenol compared to 51.5 % for 2-clorophenol. The conversion at the highest catalyst load of 8.0 g/L was 52.6 % and 52.6 % for catalyst phenol and 2-chlorophenol respectively. With increasing catalyst load, the % of conversion of both the reactants was obviously increases. However at higher loading the conversion did not change much. This may be due to the fact that although the catalyst load was varied, the amount of active phase per unit mass remains the same. Thus a small amount of catalyst load (0.4g/L) can bring a significant amount of conversion under moderate condition.

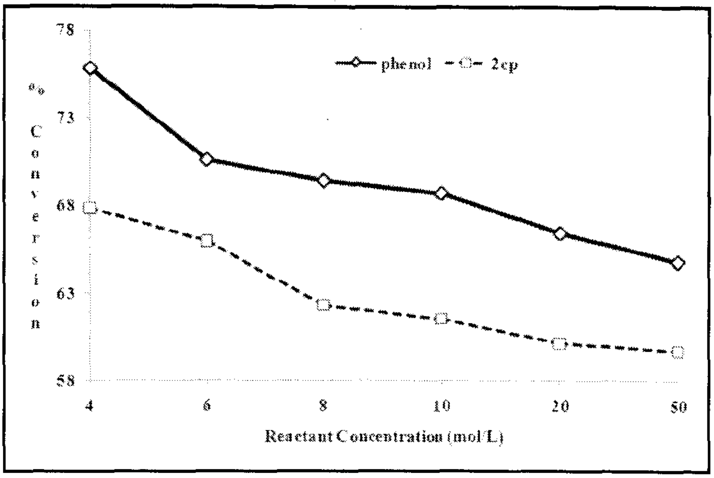


Fig. 12.5: Effect of Reactant Concentration on Phenol and 2-Chlorophenol Conversion Over the Ni(II) impregnated Catalyst for Fixed Catalyst Loading (1.0 g/L) and Time (300 min) at 323K

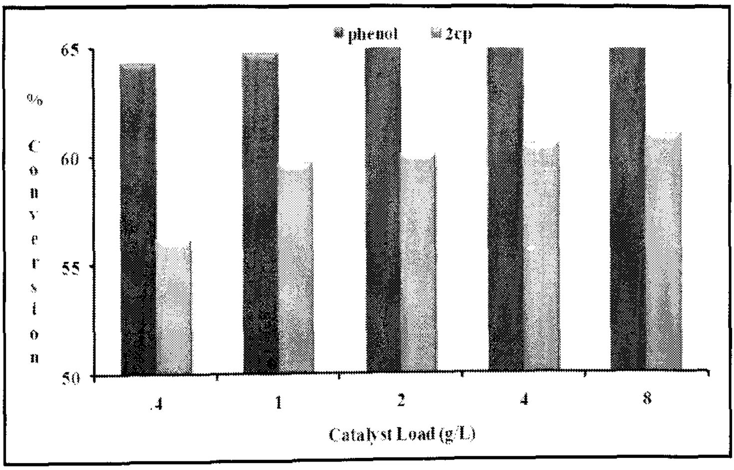


Fig. 12.6: Effect of Catalyst Load on Wet Oxidation of Phenol and 2-Chlorophenol for Reaction Time 300 min, 1.0 g/L load at 323K

Conclusion

Ni(II) incorporation into fly ash produced a supported metal catalyst having reasonable catalytic activity towards the oxidation of both phenol and 2-chlorophenol. Among the two reactants, the catalyst showed better results with phenol. Although the catalysts can bring an appreciable extent of oxidation at higher H_2O_2 concentration, but still more than 50% of conversion was observed at a lower concentration of H_2O_2 with comparatively low temperature and shorter period of time. As both the reactants are the most toxic, carcinogenic, non-biodegradable pollutants, the present work shows that catalytic wet oxidative treatment of the effluent with Ni(II)- impregnated fly ash may be a viable tertiary treatment technique for the removal of both phenol and 2-chlorophenol from water or effluent.

References

1. Adam, F., Andas, J., and Rahman I., A Study on the Oxidation of Phenol by Heterogeneous Iron Silica Catalyst, *Chemical Engineering Journal*, 2010, **165**, 658.
2. Kim, K.H. and Ihm, S.K., Heterogeneous Catalytic Wet Air Oxidation of Refractory Organic Pollutants in Industrial Wastewaters: A Review, *Journal of Hazardous Materials,* 2011, **186**, 16.
3. Fortuny, A., Miry, C., Font, J. and Fabregat, A., Three-phase Reactors for Environmental Remediation: Catalytic Wet Oxidation of Phenol using Active Carbon, *Catalysis Today*, 1999, **48**, 323.
4. Li, N., Descorme, C. and Besson, M., Catalytic Wet Air Oxidation of Aqueous Solution of 2-chlorophenol over Ru/zirconia Catalysts, *Applied Catalysis B: Environmental*, 2007, **71**, 262.
5. Castro, I.U., Stüber, F., Fabregat, A., Font, J., Fortuny, A. and Bengoa, C., Supported Cu(II) Polymer Catalysts for Aqueous Phenol Oxidation, *Journal of Hazardous Materials*, 2009, **163**, 809.
6. Matafonova, G., Philippova and N., Batoev, V., The Effect of Wavelength and pH on the Direct Photolysis of Chlorophenols by Ultraviolet Excilamps., *Engineering Letters,* 2011, **19**, 1.
7. Lim, J. W., Gan, H.M. and Seng, C.E., Bioremediation of Wastewaters containing various Phenolic Compounds by Phenol-acclimated Activated Sludge, *Desalination and Water Treatment.* 2013, 1.
8. Tasic, Z., Gupta, V.K., and Antonijevic, M. M., The Mechanism and Kinetics of Degradation of Phenolic in Wastewaters Using Electrochemical Oxidation. *Interface Journal of Electrochemisrey Science,* 2014, **9**, 3473.
9. Zhao, S., Wang, X. and Huo, M., Catalytic Wet Air Oxidation of Phenol with Air and Micellar Molybdovanadophosphoric Polyoxometalates under Room Condition, *Applied Catalysis B: Environmental.* 2010, **97**, 127.

10. Cybulski, A. and Trawczynski, J., Catalytic Wet Air Oxidation of Phenol over Platinum and Ruthenium Catalysts. *Applied Catalysis B: Environmental,* 2004, **47**, 1.
11. Yang, S., Zhu, W., Wang, J. and Chen, Z., Catalytic Wet Air Oxidation of Phenol over CeO_2-TiO_2 Catalyst in the Batch Reactor and the Packed-bed Reactor, *Journal of Hazardous Materials,* 2008, **153**, 1248.
12. Inchaurrondo, N.S., Massa, P., Fenoglio, R., Font, J., and Haure, P., Efficient Catalytic Wet Peroxide Oxidation of Phenol at moderate Temperature using a High-load supported Copper Catalyst, *Chemical Engineering Journal,* 2012, **198–199**, 426.
13. Barrault, J., Abdellaoui, M., Bouchoule, C., Majesté, A., Tatibouët, J.M., Louloudi, A., Papayannakos, N. and Gangas, N.H., Catalytic Wet Peroxide Oxidation over mixed (Al–Fe) pillared clays, *Applied Catalysis B: Environmental*, 2000, **27**, L225.
14. Britto, J. M., Oliveira, S. B. de, Rabelo, D. and Rangel M., do C., Catalytic Wet Peroxide Oxidation of Phenol from Industrial Wastewater on Activated Carbon, *Catalysis Today,* 2008, **133–135**, 582.
15. Luna, A. J., Rojas, L. O. A., Melo, D. M. A., Benachour, M. and de Sousa, J. F., Total Catalytic Wet Oxidation of Phenol and its Chlorinated derivates with MnO_2/CeO_2 Catalyst in a Slurry Reactor, *Brazilian Journal of Chemical Engineering,* 2009, **26**, 493.
16. Chaliha, S. and Bhattacharyya, K. G., Catalytic Wet Oxidation of 2-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol in Water with Mn(II)-MCM41, *Chemical Engineering Journal,* 2008, **139**, 575.
17. Atkins, P., de Paula and J. Atkins,' Physical Chemistry, *Oxford University press,* 2002, 873.
18. Sarkar, A., Rano, R., Udaybhanu, G. and Basu, A. K.., A Comprehensive Characterisation of Fly Ash from a Thermal Power Plant in Eastern India, *Fuel Processing Technology,* 2006, **87**, 259.
19. Ahmaruzzaman, M., A Review on the Utilization of Fly Ash, *Progress in Energy and Combustion Science,* 2010, **36**, 327.
20. Li, Y., Zhang, F. S. and Xiu, F. R., Arsenic (V) Removal from Aqueous System using Adsorbent Developed from a High Iron-containing Fly Ash, *Science and Total Environmental,* 2009, **407**, 5780.
21. Reinik, J., Heinmaaa, I., Kirso, U., Kallaste, T., Ritamäki, J., Boström, D., Pongrácz, E., Huuhtanen, M., Larsson, W., Keiski, R., Kordás, K. and Mikkola, J. P. Alkaline modified Oil Shale Fly Ash: Optimal Synthesis Conditions and Preliminary Tests on CO_2 Adsorption, *Journal of Hazardous Materials,* 2011, **196**, 180.

13

Synthesis and Immunological studies of Oligovalent - 1 2 -Linked Oligomannosides

Rajib Panchadhayee

Abstract

The immunostimulatory properties of - 1 2 -linked mannosides of natural origin have been studied for some time already and considerable amount of effort has gone into the synthesis and biological evaluation of such compounds. A large number of fully synthetic mannose-based structures were screened for potential use as adjuvants in allergen immunotherapy. The screened molecules have ranged from simple mono- and oligosaccharides to oligovalent clusters containing also acetylated compounds. Furthermore, the molecules were here subjected to a biological investigation where their immunostimulatory properties were evaluated by assessing their effect on the production of T_H2 type cytokine interleukin 4 and T_{reg} pro-inflammatory cytokine tumor necrosis factor (TNF).

Key words: *Saccharides, Oligomannosides, Mannosylation, Tumor Necrosis Factor (TNF), Peripheral Blood Mononuclear Cells (PBMC), Immunostimulatory Adjuvants, Immunopotent.*

Introduction

In the past decades there has been an increasing awareness of the importance of carbohydrates in biological processes [1]. The chemical synthesis of saccharides with known reactive groups and structures provides simple tools for studying ligand–receptor interactions in biological systems and such interactions often result in higher activities than their monovalent counterparts [2, 3]. Bundle and co-workers [4] have previously shown that short - 1 2 -homooligosaccharides from disaccharides up to hexasaccharides inhibit the binding of - 1 2 -mannanspecific monoclonal antibodies [4]. Divalent model compounds are the simplest form of multivalent structure. For this purpose Leino and co-warkers aimed to synthesize monovalent model compounds with corresponding divalent oligomannosides [5].

From another perspective, the chemical synthesis of β- mannosides has, until recently, remained a challenging task due to the α-directing anomeric effect and the presence of an axial substituent at the C-2 position causing steric hindrance for the incoming nucleophile. Leino and co-workers successfully used Crich protocol as a direct approach to β-selective mannosylation for the construction of several carbohydrate building blocks suitable for click coupling [6, 7].

A number of promising vaccine adjuvant candidates from a variety of natural sources, including plants, bacteria, and yeast, were identified [8]. It has previously been reported that - 1 2 -oligomannosides are capable of stimulating macrophages to produce tumor necrosis factor (TNF) [9]. It is well understood from literature that acemannan, a partially acetylated - 1 4 -mannan heteropolysaccharide, also possesses immunostimulatory properties [10]. Clustered saccharide epitopes are common in many natural glycoproteins [11]. A series of - 1 2 -linked oligovalent mannosides has been designed and synthesized by Leino and co-warkers in order to study the effects of valency, length of saccharide chain, linkage type, and reactive groups on immune cell responses [12]. A click-chemistry protocol (azide–alkyne cycloaddition) [13] was then applied to generate the oligovalencies between 2-azidoethyl glycosides and propargyl group-containing scaffolds. They also studied the biological responses of immune cells by stimulating human peripheral blood mononuclear cells (PBMC) and measuring the cytokines produced by employing Luminex technology. This technology, employing fluorescently labeled beads and a flow cytometer, enables a wide range of cytokines in one sample to be measured simultaneously [14].

Savolainen and co-workers [15] did a study where they compare TADM, CpG-ODN and MPL in human PBMC of allergic subjects and to characterize the modulatory effects of TADM on allergen induced inflammatory responses in a murine asthma model.

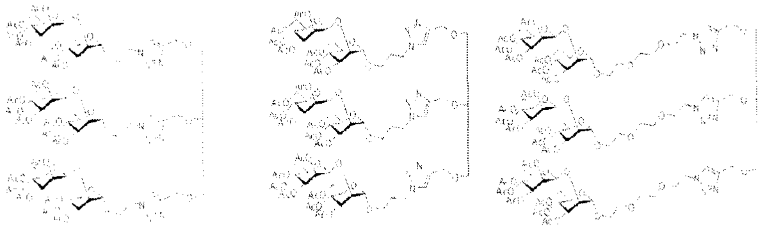

1 2 3

Fig. 13 1. The Structures of Synthesized Trivalent Compounds

Leino and co-workers also further investigated the influence of the glycocluster linker length on the immunostimulatory properties of these compounds and reported two analogues **2** and **3** of the initial lead molecule **1** showing additional variations (an additional –CH$_2$– group or a triethylene glycol based moiety) in the linker connecting the mannobioses to the triazole groups (Fig. 13.1) [16]. In addition, they disclosed detailed *in vitro* comparisons of all three active molecules **1-3** to the known immunostimulatory adjuvants CpG ODN and MPLA. Both the effect on cytokine and IgE secretion of PBMCs from patients suffering from birch allergy, after stimulating with birch allergen Bet v was examined. Finally, all three active compounds were subjected to detailed conformational studies using NMR spectroscopy combined with molecular dynamics simulations. This is a mini review where the synthesis procedure and biology of oligomannosides is described.

Results and Discussion

Synthesis of Mono- and Divalent Mannosides

Leino and co-workers have synthesized three different monovalent mannosides (4-6), along with their corresponding divalent counterparts (7-9) (Fig. 13.2) [5]. They applied click chemistry to the construction of the triazole-bridged mono- and divalent mannosides by reaction of the corresponding propargyl (propargyl alcohol in the case of the monovalent compounds) and 2-azidoethyl glycosides.

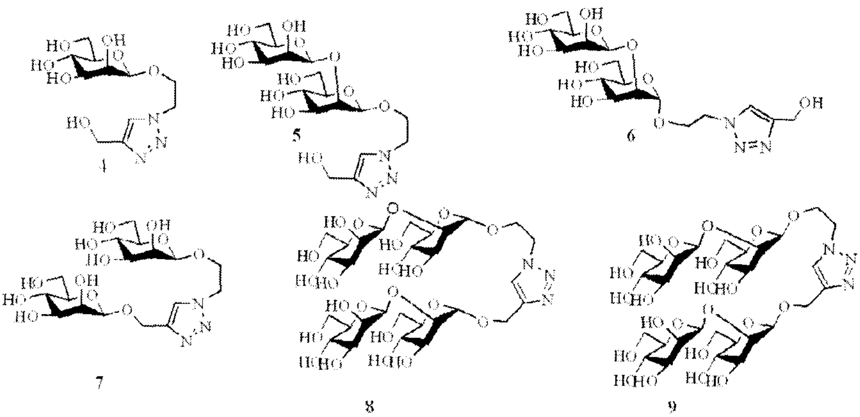
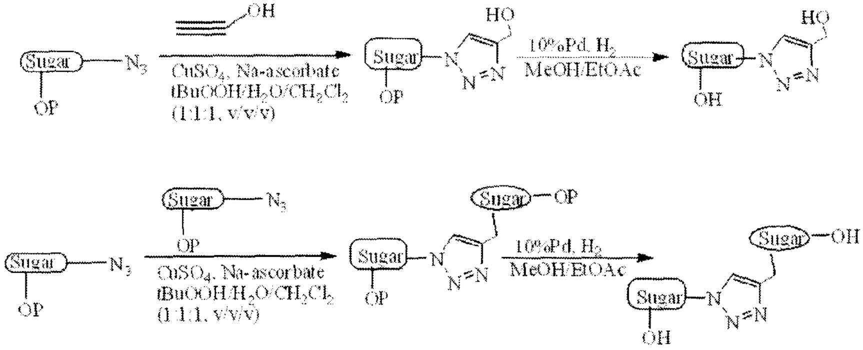
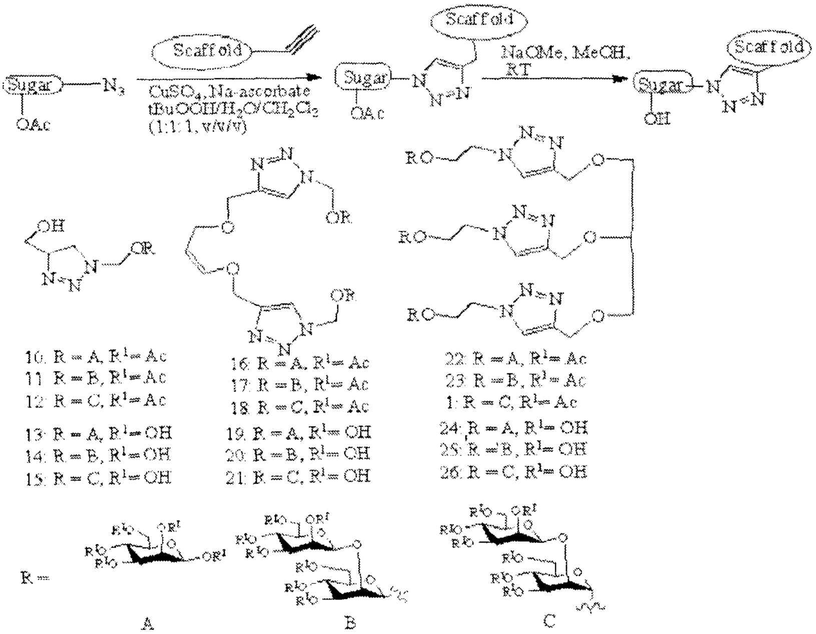


Fig. 13.2. Chemical Structures of the Mono- and Divalent Mannosides

They successfully used monosaccharide building block containing a 4,6-*O*-benzylidene group and a nonparticipating 2-*O*-(*p*-methoxybenzyl) group (PMB), as the glycosyl donor for introducing the initial β-linkage through Crich's methodology for β-mannosylation and glycosyl donor with a participating acetate group which react with 2-azidoethanol in the presence of *N*-iodosuccinimide/ TMSOTf in CH_2Cl_2 at – 40 °C afforded the desired a-glycoside in good yield. To prepare the divalent mannosides by azido-alkyne cycloaddition, they first tested the reaction by using monosaccharide building blocks. A robust click coupling reaction between 2-azidoethyl glycosides and propargyl glycoside provided the expected triazole-fused products. The coupled product was then subjected to global de-protection under hydrogenolysis in the presence of 10% Pd/C and hydrogen gas (2.76 bar) to furnish the fully de-protected divalent compounds. **(Scheme 1)**



Scheme 1: Synthesis of monovalent and divalent mannosides 4-9.



Scheme 2: Synthesis of oligovalent mannoside assemblies (10-26).

In this study, they showed that divalent mannoside **9**, with two β-linkages, showed higher inhibitory activity than its corresponding β-linked (at the reducing terminus) counterpart **8**. It is also evident that divalency appears not to be essential for inhibition as no differences in the inhibition of IgG binding with divalent compounds were observed in comparison with monovalent compounds. In contrast, the β-linked monosaccharide compound **4** and its divalent analogue **7** showed low inhibitory activity. Thus, the biological activity declines sharply in the following order: disaccharide (with two β linkages) > disaccharide (with one á and one β linkage) > monosaccharide (β-linkage).

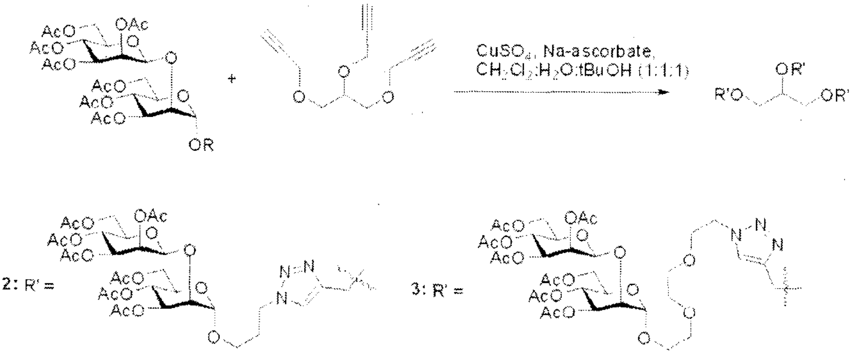
Synthesis of Oligovalent Mannoside Assemblies

In continuation their study Leino and co-workers prepared a series of oligovalent compounds ranging from mono- to penta-valent. For this purpose, three 2 azidoethyl glycosides were coupled with the five different propargyl-based scaffolds 1–5 through Huisgen 1, 3-dipolar cycloaddition. By use of this coupling reaction, 15 acetylated oligovalent variants of β-linked mannosides were synthesized. The hydroxylated analogues of these compounds were then obtained by sodium methoxide-mediated mild saponification (Scheme 2).

These mannosides induced moderate interferon-ϒ (IFN-ϒ) and tumor necrosis factor (TNF) production but not production of IL-4 and IL-5. Very strong induction of interleukin-10 (IL-10) was observed after PBMC (peripheral blood mononuclear cells) stimulation with acetylated trivalent mannobiose 1. Compound 1 is at the limit of solubility versus oligovalency, but dissolves in water to give a maximum concentration of approximately 0.4 $mgmL^{-1}$. The identified lead compound 24 showed strong immunomodulatory properties in comparison with its fully de-protected hydroxylated congener. It may be due to acetylation of carbohydrates enhances their solubility in organic solvents by disrupting intermolecular hydrogen bonding [17] and leads to an enhanced capacity to pass through cell membranes and across hydrophobic barriers in cells, leading to cell stimulation [18].

To investigate the influence of the glycocluster linker length on the immunostimulatory properties of these compounds two analogues 2 and 3 of the initial lead molecule 1 were prepared.

The two azides were coupled to a central glycerol moiety bearing three propargyl functionalities by a copper (I)-catalyzed azide-alkyne cycloaddition ("Click" reaction), which finally afforded the two new trivalent mannobioses in good to very good yields (62% and 89% for 2 and 3 respectively) (Scheme 3).



Scheme 3: Synthesis of Trivalent Mannoside Assemblies with Different Linker.

Conclusions

Here in this article a review of reported methods for synthesis of different olgovalent mannoside was described. From the study it is well understood that that the two synthesized divalent mannosides (compounds 8 and 9) share some similarities with the immunopotent and antigenic epitopes of β-(1→2)-linked *Candida albicans* mannan. The biological results demonstrate that an acetylated trivalent β-(1→2)-linked mannodisaccharide based compound, in which the disaccharide units are alinked to triazole linkers, shows considerable suppressive effects on the Th2-type allergic inflammatory response. Hence, this compound shows great promise as a potential adjuvant in the specific immunotherapy of allergies. Furthermore, it was shown that while the linker structure of the largest one of these molecules is significantly longer than in the other two, the similar folding of all of these molecules makes them almost identical in total three dimensional size. The fact that the molecules are similar in size and shape correlates well with their very similar biological activities.

References

1. Varki, A., *Glycobiology* 1993, **3**, 97–130; (b) Essentials of Glycobiology; A. Varki, R. Cummings, J. Esko, H. Freeze, G. Hart, J. Marth, Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1999.
2. Kiessling, L. L., Gestwicki, J. E. and Strong L. E., *Curr. Opin. Chem. Biol.* 02000, **4**, 696–703.
3. Kiessling, L. L., and Pohl, N. L., *Chem. Biol.* 1996, **3**, 71–77.
4. Nitz, M., Ling, C. C., Otter, A., Cutler, J. E., and Bundle, D. R., *J. Biol. Chem.* 2002, **277**, 3440–3446.
5. Mukherjee, C., Ranta, K., Savolainen, J., and Leino R., *Eur. J. Org. Chem.* 2012, 2957–2968.
6. Crich, D., and Li, H., *J. Org. Chem.* 2000, **65**, 801–805.

7. Crich, D., and Chandrasekera, N. S., *Angew. Chem.* 2004, **116**, 5500; *Angew. Chem. Int. Ed.* 2004, **43**, 5386–5389.
8. Petrovsky, N., and Cooper, P. D., *Expert Rev. Vaccines*, 2011, **10**, 523 –537.
9. Jouault, T., Lepage, G., Bernigaud, A., Trinel, P. A., Fradin, C., Wieruszeski, J. M., Strecker, G., Poulain, D., *Infect. Immun.*, 1995, **63**, 2378 – 2381.
10. (a) Womble, D., and Helderman, J. H., *Int. J. Immunopharmacol.*, 1988, **10**, 967 – 974; (b) Chinnah, A. D., Baig, M. A., Tizard, I. R., and Kemp M. C., *Vaccine*, 1992, **10**, 551 – 557.
11. (a) Lee, Y. C., and Lee, R. T., *Acc. Chem. Res.*, 1995, **28**, 321 –327; b) Lundquist, J. J., and Toone, E. J., *Chem. Rev.*, 2002, **102**, 555 –578.
12. Mukherjee, C., Ranta, K., Savolainen, J., Leino, R., *Chem. Eur. J.*, 2013, **19**, 7961 – 7974.
13. (a) Wu, P., and Fokin, V. V., *Aldrichimica Acta,* 2007, **40**, 7– 17; (b) Nepogodiev, S. A., Dedola, S., Marmuse, L., de Oliveira, M. T., and Field, R. A., *Carbohydr. Res.*, 2007, **342**, 529 – 540; (c) Ballel, L., van Scherpenzeel, M., Buchalova, K., Liskamp, R. M. J., and Pieters, R. J., *Org. Biomol. Chem.*, 2006, 1.4, 4387 –4394; d) P_rez-Balderas, F., Hernndez- Mateo, F., and Santoyo-Gonz lez, F., *Tetrahedron*, 2005, **61**, 9338 – 9348.
14. Vignali, D. A., *J. Immunol. Methods*, 2000, **243**, 243 –255.
15. Mäkinen, K., Mukherjee, C., Leino, M., Panchadhayee, R., Lehto, M., Wolff, H., Alenius, H., Leino, R., and Savolainen, J., *Allergologia et Immunopathologia*, 2016, **44**, 9-17.
16. Rahkila, J., Panchadhayee, R., Ardá, A., Jimenez-Barbero, J., Savolainen, J., Leino, R., *ChemMedChem* D.O.I. 10.1002/cmdc. 201600076.
17. Simes, J., Madureira, P., Nunes, F. M., Domingues, M. R., Vilanova, M., and Coimbra, M. A., *Mol. Nutr. Food Res.*, 2009, **53**, 1036 – 1043.
18. Witschi, M. A., and Gervay-Hague, J., *Org. Lett.*, 2010, **12**, 4312 – 4315.

14

Database of the Three Dimensional Molecular Structure of Medicinal Plant-based Molecules: A Plausible Future Resource of Khasi Traditional System of Medicine

Banteiskhem Kharwanlang

Abstract

Medicinal plant played a pivotal role in health care system since ancient time and continues to do so in developing countries in the form of traditional system of medicine. Of late, the modern drug industries are also getting dependent on medicinal plants as a source of drug candidates. The reliance of both the modern drug industry and traditional system of medicine on medicinal plant has started putting a burden on the survival of the medicinal plant. Coupled to the increasing demand is the climate change which may also hinder the existence of medicinal plant. Conservation of medicinal plant is carried out by *in situ* and *ex situ* conservation. However, such conservation does not wholly ensure conservation of the medicinal plant at the level of conserving the molecular structure of potential drug molecules present in medicinal plant. In addition, due to unforeseen calamities the conservation of medicinal plant may not be an easy task. Conservation of such molecules which have medicinal properties and as well other plant-based molecules whose therapeutic properties are untapped is a challenge and requires utmost importance. A technology which addresses the conservation of the molecular structure of potential drug candidates in medicinal plant is a necessity at this point of time. It is plausible by building a database of the three dimensional structure of such molecules found in the medicinal plant. This database could be a source of medicinal resource for the Khasi traditional system of medicine in future.

Key words: *Medicinal plants, medicinal molecules, conservation, three dimensional structure.*

Introduction

Medicinal plants have a wide spectrum of functions ranging from cosmetic to home remedy and traditional system of medicine to modern drug industry (Fig. 14.1). Turmeric, a medicinal plant in India, found its uses from cosmetics, home remedies, and traditional system of medicine to modern drug industry. Curcumin, a component of turmeric, is emerging as a potential drug candidate for treatment of various physiological and pathological conditions like cancer, diabetes, arthritis and other inflammatory associated diseases [1].

Since ancient time human beings have been using plants as a source of medicine with the field of medicine being as old as civilization itself. The reliance of human beings on plant for medicinal properties led to the development of traditional system of medicine. Traditionally humans have been taking crude extract from plant for various ailments. However, with the advance in pharmacology, plant medicinal molecules were found to be the power behind the therapeutic effect of the plant extracts. Established examples of such medicinal molecules are morphine from opium and digoxin from foxgloves [2]. Similarly in the indigenous Khasi medicinal system and local plants also play a major role. The sustenance of this system of medicine which continues to cater to the healthcare

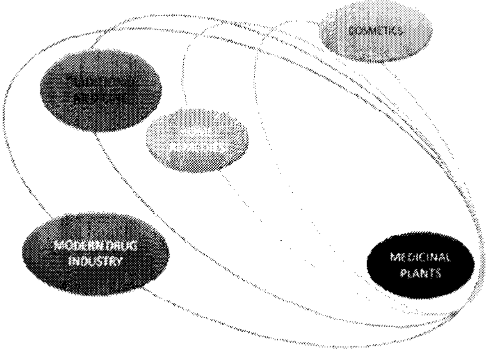


Fig. 14. 1 Medicinal plants and their usage. The usage of medicinal plants varies from home remedies to cosmetics and traditional system of medicine to modern drug industry.

of the people requires the continuous availability of medicinal plants [3]. Development of plant-based drugs by scientific research of such medicinal molecules coupled with medicinal knowledge from the traditional system of medicine is of utmost importance in the current research of drug discovery and development [4]. However, challenges faced by the medicinal plants from various factors may hinder its existence and in turn indirectly affects the indigenous healthcare and modern drug industry.

Medicinal Plants in the Squall of Challenges

The existence of medicinal plants is threatened by civilization itself. The development of civilization pushes society to capture more land for expansion, which encroaches into the ecosystem of medicinal plants. This disturbs the existence, physiology and medicinal value of medicinal plants [3]. Another threatening factor is the ever increasing demand of healthcare products and drugs derived from medicinal plants. Over harvesting of medicinal plant products may dwindle the number of these plants [5]. Lastly, the change in climate is another factor which may affect the existence of medicinal plants and medicinal molecules. Change in climate may induce the plant to adapt to the new environmental changes via changes in gene expression and metabolism. This may in turn lead to the loss of untapped drug molecules having therapeutic value. All the above factors put together exert a tremendous pressure on the existence of medicinal plants (Fig. 14.2) [6, 7].

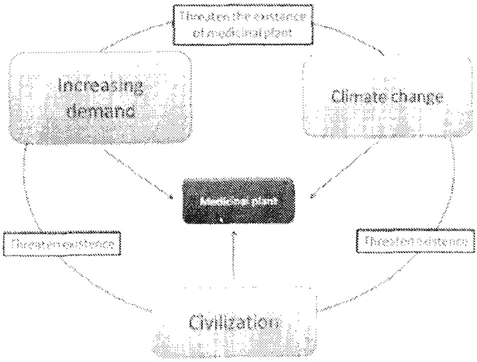


Fig. 14.2 Threats to existence of medicinal plants. Civilization, increasing demand and climate change poses serious threats to the existence of medicinal plants.

Conservation of Medicinal Plants

The conservation of medicinal plants is of utmost important and deserves attention. There are two ways of conservation techniques, which include the *in* *situ* and *ex situ* conservation (Fig. 14.3). The *in situ* conservation includes method like seed storage, DNA storage, gene bank whereas *ex situ* conservation involves creation of national park, wild life sanctuary and biosphere reserves [8]. However, these two approaches of conservation of medicinal plants do not fully address the conservation of medicinal plant at the level of conserving the potential medicinal molecules. For example, a stored seed that is required to generate a plant in the future might not produce the required medicinal molecules due to changes in environmental factors. Similarly, plants in natural habitats like biosphere reserves may lose the production of some medicinal molecules due to adaptation to climatic changes. Adaptation which may lead to variation in metabolic level may be a benefit in one way since this generates new metabolites which may have therapeutic properties, but the disadvantage is that it may also lead to a loss in potential drug candidates. Hence, structural conservation of medicinal molecules is important to meet the demand of future medicine [6, 7].

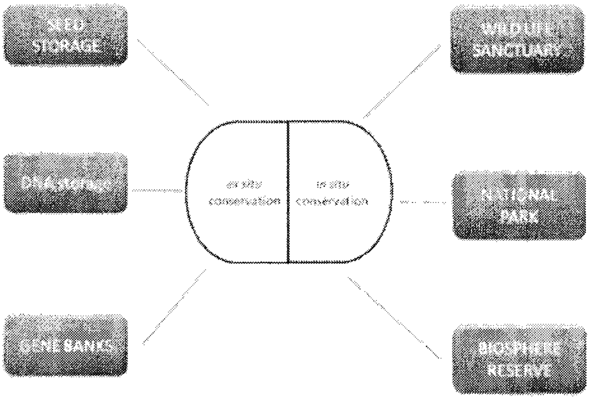


Fig. 14.3 Conservation of medicinal plants. The in situ and ex situ are the two approaches in the conservation of medicinal plants.

Complementing Solution to in situ and ex situ Conservation

The structural conservation of medicinal molecules could be a complementing solution to in situ and ex situ conservation. The elucidation of the three dimensional structure of medicinal molecules and building the database of such structures is vital. The medicinal plant molecule database ensures the conservation of medicinal molecules having therapeutic effect, conservation of plant based molecules whose potential medicinal value is presently untapped and a plausible medicinal resource of indigenous medicinal system in the future. The traditional system of medicine, like the Khasi traditional system of medicine, is highly dependent on medicinal plants. So such a database is going to push the indigenous system to adopt innovative technology in future.

Conclusion and Future Perspective

Healthcare, drug industry and traditional system of medicine are highly dependent on plant based molecules. Enduring supply of such medicinal molecules to the above clients is important for the survival of humans. Thus conservation of medicinal molecules by in situ and ex situ should be complemented by structural database of medicinal molecules (Fig. 14.4). The three conservation techniques could provide a sustaining resource for plant based medicinal molecules in future.

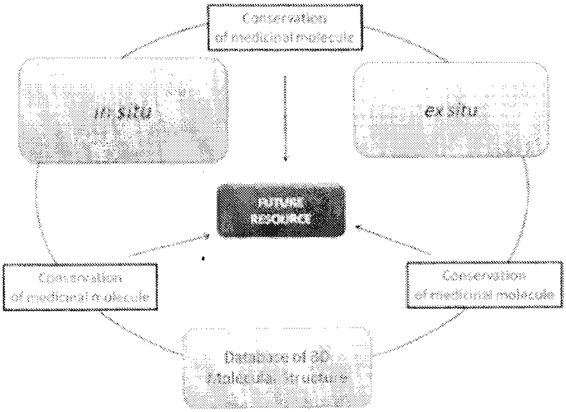


Fig. 14.4 Complementing solution for conservation of medicinal molecules. The conservation of medicinal plants by in situ and ex situ methods coupled to the conservation of the three dimensional structure of medicinal molecules by building databases could provide a possible resource of potential drug molecules in future.

Acknowledgements

The author would like to thank the Principal, St. Edmund's College & the Department of Biochemistry, St. Edmund's College for the institutional support.

References

1. Prasad, S. and Aggarwal, B. B., 2011. Turmeric, the Golden Spice, from Traditional Medicine to Modern Medicine, *Herbal Medicine, Biomolecular and Clinical Aspects*, 2nd Edition.
2. Lahlou, M., The Success of Natural Products in Drug Discovery, *Pharmacology & Pharmacy*, 2013, **4**, 17-31.

3. Lyngdoh, J. P., Syiem, D. and Mao, A. A., Pattern of Traditional Medicine usage in East Khasi Hills of Meghalaya, *Indian Journal of Traditional Knowledge*, 2014,**13**, 164-170.
4. Bai, D., Traditional Chinese Medicine and New Drug Development, *Pure & Applied Chemistry*, 1993, **65**, 1103-1112.
5. Manohar, P. R., Sustainable Harvesting of Medicinal Plants: Some thought in search for solutions Ancient Science of Life, 2012, **32**, 1-2.
6. Braam, J., Sistrunk, M. L., Polensky, D. H., Xu, W., Puruggam, M. M., Antosiewicz, D. M., Campbell, P. and Johnson, K. A., Plant Responses to Environmental Stress: Regulation and Functions of the *Arabidosis TCH genes*, *Planta*, 1996, **203**, S35-S41.
7. Ramakrishna, A. and Ravishankar, G. A., Influence of Abiotic Stress Signsls on Secondary Metabolites in Plants, *Plant Signaling & Behaviour*, 2011, **6**, 1720-1731.
8. Kasagana, V. N. and Karumuri, S. S.,) Conservation of Medicinal Plants (Past, Present & Future Trends), *Journal of Pharmaceutical Sciences and Research*, 2011, **3**, 1378-1386.

15

Characterization of Drinking Water Sources In Some Govt. Schools- A Case Study

Gitimoni Deka
Rekibuddin Ahmed

Abstract

Every society in all over the World is cautious about safe drinking water. It is widely recognized that there is a positive relationship between the health of young people and their educational achievements. Drinking plenty of water is one of the most important ways of protecting our health, and that low fluid intake is associated with a variety of health problems in our young people. School students often do not drink sufficient quantities of water during the school day to prevent dehydration, promote learning and good health. The chemistry of water is influenced by the inputs of material containing minerals their solubility and the chemical equilibrium prevailing in aqueous solution. Water pollution is one of the specific and serious problem because it is to some extent localize problem. This paper tries to understand quality of drinking water in different govt. schools with respect to some of the physical and chemical parameters. The water quality was in general found to be satisfactory with one –two exceptions.

Key words: *Drinking, Water, School Students, Good Health, Govt. School.*

Introduction

Water quality concerns are often the most important component for measuring access to improved water sources. According to the Untied Nations, more than one billion people already lack access to fresh drinking water. The water problem is global. More than 1.6 million children die annually from diseases that are largely (more than 80%) preventable with clean water. If current trends persist, by 2025 the demand for fresh water is expected to rise by 56 percent more than the amount of water that is currently available. Until fairly recently, no one imagined that water would become a valuable, expensive commodity. Acceptable quality shows the safety of drinking water in terms of its physical, chemical and bacteriological parameters (WHO, 2004) [1]. The WHO (2000) [2] reports that polluted drinking water causes about 1.8 million people die from diarrheal diseases annually worldwide. The UNDP (2008) [3] says the minimum absolute daily water need per person per day is 50 liters (13.2 gallons) which include-5 liters for drinking. However because of scarcity of drinking water, millions of people try to exist on 10 liters (2.6 gallons) a day (ADF, 2005). Many people do not have access to this vital element, which is fundamental to healthy life [4]. Providing access to safe drinking water in schools is a novel way to ensure increase in attendance in schools in developing countries, as per a research. Ensuring drinking water quality at these facilities is important because that's where children spend their day, and they are likely to drink water while they are there. Consuming enough fluids on a daily basis is important for children's health. Water is a great choice because it doesn't contain the calories, caffeine, or sugar that may be found in other beverages, and it helps nearly every part of the human body function efficiently.

Objectives

The present study has been taken to know drinking water sources available in different schools in and around Rangia town, Dist- Kamrup, Assam to understand the quality of drinking water consumed by school children during school hours. For this different parameters are to investigate the nature of drinking water sources.

Material and Methods

Drinking water samples were collected in pre-cleaned plastic bottles and are preserved as per standard methods (APHA, 1995) [5]. Altogether eight drinking water samples were collected each once in two seasons, post-monsoon (Oct-Dec) and pre-monsoon (April-May) season. The water samples from eight different schools located in and around the Rangia town were collected in previously washed and dried plastic cans and brought to the laboratory with all necessary precautions. Distilled water was used for the preparation of reagents and solutions. Colour and turbidity were noted by just seeing the samples. Physical and chemical parameters like pH, total solids, total dissolved solids, total hardness, calcium, magnesium, total alkalinity, fluoride, chloride, sulphate, arsenic, and iron have been estimated by using standard methods [5, 6, 7]. For determining fluoride, UV-Visible spectrophotometer (Hitachi 3210) was used. For determination of sulphate and phosphates, Photoelectric Colorimeter (101, Systronics) is used. The arsenic and iron were determined with atomic absorption spectrophotometer (Varian spectra AA 220) at research laboratory of Chemistry Department, Gauhati University.

Table 15.1: Description of Water Sampling Sites

Sampling Code	Water Source	Name of the school
1	Deep tube well	Baiddagarh Girls' High School
2	Deep tube well	Premada Sibanath High school
3	Tube well	Haradutta Birdutta High School
4	Tap water	Jayantipur Jogeswar High School
5	Tube well	Railway Higher Secondary School
6	Tap water	Rangia Girls' Higher Secondary
7	Tube well	Arimatta Vidyyapith (High School)
8	Tap water	Bijulinagar Vani Vidyapith (High School)

Result and Discussions

The results obtained from physico –chemical analysis of different samples collected during pre monsoon (prm) and post monsoon (pom) season are compared with drinking water standards of BIS-10500 given in Table 15.2.

Table 15.2: BIS-IS 10500 Drinking Water Quality Standard

Parameters	Highest desirable	Maximum permissible
pH	6.5-8.5	-
Alkalinity	200 mg/L	**600 mg/L**
Cl	200 mg/L	-
Total Hardness	300 mg/L	**600 mg/L**
TDS	500 mg/L	**2000 mg/L**
Sulphate	200 mg/L	**400 mg/L**
Fluoride	1.5 mg/L	-
Calcium	75 mg/L	**200 mg/L**
Magnesium	30 mg/L	**100 mg/L**
Iron	**0.3 mg/L**	**1.0 mg/L**

pH

Measurement of pH is one of the most important and frequently used tests in water chemistry. The value of pH in ground water samples of the study area ranges between 1.62-8.9 in pom and 7.3-8.0 in prm season. The seasonal variation shows the pH values fluctuating maximum during pom. Fluctuation of pH values in ground water may be due to the leaching of surface soil from surrounding areas in rainwater. However higher pH values observed suggests that carbon dioxide, carbonate-bicarbonate equilibrium is affected more due to change in physico-chemical condition [8]. The factors like air temperature bring about changes the pH of water. Most of bio-chemical and chemical reactions are influenced by the pH. The reduced rate of photosynthetic activities reduces the assimilation of carbon dioxide and bicarbonates which are ultimately responsible for increase in pH, the low oxygen values coincided with high temperature during the summer month [9].

Total Alkalinity (TA)

In present study total alkalinity ranges from 160-580 mg/L in pom and 250-600 mg/L in prm. Most of the samples was recorded more value in prm season. This is probably due to increase in bicarbonates in the water. Hujare also reported similar results [10]. Moreover, little abnormal value of alkalinity is not harmful to human being [11].

Table 15.3: pH & TA of Study Samples

pH			**Total Alkalinity**		
	pom	prm		pom	prm
1	7.27	7.37	1	160	250
2	1.62	7.84	2	220	300
3	8.1	7.77	3	240	300
4	8.2	7.53	4	300	250
5	7.97	7.3	5	580	350
6	7.7	7.81	6	400	350
7	8.6	7.4	7	460	600
8	8.9	8	8	340	400

Total hardness (TH)

All samples were found more values of total hardness in post monsoon season (range 104-480 mg/L) with exception sample no.7 which was slightly more in prm season. In pre monsoon the values were obtained 28-148 mg/L, and these all values were found within maximum permissible limit (600mg/L). According to Zoeteman, hardness above 500 mg/L are considered unacceptable for drinking purposes [12].

Calcium (Ca)

Naturally, drinking water contains calcium. In pom season, Ca was obtained 35-117 mg/L in study samples and 8.0-44.7 mg/L in prm season. The values obtained in pom season were more than prm season. This is probably less rainfall during post monsoon period. Calcium may not impair physiological reaction in man up to the level of 1800 mg/L [6].

Magnesium (Mg)

The water samples contain Mg in lower amount with one exception in sample No. 5. Like calcium, less amount of Mg is present in prm season than pom season. Sample 5 (75.35 mg/L) have more than desirable value (30 mg/L) in pom season but less than highest permissible limit (100 mg/L). All the samples have values 4.8-75.35 mg/L in pom and 1.0-12.67 mg/L in prm season.

Table 15.4: TH, Ca & Mg Values for all the Samples

TH			Ca		Mg	
Sample site	**pom**	**prm**	**pom**	**prm**	**pom**	**prm**
1	144	32	53	11.2	4.8	0.978
2	120	28	43	8	4.8	1.95
3	248	48	79	11.2	20.8	4.87
4	280	24	72	8	40	1
5	480	136	117	27	75.35	12.675
6	104	52	35	17.6	6.4	1.95
7	140	148	49	44.7	6.4	5.85
8	112	40	41	14	3.2	0.975

Total Solids (TS)

All water samples were found total solids within permissible limit. In present study total solids were BDL- 300 mg/L in pom season and BDL-200 mg/L in prm season. These values indicate the suitability of ground water for drinking purposes. It is reported that higher concentration of total solids are aesthetically unsatisfactory and may also produce distress in humans and livestock (APHA, 1989) [13].

Total Dissolved Solids (TDS)

Higher concentration of dissolved solids in drinking water indicates that water is not fit for consumption. In all samples of water TDS were within the range of BDL-100 mg/L in pom season and BDL- 100 mg/L in prm season. A TDS of less than 600 indicates good palatability (WHO, 2004) [1], and all the samples from this study were palatable.

Total Suspended Solids (TSS)

Among the eight samples only three samples (sample no. 2, 5 & 8) were found suspended solids in both the seasons. Sample 3 was free from TSS and low amount of TSS were found in Sample 1, 4 & 7 On the other hand total suspended solids were found BDL-300 mg/L in pom and BDL-200 mg/L in prm season, which were within permissible limits.

Chloride (Cl^-)

In present study chloride was obtained much more below than the desirable limit. . In potable water, the salty taste produced by chloride concentration is variable and dependent on the chemical composition of water [14]. The values of chlorides range from BDL - 2.982 mg/l in pom season and 4.8 - 9.1 mg/L in prm which was below than permissible level.

Fluoride (F^-)

Fluoride in varying concentration is present in nature. Waters with high fluoride content are found mostly in calcium-deficient ground waters in many basement aquifers, such as granite and gneiss, in geothermal waters and in some sedimentary basins. The WHO guideline value for fluoride is 1.5 mg/L and in this study one sample in prm was found high fluoride content but the same sample in pom season was low than permissible limit. Fluoride was within the range of 0.36-1.0 mg/L in pom and 1.07-2.62 mg/L in prm season. As fluoride is not a nutrient and no biochemical process in the human body needs fluoride but more fluoride content in drinking water causes teeth decay, bone deformation, yellowish brown discoloration of teeth. The information obtained from Rangia public health centre and Rangia civil hospitals indicate that no fluorosis patients have been reported/register so far.

Sulphate (SO_4^{2-})

In this study sulphate content was found very low and were within the range of BDL-11.2 mg/L in pom and BDL-0.5 mg/L in prm season. High amount of sulphate imparts bitter taste to drinking water. Uncontrolled observations implicate sulfate in drinking water at concentrations exceeding 500-700 mg/liter as a cause of diarrhea [15].

Phosphate (PO_4^{3-})

Phosphate occurs as inorganic and organic form in natural and waste water [4]. Phosphates are not toxic to people or animals unless they are present in very high levels. Digestive problem could occur from extremely high level of phosphate [16]. The phosphate content in present study was within the range of BDL- 1.1 mg/L in pom season and 0.06 – 2.5 mg/L in prm season. For phosphate, no BIS and WHO standards are available.

Table 15.5: Cl, F, SO_4, PO_4 Content of Different Samples in Study Area.

Chloride		**Fluoride**		**SO_4**		**PO_4**	
pom	**prm**	**pom**	**prm**	**pom**	**prm**	**pom**	prm
2.98	8.52	0.361	1.15	1	0.5	1.1	2.5
0	9.1	1.005	1.12	6.25	0.2	0.04	2.3
3.55	6.2	0.528	1.3	11.2	0.33	0.02	2.1
1.988	8.2	0.644	1.27	0.2	0	0.01	2.4
2.13	4.8	0.406	2.6	0.6	0.29	0.06	0.07
2.272	6.8	0.4	1.25	0	0	0.3	0.04
1.704	7.3	0.8	1.07	0.1	0.2	0.3	0.1
1.846	8.2	0.578	2.62	0.2	0.2	0	0.06

Arsenic (As)

Arsenic contamination in drinking water has been reported in different region of the world mainly in china (WHO, 2004) [1]. WHO has prescribed a provisional guideline value of As 10 ìg/L in drinking water and according to India standard drinking water specification of 1991, the highest desirable limit is 50 ìg/l and no relaxation for maximum permissible level. High concentrations of arsenic in drinking water can have an adverse effect e.g. skin cancer, cardiovascular disease, pulmonary disease, neurological disease, bladder cancer, lung cancer and cancer of the kidneys. Arsenic poisoning can also lead to an acute condition called arsenicosis (MAGC, 2001) [17].

Iron (Fe)

Iron in drinking water sources is a major problem in Assam. Iron is an essential element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and ranges from 10 to 50mg per day (The Telegraph, 2011) [18]. In pom season values were obtained 4.3-41.7 mg/L and in prm season 0.29-23.05 mg/L iron were obtained. Maximum iron was at sample 5 in both seasons. All the samples in both the season have crossed the guideline value of 0.3 mg/L. Iron has no significant health hazard but it is nuisance when present in excess. Iron in excess of 0.3 mg/L is known to cause staining of clothes and utensils [19]. Trivedy and Goel [6] also reported that iron has got little concerns as a health hazard but is still considered as a nuisance in excessive quantities.

Table 15.6: Iron and Arsenic Content of Different Samples in Study Area

	Iron			**Arsenic**	
	pom	**prm**		**pom**	**prm**
1	37.96	23.054	1	0.00359	BDL
2	6.148	1.178	2	0	BDL
3	4.509	0.289	3	0.00129	BDL
4	6.393	10.159	4	0.0019	BDL
5	41.773	15.958	5	0.00606	BDL
6	5.464	17.615	6	0.00761	BDL
7	4.355	19.749	7	0.0016	BDL
8	5.807	0.977	8	0.00362	BDL

Conclusions

After complete analysis of different parameters, results obtained indicates that only iron exceeds highest maximum limit at every locations. However, other parameters such as pH except in one location in pom season, alkalinity, TS, TDS, TSS, alkalinity, TH, calcium, magnesium, Chloride, sulphate, phosphate, fluoride, arsenic were within the permissible standard. Therefore quality of drinking water sources in different schools in and around Rangia town is good for human consumption.

Acknowledgement

We offer my sincere thanks to my teacher Prof. K.G.Bhattacharyya, Department of Chemistry, Gauhati University for giving me permission to perform some experiments in his research laboratory.

References

1. World Health Organization - Guidelines for Drinking-water Quality, World Health Organization, Geneva, **2004**
2. World Health Organization - Disinfectants and disinfectant by products (Environmental health criteria 216). Geneva: World health organization, 2000.

3. United Nations Development Program (UNDP) Millennium Development Goal Report. 2008.
4. Deka, Gitimoni and Bhattacharyya, K. G., Metal Cations present in Drinking Water samples in and around Rangia Town, Dist.- Kamrup, Assam, *Indian Journal Of Environmental Protection , Vol 29, No. 8,* ***2009****, August.*
5. APHA- American Public Health Association, Standard Methods for the Examination of Water and Waste Water, 19th Ed, Washington, D.C. 1995.
6. Trivedy, R. K. and Goel, P. K., Chemical and Biological Methods for Water Pollution Studies. Environmental Publications, Karad, India, 1984.
7. Gupta, P.K., Methods in Environmental Analysis Water, Soil and Air Agrobios Jodhpur, 2000.
8. Tiwari, R. N., Dubey,D. P. and Bharti, S. L., Hydro-geochemistry and groundwater quality in Beehar River Basin, Rewa District, Madhya Pradesh India, Proc. International Conference on Hydrology and Watershed, JN & T Hyderbad, 2009, pp.49-59.
9. Kamble, S. M., Kamble A. H. and Narke S. Y., (2009): Study of Physico-Chemical Parameters of Ruti dam, Tq. Ashti, dist. Beed, Maharashtra. J. Aqua. Biol., 2009, **24(2),** 86-89.
10. Hujare, M. S., Seasonal Variation of Physico-Chemical Parameters in the Perennial Tank of Talsande, Maharashtra. *Ecotoxicol. Environ. Monit.* , 2008, **18(3),** 233- 242.
11. Singh, T.B., Bala, I., and Singh, D., Assessment of Ground Water Quality of Panota Sahib (H.P.) *Poll res.* 1999, **18 (1),** 111-114.
12. Zoeteman, B. C. G., Sensory Assessment of Water Quality. Oxford, Pergaman Press, U.K. 198**0**.
13. APHA - American Public Health Association, Standard Methods for the Examination of Water and Waste Water, 17th Ed, Washington, D.C., 1989.
14. Garg, S.S., Water Quality of Well and Bore Well of 10 Selected Locations of Chitrakoot Region , *IJEP*, 2003, **23 (9)**, 966-974.
15. Heizer, W. D , Sandler, R. S., Seal, E. Jr, Murray, S. C., Busby, M. G., Schliebe, B.G. and Pusek S. N.., Intestinal Effects of Sulfate in Drinking Water on Normal Human Subjects, *Digestive diseases and sciences,* 1997(May), **42(5)**,1055-61,.
16. Kumar, Manoj and Avinash Puri, A review of permissible limits of drinking water, *Indian Journal of Occupational and Environmental Medicine.*, 2012, Jan-Apr; **16(1)**, 40–44.
17. MAGC Technologies www.magctech.com.
18. The Telegraph, Guwahati Water Suffers Iron Overload, 2011, 24 August.
19. Sarma, H. P., Quality of Drinking Water in the Darrang district with particular reference to Mangaldai Subdivision, Ph.D thesis, Gauhati University, Assam, 1997.

16

Assessment of physicochemical and biological characteristics of selected springs in Shillong, Meghalaya

---

Michelle Khongwir
Iasylloklang Rynjah
Larihun Jeengaph
Jasmine T. Sawian

Abstract

Contamination of our water resources is a major global problem and studies suggest that it is the leading worldwide cause of deaths and diseases. According to WHO, about 80% of all diseases in human beings are caused by the consumption of contaminated water. Shillong, the capital city of Meghalaya, has a number of aquatic bodies that drain the undulating landscape of the area. During the last few decades, there has been a phenomenal increase in disturbance and developmental activities that has caused massive damage to landscapes and biological communities. This is widely seen in the case of aquatic systems, particularly springs, which are the main source of drinking water supply for the inhabitants of the city. Once the groundwater is contaminated, its quality cannot be restored back easily. Therefore, the physicochemical and biological parameters of some of the selected springs of Shillong have been carried out. These water bodies are under constant threat and the need of the hour is for their effective management.

Key-words: *Springs, Contamination, Physicochemical Characteristics, Biological Characteristics*

Introduction

Water is a vital element for the survival of life and is regarded as the most precious natural resource that exists on our planet [1]. Water is a primary necessity for the growth of civilization, as most of the civilizations have grown near sources of water. Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs and wells [2]. The quality of any body of surface or ground water is a function of either or both natural influences and human activities. Without human influences, water quality would be determined by the weathering of bedrock minerals, atmospheric processes of evapotranspiration, and the deposition of dust and salt by wind. Others include, the natural leaching of organic matter and nutrients from soil, hydrological factors that lead to runoff and by biological processes within the aquatic environment that can alter the physical and chemical composition of water (UNEP, 2006) [3]. Unfortunately, clean, pure and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and human activities. Water from most sources is therefore unfit for immediate consumption without some sort of treatment [4].

Water quality of aquatic environment arises from a multitude of physical, chemical and biological interactions [5]. Around 30.1% of all fresh water on earth is ground water. Ground water is found in natural rock formations. These formations called aquifers are a vital natural resource with many uses. Nationally, 53% of the population relies on groundwater as a source of drinking water [6]. Groundwater is believed to be comparatively cleaner and free from pollution compared to surface water [7]. In a number of places, groundwater in the form of springs provide the main source of freshwater for drinking and other domestic uses. Springs are a concentrated discharge of groundwater that appears at the surface as a current of flowing water [8]. However, groundwater can be easily contaminated in a multitude of ways because of increasing human activities. Once groundwater is contaminated, its quality cannot be restored back easily. The pollution of groundwater has become a major environmental issue, particularly in those areas where groundwater represents the main source of drinking water [9, 10, 11]. In recent years, with the increase in human population, haphazard rapid urbanization, industrialization, technological expansion, energy utilization, waste generation and other developmental activities, most of the water bodies have become highly polluted [12]. According to WHO, about 80% of all the diseases in human beings are caused by contaminated water [13]. Consequently, a number of cases of water borne diseases have been seen as the causes of health hazards. Therefore, monitoring the quality of spring water is one of the important issues of drinking water management.

Materials and Methods

The study area is located in the city of Shillong, the district headquarter of East Khasi Hills and capital city of the state of Meghalaya, India. The altitude of the city varies between 1400-1950m above sea level and is situated at latitude 25° 35°N and longitude 91° 53°E. It has a population of 2,56,997 people (2011 census). The present study is carried out on the water quality of four springs in Shillong located in Umpling, Nongrah, Lapalang and in Mawroh. The springs are locally known as '*shyng-iar*'. The springs are relatively undisturbed, with clean and clear water. Construction of houses near to the spring is not allowed. The area around the natural springs are well-maintained and the water has been covered or channeled to cemented tank, where outlets are made through iron pipes for collection or for washing purposes. Water samples from the springs were collected in pre-cleaned polythene containers and transferred to the laboratory. The methods as outlined in the "Standard Methods for the Examination of Water and Wastewater" as prescribed by APHA (2005) [14] were adopted. Water quality parameters including temperature, pH, conductivity were measured; and acidity, alkalinity, free carbon dioxide (CO_2), hardness, chloride, calcium and magnesium content, dissolved oxygen (DO), and biological oxygen demand (BOD) were analyzed for each sample.

Results and Discussion

The contamination of water bodies by various developmental activities is a major environmental problem in Meghalaya. Assessment of the physicochemical characteristics of water samples not only reflects the quality of an aquatic ecosystem but also its biological diversity [5,16]. All the water samples collected from the different springs were clear, colourless and odourless. The physicochemical and biological properties of water in various surface water samples were analyzed and presented in Table 16.1.

Table 16.1: Physicochemical Parameters of Spring Water

Parameter	Umpling Spring	Nongrah Spring	Lapalang Spring	Mawroh Spring	SD
Temperature (°C)	7.10	6.50	6.37	8.13	0.80
pH	6.86	6.57	6.95	7.14	0.23
Conductivity(µS/cm)	96.76	74.50	97.50	65.26	16.81
CO_2(mg/l)	12.46	11.00	13.20	14.66	1.52
Acidity (mg/l)	16.66	27.33	17.33	21.33	4.89
Total Alkalinity (mg/l)	14.66	20.00	15.33	29.33	6.76
Total Hardness (mg/l)	46.00	33.33	48.00	20.00	12.96
Chloride (mg/l)	7.16	10.46	7.26	14.43	3.42

Calcium (mg/l)	19.60	12.27	20.80	5.67	7.03
Magnesium (mg/l)	6.42	5.12	6.61	3.50	1.43
Dissolved Oxygen (mg/l)	18.73	19.66	28.93	17.26	5.28
BOD (mg/l)	8.51	10.44	13.71	7.04	2.88

Temperature

Temperature is the basic physical parameter, which alters the characteristics of water quality. It affects the physical and chemical properties of water and affects the aquatic vegetation, organisms and their biological activities. The values of temperature of spring water at the sampling sites ranged from 6.50^{0}C at Nongrah to 8.13^{0}C at Mawroh. The temperature values may shows significant variation, however the lower temperature may be due to the shading effect of trees along the periphery of the springs. High concentration of degradable carbonaceous and nitrogenous substances releases energy during the process of decomposition and may lead to increase in water temperature.

pH

The pH of spring water from the sampling areas was found to be slightly acidic to neutral and is in the range of 6.57 at Nongrah to 7.14 at Mawroh. The pH of spring water is found to be within the WHO (1993) [17] maximum permissible limit of pH 6.5 – 8.5, and thus the water can be used for drinking and domestic purposes. Low pH values recorded in springs may be due to saturation with carbon dioxide as indicated in studies by Byamukama *et al.* [18].

Conductivity

Acid, bases and salts in water makes it a good conductor of electricity – such substances are called electrolytes which dissociate into positive ions and negative ions and impart conductivity. The electrical conductivity of spring water in all the sampling sites were well within the WHO (1993) [17] maximum permissible limit of 400μS/cm. The values of conductivity show variation from a range of 65.26μS/cm (Mawroh) to 97.5μS/cm (Lapalang). The relatively lower values of conductivity observed in spring water samples may be due to bicarbonate and calcium ions present in the rocks there and indicate the suitability of water for domestic and agricultural use.

Free CO_2

Free carbon dioxide content of water is regarded as an important index of the deterioration of water quality and is a valuable measure of CO_2 concentration in various aquatic ecosystems. CO_2 content in water in free form and as bicarbonates is very important for photosynthetic activities of green plants which is responsible for production of food and oxygen which is essential for fishes and other aquatic animals. The free carbon dioxide content of spring water from the sampling areas was found to be low and is in the range of 11.00 mg/l (Nongrah) to 14.66mg/l (Mawroh).

Acidity

It is the capacity of water to neutralize a strong base to a fixed pH. It is an aggregate property of water due to several specific substances but chiefly it is due to the CO_2 dissociating from carbonic acid. Acidity affects chemical and biological process taking place in water. Spring water from Nongrah possessed higher values of acidity (27.33mg/l), whereas the lowest value was noted at Umpling (16.66mg/1). Addition of organic acids, tannic acids, mineral acid and hydrolyzing salts, are also responsible to increase acidity level in water. In unpolluted water i.e. at the spring site, dissolved CO_2 is the main source of acidity. Determination of acidity is significant as it causes corrosion and influences the chemical and biochemical reactions [19].

Total Alkalinity

Total alkalinity is regarded as an important index of biological productivity in an aquatic ecosystem. A number of bases such as carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates and borates contribute to the alkalinity. In spring water, alkalinity is raised where water traverses through calcareous rocks. In the present investigation, total alkalinity of spring water ranged from 14.66mg/l (Umpling) to 29.33mg/l (Mawroh) in the different sampling sites. Total alkalinity in spring water may be due to the presence of bicarbonates of calcium and magnesium in the springs. The WHO (1993) [17] acceptable limit for Total Alkalinity is 200 mg/l; beyond this limit taste may become unpleasant.

Total Hardness

Hardness of water is due to dissolved Ca and Mg carbonates and bicarbonates as well as ions of other metals like Sr, Fe, Al, Zn and Mn which are capable of precipitating soap. Hard water does not form lather with soap as the dissolved calcium and magnesium carbonates and bicarbonates, present in hard water precipitates soap. From the present study, it is observed that spring water ranged from 20.00mg/l at Mawroh to 48.00mg/l at Lapalang. Hardness has got no adverse effect on human health [20]. Hardness of spring water in the sampling areas was well within the WHO (1993) [17] maximum permissible limit of 500 mg/l. Water with hardness above 200 mg/l may cause scale deposition in the water distribution system and more soap consumption. Hardness is usually not regarded as pollution because it does not harm the health in a major way.

Chloride

Chloride is regarded as a valuable indicator for water quality. It behaves as a conservative ion in most aquatic environment but plays a metabolically active role in the photosynthesis of aquatic plants and phosphorylation in autotrophs. A high concentration of chloride is an indicator of pollution. The salty taste produced in potable water is due to presence of chloride ions. High chloride content in irrigation water gradually leads to accumulation of salt and adversely affects soil property. Lower value of chloride noted at the sampling sites which ranged from 7.16 to 14.43mg/l, maybe due to relatively undisturbed nature of the area. The lesser concentrations of chloride in the spring water depict the low level of pollution of animal origin [21].

Calcium

Calcium is one of the most abundant substances found in natural water. It is present in higher quantities in rocks from where it is leached to contaminate water. High concentration of calcium in water is not desirable as it suppresses the formation of lather with soap. The calcium content values shows variation within the range from a minimum of 5.67mg/l at Mawroh to a maximum of 20.80mg/l at Lapalang. CO_2 is converted into carbonic acid that converts soluble carbonates into soluble bicarbonates. These carbonates react with calcium to form $CaCO_3$ hence leading to water hardness.

Magnesium

Magnesium is a common constituent of natural water and occurs with calcium. Its concentration is lower than calcium. Calcium and magnesium are the most abundant cations found in surface waters and hence, they are important contributors to the total hardness of the medium. In the present study, concentration of magnesium in spring water ranged from 3.50mg/l at Mawroh to 6.61mg/l at Lapalang.

Dissolved Oxygen (DO)

The concentration of Dissolved Oxygen in freshwater depends on the physical, chemical and biological process in an aquatic ecosystem. Since DO is essential for the biota of an ecosystem and for the aerobic degradation of organic pollutants, the measurement of DO is important in wastewater treatment. Non-polluted surface waters are generally saturated with DO. The DO concentration of spring water ranged from 17.26mg/l at Mawroh to 28.93mg/l at Lapalang. The values obtained are in agreement with the WHO (1993) [17] permissible limit of dissolved oxygen is >5 mg/l.

Biological Oxygen Demand (BOD)

Biological Oxygen Demand is the major criteria used in water pollution control where organic loading must be restricted to maintain desired DO [22]. The values of BOD of spring water in the sampling areas ranged from 7.04mg/l at Mawroh to 13.71mg/l at Lapalang. Low BOD content of spring water indicates that the water was free from organic pollution. According to WHO, the value of BOD should not exceed 30mg/l.

Table 16.2: Comparative Estimation of Values with WHO Standards

Parameter	Range	WHO Standards	Inference
Temperature (^{0}C)	9.10 to 14.23	-	Potable
pH	6.57 to 7.14	6.5 – 8.5	Potable
Conductivity (μS/cm)	65.26 to 97.57	400 μS/cm	Potable
CO_2 (mg/l)	11.00 to 14.66	-	Potable
Acidity (mg/l)	16.66 to 27.33	-	Potable
Total Alkalinity (mg/l)	14.66 to 29.33	200mg/l	Potable
Total Hardness (mg/l)	20.00 to 48.00	500mg/l	Potable
Chloride (mg/l)	7.16 to 14.43	250mg/l	Potable
Calcium (mg/l)	5.67 to 20.80	200mg/l	Potable
Magnesium (mg/l)	3.50 to 6.61	-	Potable
DO (mg/l)	17.26 to 28.93	>5.0mg/l	Potable
BOD (mg/l)	7.04 to 13.71	< 30mg/l	Potable

Drinking water quality guidelines and standards are designed to enable the provision of clean and safe water for human consumption, thereby protecting human health as well as the environment. The guidelines are usually based on scientifically assessed acceptable levels of toxicity to either humans or aquatic organisms [23]. From the results of the physicochemical and biological analysis of the present study it can be concluded that all the parameters under study lie within the potability range of the World Health Organization (WHO).

Conclusion

Spring water is a relatively safe source of potable water compared to other unprotected surface water sources e.g. rivers, lakes, rainwater etc. In the present study, the assessment of the various physicochemical and biological characteristics of water from the four springs located in Shillong was found to be acceptable according to the guidelines for drinking water provided by the World Health Organization (WHO). The low level observed for some parameters may be attributed to low level of industrialization, lack of proximity to possible source of contaminants and possibly far reaching water table in the study areas. However, there may be chances of contamination of water after collection, or during transportation and storage; therefore the water may require treatment such as boiling or treatment with hypochlorite solution to eliminate most microbial contaminants before drinking. Variations in some physiochemical parameters suggest that there is the need for continuous monitoring of the spring water quality to determine any change in the level of pollution at the sources. Microorganisms might also be responsible for changes in water properties, thus further studies may be conducted to determine the presence of bacterial contaminants, including the presence of heavy metals, which would otherwise inhibit the use and consumption of the spring waters. Therefore, efforts should be made to maintain the quality of spring water by protecting the springs and their surroundings from contamination by humans and animals.

References

1. Sawian. J.T., Lyndem, F.G., Laloo, R.C. and Jeeva, S., Water Pollution in Relation to Human Health. In: Gupta, K.R., Jankowska, M.A., Bosselmann, K, and Maiti, P. (eds.), Global Environment: Problems and Policies, Volume **3**, Atlantic Publishers and Distributers (P) Ltd., New Delhi, 2008, 108-124.
2. Kolade, O.A., Shallow Wells as Supplementary Sources of Water in Nasarawa, Department of Geography and Planning, University of Jos, Nigeria, 1982, 8-11.
3. United Nations Environment Program (UNEP), Water Quality for Ecosystem and Human Health, United Nations Environment Program/Global Environment Monitoring System (UNEP/GEMS) Program, 2006, 1-132.
4. Raymond, F., Le Problame dis ean dans le monde (Problems of Water), EB and Sons Ltd., 1992, 123-126.
5. Dezuane, J., Handbook of Drinking Water Quality, Indiana University Press, 1979, 3-17.
6. Santosh, P. and Revathi, D., Hydrogeochemical Analysis of Ground Water Parameters in Coimbatore District, Tamil Nadu, India, *Research Journal of Chemical and Environmental Sciences*, 2004, **2(2)**, 89-93.
7. Agarwal, R., Physico-Chemical Analysis of Some Groundwater Samples of Kotputil Town Jaipur, Rajasthan. *International Journal of Chemical, Environmental and Pharmaceutical Research.*, 2010, 111-113.
8. Todd, D. K., Ground Hydrology, 2nd edition. Wiley, New York. 1980.
9. Adeyemi, O., Oloyede, O. B. and. Oladiji, A.T., Physicochemical and Microbial Characteristics of Leachate-Contaminated Groundwater. *Asian Journal of Biochemistry*. 2007, **2**, 343-348.

10. Akpoveta, O.V., Okoh, B.E. and Osakwe, S.A., Quality Assessment of Borehole Water used in the Vicinities of Benin, Edo State and Agbor, Delta State of Nigeria. *Current Research in Chemistry.*, 2011, **3**, 62-69.
11. Uffia Dan, I., Ekpo, F.E. and Etim, D. E., Influence of Heavy Metals Pollution in Borehole Water Collected within Abandoned Battery Industry, Essien Udim, Nigeria. *Journal of Environmental Science and Water Resources*, 2013, **2(1)**, 022-026.
12. Rajput, S.I., Waghulade, G.P. and Zambare, S.P., A Study on Physico-Chemical Characteristics of Water from Right Canal of Hatnur Reservoir of Jalgaon, Maharashtra State, *Ecology Environment and Conservation*, 2004, **10(2)**, 171-173.
13. Kavitha, R. and Elangovan, K., Ground Water Quality Characteristics at Erode District, T.N., India, *International Journal of Environmental Science*, 2010, **1**, 2.
14. APHA. Standard Methods for the Examination of Water and Wastewater. 21st edition. APHA, AWWA, WPCF, Washington DC, 2005.
15. Ghavzan, N.J., Gunale, V.R. and Trivedy, R.K., Limnological Evaluation of an Urban Freshwater River with Special Reference to Phytoplankton, *Pollution Research.*, 2006, **25**, 259-268.
16. Tas, B. and Gonulol, A., An Ecological and Taxonomic Study on Phytoplankton of a Shallow Lake, Turkey. *Journal of Environmental Biology*, 2007, 439-445.
17. WHO (World Health Organization), Guidelines for Drinking Water Quality, 2nd edition. Geneva., 1993.
18. Byamukama, D., Kansime, F., Mach, L. and Farnleitner, A., Determination of *Escherichia coli* Contamination with Chromocult coliform Agar showed a High Level of Discrimination Efficiency for Differing Pollution Levels in Tropical Waters of Kampala, Uganda, *Applied Environmental* Microbiology, 1999, **66**, 864–868.
19. Kulkarni, J. R. and Shrivastava, V. S., Physical and Chemical Investigative for the Assessment of Pollution in and around Industrial Area, *Indian Journal of Environmental Protection.* , 2000, **20 (4)**, 252- 256.
20. Wilson, A., Parrott, K. and Ross, B. Household Water Quality - Water Hardness, Virginia Cooperative Extension. 1999, 356-490.
21. Thresh, J. C., Suckling, E.V. and Baele, J. F., The Examination of Water Supplies. 6th edition, Taylor, E.W. (ed). J. and A. Churchill, London. 1949.
22. Hussain, J., Hussain, I. and Arif, M., Characterization of Textile Wastewater. *Journal of Industrial Pollution Control*, 2004, **20(1)**, 137-144.
23. Okechukwu, M.E., Ogwo V., Onuegbu, C.U. and Mbajiorgu, C.C., Water Quality Evaluation of Spring Waters in Nsukka, Nigeria, *Hydrology for Disaster Management, Special Publication of the Nigerian Association of Hydrological* Sciences, 2012.

17

Impact of an Oil Refinery on Distribution of SO_2, NO_2, and O_3 Concentrations: A Case Study from Golaghat District, Assam

Devolakshi Handique
Krishna G Bhattacharyya

Abstract

Ambient concentrations of sulphur dioxide (SO_2), nitrogen dioxide (NO_2), and ozone (O_3) were measured at 4 sampling stations around the 3 million tonne oil refinery at Numaligarh in Golaghat district of Assam and a fifth sampling station near the Kaziranga National Park at an aerial distance of around 20 km from the refinery. Samples were analyzed by standard techniques. Concentrations of SO_2, NO_2, and O_3 were determined to investigate their distribution and source characterization. The sampling showed an average concentration of NO_2 of 8 µg/m^3 (maximum 12.52 µg/m^3) in winter, and 6.2 µg/m^3 (maximum 10.06 µg/m^3) in summer; while the average winter concentration for SO_2 was 10.42 µg/m^3 (maximum 13.61 µg/m^3), and summer concentration was 5.23 µg/m^3 (maximum 9.18 µg/m^3). The maximum ozone concentrations were 44.32 µg/m^3 in summer and 36.91 µg/m^3 in winter. The results showed that NO_2 and SO_2 concentrations near the refinery and the highway areas were two to three times higher compared with the residential and forest areas in both summer and winter. The measurements did not indicate any significant impact of the refinery on ambient air quality with respect to SO_2, NO_2, and O_3. The results of this work have helped in an understanding of the air quality in an ecologically fragile area.

Key words: *SO_2, NO_2, O_3, Ambient Air*

Introduction

Air pollution now a day's is a global problem. Mostly all the developing nations are affected by atmospheric pollution in terms of human health [1], climate change [2] and loss of bio-diversity [3]. In 21st century air pollution is a great challenge to be considered. Gaseous pollutants, like O_3, NO_x and SO_2 and particulate matter (PM), have been recognized as key environment problem in many cities around the world. Air Quality in cities, mainly affected by photochemical oxidants [4]. Vehicle emissions is the cause for the primary pollutant such as hydrocarbons, NO, CO, and some trace elements, as well as the secondary pollutants such as O_3 formed by the photochemical reactions of some primary pollutants in the atmosphere, such as NO_2, NO [5]. Secondary pollutants like O_3 are more hazardous than the pollutants originated from the combustion with respect to the adverse effects on the human health and the ecosystem [6]. Ozone is produced in the troposphere as a consequence of interaction of meteorological conditions, sunlight, nitrogen oxides (NO_x), O_2 and volatile organic compounds (VOCs) [7-9]. The relation between ozone and its two main precursors, NO_x (NO and NO_2) and volatile organic compounds (VOC), represents one of the major scientific challenges associated with urban air pollution [7]. The formation of ground level ozone depends on the intensity of solar radiation, the absolute concentrations of NO_x and VOCs, and the ratio of NO_x and VOCs [8, 9].

NO_x is produced formed in the atmosphere from both natural and anthropogenic sources, involving naturally occurring nitrogen and volatile organic compounds (VOCs) by the action of lightning [10]. However, NO_x emissions from anthropogenic sources exceed natural sources [11]. Increased combustion of fossil fuels and exhaust fumes may be extremely pervasive, causing serious environmental degradation, illness and deaths [12, 13].

Passive sampling has become a widely accepted technology throughout the world for environmental sampling as evidenced by many regulatory guidelines, manuals and protocols published by various environmental standard policy makers worldwide. The paper presents an assessment of NO_2, SO_2, and O_3 levels measured by passive sampling in Numaligarh, an important oil refinery town of Assam. The main objective is to define the spatial differences in the concentrations of the pollutants of interest and their possible sources. Moreover, we attempted in the present study to collect enough and significant data to estimate air quality trends in Numaligarh and Kaziranga atmosphere. Different metrological parameters (ambient temperature, relative humidity and wind speed and direction) and their influence on the air quality were also monitored during the sampling period.

Methodology

Study Area

Numaligarh is a town in the Golaghat District of Assam, India. It is situated at the distance of 32 km from Golaghat town (26°38'N, 93°45'E). It has well established traffic networks because here a medium oil refinery is situated, resulting in high population density as well as dense urban and infrastructural network. At aerial distance of 10 km Kaziranga National Park is situated which is famous for one horned rhinoceros.

There were five monitoring locations chosen along transect representing different levels of pollution in order to have a comprehensive study of spatial distribution of SO_2, NO_2 and O_3. The gradient extended over a distance of approximately 10 km within the boundaries of the city. In the 10 km study area around the Numaligarh Refinery, the ambient air quality was monitored in two season in a year in 5 sampling stations. The GPS locations are given in Table 17.1 below. The selection of parameters and the sampling techniques were as per Ministry of Environment & Forests (MoEF) / Central Pollution Control Board guidelines.

Table 17.1: Location of Monitoring Station

S/N	Location	Direction	Distance (km)	Latitude (N)	Longitude (E)
S1	Close to the front gate of the Refinery	—	0	26°3522.33	93°46242.73
S2	NRL township [The Butterfly Park]	N	2	26°35231.53	93°44241.53
S3	Raw water intake point, the Dhansiri River	E	2	26°35225.73	93°482253
S4	Near NH39 bypass (Rangbang Village)	S	4	26°332 42.13	93°462363
S5	Agartoli, Kaziranga National Park	SW	10	26°39217.73	93°48247.33

Climate Characterization

Numaligarh town is located in the Golaghat district of Assam. This town has also a tropical climate, seasonally humid with maritime influence. There are two climatic periods, the summer period from April to June and winter period from November till January. In this town, the annual daily average temperature is 23 °C. The daily average temperature peaks in the summer period in June at around 28.3 °C and reaches its minimum of 13.2°C in December. The relative humidity is high; its average value is ranges from 36-87%. During summer due to monsoon climate humidity is more. It lies around 60-74%. During winter the values lies around 40-52%. The prevailing wind direction is north-north eastern and second prevailing is south eastern. The average wind speed is around 2.1 m/s - 5.7 m/s throughout the year.

Sampling of SO_2, NO_2 & O_3

Sampling campaign was conducted one year i.e. 2014-15 in Numaligarh area and done twice in a week and eight times in a month. The sampling periods are summer and winter respectively. Summer period sampling was done in month of April, May and June where as winter in the month of November, December and January.

Sampling was done by means of Envirotech dust sampler APM 460 NL for all the three pollutants.

Analytical procedure for SO_2, NO_2 and O_3

Sulphur dioxide from air is absorbed in a solution of potassium tetrachloromercurate (TCM). The complex is made to react with para rosaniline and formaldehyde to form the intensely coloured pararosaniline methyl sulphonic acid. Absorbance is measured at 560 nm. Ambient nitrogen dioxide is collected by bubbling air through a solution of sodium hydroxide and sodium arsenite. The concentration of nitrite ion produced during sampling is determined calorimetrically by reacting the nitrite ion with phosphoric acid, sulphanilamide, and N-(1-naphthyle)-ethylenediammine dihydrochloride (NEDA) and measuring the absorbance of the highly coloured azo dye at 540 nm. For ozone potassium iodine is taken as absorbing solution. The iodine is determined spectrophotometrically by measuring the absorption of tri-iodide ion at 352nm.

Reagents

Analytical grade reagents were. For making chemical solution and dilution, de ionised water having conductivity less than 0.15 mS cm^{-1} was used. The extraction solution for SO_2 is prepared by dissolving 10.86 g mercuric chloride (Loba Chemie, India), 0.066 g EDTA (Qualigens fine chemicals) and 6 g potassium chloride (Qualigens fine chemicals) and make up to 1L. For nitrogen dioxide was prepared by mixing 4 gm of sodium hydroxide (Avantor performance materials, India) and 1 gm of sodium arsenite (Loba Chemie, India). For ozone by mixing 13.6 g of potassium dihydrogen phosphate (Merck Mumbai), 14.2 g of disodium hydrogen phosphate (Loba Chemie, India) and 10 g of potassium iodide (RFCL limited India) and make up to 1 L. Accurate weighing of chemical was performed on Kern Electronic Balance Model Type ABS 220-4 no WB 1110567.

Results and Discussion

Sulphur Dioxide Concentration in the Winter and Summer Season

Sulphur dioxide concentrations varied between 5.84–8.71 µg/m³ in summer and 12.62–13.6 µg/m³ in winter in the refinery site (S1). Similarly for township area (S2), the values varied between 2.67-4.80 µg/m³ in summer and 5.22 µg/m³ in winter. The values at S3 lie between 1.78-3.57 µg/m³ in winter, 1.14-1.91 µg/m³ in summer as given in below Table 17.2. At the National Highway Bypass (S4), the values show ranges of 1.94 to 8.58 µg/m³ in summer and 3.94 to 9.88 µg/m³ in winter. The site S5 (Agartoli, Kajiranga) does not show any significant result in this regard.

The wide range of the results could be attributed to the locations of the sources and their different pollutant loadings. Maximum SO_2 concentration, which was measured as 14.2 µg/m³ near the refinery in winter, did not exceed the limit value (100 mg/m³) of the National Ambient Air Quality Standards (NAAQS) set by the Ministry of Environment and forests, Government of India [14].

Table 17.2: Temporal and Site Specific Variation of SO_2 in Winter and Summer Season

Month	November	December	January	April	May	June
avgS1	12.62	12.62	13.6	5.84	8.32	8.71
avgS2	5.22	5.22	5.22	2.67	4.8	3.66
avgS3	1.78	1.78	3.57	1.14	1.91	1.66
avgS4	7.00	7.00	9.88	1.94	8.05	8.50
avgS5	0.933	0.933	2.9	0	1.9	1.43
Tem⁰C	13⁰	14⁰	15⁰	29⁰	29⁰	30⁰

Sulfur dioxide levels measured in winter and summer seasons were assessed comparatively (Fig. 17.1) to evaluate the effects of the meteorological conditions and the combustion processes on SO_2 pollution. The winter levels were higher than the summer levels for all the sampling points.

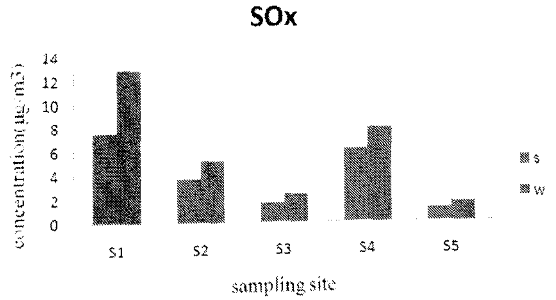


Fig. 17.1: Winter and Summer Level Concentration of Sulphur Dioxide

Nitrogen Dioxide Concentration in the Winter and Summer Season

NO_2 ground level concentrations (GLC) in the winter were between 2.9-10.48 μg/m^3 (Table 17.3). These concentrations do not exceed both the target level of 80 mg/m^3 given in the NAAQS. In the summer, on the other hand, ambient air NO_2 levels varied between 1.1 and 8 μg/m^3 (Table 17.3). The winter levels were higher than the summer levels for all the sampling points.

Table 17.3: Temporal and Site Specific Variation of NO_2 in Winter and Summer Season

Month	November	December	January	April	May	June
avgS1	10.48	10.48	7.54	6.2	6.3	8
avgS2	6.05	6.05	4.15	3.5	2.3	3.2
avgS3	3.76	3.76	3.04	2.7	1.8	1.1
avgS4	5.96	5.96	5.16	4.8	2.1	4.3
avgS5	2.9	2.9	2.82	2.9	2.9	2.82
Tem^{0}C	13^0	14^0	15^0	29^0	29^0	30^0

Concentration unit is μg/m^3.

The results obtained for NO_2 are originate mainly from the combustion of fossil fuels and have similar sources including vehicles, residential heating, chemical processes, etc. Due to the small area and population density, the large amount of traffic (5,000 vehicles/day on S4 highway, 3,000 vehicles/day on S2 highway, and 2,401 vehicles/day on S1 highway) has led to residential areas being

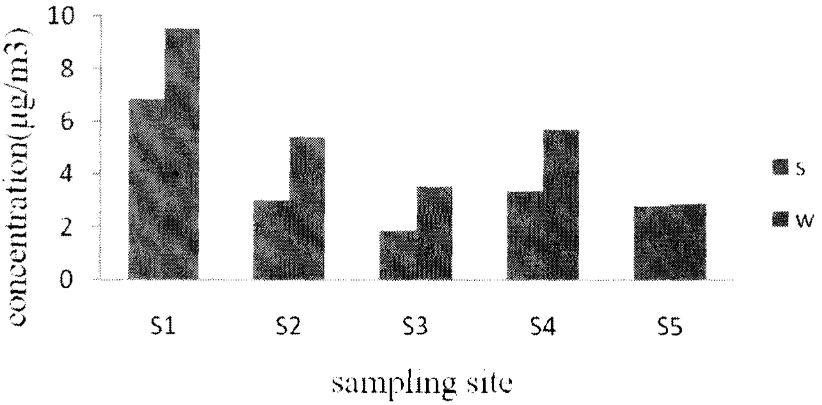


Fig. 17.2: Winter and Summer Level Concentration of NO_2

near the heavy traffic and industrial fields in Numaligarh. NO_2 concentrations are high not only in the areas close to the highways of S2 and S4 , but also in the S3 point, proving that the traffic emissions and the industrial activities are the most important sources determining the NO_2 levels measured in the study area. Chemical reactions, dispersion, and dilution processes result in spatial and temporal variations in concentrations of air pollutants. Due to its short life-time, the distribution of NO_2 is strongly heterogeneous with highest abundances in industrial areas [15]. As a result of which the increasing concentration was observed in the refinery and the bypass area. It is shown that the natural gas combustion in the industrial plants is the most significant source of NOx pollution [16].

Ozone Concentration in the Winter and Summer Season

The lowest average GLC O_3 concentration in winter season was 4.5 µg/m³ detected at the S3 sampling site, where the air exits the sampling zone and the highest value was 36.9 µg/m³ measured at the S4 site, where the clean air arrives at this area (Table 17.4). As can be seen from these results, the O_3 could not be produced in this zone. The high concentrations observed at the S1 site can be due to the emission from refinery. Ozone concentrations in all sites during winter were measured between 4.5 and 36.9 µg/m³ (Table 17.4), which are below the limit values set for both human health and the development of the plants. It is known that the photochemical reactions occurring in the atmosphere play a significant role in ozone formation; therefore, ozone concentrations in summer are generally higher than those in winter due to the higher temperatures and light intensity promoting these reactions, making ozone as a typical summer pollutant [17, 18].

Table 17.4: Temporal and Site Specific Variation of O_3 in Winter and Summer Season

Month	November	December	January	April	May	June
avgS1	23.8	23.6	30.7	40.9	37.1	10.2
avgS2	7.6	17.8	29.2	32.9	32.7	20.4
avgS3	4.5	4.7	5	20	27.9	19
avgS4	12.5	22.7	30.1	38.5	35.8	11.2
avgS5	4.7	5	4.3	20.5	19.8	19.2
Tem⁰C	130	140	150	290	290	300

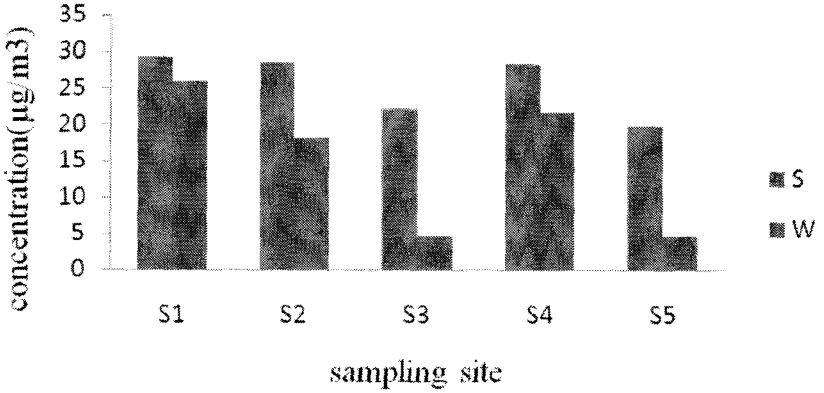


Fig. 17.3: Winter and Summer Level Concentration of Ozone

In the study, ozone concentrations in summer varied between 10.13 and 54.33 µg/m^{3}as given in Table 17.4 above. All the ozone concentrations measured in the study was below the limit value of 120 µg/m^3 (daily average) given in NAAQs directives. Seasonal distribution diagram for ozone pollution in summer and winter in the study area are shown in above Fig. 17.3. It is observed that the ozone levels are generally low in the residential i.e. S2 (township) and the S3 (intake) areas, and high in the S4 (bypass) areas.

During the noon hours, the sun rays increased greatly and the photochemical processes that produce O_3 dominated, especially after the sunrise. Oxygen atoms produced in the photolysis of NO_2 could react with O_2 and to produce O_3 through the chemical reactions [19]. The reduction in O_3 level is mainly due to the decrease of solar radiation which then would lower the level of the photochemical production. During winter the intensity of solar light is less. Ozone concentration therefore is less in the winter season than the summer.

Conclusion

The aim of this work is to determine the pollutant level of sulphur dioxide, Nitrogen dioxide and ozone in ambient air. This gave a good knowledge about the ground level concentration of these pollutants. The concentration of sulphur dioxide, nitrogen dioxide is found higher in winter than the summer whereas the ozone concentration is found high in summer than the winter. It can be seen that the maximum increase are all very minimal. The contour shows that in most of the study area, the increase in GLC of the pollutant is negligible. Only in some small packet, there is some marginal increase. The results of this work will definitely help in expanding the knowledge base and understanding air quality in an ecologically fragile area.

References

1. Anderson, J.O., Thundiyil, J.G and Stolbach, A., Clearing the air: a Review of the Effects of Particulate Matter Air Pollution on Human Health, *Journal of Medical Toxicology*, 2012, **8**, 166-175.
2. Kan, H., Chen, R. and Tong, S., Ambient Air Pollution, Climate Change, and Population Health in China, *Environmental International*, 2012, **42**,. 10–19.
3. Lovett, G.M., Tear, T.H., Evers, D.C., Findlay, S.E.G, Cosby, B.J., Dunscomb, J.K., Driscoll, C. T. and Weathers, K.C., Effects of Air Pollution on Ecosystems and Biological Diversity in the Eastern United States, *Annals of New York Academy of Sciences*, 2009, **1162**,. 99–135.
4. Han, S., Bian, H., Feng, Y., Liu, A., Xiangjin Li, X., Zeng,F. and Zhang, X., Analysis of the Relationship between O_3, NO and NO_2 in Tianjin, China, *Aerosol and Air Quality Research*, 2011, **11,** 128–139.
5. Wang, G, Bai, S. and Ogden, J. M., Identifying Contributions of On-road Motor Vehicles to Urban Air Pollution Using Travel Demand Model Data, *Transport Research part D*, 2009, **14,** 168.
6. Wang, X., Lu, W., Wang, W. and Leung, A., A Study of Ozone Variation Trend within Area of Affecting Human Health in Hong Kong, *Chemosphere*, 2003, **52**, 1405.
7. Khoder, M.I., "Diurnal, seasonal and weekdays–weekends variations of ground level ozone concentrations in an urban area in greater Cairo", *Environmental Monitoring and Assessment*, 2009, **149**, 349–362.
8. Tong, N.Y.O., Leung, D.Y.C. and Liu, C.-H., A Review on Ozone Evolution and its Relationship with Boundary Layer Characteristics in Urban Environments, *Water, Air, Soil Pollution*, 2011, **214**, 13–36.
9. Mahapatra, A., Prediction of Daily Ground-level Ozone Concentration Maxima over New Delhi, *Environmental Monitoring and Assessment*, 2010, **170**, 159–170.
10. Brown, S.S., Ryerson, T.B., Wollny, A.G., Brock, C.A., Peltier, R., Sullivan, A.P., Weber, R.J., Dube, W.P., Trainer, M., Meagher, J.F., Fehsenfeld, F.C. and Ravishankara, A.R. Variability in Nocturnal Nitrogen Oxide Processing and Its Role in Regional Air Quality. *Science*, 2006, **311**, 67–70.
11. Godish, T., *Air Quality*, Lewis Publishers, 2004.
12. Ricciardolo, F.L.M., Sterk, P.J., Gaston, B. and Folkerts, G., Nitric Oxide in Health and Disease of the Respiratory System, *Physiological Review*, 2004, **84**, 219–234.
13. Gurjar, B.R., Butler, T.M., Lawrence, M.G and Lelieveld, J., Evaluation of Emissions and Air Quality in Megacities, *Atmospheric Environment*, 2008, **42**, 1593–1606.
14. Odlyha, M., Foster, G M., Cohen, N. S., Sitwell, C., and Bullock, L., Microclimate, Monitoring of Indoor Environments Using Piezoelectric Quartz Crystal Humidity Sensors, *Journal of Environmental Monitoring*, 2000, **2**, 127"131.
15. Etin, S. C., Karademir, A., Pekey, B. and Ayberk, S., Inventory of Emissions of Primary Air Pollutants in the City of Kocaeli, Turkey, *Environment Monitoring Assessment*, 2007, **128**, 165.

16. Isaksen, I. S. A., Granier, C., Myhre, G., Berntsen, T. K., Dalsøren, S. B. and Gauss, M., Klimont, Z., et al., Atmospheric Composition Change: Climate–Chemistry Interactions, *Atmospheric Environment,* 2009, **43**, 5138.

17. Tu, J., Xia, Z.-G., Wang, H. and Li, W., "Temporal Variations in Surface Ozone and Its Precursors and Meteorological Effects at an Urban Site in China", *Atmospheric Research*, 2007, **85**, 310.

18. Ooka, R., Khiem, M., Hayami, H., Yoshikado, H., Huang, H. and Kawamoto, Y., Influence of Meteorological Conditions on Summer Ozone Levels in Central Kanto Area of Japan, *Procedia Environmental Sciences*, 2011, **4**, 138.

19. Reddy, B.S., Kumar, K.R., Balakrishnaiah, G., Gopal, K.R., Reddy, R.R., Sivakumar, V., Lingaswamy, A.P., Arafath, S.Md., Umadevi, K., Kumari, S.P., Ahammed, Y.N. and Lal, S. Analysis of Diurnal and Seasonal Behavior of Surface Ozone and Its Precursors (NOx) at a Semi-Arid Rural Site in Southern India, *Aerosol Air Quality Research,* 2012, **12**, 1081–1094.

18

Characterization of Phytase Isolated From Rice bean Cotyledons (Vigna umbellata Thunb.) With Respect to Influence of Different Factors

K. Belho
P.K. Ambasht

Abstract

Phytase isolated from rice bean has been characterized with respect to influence of different factors. Ag^- showed inhibition at 0.7 mM, but had no effect at 0.25 mM. At 0.7 mM, some divalent cations showed activation in the following order: $Cr^{3-} > Co^{2-} > Ni^{2-} > Mg^{2-} > Ca^{2-}$ while some others inhibited the enzyme $Pb^{2-} > Cu^{2-} > Fe^{2-} > Zn^{2-} > Mn^{2-}$. Vanadate and molybdate anions were mild inhibitors while fluoride and Pi were strong inhibitors. The latter showed uncompetitive inhibition. Metal chelators had no effect on phytase activity suggesting it to be not a metalloenzyme. Enzyme was not membrane bound as detergents had no effect on phytase activity. Results of incubation of enzyme with group specific reagents rule out the presence of thiol, tryptophanyl and tyrosyl residues at active site. β-mercaptoethanol and PMSF stabilized the enzyme. Phytase showed resistance against proteolysis.

Keywords: *Rice bean, Phytate, Phytase*

Abbreviations: BSA, bovine serum albumin; EDTA, ethylene diamine tetra-acetic acid; PMSF, Phenylmethane sulphonyl fluoride; SDS, Sodium dodecylsulphate.

Introduction

Phytase catalyzes hydrolysis of phytic acid (*myo*-inositol*hexakis*phosphate) to orthophosphate and *myo*-inositol*pentakis*phosphate [1]. Depending on the location of the first hydrolyzed phosphate, there are two types of phytases namely: 3-phytase (EC 3.1.3.8 *myo*-inositol*hexakis*phosphate 3-phosphohydrolases) and 6-phytase (EC 3.1.3.26 *myo*-inositol*hexakis*phosphate 6-phosphohydrolases) [2]. On the basis of pH optima, phytases are acidic [3] or alkaline [4].

In higher plants, phytase occurs in grains, seeds and pollens. Phytase plays important roles in the physiology of plants. It is responsible for the degradation of phytate during the germination and mobilizes phosphates, minerals, and *myo*-inositols for the growth and development [5]. Legumes are good source of proteins, carbohydrates, fiber, minerals, and water-soluble vitamins; however, their phytate contents can limit the availability of minerals [6]. Human beings are monogastric animals, lack or have insufficient amount of phytate degrading enzymes in their gastrointestinal tract [7]. Rice bean (*Vigna umbellata* Thunb.) is a legume, belongs to the family brassicaceae and is rich in vitamin B with a considerable amount of K, Mg, Ca, Fe, Zn, Cu, Mn and P [8]. With easy cultivation of rice bean, its nutritional properties can be of great aid for improving the nutrition among the economically marginalized people and livestock [9].

Phytase which was purified from 72 h germinated rice bean cotyledon was used [10]. In the present paper, we report the influence of different factors on rice bean phytase activity.

Materials and Methods

Sodium phytate, trypsin, pepsin, Tween 20 and Tween 80 were from Sigma Aldrich, USA. The other chemicals were of AR grade from SRL, Sd Fine, HiMedia, India. All solutions were prepared in de-ionized water from Milli-Q system (Millipore, USA). Phytase from rice bean with specific activity 2.22 U/mg protein was used.

Phytase activity was assayed at 40 °C [11]. The assay buffer was 0.1 M sodium acetate buffer, pH 4.0. One enzyme unit is defined as the amount of enzyme that catalyzes liberation of one µmole of phosphate per min under the assay condition. Protein was quantified using BSA as standard [12].

The effect of various cations and anions on rice bean phytase activity has been studied at two different concentrations (0.25 and 0.7 mM). Phytase was first dialyzed against 50 mM sodium acetate buffer pH 5.0 containing 1.0 mM EDTA and was used in the study of ions effect. The solutions were prepared in assay buffer.

The other effectors were tested for their effects on un-dialyzed phytase. Chelators were also tested at two concentrations (0.25 and 0.7 mM). The effects of detergents like Triton X 100, Tween 20 and 80 on phytase activity was studied at 0.1 and 1 % and SDS at 0.1 and 1.0 mM.

The effect of group specific reagents like iodoacetate, N-ethylmaleimide, N-bromosuccinimide and iodine on phytase was tested by taking aliquots from each solution after incubation for 1h at 4 °C. Similar procedure was also followed for additives like PMSF (0.1 and 1.0 mM) and β-mercaptoethanol (0.1 and 1.0 %).

For the study of effect of proteolytic enzymes on phytase, the latter was incubated with pepsin (0.1 mg/mL) in 50 mM glycine-HCl buffer at pH 2.5 and with trypsin (0.1 mg/mL) in 50 mM Tris-HCl buffer, pH 8.0 at 37 °C. Enzyme aliquots were withdrawn and assayed for phytase residual activity.

For inhibition studies, in one set of experiments, concentration of inorganic phosphate was varied from 0.1 to 2 mM keeping the sodium phytate concentration fixed. The % relative activity versus the concentration of Pi was then plotted. In another set of experiment, the phytate concentration was varied, in the absence and in the presence of Pi (0.8 mM). Lineweaver Burk plot was made.

Result and Discussion

The results of effect of cations, anions and chelators on phytase activity have been described in Table 18.1. It is evident that among monovalent cations, none of the tested cations showed any effect at 0.25 mM. In most reports, monovalent cations did not show any effect on phytase activity [3,13].

Table 18.1: Effect of Cations, Anions and Chelating Agents on Rice Bean Phytase Activity

Effectors	**% Relative activity**	
	(0.25 mM)	**(0.7 mM)**
Control	100.00	100.00
$(NH_4)_2SO_4$	98.66±0.8	113.12±5.74
LiCl	96.26 ±3.24	100.92±1.16
NaCl	102.81±7.43	100.92±2.19
KCl	95.66±0.97	101.7±0.87
$AgNO_3$	99.66±0.80	62.09±2.91
$CaCl_2$	96.84±4.36	115.68±2.49
$MgCl_2$	97.61±2.88	118.22±0.87
$CdCl_2$	100.7±4.08	103.99±1.35
$BaCl_2$	103.15±0.94	110.05±3.49
$NiSO_4$	118.99±7.22	121.68±1.35
$CoCl_2$	124.3±4.18	133.2±1.09

Effectors	**% Relative activity**	
	(0.25 mM)	**(0.7 mM)**
$MnCl_2$	66.63±0.91	82.81±0.78
$ZnCl_2$	70.96±5.34	83.5±3.39
$FeSO_4$	80.45±5.13	59.85±1.51
$FeCl_3$	42.92±1.35	34.34±5.39
$SrCl_2$	102.40±0.4	118.21±0.87
$Cr_2(SO_4)_3$	125.79±1.29	172.89±2.5
$Pb(NO_3)_2$	41.36±0.83	NA*
$CuSO_4$	80.34±0.96	54.12±1.64
NH_4VO_3	84.86±0.59	67.17±3.11
NaH_2PO_4	64.73±1.21	56.86±4.47
NaF	72.18±3.61	64.80±1.16
Na_2MoO_4	82.38±2.1	67.45±2.84
Inositol	57.54±4.94	44.46±3.74
Chelators		
Sodium citrate	98.54±0.95	100.14±1.37
Sodium potassium tartarate	98.54±0.95	100.14±1.4
Ammonium oxalate	99.28±0.89	99.57±0.43
EDTA	99.29±0.89	99.57±0.43

*NA "No Activity"

At 0.7 mM, however, a very nominal increase in rice bean phytase activity was observed in the presence of ammonium sulphate. Ammonium ions brought activation in rapeseed phytase [14]. Ag^+ strongly inhibited rice bean phytase at higher concentration like some other phytases [14,15].

The salts of II group elements like $MgCl_2$, $CaCl_2$, $SrCl_2$ and $BaCl_2$ showed no effect on rice bean phytase activity at 0.25 mM. Mg^{2+} and Ca^{2+} had no effect on some other phytases [3, 16, 17]. At 0.7 mM, however, Mg^{2+}, Ca^{2+} and Sr^{2+} showed mild activation to rice bean phytase. Ca^{2+} in most cases showed activation [1, 4, 18, 19]. The possible reason may be the removal of Pi in the form of calcium phosphate, thereby reducing the inhibition due to Pi. Mg^{2+} has also been reported to activate phytase [19,20]. There is only one report where Sr^{2+} has an activating effect on phytase [21]. Rice bean phytase was strongly inhibited in presence of lead nitrate similar to phytase from canola seed [20]. Though lead belongs to group IV of Periodic Table, forms Pb^{2+} due to inert pair effect.

In the presence of salts of transition metals like $NiSO_4$, $CoCl_2$ and $Cr_2(SO_4)_3$, rice bean phytase showed activation at both 0.25 and 0.7 mM. This is the first report on chromium being an activator of phytase. Phytase activity activation in the presence of Co^{2+} has been reported earlier [14,20].

Rice bean phytase was strongly inhibited in the presence of salts of some other transition metals like $MnCl_2$ and $ZnCl_2$, at 0.25 mM. Mn^{2+} also inhibited oat phytase [16]. In the presence of $FeSO_4$ and $CuSO_4$, inhibition was observed at 0.25 mM, which increases further at 0.7 mM. $FeCl_3$ showed a very strong inhibition at both concentrations. The strong inhibition in the presence of $FeCl_3$ and $FeSO_4$ may be because of the formation of the complex of Fe-phytate reducing the phytate concentration [16].

Anions like vanadate and molybdate showed mild inhibition towards rice bean phytase activity like some other reports [14,15,22]. Strong inhibition in rice bean phytase activity was observed in the presence of fluoride and Pi like other reports [3,14,15,17,22]. Inhibition in the presence of fluoride is a characteristic feature of acid phytases [23,24]. Pi being a product of the reaction, therefore, is expected to inhibit the activity.

From the results of effect of chelators on rice bean phytase, it is evident that none of them had any effect like in other reports [16,17, 25] suggesting that rice bean phytase is not a metalloenzyme. The results of effect of detergents on rice bean phytase activity have been summarized in Table 18.2.

Table 18.2: Effect of Detergents on Rice Bean Phytase Activity

Detergent	**% Relative activity**	
	(0.1 %)	**(1.0 %)**
Control	100.0	100.0
Triton X-100	98.11±2.96	100.32±0.79
Tween 20	97.78±2.31	100.92±2.7
Tween 80	97.25±0.56	99.26±3.92
	0.1 mM	1.0 mM
SDS	100.39±1.93	103.17±3.86

In the presence of non-ionic and ionic detergents, there was no effect on rice bean phytase activity. There are reports of activation in phytase from pollen of *Lilium longiflorum* and *Typha latifolia* in the presence of Triton X-100 suggesting their association with membrane [26,27]. The present results suggest that rice bean phytase is not associated to any membrane structure. The results of effect of incubation of group specific reagents and additives are summarized in Table 18.3.

Table 18.3: Effect of incubation of group specific reagents and additives on rice bean phytase activity

Group specific reagentsand additives	**% Residual activity**	
Control	100.00	
Group specific reagent	**(0.1 mM)**	**(1.0 mM)**
N-Bromosuccinimide	99.49±5.26	99.32±1.07
Iodoacetate	98.99±1.07	98.90±3.64
N-Ethylmaleimide	105.37±1.21	99.43±3.27
Iodine	99.94±1.39	97.61±2.52
Additive		
PMSF	99.65±0.97	98.76±0.89
	(0.1 %)	**(1.0 %)**
β- Mercaptoethanol	100.88±4.63	108.49±2.38

There was no loss in enzyme activity in the presence of any of the above group specific reagents. The results therefore rule out the presence of thiol,

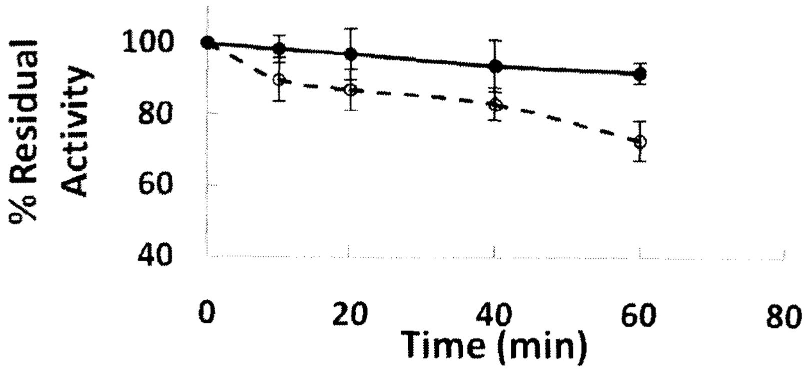


Fig. 18.1: A plot showing effect of time of incubation of trypsin (o??o) and pepsin (o······o) on residual rice bean phytase activity. The values used are the mean values along with its SD of three independent experiments.

tryptophanyl and tyrosyl residue at the active site of rice bean phytase. Some other phytase also had no effect of SH-reagents [16,17,25,28]. In the presence of β-mercaptoethanol and PMSF, no loss in enzyme activity was observed and may have a stabilizing effect. PMSF is used as a protease inhibitor, while β-mercaptoethanol keeps the reducing environment and prevents any loss due to oxidants. The results of effect of different proteases on rice bean phytase are shown in Fig. 18.1.

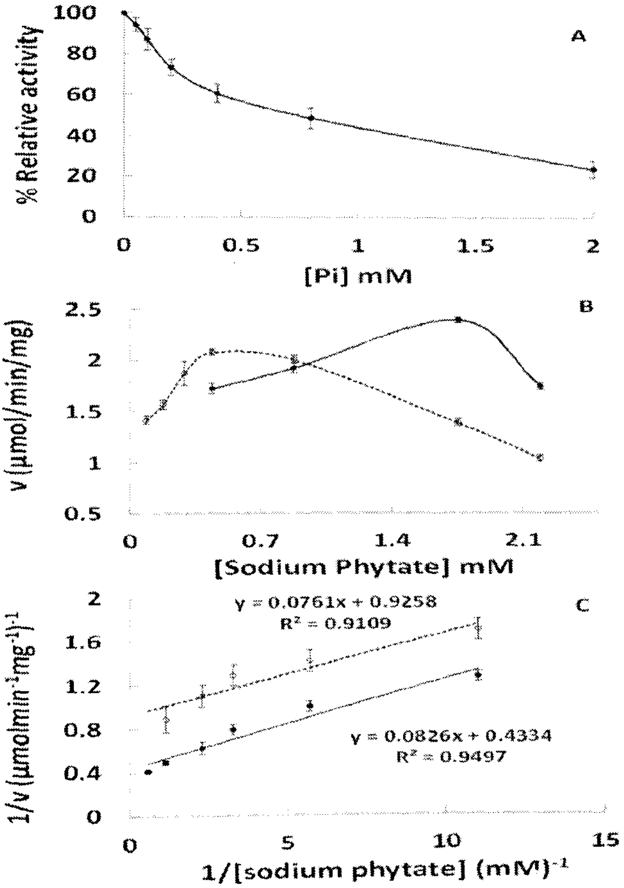


Fig. 18.2: Effect of variation of Pi on rice bean phytase activity; Fig. 18.2 B: Graphical representation of rice Bean phytase activity in the absence (o??o) and in presence of inorganic phosphate (0.8 mM) (o······o). Fig. 18.2 C: Lineweaver Burk plot of rate of reaction catalyzed by rice bean phytase versus [sodium phytate] in presence (o······o) and absence (o??o) of Pi. The values used are the mean values with its SD of three independent sets conducted.

It showed a good retention of activity even after 1 h of incubation with trypsin (91 %) and pepsin (72 %). Some other phytases also show resistance against proteolytic enzymes [29].

The results of effect of variation of Pi on phytase activity at fixed [phytate] are shown in Fig. 18.2. It is evident that 50 % inhibition was observed at 0.8 mM Pi. The results of effect of variation of [phytate] in the absence and presence of Pi (0.8 mM) are shown in Fig. 18.2 B. In the absence of any external Pi, there was inhibition above 2 mM phytate. In the presence of 0.8 mM Pi, the inhibition was observed beyond 0.5 mM phytate, suggesting that phytase activity may be regulated depending upon phosphate reserves. The Lineweaver Burk plot in the absence and presence of Pi (0.8 mM) is shown in Fig. 18.2 C and it is evident that Pi acts as an uncompetitive inhibitor. The values of slopes in the absence (0.082) and in the presence of Pi (0.076) are very close, the ratio being 1.08. The apparent K_m and V_{max} were 0.082 mM and 1.08 ìmol/min/mg protein, respectively. The values of K_i determined from apparent K_m and V_{max} were 0.69 mM, 0.74 mM, respectively. The value of K_i is almost more than three times the K_m of sodium phytate (0.19 mM). The present report is the first report on Pi being an uncompetitive inhibitor. In most cases, it acts as a competitive [3,30,31] or non-competitive inhibitor [20].

Conclusion

Pb^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} and Fe^{3+} showed inhibition which may be attributed to the formation of precipitation due to the interaction between substrate with the metal ions. The end products and their analogues were involved in the regulation of activity. Overall, the characteristics displayed by phytase from rice bean suggest that it will be favourable for application in aquaculture for improving the nutrition. It can also be immobilized to different matrices for improving its stability with respect to pH and temperature.

Acknowledgement

Maulana Azad National Fellowship in the form of Senior Research Fellowship to K. Belho is gratefully acknowledged.

References

1. Laboure, A.-M., Gagnon,J. and Lescure, A.- M., Purification and characterization of phytase (*myo*-inositolhexakisphosphate phosphohydrolase) Accumulated in Maize (*Zea mays*) seedlings during Germination, *Biochemical Journal,* 1993, **295**, 413.
2. Dixon, M. and Webb, E.C., Enzymes. Longman Group Ltd., ISBN 0-582-46217-7, 1979.
3. Andriotis, V. M. E., and Ross, J. D., Isolation and Characterisation of Phytase from dormant *Corylus avellana* Seeds, *Phytochemistry*, 2003, **64**, 689.

4. Jog, S.P., Garchow, B.G., Mehta, B.D. and Murthy, P.P.N., Alkaline Phytase from Lily Pollen: Investigation of Biochemical Properties, *Archives of Biochemistry and Biophysics,* 2005, **440**, 133.
5. Reddy, N. R., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K., Phytate in Cereals and Legumes, Boca Raton, FL: CRC Press Inc., 1989.
6. Frias, J., Doblado, R., Antezana, J.R. and Vidal-Valverde, C., Inositol Phosphate Degradation by the action of Phytase Enzyme in Legume Seeds, *Food Chemistry,* 2003, **81**, 233.
7. Brinch-Pedersen, H., SØrensen, L. D. and Holm, P. B., Engineering Crop plants: Getting a Handle on Phosphate, *TRENDS in Plant Science,* 2002, **7**, 118.
8. Katoch, R., Nutritional Potential of Rice Bean (*Vigna umbellata*): An Underutilized Legume, *Journal of Food Science,* 2013, **78**, C8.
9. Kaur, M., Effect of Domestic Processing and Amino Acid Composition of Rice Bean (*Vigna umbellata*), *Indian Journal of Applied Resources,* 2015, **5**, 280.
10. Belho, K., Nongpiur, S. R., and Ambasht, P. K., Purification and Partial Characterization of Phytase from Rice Bean (*Vigna umbellate* Thunb.) germinated seeds, *Journal of Plant Biochemistry &* Biotechnology, 2015, DOI: 10.1007/s13562-015-0333-7.
11. Heinonen, J. K. and Lahti, R. J., A New and Convenient Colorimetric Determination of Inorganic Orthophosphate and its Application to the Assay of Inorganic Pyrophosphatase, *Anaytical Biochemistry,* 1981, **113**, 313.
12. Bradford, M. M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein utilizing the Principle of Protein-dye Binding, *Analytical Biochemistry,* 1976, **72**, 248.
13. Vohra, A. and Satyanarayana, T., Purification and Characterization of a Thermostable and Acid-stable from *Pichia anomala, World Journal of Microbiology and Biotechnology,* 2002, **18**, 687.
14. Mahajan, A. and Dua, S., Nonchemical Approach for Reducing Antinutritional Factors in Rapeseed (*Brassica campestris* var. Toria) and Characterization of Enzyme Phytase, *Journal of Agricultural and Food Chemistry,* 1997, **45**, 2504.
15. Nakano, T., Joh, T., Tokumoto, E. and Hayakawa, T., Purification and Characterization of Phytase from Bran of *Triticum aestivum* L. cv. Nourin #61, *Food Science and Technology Research,* 1999, **5**, 18.
16. Greiner, R. and Alminger, M. L., Purification and Characterization of a Phytate-degrading Enzyme from Germinated Oat (*Avena sativa*), *Journal of the Science of Food and Agriculture,* 1999, **79**, 1453.
17. Greiner, R., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M. M., and Goyoga, C., Purification and Characterization of a Phytate-degrading Enzyme from Germinated faba beans (*Vicia faba* var. Alameda), *Journal of Agricultural and Food Chemistry,* 2001, **49**, 2234.
18. Gibson, D.M. and Ullah, A.H.J., Purification and Characterization of Phytase from Cotyledons of Germinating Soybean Seeds, *Archives of Biochemistry and Biophysics,* 1988, **260**, 503.

19. El-Toukhy, N. M.K., Youssef, A. S. and Mikhail, G.M., Isolation, Purification and Characterization of Phytase from *Bacillus subtilis* MJA. *African Journal of Biotechnology*, 2013, **12**, 2957.
20. Houde, R.L. and Alli, I., Kermasha Purification and Characterization of Canola Seeds (*Brassica* sp.) phytase, *Journal of Food Biochemistry*, 1990, **14**, 331.
21. Scott, J. J. and Loewus, F., A Calcium Activated Phytase from Pollen of *Lilium longiflorum*, *Plant physiology*, 1986, **82**, 333.
22. Li, M.G., Osaki, M., Honma, M. and Tadano, T., Purification and Characterization of Phytase Induced in Tomato Roots under Phosphorus-deficient Conditions, *Soil Science and Plant Nutrition*, 1997, **43**,179.
23. Ullah, A.H.J. and Sethumadhavan, K., Differences in the Active Site Environment of *Aspergillus ficuum* Phytases, *Biochemical and Biophysical Research Communications*. 1998, **243**, 458.
24. Crans, D.C., Smee, J.J., Gaidamuskas, E. and Yang, L., The Chemistry and Biochemistry of Vanadium and the Biological Activities Exerted by Vanadium Compounds, *Chemistry Reviews*, 2004, **104**, 849.
25. Greiner, R., Konietzny, U. and Jany, K.D., Purification and Properties of a Phytase from Rye., *Journal of Food Biochemistry*, 1998, **22**, 143.
26. Hara, A., Ebina, S., Kondo, A. and Funaguma, T., A New Type of Phytase from Oollen of *Typha latifolia* L., *Agricultural and Biological Chemistry*, 1985, **49**, 3539.
27. Lin, J-J., Dickinson, D.B. and Ho, T-H.D., Phytic Acid Metabolism in Lily (*Lilium longiflorum* Thunb.) Pollen, *Plant Physiology*, 1987, **83**, 408.
28. Greiner, R., Purification and Characterization of Three Phytases from Germinated Lupine Seeds (*Lupinus albus var. Amiga*), *Journal of Agricultural Food Chemistry*, 2002, **50**, 6858.
29. Zhang, G Q., Dong, X. F., Wang, Z. H., Zhang, Q., Wang, H. X. and Tong, J. M., Purification, Characterization and Cloning of a Novel Phytase with Low pH Optimum and Strong Proteolysis Resistance from *Aspergillus ficuum* NTG-23, *Bioresource Technology*. 2010, **101**, 4125.
30. Lim, P. E. and Tate, M. E., The phytases. II. Properties of Phytase Fractions F1 and F2 from Wheat Bran and the *myo*-inositol Phosphates produced by fraction F2, *Biochimica et Biophysica Acta*, 1973, **302**, 316.
31. Chang, R. and Schwimmer, S., Characterization of Phytase of Beans (*Phaseolus vulgaris*), *Journal of Food Biochemistry*, 1977, **1**, 45.

19

Isotherm and Kinetic Studies on Adsorption of Fe(III) by Sand and Rice Husk Mixture

Leena Deka
K G Bhattacharyya

Abstract

Sand and rice husk are two locally available and low cost materials. The feasibility of the mixture of sand and rice husk as an adsorbent for the removal of Fe(III) from aqueous solutions was investigated. For the removal process, batch adsorption technique was used. The effects of metal ion concentration and contact time on adsorption efficiency, under a constant temperature of 303 K and normal pH were studied. The experimental results were analyzed using Langmuir and Freundlich adsorption isotherm models. Kinetic studies showed that a pseudo-second order model was more suitable than the pseudo first order model. It is concluded that sand- rice husk mixture can be used as an effective adsorbent for removing Fe(III) from aqueous solutions.

Keywords: *Batch Adsorption, Mixture, Sand, Rice Husk, Isotherm, Kinetic.*

Introduction

Iron occurs naturally in water. It is an essential element which takes part in the transportation of oxygen in the blood. At concentrations found in most natural waters, and at concentrations below the aesthetic objective, iron is not considered a health risk. When the concentration of iron in water exceeds a certain level, it causes some aesthetic and economic problems. The recommended Secondary Maximum Contaminant Level (SMCL) determined by the US EPA for iron in drinking water is 0.3 mg/L [1, 2]. Water contaminated with iron has a disagreeable taste. Beverages and vegetables cooked in water loaded with iron have dispelling taste and appearance. Iron stains laundry, utensils, cookware etc. which is not easily removable by soaps and detergents. Iron precipitates in water blocks pipes and sometimes water supply or water softening equipment must be replaced. Drinking water overloaded with iron may cause vomiting, bleeding and liver problems. Therefore, iron needs to be removed from water to make it drinkable and usable. There are many techniques used to remove dyes or metal ions from wastewaters which includes chemical precipitation, solvent extraction, ion exchange, membrane separation and adsorption [3-8].

Adsorption process involves in the removal of specific components from wastewater by adsorption onto solid surfaces. Adsorption is a more feasible technique as it involves low initial cost, simple design, is easy to operate and insensitive towards toxic substances [9]. Biological materials prove to be good adsorbents due to their morphology and surface functional groups and biodegradability [10]. They have sorption capacity similar to synthetic materials and have lower susceptibility to interference. Biosorbents are more advantageous due to their cost effectiveness and relative abundance [11]. There have been several reports on the use of various low cost and easily available adsorbents like rice husk [10, 12], saw dust [13], coir pith [14], etc to remove heavy metals and organic pollutants. Rice husk is a by-product during rice-milling process and is produced in large quantities. Rice husk is enriched with floristic fibre, protein and some functional groups such as carboxyl, hydroxyl and amidogen, etc.[15]. The presence of these groups makes adsorption processes possible. So, rice husk is low cost and easy to obtain [16]. The rural and semi-urban population of Assam, uses local household iron filter units which are made-up of local materials such as wooden charcoal, river sand and gravel [17]. Sand is widely used for iron removal purposes. Sand was used for removing dyes like coomassie blue, malachite green and safranin orange from aqueous solutions [18]. In this study, sand and rice husk are mixed together to investigate its adsorptive capacity for iron removal from water.

Materials and Methods

- **Adsorbent Material**

  Sand and rice husk were collected from local sources. Collected sand and rice husk were washed thoroughly with distilled water, oven dried and sieved and the fractions between 0.595 mm and 0.063 mm were chosen. The sand is mixed with rice husk in 2:1 proportions.

- **Chemicals**

  Anhydrous $FeCl_3$ (Qualigens, mol. wt = 162.21 g) was dissolved in distilled water to prepare the synthetic adsorbate solutions and the stock solution was diluted with distilled water for each required concentration of Fe(III).

- **Adsorption Experiments**

The adsorption experiments are carried out by mixing a fixed amount of the adsorbent with 20 ml of aqueous Fe(III) solution in Erlenmeyer flasks. The mixture was agitated in a thermostatic water bath shaker (NSW, Mumbai, India) for a pre-determined time interval. The mixture is then filtered and Fe(III) remaining unadsorbed in the supernatant liquid is determined spectrometrically (Flame Atomic Absorption Spectrometer, Perkin Elmer).

The adsorbed amount of Fe(III) and adsorption % was calculated by Equations (i) and (ii), respectively.

$$q_t = (C_o - C_t)V/m \qquad \text{(i)}$$

$$\text{Adsorption percentage} = \{(C_o - C_t) \times 100\} \div Co \qquad \text{(ii)}$$

Where, q_t is the amount of Fe(III) adsorbed by the sand (mg/g), m is the mass of sand(g) and V is the volume of Fe(III) solution (L), C_0 is the initial Fe(III) concentration (mg/L), C_t is the residual Fe(III) concentrations (mg/L) after the batch adsorption at time, t.

- **Effect of Contact Time on the Removal of Fe(III)**

Standard solutions of Fe(III) of concentration 50 mg/L, were prepared. To 20 ml of each solution sand-rice husk mixture was added in 100 ml conical flasks for a contact time of 360 minutes. After that the solution was filtered and analyzed with atomic adsorption spectrophotometer (AAS) to determine the equilibrium time.

- **Effect of pH on the Removal of Fe(III)**

The effect of pH on the removal of Fe(III) was performed by transferring 20 ml of standard solution(50mg/l) of Fe(III) solution into different 100ml conical flasks and then adjusting the pH of the solution to different pH (2.0, 2.5, 3.0, 3.5, 4.0). The adsorbent mixture was weighed into the flask, corked and labelled. The flasks were agitated in the shaker for 150 minutes to attain equilibrium. Thereafter, the content of each flask was filtered and the residual metal ion concentration (Ce) was analyzed using AAS.

- **Effect of Dose on the Removal of Fe(III)**

Adsorption of Fe(III) was investigated at various dosage ranged from 0.5 g sand mixed with rice husk in the range of 0.5-2.5 gram per litre of Fe(III) solution in the ratio of 1:1, 1:2, 1:3, 1:4, 1:5. The initial concentration of Fe(III) was 50 mg/l. The mixture was agitated at 30°C on a water bath shaker for 150 minutes. After this, the solutions were filtered and analyzed with AAS.

- **Effect of Initial Concentration of Fe(III) on the Removal Percentage**

  To 20 ml of prepared standard solutions of different concentration ranging from (5, 10,20, 30, 40, 50, 80) mg/L fixed amount of adsorbent was added. These were then treated and analyzed as done previously.

- **Adsorption Isotherm**

  The adsorption process was studied by Langmuir and Freundlich isotherm models. The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites.

  According to the Langmuir adsorption isotherm, when the adsorbate occupies a site, no adsorption can further take place at that site. All sites are equivalent energetically and no interaction occurs between molecules adsorbed on neighbouring sites [19].

  The linearized Langmuir equation can be described as follows:

  $C_e/q_e = C_e/q_m + 1/bq_m$

  where C_e (mg/l) is the concentration of the Fe(III) solution at equilibrium, q_e (mg/g) is the amount of Fe(III) adsorbed at equilibrium, q_m is the maximum adsorption capacity, b is the Langmuir constant related to the affinity of the binding sites and energy of adsorption.

  Similarly, the Freundlich isotherm describes adsorption onto a heterogeneous surface and suggests a multilayer adsorption. After filling the adsorption centres of an adsorbent, adsorption energy decreases exponentially [20]. The linearized Freundlich isotherm can be presented as follows:

  $\log q_e = \log K_F + (1/n)\log C_e$

where K_F and n are the Freundlich constants related to the adsorption capacity and the adsorption intensity of the adsorbent, respectively. 1/n is the heterogeneity factor and n is a measure of the deviation from linearity of adsorption. The adsorption capacity (K_F) and the adsorption intensity (1/n) are directly obtained from the slope and the intercept of the linear plot of log qe versus log Ce. The higher fractional values of 1/n signify that strong adsorption forces are operative on the system. The magnitude of 1/n also gives indication of the favourability and capacity of the adsorbent/adsorbate system. The value 1/n, between 0 and 1, represents favourable adsorption.

- **Kinetics Study**

  The pseudo first-order Lagergren plots were obtained by plotting ln (qe – qt) vs. time according to the [21]:

  $\ln (q_e - q_t) = \ln (q_e) - (k_1/2.303)\, t$

where qe and qt were the amounts adsorbed per unit mass at equilibrium and at any time t, and k_1 was the first order adsorption rate constant.

The second order kinetics(22) governed by the equation:

$$t / q_t = 1/(k_2 q_e^2) + (1/q_e) t$$

where k_2 is the rate coefficient of adsorption (g mg^1 min^{-1}), q_e the amount of ions adsorbed at equilibrium (mg g^{-1}) and q_t (mg g^{-1}) the amount of ions adsorbed onto the surface at time, t. The value of qe and k_2 can be determined by the slopes and intercepts of the straight line of the plots 't/qt vs t', respectively.

Results and Discussion

- **Influence of Contact Time**

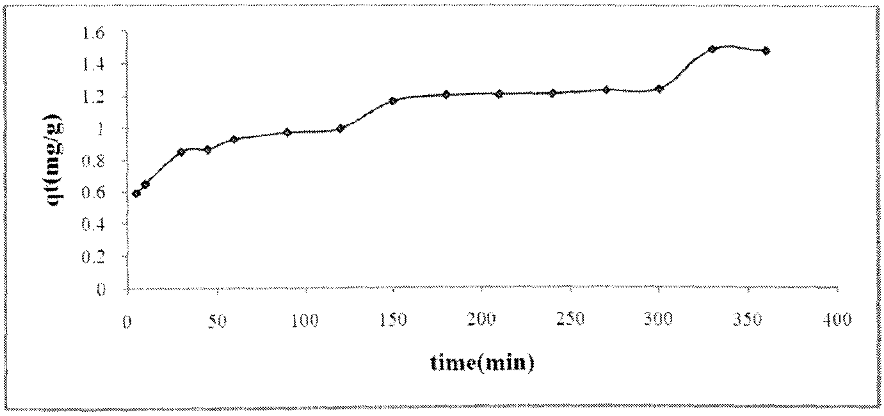


Fig.19.1: Effect of Contact Time on Adsorption

The adsorption is rapid in the first 60 minutes for sand- rice husk mixture after which it slowly attains equilibrium at ~ 150 min (Fig. 19.1).

At the initial stage, a large number of vacant surface sites are available for adsorption. However, after a lapse of time the remaining vacant surface sites are difficult to be occupied due to repulsive forces between the solute species on the solid and bulk phases [23].

- **Influence of pH**

The adsorption percentage of Fe(III) increased sharply on increasing solution pH values from 2.0 to 3.0 from 51.03% to 96.46% for sand and rice husk mixture (2:1).

Adsorption experiments at pH greater than 4.0 was not done as the metal get precipitated as Fe(III)-hydroxide, which introduces doubts in interpreting the results.

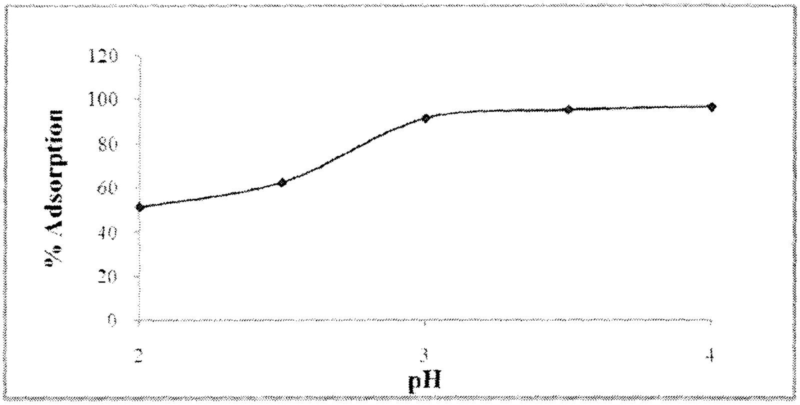


Fig. 19.2: Effect of pH on Adsorption

At very low pH, the hydrogen ions surround the surface of sorbent and compete with Fe(III) ions for the binding sites resulting in low adsorption. As the pH increase, acidity decreases and the competition for the adsorption sites decreases [24].

- **Influence of Adsorbent Dose**

  Adsorption percent increased on increasing amount of sand and adsorbent mixture from 1 g/L to 3 g/L from 64.8 % 96.99 % for sand-rice husk mixture.

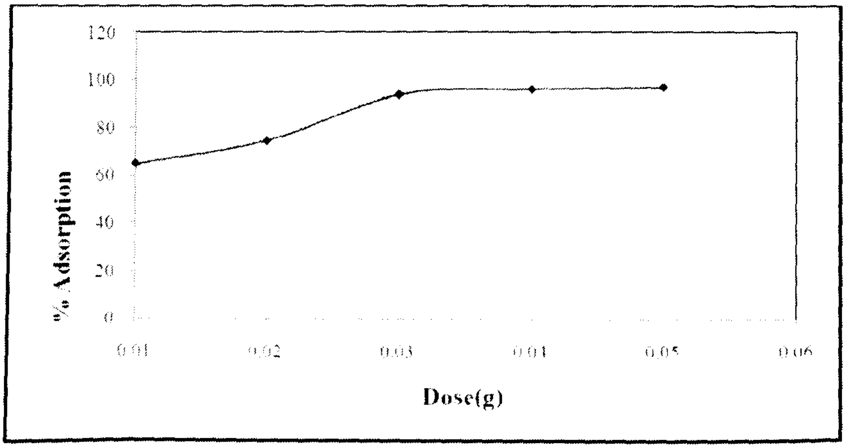


Fig.19.3: Effect of Adsorbent Dose

Adsorption percentage increases with increase in adsorbent dosage which is due to the availability of larger surface area and more adsorption sites [25]. On increasing adsorbent dosage leads to an aggregation of adsorbent particles and increase in diffusion path length [26]. On increasing amount of sand and sand-rice husk mixture, adsorption percent increased. The increase in the adsorption percentage with an increase in adsorbent dosage is due to the availability of larger surface area and more adsorption sites.

- **Influence of Initial Fe(III) Concentration**

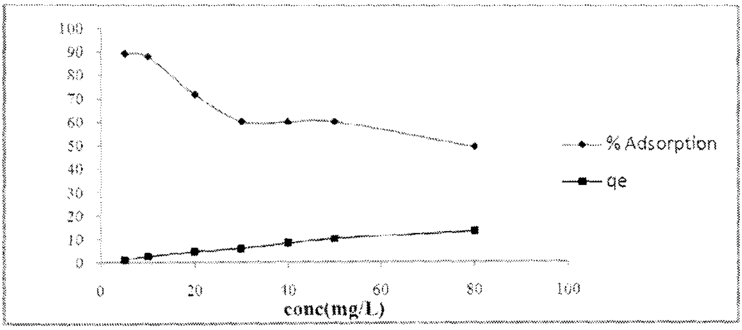


Fig.19.4: Effect of Initial Concentration of Fe(III) on Adsorption

On increasing initial Fe(III) concentrations from 5 mg/L to 80 mg/L, adsorption percentage decreased from 89.29% to 49.35 % for sand-rice husk mixture (Fig. 19.4). It is well known, adsorbed amount of adsorbate is a function of its equilibrium concentration. Hence, increasing of adsorbed amount (q_e) of Fe(III) ions was observed by increasing of initial Fe(III) concentrations. But, adsorption percent was decreased by increasing of initial Fe(III) concentrations. This is due to saturation of adsorption sites on the sand [27].

- **Isotherm Studies**

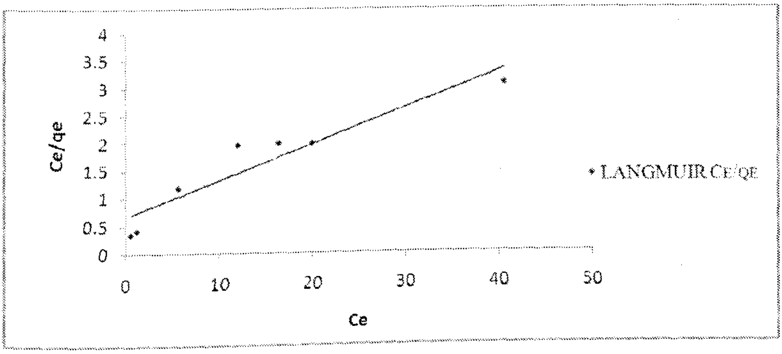


Fig.19.5: Langmuir Adsorption Isotherm of Fe(III) Ions Adsorption on Sand-rice Husk (2:1) Mixture

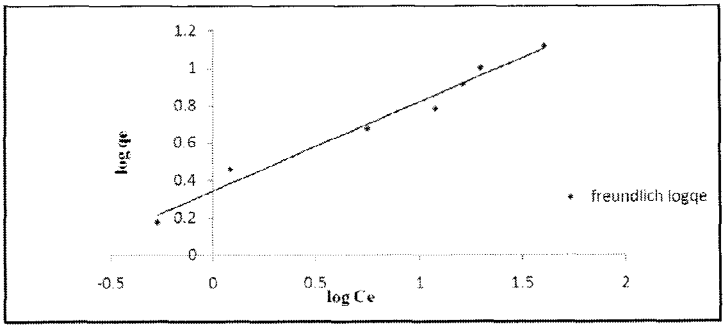


Fig.19.6: Freundlich Adsorption Isotherm of Fe(III) Ions on Sand-rice Husk(2:1) Mixture

Table 19.1: Results of Fe(III) Adsorption Isotherms at 30°C

Langmuir	
q_m (mg/g)	15.128
b (L/mg)	0.04393
r	0.9418
Freundlich	
K_F(mg/g)(L/g)$^{1/n}$	2.198
1/n	0.4736
r	0.988

The Langmuir isotherm plot (Fig. 19.5) have high linearity (r ~ 0.94) and yield good Langmuir monolayer adsorption capacity, q_m (Table 19.1). The positive values of the Langmuir equilibrium coefficient, b indicates that the adsorbate-adsorbent interactions proceed in the direction of formation of the adsorption complex and hence, adsorption is the resultant process.

The Freundlich plot is linear with good regression coefficients (r ~ 0.98, Fig.19.6). The adsorption coefficients obtained from the plots are presented in Table 19.1. The Freundlich adsorption intensity, 1/n, at 303 K, was found to be 0.4736 (by definition, the adsorption intensity, 1/n, should have values less than unity for favourable adsorption).

- **Kinetics Studies**

The results of kinetics study are listed in the table below:

Table 19.2: The Adsorption Kinetic Model Rate Constants for Adsorption of Fe(III) ions on Sand-rice-husk Mixture

Temp (K)	Experimental qe(mg/g)	Kinetic model	Parameter	
303 K	1.167	First order	$k_1 \times 10^2 (min^{-1})$	1.04
			r	0.95
			q_e	0.517
			% deviation	55.7
		Second order	$k_2 \times 10^2$ $(g\ mg^{-1} min^{-1})$	15.81
			r	0.99
			q_e	1.04
			% deviation	10.88

The value of k_1 obtained from the Lagergren plots (r~ 0.95) at 303 K indicates first order to be the likely mechanism. However, linearity of the Lagergren plots do not necessarily assure a first order mechanism due to the inherent disadvantage of correctly estimating the equilibrium adsorption capacity, q_e. The q_e values obtained from the Lagergren plots differed from the experimental q_e values by 55.70% (at 303 K). The large deviation do not support first order kinetics. The second order plot shows better linearity ~ 0.99 (Fig. 19.8). The rate coefficient value reveal that the affinity of the sand-rice husk mixtures towards Fe(III). A comparison of q_e values (experimental and those obtained from the slopes of the second order plots) shows a better agreement which indicates a probable second order mechanism for the adsorption process.

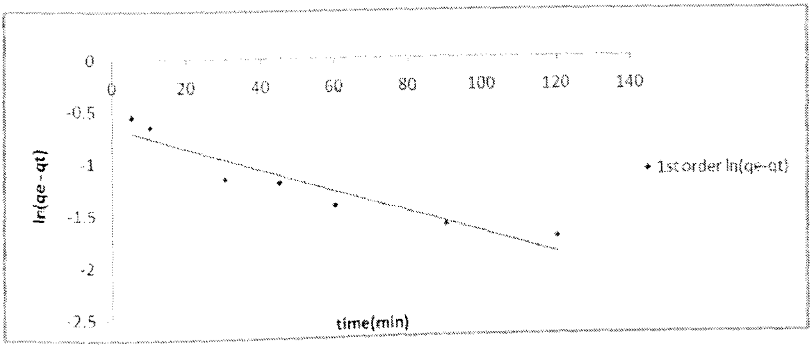


Fig.19.7: Lagergren Plot for Adsorption of Fe(III) on Sand-rice husk (2:1) Mixture at 303 K

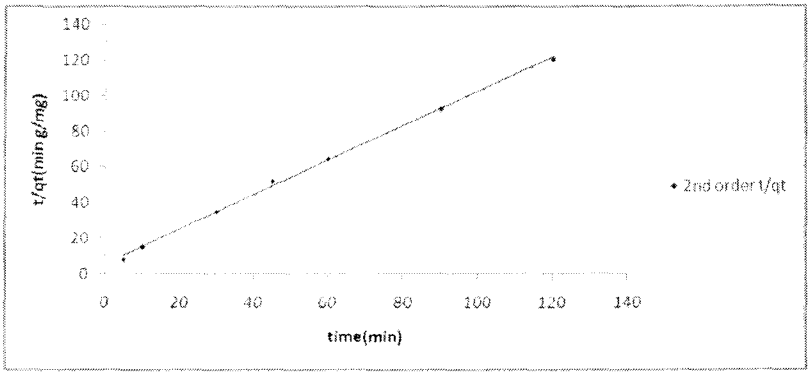


Fig. 19.8: Second Order Plot for Adsorption of Fe(III) on Sand-rice husk(2:1) Mixture at 303K

Conclusions

This study shows that sand- rice husk mixture is a suitable adsorbent for the removal of Fe(III) from aqueous solution due to its high capacity adsorption, availability, and low cost. The Fe(III) removal was pH-, adsorbent amount-, and contact time-dependent. The adsorption data fit Langmuir and Freundlich isotherms. The adsorption follows Langmuir isotherm better than Freundlich isotherm. The positive values of the Langmuir equilibrium coefficient, b indicates that the adsorbate-adsorbent interactions proceed in the direction of formation of the adsorption complex and hence, adsorption is the resultant process. The monolayer adsorption capacity, q_m is found to be 15.12 mg/g for sand-rice husk mixture. Fe(III) adsorption follows second order kinetics. The second order plot shows good linearity ~ 0.99 and the rate coefficient, k_2 is 15.81 x 10^{-2} g mg-1 min-1 at 303 K.

References

1. BDWG, Iron in drinking water, Bureau of Drinking Water and Groundwater, Wisconsin Department of Natural Resources, 2001, PUB-DG-035.
2. USEPA, Drinking Water Standards and Health Advisories, United States Environmental Protection Agency, (http://www.epa.gov/OST), Office of Water, Washington, D.C., 2000, EPA 822-B-00-001.
3. Teker, M., Imamoglu, M., Saltabas, O., Adsorption of Copper and Cadmium Ions by Activated Carbon from Rice Hulls, *Turkish Journal of Chemistry*, 1999, **23(2)**, 185-191.
4. Bayo, J., Esteban, G., Castillo, J., The Use of Native and Protonated Grapefruit Biomass (*Citrus paradisi* L.) for Cadmium(II) Biosorption: Equilibrium and Kinetic Modeling, *Environmental Technology*, 2012, **33**, 761.

5. Hejazifar, M., Azizian, S., Adsorption of Cationic and Anionic Dyes onto the Activated Carbon Prepared from Grapevine Rhytidome, *Journal of Dispersion Science and Technology*, 2012, **33**, 846-853.
6. Marwani, H.M., Albishri, H.M., Soliman, E.M., and Jalal, T.A., Selective Adsorption and Determination of Hexavalent Chromium in Water Samples by Chemically Modified Activated Carbon withTris(hydroxymethyl) Aminomethane, *Journal of Dispersion Science and Technology*, 2012, **33**, 549.
7. Teker, M., Saltabas, O., and Imamoglu, M., Adsorption of Cobalt by Activated Carbon from the Rice Hull, *Journal of Environmental Science and Health*, 1997, **A32**, 2077-2086.
8. Azizian, S., Niknam, Z., Rombi, E., Adsorption of Pentafluorophenol onto Powdered, Granular, and Cloth Activated Carbons, *Journal of Dispersion Science and Technology*, 2012, **33**, 206.
9. Meshko V., Markovska L., Mincheva M., Rodrigues A.E., Adsorption of Basic Dyes on Granular Activated Carbon and Natural Zeolites, *Water Research*, 2001, **5**, 3357–3366.
10. Ngah, W., and Hanafiah, M., Removal of Heavy Metal Ions from Wastewater by Chemically Modified Plant Wastes as Adsorbents: A Review, *Bioresource Technology* , 2008, **99**, 3935–3948.
11. Gonzalez, M. H., Araujo G. C. L., Pelizaro C. B., Menezes E. A., Lemos S. G., DeSousa G. B., and. Nogueira A. R. A, Coconut Coir as Biosorbent for Cr(VI) Removal from Laboratory Wastewater, *Journal of Hazardous Materials*, 2008, **159**, 252–256.
12. Ajmal. M. R., Rao A. K., Anwar S., Ahmad J. and Ahmad R., Adsorption Studies on Rice Husk: Removal and Recovery of Cd(II) from Wastewater, *Bioresources Technology*, 2003, **86**, 147–149.
13. Sharma, N., Kaur K., and Kaur S., Kinetic and Equilibrium Studies on the Removal of Cd^{2+} Ions from Water using Polyacrylamide Grafted Rice (Oryza sativa) Husk and Tectonia grandis Saw Dust, *Journal of Hazardous Materials*, 2009, **163**, 1338–1344.
14. Makehelwala, M., Jinadasa K. B. S. N. , Tanaka N., Weerasooriya R., Bandara A., Adsorption Mechanism of Cr(VI) onto Coir Pith, *Bioremediation Journal*, 2009, **13**, 188–197.
15. Tarley, C. R. T, Zezzi Arruda, M. A., Biosorption of Heavy Metals using Rice Milling by-products, Characterization and Application for Removal of Metals from Aqueous Effluents, *Chemosphere*, 2004, **54**, 987–995.
16. Zoua, W., Hana, P., Lia, Y., Liub ., Hea, X. and Hana, R., Equilibrium, Kinetic and Mechanism Study for the Adsorption of Neutral Red onto Rice Husk, *Desalination and Water Treatment* , 2009, **12**, 210–218.
17. Ahamad, K. U. and Jawed, M., Breakthrough Column Studies for Iron(II) Removal from Water by Wooden Charcoal and Sand: A Low Cost Approach, *International Journal of Environmental Research*, 2011, **5(1)**, 127-138.
18. Rauf, M. F., Bukallah, S. B. and Hamour, F.A., Nasir A.S, Adsorption of Dyes from

Aqueous Solutions onto Sand and Their Kinetic Behavior, *Chemical Engineering Journal*, 2008, **137**, 238–243.

19. Langmuir, I., The Adsorption of Gases on Plane Surfaces of Glass, Mica and Platinum, *Journal of the American Chemical Society*, 1918, **40**, 1361–1403.
20. Freundlich, H. M. F., Over the Adsorption in Solution, *Journal of Physical Chemistry*, 1906, **57**, 385–471.
21. Lagergren, S., About the Theory of so-called Adsorption of Soluble Substances. Kungliga SvenskaVetenskapsakademiens. Handlingar, 1898, 24.
22. Ho., Y. S. and Mckay, G., Batch Lead (II) Removal From Aqueous Solution By Peat: Equilibrium and Kinetics, Trans IChemE, 1999, **77**, 165.
23. Almasi, A., Omidi, M., Khodadadian, M., Khamutian, R. and Gholivand, M. G., Lead(II) and Cadmium(II) Removal from Aqueous Solution using Processed Walnut Shell: Kinetic and Equilibrium Study, *Toxicological & Environmental Chemistry*, 2012, **94(4)**, 660–671.
24. Bhattacharyya, K. G and Gupta, S. S, Adsorption of Fe(III) from Water by Natural and Acid Activated Clays: Studies on Equilibrium Isotherm, Kinetics and Thermodynamics of Interactions, *Adsorption* , 2006, **12**, 185–204.
25. Yuan, J., Hu, M., Zhou, Z. and Wang L., Kinetic and Thermodynamic Behavior of the Batch Adsorption of Phosphate from Aqueous Solutions onto Environmental Friendly Barbecue Bamboo Charcoal, *Desalination and Water Treatment*, 2013, **52**, 7248-7257.
26. Mehmet, E. A., Dursun, S., Ozdemir, C. and Karatas M., Heavy Metal Adsorption by Modified Oak Sawdust: Thermodynamics and Kinetics, *Journal of Hazardous Materials*, 2007, **141**, 77–85.
27. Imamoglu, M., Adsorption of Cd(II) Ions onto Activated Carbon Prepared from Hazelnut Husks, *Journal of Dispersion Science and Technology*, 2013, **34**, 1183–1187.

20

Degradation of Rhodamine B Dye from Water using ZnO as Catalyst

Moutusi Das
Krishna G Bhattacharyya

Abstract

Rhodamine B is a cationic xanthene dye widely used in the printing, textile, and photographic industries. It is important to destroy this dye before it enters natural water sources. In the present work, catalytic wet oxidation of the dye was carried out with commercial ZnO and its acid-treated form as the catalysts. Acid treatment was carried out with 0.5, 0.75, 1.0 N H_2SO_4. The catalysts were characterized by XRD and SEM measurements, BET surface area and pore volume determination. Oxidation in aqueous solution was carried out with reaction time, catalyst load, dye concentration. pH and temperature as the process variables. It was found that 1.0 N acid activated ZnO could bring about 75.0 % oxidation of the dye at room temperature. The results demonstrated that ZnO can play an important role in oxidation of non-biodegradable dyes in water.

Keywords: *Rhodamine B Oxidation; Dye Removal through Oxidation; ZnO; Acid-treated ZnO.*

Introduction

The textile industry produces large number of colored wastewater that contains high levels of organic matter [1]. Treatment of this colored wastewater from textile is a serious problem today. In general, the methods for the treatment of wastewater containing dyes can be divided into two main groups [2]: first, the chemical or physical methods of dye removal, which refer to the process called decoloration and, second, dye removal by means of biodegradation. Among them, the more intense used method is physical/chemical in order to remove dyes from the wastewater [3,4]. Physical treatment of colored wastewater requires a post-treatment to remove newly secondary waste. Among chemical methods, advanced oxidation processes (AOPs) are a class of processes based on generation of hydroxyl radicals, highly reactive species, which promote the oxidation of hazardous organic compounds [5]. Therefore, AOPs represent a potential alternative method able to decolorize and to reduce recalcitrant colored wastewater load. Wet oxidation is considered as one of the most promising and simplest techniques for partial oxidation of parent pollutants into more biologically amenable intermediates [6]. An attractive alternative to wet oxidation is catalytic wet air oxidation, which may be effectively employed for total destruction of pollutants present in waste water.

The present work reports the removal of Rhodamine B dye using ZnO and its acid treated form as catalysts. The oxidation was observed by changing the reaction time, dye concentration, catalyst load, pH and temperature of the medium. The degradation of the reaction was determined by UV-vis spectrophotometer.

Materials and Methods

Chemicals

The basic catalytic materials in this work were ZnO (Oster pharmaceutical), H_2SO_4 (Merck Mumbai), Rhodamine B (Merck Mumbai). All chemicals were of analytical grade and used without further purification.

Preparation of Acid Treated Catalysts

The acid treated catalysts were prepared by treating the metal oxides with 0.5, 0.75, 1.0 N H_2SO_4. 15 g of the material was activated with 100 mL acid (H_2SO_4) for 4 h at room temperature in a batch reactor. The material was separated by filtration and was washed with distilled water till the washings were neutral. The material was dried at 373 K in an air oven for 5 h and was preserved. Altogether four catalysts were tested in this work: (i) raw ZnO (Z), (ii) 0.5 N acid-activated ZnO (Z1), (iii) 0.75 N acid-activated ZnO (Z2) and (iv) 1.0 N acid-activated ZnO (Z3).

Characterization of the Catalysts

The catalysts were characterized by measuring (i) the crystallinity of the phases by XRD (Bruker D8 Advance diffractometer equipped with a scintillation counter) (ii) the surface topography by scanning electron microscopy (SEM) (JEOL JSM-6360), and (iii) the surface area and pore volume by BET adsorption isotherms (Micromeritics Tristar Surface Area and Porosity Analyzer).

Experimental Procedure for Wet Oxidation of the Dye

The catalytic oxidation was carried out in a water bath shaker (NSW, New Delhi, India) with 20 mL of Rhodamine B in capped 50 mL Erlenmeyer flasks. After a reaction run was completed, the mixture was centrifuged (Remi Research Centrifuge, R24) and the unconverted reactant was estimated in the supernatant layer spectrophotometrically (Hitachi UV-visible U3210).

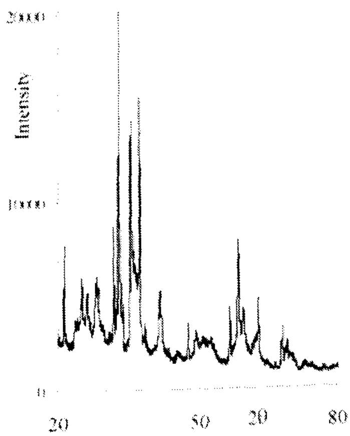
Results and Discussion

Characterization of the Catalysts

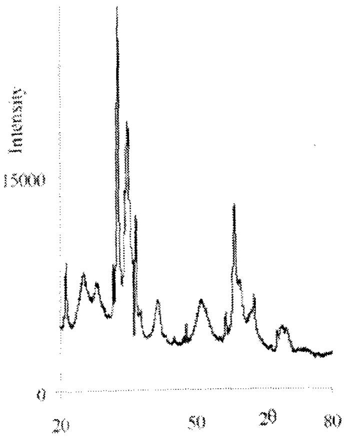
XRD Measurements

ZnO yielded major XRD bands at 2θ = 31.76^0, 36.26^0, 38.71^0, 47.60^0, 56.66^0, 62.83^0, 67.93^0, 73.03^0, 77.55^0 (Figure 1) for the raw ZnO (Z), These could be assigned to reflections from (100), (002), (101), (102), (110), (103), (112), (004) and (202) Bragg planes with 'd' of 2.81, 2.47, 2.32, 1.90, 1.62, 1.47, 1.37, 1.29, 1.23 (Table I). The reflections can be indexed as those due to hexagonal Wurtzite structure of ZnO. Wang et al. (2007) have found similar XRD results for ZnO nano powder within 2θ range of 20 " 80^0 [7].

The bands shifted slightly towards lower angles after acid treatment of ZnO in Z1 (0.50 N H_2SO_4 treated ZnO), Z2 (0.75 N H_2SO_4 treated ZnO) and Z3 (1.00 N H_2SO_4 treated ZnO) catalysts. The intensities of the diffraction bands also increased after acid treatment. The acid treatment generally enhanced the crystanillity of the catalysts in the order of Z3 > Z2 > Z1 > Z.



Z



Z1

showed the surface area of ZnO nano particles to be between 8.28 – 43.76 m^2/g depending on temperature values much higher than those obtained in this work for ordinary commercial ZnO [10]. Reduction in the size of oxide particles after acid treatment was observed. ZnO showed a decrease in size after acid treatment. The acid activation had a very pronounced effect on size reduction of the catalysts resulting in huge increase in surface area and pore volume.

Table 20.2: BET Surface area, Pore Diameter and Pore Volume of ZnO Catalysts

ZnO Catalysts	BET Surface area (m^2/g)	Average pore diameter (nm)	Pore volume x 10^2 (cm^3/g)
Z	8.57	19.73	1.87
Z1	10.01	19.08	4.28
Z2	11.59	15.77	5.68
Z3	17.02	6.92	6.80

Wet Oxidation of Rhodamine B

Effects of Reaction Time

The oxidation reactions were carried out with raw and acid treated oxides as catalysts. All the reactions were carried out with aqueous dye solutions of concentration 1.0 mg/L at room temperature (303K) and under the atmospheric pressure. The reaction time was varied from 15 to 300 min and the oxidative degradation of the dyes was monitored after each time interval. Each set of reactions was done in triplicate and it was found that the results deviated from each other only by 1 – 3 %.

Rhodamine B (1.0 mg/L) was oxidized with raw and acid treated ZnO catalysts (2.5 g/L) with the reaction time varying from 15 – 300 min. The conversion of the dye increased from 20.0 – 38.9 % for raw ZnO (Z), 24.7 – 64.8 % for 0.5 N acid treated ZnO (Z), 35.5 – 68.8 % for 0.75 N acid treated ZnO (Z) and 41.6 – 75.0 % for 1.0 N acid treated ZnO (Z). The effects of reaction time on Rhodamine B oxidation are shown in Fig. 20.3. It was observed that the reaction was very fast in the first 30 min and slowed down with further increase in the reaction time and near equilibrium conditions were reached after 240 min.

It was observed that the reactions were very fast and considerable conversion of the dyes was possible in a relatively shorter time interval. Acid treated ZnO gave much better results in dye oxidation and it was found that the catalytic activity was dependent on the strength of the acid.

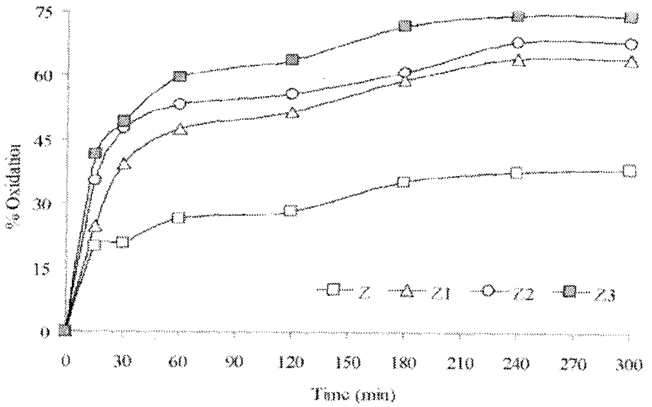


Fig. 20.3: Effects of Reaction Time on Oxidation of Rhodamine B (1.0 mg/L) with ZnO Catalysts (Z, Z1, Z2 and Z3; 2.5 g/L) at Room Temperature (303K)

Effects of Catalyst Load

The effect of catalyst load was observed by changing the catalyst load from 0.5 – 1.0 g/L for the oxidation of Rhodamine B dye. The change in oxidation was given in the Table 20.3 . The effects of catalyst load on Rhodamine B conversion were only of limited nature, the increase remaining within ~ 10 % for 15-fold increase in the catalyst load. After an amount of 2.5 g/L the oxidation become almost constant. Similar observations have been made earlier by Fatimah et al. (2011). They found complete degradation of Methylene Blue (10.0 mg/L) in case of ZnO/montmorillonite photocatalyst with a catalyst load of 5.0 g/L. The conversion remained nearly constant at increased loadings [11]. In case of their photocatalytic reactions, the authors suggested that higher loadings made the solution turbid reducing light penetration and therefore, the conversion could not increase any further.

Table 20.3: Degradation of Rhodamine B (1.0 mg/L) with different ZnO loads at 303 K

Catalysts	Total degradation (%) at Catalyst load (g/L)			
	0.5	2.5	5.0	7.5
Z	30.3	38.9	39.5	39.9
Z1	55.7	64.8	65.3	66.9
Z2	59.9	68.8	69.8	70.6
Z3	66.5	75.0	75.4	76.7

Effects of Dye Concentration

ZnO catalysts (raw and acid treated; 2.5 g/L) could bring about oxidative degradation of Rhodamine B in the concentration range of 1.0 – 100.0 mg/L. It was observed that with the increase in dye concentration, % oxidation decreases and the decrease in oxidation was found in the range of 38.9 – 9.1 % for raw ZnO (Z), 64.8 – 26.0 % for Z1, 68.8 – 27.5 % for Z2 and 75.0 – 31.6 % for Z3. The decrease in oxidation with dye concentration was shown in the Fig. 19.4. Meena et al. (2009) gave the reason of decrease in oxidation is that at higher concentration, there will be stiff competition among the reactant molecules for attachment to active sites on the catalyst surface resulting in decrease in the rate of degradation [12].

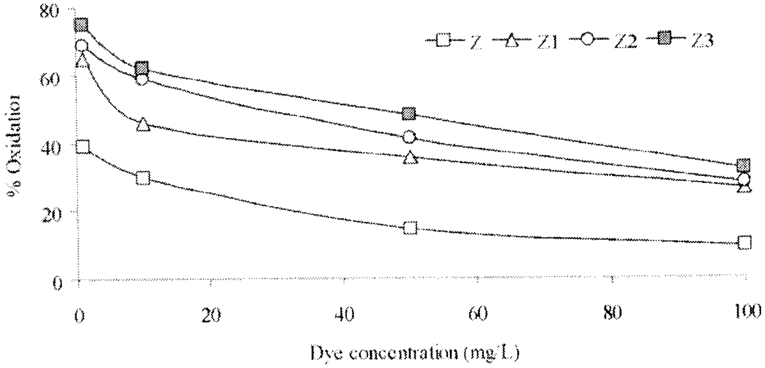


Fig. 20.4: Effects of Dye Concentration on Oxidation of Rhodamine B with ZnO Catalysts (Z, Z1, Z2 and Z3; 2.5 g/L) at Room Temperature (303K)

Effects of pH on Dye Oxidation

Dye oxidation was carried out over the pH range of 2.0 to 12.0 to find out if the pH had any influence on the oxidation reaction. Only two sets of catalysts were used the raw metal oxides and 1.00 N acid treated metal oxides and in both the cases, Rhodamine B of concentration 10.0 mg/L was taken and the reaction time was uniform 5 h. The trends in the dye oxidation with increasing pH were similar for both the catalysts and it was generally observed that in basic medium, the dye show maximum oxidation. The % oxidation with raw ZnO (Z) were 27.3 and 38.7 % at pH 2.0 and 12.0 respectively and 55.7 and 69.5 % with 1.0 N acid treated ZnO (Z3) at pH 2.0 and 12.0 respectively. The results of oxidation are shown in Fig. 20.5. With the increase of pH, the catalyst surface will have OH^- ions on it that will facilitate adsorption of the dye cations on the surface and subsequent conversion to simpler molecules. Li et al. (2006) suggested that increase in oxidation was obviously favoured by the presence of hydroxyl radicals in the basic medium [13].

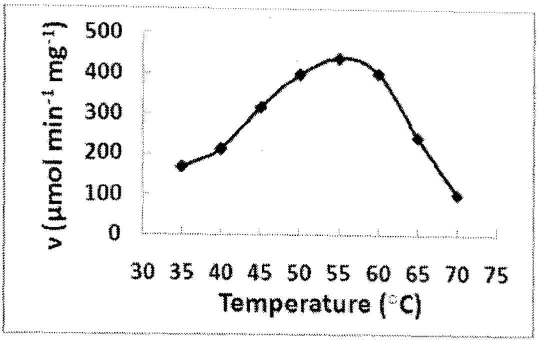


Fig. 20.5: Oxidation of Rhodamine B (10.0 mg/L) with 2.5 g/L of Z and Z3 at different pH under Room Temperature and Atmospheric Pressure (time 5 h)

Effects of Temperature

The catalytic efficiency of ZnO catalysts for Rhodamine B (10 mg/L) oxidation increased when the reaction temperature was raised from 298 – 313 K. It was observed that the % oxidation increased with increase the temperature of the medium. The increase in % oxidation are 25.4 – 35.9 % for raw ZnO (Z), 41.3 51.7 % for 0.5 N acid treated ZnO (Z1), 56.2 – 65.3 % for 0.75 N acid treated ZnO (Z2) and 59.1 – 67.8 % for 1.0 N acid treated ZnO (Z3). There was almost a linear increase in the dye oxidation capacity with temperature for ZnO catalysts as shown in Fig. 20.6. With increase in temperature, the mobility of the dye molecules increases which resulted in the increase of oxidation of the dye. Yuan *et. al.* (2011) have found a similar results of reaction temperature in the oxidation of Safranin T using metal oxide (macroporous ZnO/MoO$_3$/SiO$_2$) hybrid catalyst in the temperature range of 293- 308 K [14].

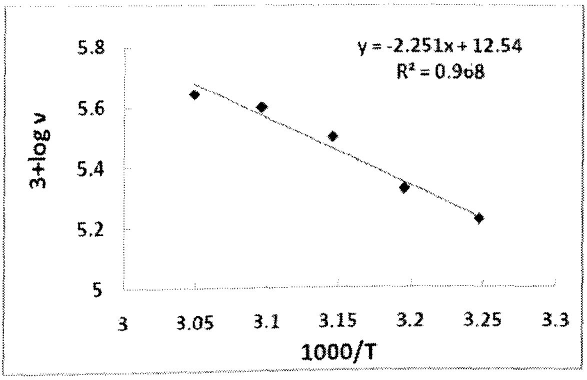


Fig. 20.6: Effects of reaction temperature on Rhodamine B oxidation (dye 10.0 mg/L, Z, Z1, Z2 and Z3 catalysts 2.5 g/L, pH as prepared (6.7), pressure 1 atm, time 5 h)

Conclusions

From the above discussion, it was concluded that acid treated ZnO could act as a good catalyst than that of untreated ZnO. 1.0N acid treated ZnO is more active than other catalysts. As the oxidation of the dye showed a high degradation, so this is very useful study for degradation of dye in aqueous medium under normal temperature. As the color of the dye became less intense after oxidation, so, it was less harmful than the original one.

Acknowledgement

The author, Moutushi Das, is grateful to BSR sponsored UGC RFSMS scheme for providing the financial support during the research work.

References

1. Emami, F., Tehrani-Bagha, A.R., Gharanjig K., Menger, F.M., Kinetic Study of the Factors Controlling Fenton-promoted Destruction of a Non-biodegradable Dye, *Desalination,* 2010, **257**, 124.
2. Slokar, Y.M. and Marechal, A.M.L., Methods of Decoloration of Textile Wastewaters, *Dyes Pigments*, 1998, **37**, 335.
3. Georgiou, D., Melidis, P., Aivasidis, A. and Gimouhopoulos, K. Degradation of azo-reactive dyes by ultraviolet radiation in the presence of hydrogen peroxide. *Dyes Pigments*. 2002, **52**, 69.
4. Peternel, I.T., Koprivanac, N., Bozic, A.M.L., Kusic, H.M. Comparative study of UV/TiO_2, UV/ZnO and photo-Fenton processes for the organic reactive dye degradation in aqueous solution. *Journal of Hazardous Materials*. 2007, **148,** 477.
5. Gogate, R., Pandit, B. A review of imperative technologies for wastewater treatment. I. Oxidation technologies at ambient conditions. *Advance Environmental Research*. 2004, **8**, 501.
6. Verenich, S., Laari, A., Nissen, M., Kallas, J. Combination of coagulation and catalytic wet oxidation for the treatment of pulp and paper mill effluents. Water *Science and Technology*. 2001, **44**, 145.
7. Wang, H., Xie, C., Zhang, W., Cai, S., Yang, Z., Gui, Y. Comparison of dye degradation efficiency using ZnO powders with various size scales. *Journal of Hazardous Materials*. 2007, **141**, 645.
8. Sanoop, P.K., Anas, S., Ananthakumar, S., Gunasekar, V., Saravanan, R., Ponnusami, V. Synthesis of yttrium doped nanocrystalline ZnO and its photocatalytic activity in methylene blue degradation. *Arabian Journal of Chemistry*. 2012, http://dx.doi.org/10.1016/j.arabjc.2012.04.023
9. Maciel, A.V., Job, A.E., Mussel, W.N., Pasa, V.M.D. Pyrolysis and auto-gasification of black liquor in presence of ZnO: An integrated process for Zn/ZnO nanostructure production and bioenergy generation. *Biomass and Bioenergy*. 2012, **46**, 538.

10. Reddy, A.J., Kokila, M.K., Nagabhushana, H., Rao, J.L., Shivakumara, C., Nagabhushana, B.M., Chakradhar, R.P.S. Combustion synthesis, characterization and Raman studies of ZnO nanopowders. *Spectrochimica Acta, Part A*. 2011, **81**, 53.

11. Fatimah, I., Wang, S., Wulandari, D. ZnO/montmorillonite for photocatalytic and photochemical degradation of methylene blue. *Applied Clay Science*. 2011, **53**, 553.

12. Meena, R.C., Pachwarya, R.B. Photocatalytic degradation of model textile azo dyes in textile wastewater using methylene blue immobilized resin dowex-11. *Journal of scientific and industrial research.* 2009, **68**, 730.

13. Li, J., Li, L., Zheng, L., Xian, Y., Jin, L., Photoelectrocatalytic degradation of rhodamine B using Ti/TiO_2 electrode prepared by laser calcination method. *Electrochimica Acta.* 2006, **51**, 4942.

14. Yuan, M., Wang, S., Wang, X., Zhao, L., Hao, T. Removal of organic dye by air and macroporous ZnO/MoO_3/SiO_2 hybrid under room conditions. *Applied Surface Science*. 2011, **257**, 7913.

21

Effect of Root Extract of Clitoria Ternatea on Some Biochemical Parameters in Ovariectomised Mice

---

Manalisha Deka
Pranabjyoti Das

Abstract

Use of plant for curing various ailments is as old as human civilization. In recent times, emphasis on plant research has increased and collection as well as evaluation of the traditional use of plants as medicine gets prime importance in the present day scenario. *Clitoria ternatea* is a vigorous, strongly persistent, herbaceous perennial legume. Almost all parts of this plant are reported to have medicinal properties. The root of the plant is reported to have anti-diarrheal, Anti-histamic, anti-diabetic, cholinergic activity etc. Traditionally the root has been using for the treatment of many diseases like leucorrhoea, diarrhea, urinary problems, diuretic, impotency, infertility stomach trouble etc. The present work was designed to estimate some biochemical parameters of uterus of ovariectomised mice after treatment with *Clitoria ternatea* root extract. Adult female mice were used in this study. Mice were subjected to bilateral ovariectomy and treated with methanol root extract for 7 consecutive days. Biochemical estimation of uterine protein, glycogen and serum cholesterol were done using standard protocols. Results showed increased level of uterine protein, no change in uterine glycogen and decreased level of serum cholesterol. It was concluded that root extract of *Clitoria ternatea* may have some estrogenic property.

Key words: *Plant Based Drugs, Ovariectomised Mice, Clitoria ternatea Linn.*

Introduction

Evidences of the use of plants for medicinal purposes date as far back as 60,000 years ago in both western and eastern cultures; in both developed and undeveloped countries [1]. WHO defines traditional medicine as: "*The health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being*" (WHO, 2008) [2]. Almost all developing countries rely on traditional medicine for curing primary health disorders. In India also traditional medicine is being practiced by different traditional medicine men and also by household women. These practices are the inherited ones and have no scientific documentations because most of these medicine men do not have formal training on this kind of practice. The present investigation was carried out on a plant namely *Clitoria ternatea* L commonly known as Aparajita. This plant belongs to the genus Clitoria of family Fabaceae which comprises 60 species distributed mostly within the tropical belt with a few species found in temperate areas [3]. The plant *C. ternatea* L. is a strongly persistent, herbaceous perennial legume having twining fine stems. This plant is a very well-known Ayurvedic medicine used for different ailments, which has been investigated scientifically in considerable detail. Traditional use of the plant has been reported on skin diseases, worm Infestation, retro auricular adenitis, tonsillitis, migraine, appetizer vermicide, gout, habitual abortion, promote intellect, burning sensation in vagina and infertility. Many workers have been reported medicinal values of the plant scientifically. Some of these include anti-helmintic [4], anti hyperglycemic [5], anti-inflammatory [6], anti-diarrhoeal [7], anti-oxidant [8], hepatoprotective [9], immunomodulatory [10] etc.

This plant has been using for the treatment of gynecological disorders by the traditional healers of Barpeta district of Assam. In this area three flower colour varieties of the plant is available. In our study roots of blue flower variety was used which has been using against infertility in this area. For normal reproduction it is essential to have normal estrogen level. There is a wide variety of natural or chemical components exhibiting estrogenic activity. The natural estrogens include ovarian estrogenic steroids and phytoestrogens. Extensive research has made to identify the types of phytoestrogens. Kenneth Setchell, began studying phytoestrogens in the early 1980s. Different investigators worked differently to investigate the actions of phytoestrogens on animals. Kaldas and Hudges (1989) [11], found that, there is a marked increased in weight of uterus relative to body weight. Equol is the main estrogenic compound in the blood of sheep grazing Sub-terranean clover. In immature three week old female rats 5mg equol injected sub-cutaneously increased uterine weight [12]. A variety of phytoestrogens have been identified which bind to the estrogen receptor, and these could induce estrogen actions [13]. Estrogenic activity of many plants and chemicals has been evaluated on rodent. Rodent uterotropic assay is one of the best experiments to investigate estrogenic potency. As the plant under investigation has been using against gynecological disorders and estrogen is necessary for the treatment of such disorders, the present study was designed to investigate the estrogenic effect of the plant on ovariectomised mice.

Materials and Methods

Plant Sample Collection and Identification

The present study was designed to investigate the effect of roots of *Clitoria ternatea* L. on ovariectomised mice. For this roots were collected from local household gardens and washed properly and dried in shed to make powered form in a mechanical grinder. For scientific identification of the plant, whole plant was collected, prepared herbarium and submitted in the department of Botany, Gauhati University, Assam and a voucher no -09193 was collected against the submitted herbarium for future references.

Preparation of Methanol Root Extract

Powdered root was run in a Sohxlet apparatus for 18 hrs continuously in methanol. Methanol was then evaporated in a rotary evaporator and the extract was collected in the form of a paste and used in the present study.

Animals and Animal Procedures

To investigate the effect of the plant, rodent uterotrophic assay was carried out in adult ovariectomised female mice [14,15]. For this adult female mice (C3H) weighing approx 25g showing normal estrus cycle were selected. They were acclimatized for 10 days with same food and water. Then they were subjected to bilateral ovariectomy under ketamine-xylazine (1:2) anesthesia. Ovarictomised mice were kept under constant observation for at least 14 days. Then they were divided into four groups viz. control, positive control, test-1 and test-2 each group having 5 animals. Control group received normal saline, positive control group received 1µg/kg body weight of synthetic estradiol, test1 and test-2 received 200 and 400mg root extract per kg body weight of each mouse for 7 consecutive days. All the treatments were done through oral route except the positive control group, where estradiol was injected subcutaneously. On day 8th all the mice were sacrificed and blood and intact uterus were collected for the biochemical tests.

Biochemical tests

Uterine protein and glycogen were estimated following the method of Lowry *et al.*, 1951 [16] and Seifter *et al.*, 1950 [17] respectively. For this uteri were removed from each mouse after sacrifice. The uteri were divided into two parts and one part was used to estimate protein and other part was used to estimate glycogen level. Blood was collected from heart by routine procedure. Serum was collected from blood to estimate serum cholesterol. Serum cholesterol was estimated with in-vitro diagnostic kit of Crest Bio-systems by CHOD/PAP method.

Results and Discussions

Effect on Uterine Protein Content

Analysis of uterine protein content revealed increase in the level of uterine protein in all the treatment groups. Of all the treated animals, animals exposed to Estradiol exhibited maximum increase (67.5 ± 3.75 mg/g tissue) in uterine protein content, while the control group exhibited lowest amount of (26.5 ± 2.45 mg/g). In the animals treated with root extract, uterine protein content was increased with increasing concentration of the extract. Uterine protein was recorded as 33.44 ± 2.84 mg and 40.2 ± 2.97 mg/g tissue respectively for 200 mg and 400 mg of root extract. Data presented here are the average of all the animals in a particular group with standard error. Data were tested with student's t-test and each group of the treatment was compared with the control group. The increase in the uterine protein was statistically significant in all the experimental groups in different significant levels (Fig. 21.1).

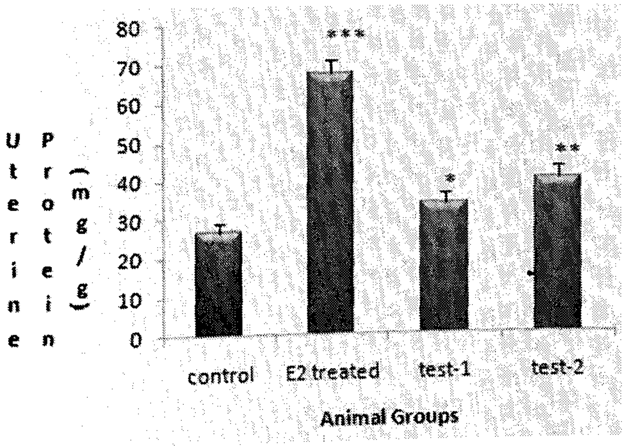


Fig. 21.1: Effect of root extract on Uterine Protein content (mg/g) of adult ovariectomized mice. Here **P<0.01 and ***P<0.001, by comparing with the control group with Student t-test

Synthesis of protein was reported to be stimulated by estradiol [18,19]. Plants having estrogenic potency have been reported to increase the uterine protein in ovariectomized mice. Methanol extract of *Careya arborea* Roxb., roots when administered to adult ovariectomized mice for 14 days increased the uterine protein level [20]. Hexane extract of whole plant of *Ferula jaeschkenana* when administered to adult ovariectomized rats for 7 days significantly increased the uterine protein content [21]. Therefore, increase in total uterine protein content firmly established that the root extract has estrogenic activity.

Effect on Serum Cholesterol Level

Fig. 21.2 shows the changes in serum cholesterol level in the treated ovariectomized mice. There was decrease in the serum cholesterol level in all the experimental groups when compared to control. The serum cholesterol level was found 167.7 ± 6.73 mg/dl in control, 122.5 ± 5.67 mg/dl in Estradiol treated group, 167.1 ± 6.2 mg/dl in test-1 and 144.2 ± 5.58 mg/dl in test-2 group. It was found that the level of cholesterol decreased in all the treatment groups when compared to the control. This decrease was statistically analyzed using student t-test and found that, there was no significant difference in the serum cholesterol level between control and test-1 group. On the other hand, there was significant decrease of serum cholesterol level in the Estradiol and test-2 groups at *p<0.001.

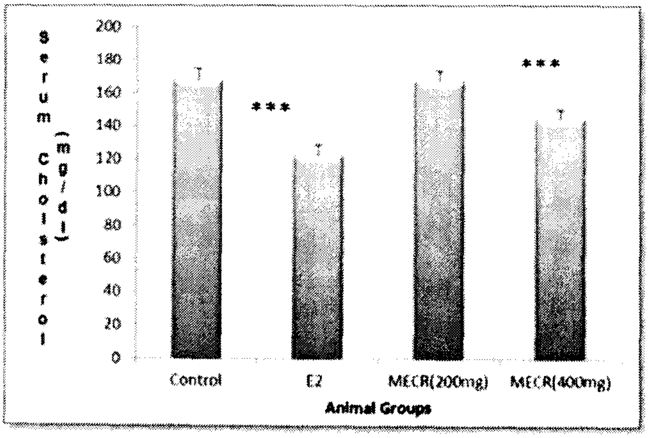


Fig. 21.2: Changes in the Serum Cholesterol level after treatment with root extract on adult ovariectomized mice (mg/dl) for 7 consecutive days. Data are expressed as Mean ± SEM, (n=5). Significance test was done by using Student t-test where ***P< 0.001 and no asterisk denotes non- significant

Carrol & Kurowska, 1995 [22] and Sirtori *et al.*, 1995 [23] have reported lower level of total blood cholesterol after consumption of soy products in both animals and humans. As the present study reveals almost same results as with other workers who reported the estrogenic property of plant with the reduction of serum cholesterol level, it can be established that the root extract of the selected plant in the present investigation may have estrogenic activity.

Effect on Uterine Glycogen Content

Results of effect of root extract on uterus of ovariectomised mice are shown in the Fig. 21.3. There was no significant change in uterine glycogen content in the experimental animals. Control group showed 3.96 ± 0.22 mg, estradiol treated group showed 0.93 ± 0.02 mg, test-1group showed 3.35 ± 0.22 mg and test-2 group showed 3.22 ± 0.22 mg per gram tissue of uterus. Data were analyzed statistically and found no significant difference in control and extract treated groups.

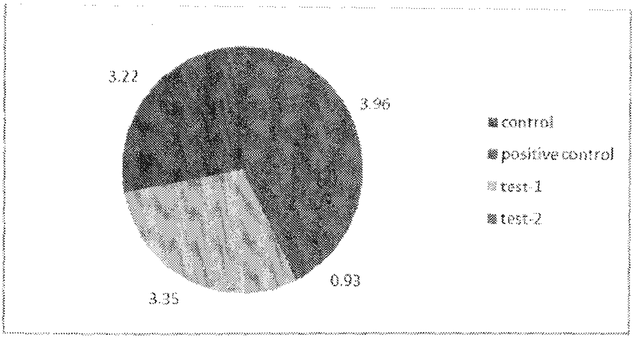


Fig. 21.3: Effect of Root Extract on Uterine Glycogen of Ovariectomised Mice

Reduction in the uterine glycogen content suggested the possibility of active glycogenolysis [24]. In a study, Reddy *et al.* (1993) reported that glycogen in muscle tissues of *Channa punctatus* reduced when they exposed to phenol which is an estrogenic compound . In the present investigation there was a visible reduction in the uterine glycogen content in positive control group. While there was reduction in uterine glycogen in the root extract treated groups when compared to control but it was not a significant reduction. This result indicated possible estrogenic activity of the root extract but it may have much lower potency than estradiol which was used as positive control in the present investigation.

Conclusions

The present investigation revealed increased level of uterine protein, decreased serum cholesterol but no change in uterine glycogen content. It can be concluded from this study that the plant under study may have some estrogenic potency which is helpful in the treatment of gynecological disorders. Further study is necessary to investigate the estrogenic effect of the plant both in in-vivo and in-vitro methods.

References

1. Williams, M.G, Simon, O.R. and West, M.E., The Past and Present Use of Plants for Medicines, *West Indian Medical Journal*, 2006, **55 (4)**, 217.
2. WHO, "Traditional Medicine", World Health Organization, Geneva. (From web site) 2008, Fact sheet no. 134.
3. Gupta, G.K., Chahal, J. and Bhatia, M., *Clitoria ternatea* (L.): Old and New Aspects, *Journal of Pharmacy Research*, 2010, **3(11),** 2610.
4. Nahar, K., Rahman, M.A., Most. Parvin, N. and Sarwar, S., Evaluation of Anthelmintic Activity of Aqueous Leaf Extract of Clitoria ternatea Linn., *Stanford Journal of Pharmaceutical Sciences,* 2010, **3(1),** 46.
5. Daisy, P., Kanakappan Santoshand and Rajathi, M.., Anti-hyperglycemic and Antihyperlipidemic Effects of Clitoria ternatea Linn. in alloxan-induced Diabetic Rats, *African Journal of Microbiology Research*, 2009, **3 (5)**, 287.
6. Devi, B.P., Boominathan, R. and Mandal, S.C., Anti-inflammatory, Analgesic and Antipyretic Properties of Clitoria Ternatea Root, *Fitoterapia,* 2003, **74**, 345.
7. Upwar, N., Patel, R., Waseem, N. and Mahobia, N.K., Evaluation of Anti-diarrhoeal Activity of the Root of Clitoria ternatea Linn., *International Journal of Pharmaceutical Sciences Review and Research,* 2010, **5(1)**, 131.
8. Sarumathy, K., Dhana, M.S., Rajan, Vijay, T. and Jayakanthi J., Evaluation of Phytoconstituents, Nephro-protective and Antioxidant Activities of Clitoria ternatea, *Journal of Applied Pharmaceutical Science,* 2011, **01 (05),** 164.
9. Solanki, Y.B. and Jain, S. M., Hepato-protective Effects of Clitoria ternatea and Vigna mungo against Acetaminophen and Carbon Tetrachloride-induced Hepatotoxicity in Rats, *Journal of Pharmacology and Toxicology*, 2011, **6(1)**, 30.
10. Yogendrasinh, Solanki, B. and Jain, S.M., Immunomodulatory Activity of Ayurvedic Plant Aparajita (Clitoria ternatea L.) In Male Albino Rats, *Global Journal of Science Frontier Research*, 2010, **10 (3)**, 2.
11. Kaldas, R.S. and Hughes, Jr. C.L., Reproductive and General Metabolic Effects of Phytoestrogens in Mammals, *Reproductive Toxicology,* 1989, **3**, 81.
12. Tang B.Y. and Adams N. R., Oestrogen Receptor and Metabolic Activities in the Genital Tract after Ovariectomy of Ewes with Permanent Infertility Caused by Exposure to Phytoestrogens, *Endocrinology*, 1981, **89**, 365.
13. Davis, S, R., Dalais, F. S,, Simpson, E. R., and Murkies, A. L., Phytoestrogens in Health and Disease, *Recent Progress in Hormone Research*, 1999, **54**, 185.
14. Kalita, J.C., Haque, A., Kalita, E., Sharma, R. and Rahman, M. R., Anti-Fertility Effects of the Methanolic Root Extract of Careya Arborea Roxb in Albino Mice, *The Bioscan,* 2011, **6(4)**, 701.
15. Peyghambari, F., Salehnia, M., Moghadam, M.F., Valujerdi, M.R. and Hajizadeh, E., The Changes in morphology and morphometrical indices of endometrium of ovariectomized mice in response to exogenous Ovarian Hormones, *Iranian Journal of Reproductive Medicine*, 2008, **6(3)**, 125.

16. Lowry, O. H. N., Rosenbough, J., Farr, A. L. and Randall, R. J., Protein Measurement with Folin Phenol Reagent, *Journal of Biological Chemistry*, 1951, **193**, 261.
17. Seifter, S., Daylon, S., Novic, B. and Muntwyler, E., An Indirect Method for Glycogen in tissue of Low Glycogen Content - The estimation of Glycogen with the Anthrone Reagent, *Archives in Biochem*, 1950, **25**, 191.
18. Takeda, A., Takashashi, N. and Shimizu, S., Identification and Characterization of an Estrogen Inducible Glycoprotein (uterine secretory protein) Synthesized and Secreted by Uterine Epithelial Cells, *Endocrinology,* 1988, **122**, 105.
19. Ireland, J. S., Mukku, V. R., Robison, A. K. and Stancel, G. M., Stimulation of Uterine Deoxyribonucleic acid Synthesis by 1,1,1,-trichloro-2-(*p*-Chlorophenyl)-2-(*o*-Chlorophenyl) ethane (*o, p2*-DDT), *Biochemical Pharmacology,* 1980, **29**, 1469.
20. Kalita, J.C., Haque, A., Kalita, E., Sharma, R. and Rahman, M. R., Anti-Fertility Effects of the Methanolic Root Extract of Careya Arborea Roxb in Albino Mice, *The Bioscan,* 2011, **6(4)**, 701.
21. Pathak, S. and Prakash, A. O., Effect of Hexane Extract of Ferula Jaeschkeana on the Uterus of Adult Ovariectomised Rats, *Ancient Science of Life.*, 1992, **12,** 231.
22. Carrol, K. K. and Kurowska, E. M., Soy Consumption and Cholesterol Reduction: Review of Animal and Human Studies, *Journal of Nutrition,* 1995, **125(3)**, 594S.
23. Sirtori, C.R., Lovati, M.R., Manzoni, C., Monetti, M., Pazzucconi, F. and Gatti, E., Soy and Cholesterol Reduction: Clinical Experience, *Journal of Nutrition,* 1995, **125(3),** 598S.
24. Reddy, M.M., Kumar, V. A., Reddy, P. S. and Reddy, S. L. N., Phenol Induced Metabolic Alterations in the Brain and Muscle of a Freshwater Fish Channa Punctatus during Sub-lethal Toxicosis, *Journal of Ecotoxicology and Environmental Monitoring,* 1993, **3(1)**, 13.

22

Characterization of Xylanase from *Trichoderma longibrachiatum*

Lashngain R. Kharkongor
P.K. Ambasht

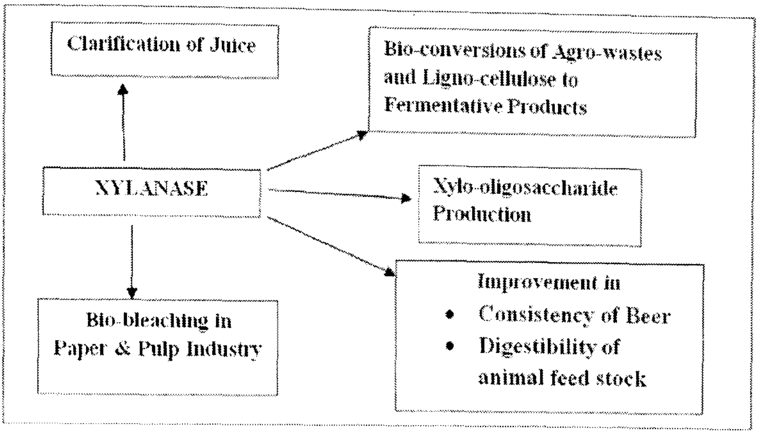
Abstract

Xylanase (EC 3.2.1.8 Endo-β-1, 4-D-xylanase) catalyzes the hydrolysis of xylan to its reducing sugar residues and has wide industrial applications. In the present study, commercially available *Trichoderma longibrachiatum* xylanase (specific activity 440.8 Umg^{-1}) and beechwood xylan were used. The optimal pH was 6.0. The optimal temperature was 55 °C. The enzyme still had more than 90 % activity at 60 °C, but drops down to less than 25 % at 70 °C. The energy of activation determined from Arrhenius plot was 42.85 kJ mol^{-1}. When the enzyme was incubated at 50, 55 and 60 °C for 10 min retained 97.0, 67.0 and 17.0 % residual activities, respectively. From the Lineweaver Burk plot, the values of K_m and V_{max} were 2.0 mg mL^{-1} and 1000 μmol $min^{-1}mg^{-1}$, respectively.

Keywords: *Xylanase, Xylan, Activation energy*

Introduction

Xylan, which comprises 20-35 % by weight of wood and agricultural wastes, is the major component of the hemicellulose portion of plant cells. Xylan therefore represents a significant resource of renewable biomass which can be utilized as a substrate for the preparation of many useful products such as fuels, solvents, and chemicals. Endo- β-1,4-xylanase (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) due to their high specificity hydrolyzed β-1, 4-glycosidic lingkages present in xylan and hence, is a crucial enzyme component of microbial xylanolytic system [1, 2]. In recent years, many kinds of xylanases have been isolated from various microorganisms including bacteria, actinomyces, fungi and yeast [3,4,5,6].



Scheme 1: Applications of Xylanase.

Xylanase has potential application in industrial processes [7.8.9]. Xylo-oligosaccharides have stimulatory effects on the selective growth of human intestinal *Bifdiobacteria* and are frequently defined as probiotics [10]. Many microorganisms are known to produce different types of xylanases. Among them, the fungi *Trichoderma*; a saprophyte, which belongs to ascomycetes is a good source of cellulose and xylan hydrolyzing enzymes [11]. Xylanase from *Trichoderma longibrachiatum* is commercially available and in the present paper, we describe its characterization with respect to kinetic properties. The results will be useful for its future industrial applications. There are reports on xylanase purification and production from *T. longibrachiatum* [12,13,14].

Materials and Methods

Xylan from Beechwood and endo-β-1,4-xylanase (*Trichoderma longibrachiatum*) were from Sigma Aldrich, USA. All other chemicals were of AR grade procured from SRL, HiMedia Laboratories, Sd Fine chemicals, India. All solutions were prepared in de-ionized water from Milli-Q system (Millipore, USA).

Xylanase Activity Assay and Protein Quantification

Xylanase activity was assayed according to Bailey *et. al.* [15]. A 0.2 % (w/v) xylan solution was prepared in 0.05 M sodium acetate buûer, pH 6.0. DNS reagent was prepared by transferring 5.0 g 3,5-dinitrosalicylic acid, 0.25 g sodium sulphite, 100 g potassium sodium tartarate, 5.0 g NaOH and 1.0 g phenol in a beaker and raising the final volume to 500 mL with de-ionized water. The 2.0 mL assay mixture contained 1.8 mL xylan solution maintained at 55 °C and the reaction was initiated by addition of an aliquot (0.2 mL) of suitably diluted xylanase. After an incubation of 5 min, the reaction was stopped by addition of 3.0 mL dinitrosalicylic acid (DNS) reagent and was kept in boiling water bath for 5 min. Tubes were brought to room temperature and absorbance was recorded at 540 nm using Varian 50 Spectrophotometer. The amount of reducing sugar liberated was determined according to Miller [16] using xylose as standard. One unit (U) of xylanase activity was expressed as amount of enzyme responsible for release of 1 μmol of reducing sugar (xylose equivalent) in 1 min.

Protein was quantified using BSA as a standard, according to Bradford [17].

Spectral Characterization

The spectrum of xylanase was recorded in the ultraviolet region using Varian 50 spectrophotometer. The fluorescence spectrum of enzyme was recorded in Varian Cary Eclipse fluorescence spectrophotometer. The excitation wavelength was 293 nm and emission was between 300 to 380 nm.

Kinetic Characterization

The rate of xylanase catalyzed reaction was studied by varying the pH using different buffers (50 mM sodium acetate buffer, pH 4.0-6.5; 50 mM Tris-HCl buffer, pH 7.0) at enzyme saturating concentrations of xylan. From the data, a plot of rate of reaction versus pH was made.

The rate of xylanase catalyzed reaction was studied by varying the concentration of xylan (0.36 mg to 2.16 mg). A stock solution of 1% xylan was prepared in assay buffer and was used. From this data, Lineweaver Burk plot (1/v versus 1/ [Xylan]) was made to determine value of Michaelis constant (K_m) and V_{max}.

The effect of variation of temperature on xylanase catalyzed reaction was studied in the range of 35 to 70 °C at pH 6.0 using assay buffer. From the data, a plot of rate versus temperature was made to determine optimum temperature. Further, the data was used to make Arrhenius plot for the determination of energy of activation.

Thermal denaturation of xylanase was carried out at 50, 55 and 60 °C. The enzyme solutions were maintained at a particular temperature, in different tubes. Enzyme aliquots were withdrawn at different time intervals, chilled in ice and activity was assayed. The value of percent residual activity was computed and suitable plots were made to analyze results.

Results and Discussion

Xylanase Assay

The xylanase activity was assayed in the present study according to Bailey *et. al.* [15], which is quite recent method. There are some reports where the reducing sugar content was determined according to Somogyi [18] and Royer and Nakas [12]. Further, in the present study, beechwood xylan was used as was done by Ding *et. al.* [19[and Ai *et. al.* [20]. In other reports, source of xylan was different like larch [12], birchwood [20, 21], oat spelt [20], corn-cob [19], cotton husk-seed [19].

Spectral Characterization

The results of ultraviolet and fluorescence spectrum are shown in Fig. 22.1 and 22.2, respectively.

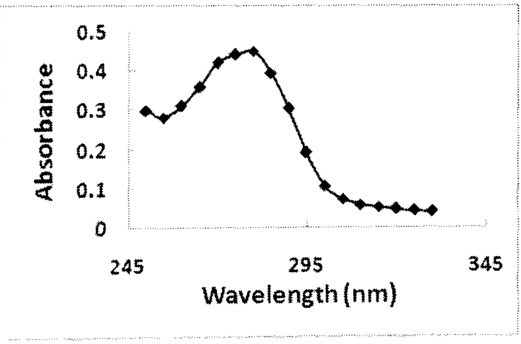


Fig. 22.1: Ultraviolet Spectrum of Xylanase

In the ultra-violet spectrum, the enzyme showed maximum absorbance at 280 nm. The absorbance $A_{280/260}$ ratio was 1.45. It suggests that the enzyme was

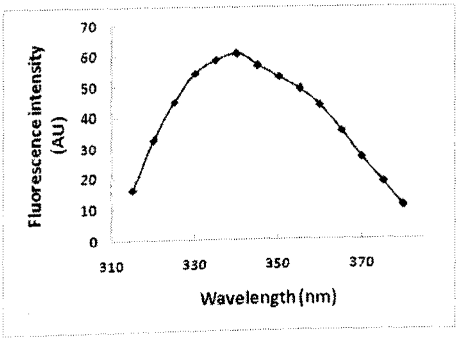


Fig. 22.2: Fluorescence Spectrum of Xylanase with Excitation Wavelength at 293 nm

free from nucleotides. The emission maximum in the fluorescence spectrum was observed at 340 nm. The results suggest that Trp residues are there on the surface of enzyme. There are however, no other reports on spectral characterization of xylanase from *T. longibrachiatum*.

Kinetic Characterization

The results of the effect of pH on xylanase catalyzed reaction are shown in Fig. 22.3. Xylanase was stable in the pH range 4.5 to 7.0. The enzyme was quite active at pH 5.5 (94 %), but the optimum pH was 6.0. The optimum pH of the *T. longibrachiatum* xylanase is close to some other microbial xylanases [20-24]. The enzyme activity dropped thereafter but at pH 6.5 more than 80 % activity was retained. There is a report in which the pH optimum was not sharp and was between pH 5.0 and 6.0 [13].

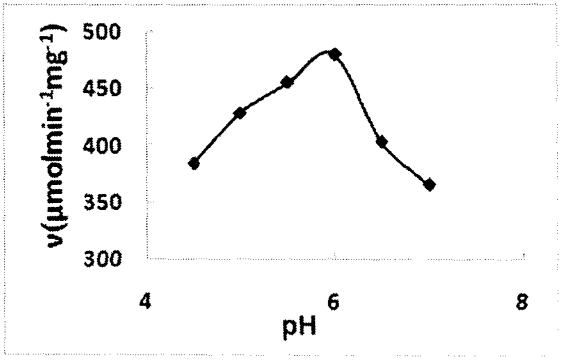


Fig. 22.3: The Effect of pH on Xylanase Catalyzed Reaction Rate

Lineweaver Burk plot of the data of effect of xylan concentration variation on rate of xylanase catalyzed reaction is shown in Fig. 22.4. The value of K_m was found to be 2 mg mL^{-1}. The values of K_m are close to Madakbas *et. al.* [25] or

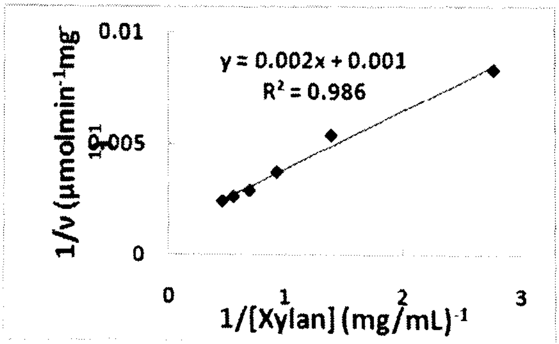


Fig. 22.4: Lineweaver Burk plot on Xylanase Catalyzed Reaction

lower [22] in comparison to some other microbial xylanases. The K_m value was on a higher side (10.14 mg mL^{-1}) with oat spelt xylan [13]. In one case, Hanes plot was used for determination of K_m [12]. The lower value of K_m indicates that xylanase has stronger affinity for xylan. The value of V_{max} in the present study was 1000 µmol $min^{-1}mg^{-1}$ protein which is higher than earlier report [12].

The results of effect of temperature on xylanase catalyzed reaction rate are shown in Fig. 22.5. The optimum temperature for xylanase was 55 °C. The enzyme was active at 50 and 60 °C with 90.5 and 91.3 % activity. The activity dropped beyond 60 °C and at 65 °C, only 55 % activity was retained. Variation in optimum temperature has been observed among microbial xylanases like 45 °C [13], 50 °C [21,22,25], 60 °C [20], 75 °C [23], 90 °C [24]. The Arrhenius plot for the data is shown in Fig. 22.6. The energy of activation was determined to be 10.3 kcal mol^{-1} (42.85 kJ mol^{-1}). A very high value of activation energy (150.1 kJ) was reported in xylanase from *Melanocarpus albomyces* [26].

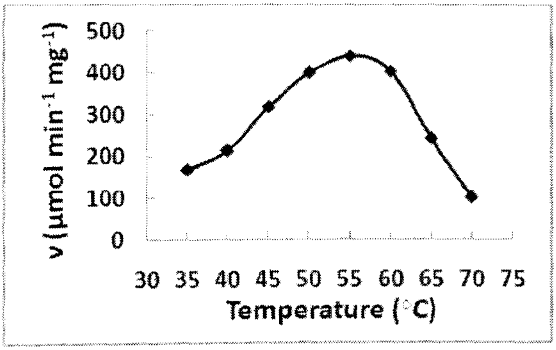


Fig. 22.5: Effect of Temperature on Xylanase Catalyzed Reaction Rate

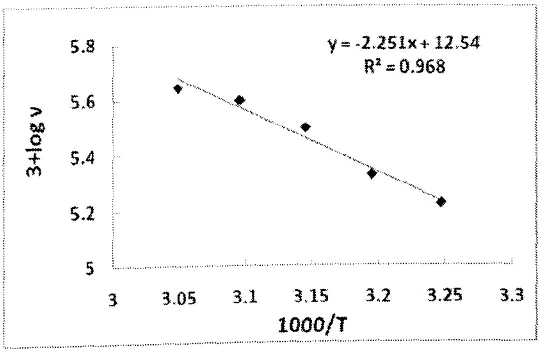


Fig. 22.6: Arrhenius Plot of Xylanase Enzyme Catalyzed Reaction at Different Temperatures

The results of thermal denaturation of xylanase are shown in Fig. 22.7 and Fig. 22.8. Single exponential decay was observed at 50 °C. The result suggests that the loss in activity follows first order kinetics. The enzyme was very active with more than 65 % activity after 4 h. The result of thermal denaturation at the sub-optimal temperature (50 °C) is in coherence with effect of temperature on xylanase activity (94 % activity). The result of thermal denaturation at 55 °C (optimal temperature) was quite different. The enzyme lost more than 50 % activity after 20 min when kept at 55 °C. The $t_{1/2}$ was 15 min with $k_{inactivation}$ 0.0413 min^{-1}. More than 90 % xylanase activity was lost when the enzyme was subjected to thermal denaturation at 60 °C after 20 min.

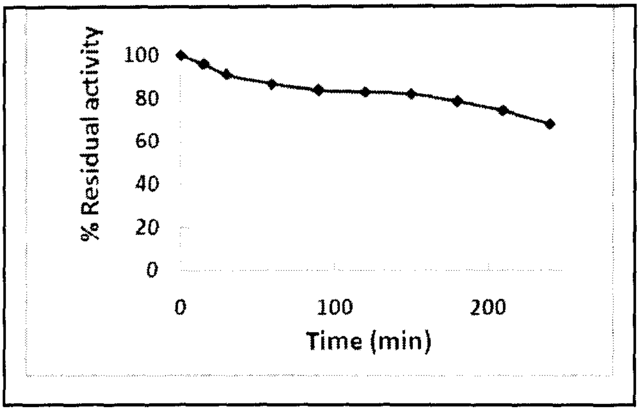


Fig. 22.7: Thermal Denaturation of Xylanase at 50 °C

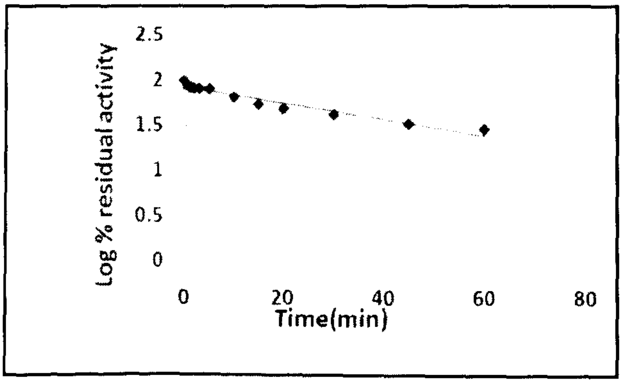


Fig. 22.8: Thermal Denaturation of Xylanase at 55 °C

Conclusion

The above characteristic study of Xylanase from *T. longibrachiatum* showed promising properties which can be applied for mass production of xylooligosaccharide as well as its application in animal feed. It can be also immobilized by using different matrices to improve its physio-chemical properties.

Acknowledgement

L. R. Kharkongor is grateful to The University Grants Commission, New Delhi for the Rajiv Gandhi National Fellowship (RGNF-ST) in the form of JRF.

References

1. Sunna, A. and Antranikian, G, Xylanolytic Enzymes from Fungi and Bacteria, *Critical Reviews in Biotechnology,* 1997, **17**, 39.
2. Kapoor, M. and Kuhad, R.C., Immobilization of Xylanase from *Bacillus pumilus* Strain MK001 and its Application in Production of Xylo-oligosaccharides, *Applied Biochemistry and Biotechnology*, 2007, **142**, 125.
3. Garg, S., Ali, R. and Kumar, A., Production of Alkaline Xylanase by an Alkalo-thermophilic Bacteria, *Bacillus halodurans* MTCC 9512 Isolated from Dung, *Current Trends in Biotechnology and Pharmacy*, 2009, **3**, 90.
4. Bai, Y., Wang, J., Zhang, Z., Yang, P., Shi, P. and Luo, H. A New Xylanase from Thermoacidophilic *Alicyclobacillus sp.* A4 with Broad-range pH Activity and pH Stability, *Journal of Industrial Microbiology and Biotechnology,* 2010, **37**, 187.
5. Menon, G., Mody, K., Keshri, J. and Jha, B., Isolation, Purification, and Characterization of Haloalkaline Xylanase from a Marine *Bacillus pumilus* Strain GESF-1, *Biotechnology and Bioprocess Engineering*, 2010, **15**, 998.
6. Pal, A. and Khanum, F., Production and Extraction Optimization of Xylanase from *Aspergillus niger* DFR-5 through Solid-state Fermentation, *Bioresource Technology*, 2010, **101**, 7563.
7. Subramaniyan, S. and Prema, P., Biotechnology of Microbial Xylanases: Enzymology, Molecular Biology, and Application, *Critical Reviews in Biotechnology*, 2002, **22**, 33.
8. Wang, C.Y., Chan, H., Lin, H.T. and Shyu, Y.T., Production, Purification and Characterization of a Novel Holostable Xylanase from *Bacillus sp.* NTU-06, *Annals of Applied Biology*, 2010, **1**,187.
9. Ouyang, J., Wang, S., Wang, Y., Li, X., Chen, M., Yong, Q. and Yu, S., Production of a *Trichoderma reesei* QM9414 Xylanase in *Pichia pastoris* and its Application in Biobleaching of Wheat Straw Pulp, *World Journal of Microbiology and Biotechnology*, 2011, **27**, 751.
10. Beg, Q.K., Kapoor, M., Bhushan, B. and Hoondal, G. S., Microbial Xylanases and their Industrial Applications: A Review, *Applied Microbiology and Biotechnology*, 2001, **56**, 326.
11. Wong, K.K.Y. and Saddler, J.N., *Trichoderma* xylanases, their Properties and Application, *Critical Reviews in Biotechnology*, 1992, **12**, 413.
12. Royer, J.C. and Nakas, J.P., Purification and Characterization of Two Xylanases from *Trichoderma longibrachiatum, European Journal of Biochemistry,* 1991, **202**, 521.

13. Chen, C., Chen. J.L. and Lin, T.Y., Purification and Characterization of a Xylanase from *Trichoderma longibrachiatum* for xylooligosaccharide Production, *Enzyme and Microbial Technology*, 1997, **21**, 91.
14. Azin, M., Moravej, R. and Zareh, D., Production of xylanase by *Trichoderma longibrachiatum* on a Mixture of Wheat Bran and Wheat Straw: Optimization of Culture Condition by Taguchi Method, *Enzyme and Microbial Technology*. 2007, **40**, 801.
15. Bailey, M.J., Biely, P. and Poutanen, K., Inter-laboratory Testing of Methods for Assay of Xylanase Activity, *Journal of Biotechnology*, 1992, **23**, 257.
16. Miller, G.L., Use of Dinitrosalicyclic Acid Reagent for the Determination of Reducing Sugars, *Analytical Chemistry*, 1959, **31**, 426.
17. Bradford, M. M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding, *Analytical Biochemistry*, 1976, **72**, 248.
18. Somogyi, M., Notes on Sugar Determination, *Journal of Biological Chemistry*, 1952, **195**, 19.
19. Ding, C.H., Jiang, Z.Q., Li, X.T., Li, L.T. and Kusakabe, I., High Activity Xylanase Production *Streptomyces olivaceovirdis* E-86, *World Journal of Microbiology and Biotechnology*, 2004, **20**, 7.
20. Ai, Z., Jiang, Z., Li, L., Deng, W., Kusakabe, I. and Li, H., Immobilization of *Streptomyces olivaceovirdis* E-86 xylanase on Eudragit S-100 for xylo-oligosaccharide production, *Process Biochemistry*, 2005, **40**, 2707.
21. Akdemir, Z.S., Demir, S., Kahraman, M.V. and Apohan, N. K., Preparation and Characterization of UV-curable Polymeric Support for Covalent Immobilization of Xylanase Enzyme, *Journal of Molecular Catalysis B: Enzymatic*, 2011, **68**, 104.
22. Fialho, M.B. and Carmona, E.C., Purification and Characterization of Xylanases from *Aspergillus giganteus*, *Folia Microbioligica*, 2004, **49**, 13.
23. Jiang, Z.Q., Yang, S.Q., Tan, S.S., Li, L. T. and Li, X.T., Characterization of a Xylanase from the Newly Isolated Thermophilic *Thermomyces lanuginosus* CAU44 and its Application in Bread Making, *Letters in Applied Microbiology*, 2005, **41**, 69.
24. Li, L., Zhu, Y., Huang Z., Jiang, Z. and Chen, W., Immobilization of the Recombinant Xylanase B (Xyn B) from the Hyperthermophilic *Thermotoga maritime* on Metal-chelate Eupergit C 250 L, *Enzyme and Microbial Technology*, 2007, **41**, 278.
25. Madakbas, S., Danis, O., Demir, S. and Kahraman, M.V., Xylanase Immobilization on Functionalized Polyaniline Support by Covalent Attachment, *Starch*, 2013, **65**, 146.
26. Gupta, G, Sahay, V., and Gupta R. K., Thermal Stability and Thermodynamics of Xylanase from *Melanocarpus albomyces* in Presence of Polyols and Salts, *Bioresources*, 2014, **9**, 5801.

23

Study of Ground Water Quality in the Chirang District of Assam

Valentina Basumatary
K. G Bhattacharyya

Abstract

Ground water is the main source of drinking water in Chirang. A total of 14 different groundwater (well water) samples were collected from different stations. From the laboratory analysis of these samples it was found that iron (Fe) concentration was highest in the region compared to other trace metals ;while the metals like Cd, Cr, Cu, Mn, Ni, Pb and Zn were found to be within the WHO desirable drinking water limit. The water pH was found to be in the neutral range (5.6-6.4). Electrical conductance was seen in the range of 0.20-0.35mS/cm . The highest TDS concentration was found to be 243 mg/L. Fluoride was seen to be within the desirable limit (WHO 2011 is 1.5 mg/L).

Keywords: *Chirang, Concentration, Desirable, Drinking, Groundwater.*

Introduction

Groundwater resources are of major concern and vital to the development of a country. Intensive urbanisation and agricultural development has created high demand on groundwater resources. The composition of groundwater depends not only on natural factors such as composition of precipitation, geological structure and mineralogy of the watersheds and aquifers, geological processes within the aquifer, but also on human activities, which can alter these fragile groundwater system, either by polluting them or by changing the hydrological cycle [1]. Some of these are used for drinking water, and about 80% of the water is used for irrigation. The agricultural production that is based essentially on flood irrigation and potential application of fertilizer to improve the soils has permanently increased the risk of water scarcity and groundwater quality deterioration [2]. But the groundwater today is facing serious problem due to addition of fertilizers and pesticides for improvement of agricultural production [3].

The toxic heavy metals enter into the environment through improper disposal of used lead accumulators, corrosion of household plumbing systems, corrosion of galvanized pipes, runoff from waste batteries, and paint effluents. In particular, Hg and Pb interfere with the central nervous system [4].

Materials and Methods

Study Area and Sampling Procedure

A total of 14 groundwater (ring well) samples were collected from the agricultural areas of Chirang. The sampling was done in the month of April 2014. Geographical locations were recorded with the help of GPS (Geographical Positioning System) in each of the sampling locations for easy identification. Random daytime sampling or 30-minute stagnation samples were taken in order to provide reasonably representative results. Samples were collected in pre-cleaned polyethylene containers. These containers were pre-cleaned by soaking in a

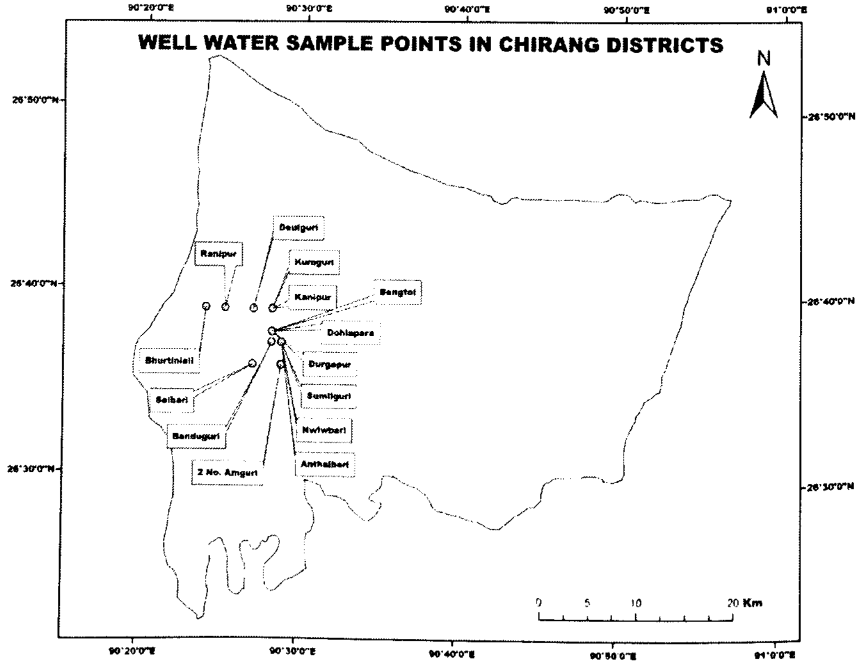


Fig. 23.1: Location of Well Water Sample Points

solution of 10 % nitric acid and rinsed with lab water. The water samples were collected randomly. A total of 14 well water samples are collected and numbered serially from GW1 to GW14 (Table 23.1).

Sample Preservation

In order to prevent adsorption losses, samples for the analysis of total arsenic were preserved by acidification to $pH < 2$. For As measurement, hydrochloric acid is the choice for sample preservation prior to analysis and ISO 11969:1996 (HGAAS technique) prescribes the addition of 20 ml of 50 % hydrochloric acid to each litre of water sample.

Filtration of Water Samples

Environmental water samples contain significant amounts of suspended matter. For this reason all water samples after collection are filtered through a 0.45 μm membrane filter as soon as possible and prior to acid preservation. This is basically done for the determination of dissolved metals.

Methodology

All the water quality parameters are determined according to standard procedures laid down by American Public Health Association (APHA). Arsenic is determined with atomic absorption spectrometry (AAS) with a hydride generator system (HGAAS) for atomization. Other trace metals are determined with flame AAS (PerkinElmer Analyst 220).

Table 23.1: GPS Record of the Sampling Locations

SL NO	VILLAGES	COORDINATES						HEIGHT
		LATITUDE			LONGITUDE			(m)
		D	M	S	D	M	S	
GW1	Anthaibari	26p	37	24.4	090p	29	03.7	87
GW2	Salbari 26p	36	16.5	090p	27	49.1	80	
GW3	Banduguri	26p	37	44.0	090p	28	26.4	79
GW4	Kumguri 26p	39	22.7	090p	28	19.3	86	
GW5	Kanipur 26p	39	39.8	090p	28	56.0	85	
GW6	Durgapur	26p	38	55.8	090p	28	46.1	84
GW7	Ranipur 26p	39	46.4	090p	25	48.2	86	
GW8	Bhurtiniali	26p	39	54.1	090p	24	19.0	106
GW9	Deulguri 26p	39	05.4	090p	27	59.1	105	

GW10	Dohlapara	26p	38	13.2	090p	28	53.4	83
GW11	Nwlwbari	26p	37	44.2	090p	29	17.9	89
GW12	2 no Amguri	26p	36	08.5	090p	29	33.3	90
GW13	Sumliguri	26p	37	53.3	090p	29	02.3	95
GW14	Bengtol 26p	38	25.7	090p	28	32.9	120	

Results and Discussion

The pH of the groundwater samples was found to be ranging from 5.60 – 6.44 (well water) and with mean of 6.1.While, the WHO guideline concentration of pH in drinking water is 7-8.5 [5]. Therefore the pH was found to be within the desirable limit. The electrical conductivity ranges from 0.02-0.35 mS/cm . TDS is the concentrations of all dissolved minerals in water indicate the general nature of salinity of water [6]. The TDS in the region was found to be below 500 mg/L. Bicarbonate concentration ranges from 16.7- 133.3mg/L. The dissolution of gases and minerals, particularly CO_2 and CO_3 compounds in the atmosphere and in the unsaturated zone during precipitation and infiltration, would impart the observed HCO_3^- water type. Further, feldspar is among the major minerals present in the region and kaolinization of K-feldspar and consequent production of bicarbonate ions can also be explained by this factor. The geo-chemical reaction may be expressed as:

$$2\text{K-Feldspar} + 2CO_2 + 11H_2O \rightarrow \text{Kaolinite} + 2K^+ + 2HCO_3^- + 2H_2SiO_4 \text{ [7]}$$

Excess concentration of Cl in drinking water gives a salty taste and has a laxative effect in people not accustomed to it [8]. Chloride in the well water ranges from 7.1 mg/l to 36.9 mg/l. High level of sulphate in groundwater causes gastrointestinal irritation. 250mg/L is the WHO limit for drinking water [9] Sulphate concentration ranges from 10.1mg/L to 69.3 mg/L. The occurrence of nitrate in groundwater is an anthropogenic pollutant contributed by nitrogenous fertilizers, industrial effluents, human and animal wastes through biochemical activity [10]. Nitrate in the well water ranges from 2.62 mg/l to 49.21 mg/l. The presence of vast paddy cultivation in the study area suggest that agricultural runoff is the probable source for this concentration. Easy solubility is also responsible for phosphates finding their way into water from animal waste, runoff from agricultural land due to fertilizer use, and detergent-filled domestic wastewater [11] The phosphate concentration ranges from 2.51 mg/L to 5.02 mg/L (ring well).

The fluoride concentration in the region was within the WHO 2011 desirable limit (1.5 mg/L). Worldwide statistical evidence shows that the people affected by excessive fluoride concentration were found to be much more than that affected by excessive arsenic. People exposed to very high amount of fluoride are affected with dental and/or skeletal fluorosis, which can lead to debilitating paralysis [12].

The mean Ca and Mg concentration in the region are 17.6 mg/L and 2.8mg/L respectively. High concentrations of Ca and Mg in the water are attributed to the weathering of crystalline dolomitic limestones and Ca-Mg silicates (amphiboles, pyroxenes, olivine, biotite and others) [13]. The World Health Organisation (2004) has identified that water with a hardness of 200 mg/L or higher (measured as calcium carbonate) will produce scale and soft water with a value of 100 mg/L (as calcium carbonate) or less will have a low buffering capacity and be more corrosive to pipes [14]. It is found the total hardness of all the samples are below 100 mg/L. Potassium in groundwater is mostly due to weathering of orthoclase and microcline. Potassium is found to be in lesser amount than Na in groundwater, this is because K-minerals have higher resistance to weathering[15]. The sodium was found to be 0.7-30 mg/L and potassium was 0.1-24.3 mg/L.

Fe and Mn both are essential constituents of plant and animal metabolism. The Iron is present in the environment due to human activities. Industrial wastes and mine drainage waters are two common anthropogenic sources of iron. The ground water containing iron at concentration greater than 2 mg/l cause straining of clothes and plumbing fixtures and impart bad taste and colour to water [16]. The Fe concentration ranges from 6.09 mg/L to 27.56 mg/L (above the desirable limit). While the Mn was mostly within the desirable limit (WHO 2011 is 0.4 mg/L). The metals like Cd, Cr, Cu, Ni and Pb were found to be within the WHO 2011 desirable limit of drinking water standard. Zn in the ring-well was 0.111-0.277 mg/L. Since Zn is required for physiological processes in both plant and animals, WHO has not given any guideline value. But even though excessive amount of Zn can be harmful.

Table 23.2: Ranges of Analytical Data of the Groundwater (ring wells) Samples

Sites	pH	EC mS/cm	TDS mg/L	Na^+ mg/L	K^+ mg/L	HCO_3^- mg/L	Cl^- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	PO_4^{3-} mg/L	F^- mg/L
GW1	5.7	0.070	63.0	20.20	0.10	16.7	14.2	28.7	2.6	2.7	BDL
GW2	5.7	0.350	43.0	29.70	20.70	33.3	36.9	53.6	12.3	2.8	0.8
GW3	6.1	0.100	53.0	6.70	3.10	33.3	19.9	10.1	14.5	2.6	1.2
GW4	6.4	0.130	93.0	23.00	8.10	83.0	19.9	34.0	6.6	5.0	0.9
GW5	6.1	0.050	29.0	24.20	0.20	25.0	8.5	11.2	6.0	3.5	0.6
GW6	6.1	0.090	93.0	4.60	1.40	16.7	12.8	41.6	27.2	2.8	1.4

GW7	6.0	0.020	36.0	25.90	0.80	33.3	8.5	23.4	5.6	2.8	0.7
GW8	5.6	0.080	103.0	16.80	0.10	16.7	8.5	31.8	49.2	3.5	0.4
GW9	6.3	0.140	9.0	0.70	0.20	133.3	15.6	37.7	9.5	2.6	1.2
GW10	6.4	0.030	66.0	28.90	24.30	16.7	8.5	35.7	3.2	2.6	1.3
GW11	6.3	0.140	26.0	2.90	17.40	49.0	14.2	69.3	25.1	3.4	1.1
GW12	6.2	0.310	139.0	24.10	18.70	133.3	25.6	56.6	3.4	2.5	1.4
GW13	5.9	0.050	9.0	13.70	22.40	33.3	7.1	35.3	9.3	3.8	0.9
GW14	6.0	0.350	243.0	30.00	15.30	50.0	34.1	36.0	9.5	3.3	0.6
MIN	5.6	0.020	9.0	0.70	0.10	16.7	7.1	10.1	2.6	2.5	BDL
MAX	6.4	0.350	243.0	30.00	24.30	133.3	36.9	69.3	49.2	5.0	1.4
MEAN	6.1	0.136	71.0	17.96	9.49	48.1	16.7	36.1	13.1	3.1	0.9
STDEV	0.3	0.115	62.1	10.46	9.71	40.3	9.6	16.2	12.8	0.7	0.4

Table 23.3: Concentration of Metals (mg/L) in Groundwater (ring well) Samples

Sites	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ca	Mg
GW1	0.01	BDL	0.01	10.86	0.31	0.03	BDL	0.17	BDL	BDL
GW2	BDL	BDL	0.00	12.29	0.15	0.02	BDL	0.11	35.3	3.9
GW3	0.01	BDL	0.03	12.73	0.13	0.07	BDL	0.28	12.8	2.9
GW4	0.00	BDL	0.01	7.15	0.06	0.07	BDL	0.24	24.1	4.9
GW5	BDL	BDL	0.00	8.14	0.12	0.04	BDL	0.16	16.0	3.9
GW6	BDL	BDL	0.01	13.90	0.02	0.10	BDL	0.14	16.0	2.0
GW7	BDL	BDL	0.01	8.94	0.75	0.02	BDL	0.22	BDL	BDL
GW8	BDL	0.07	0.00	27.56	0.56	0.13	BDL	0.20	14.4	BDL
GW9	BDL	0.13	0.00	6.09	BDL	BDL	BDL	0.18	16.0	2.0
GW10	BDL	0.26	0.00	7.75	BDL	BDL	BDL	0.12	24.1	4.9
GW11	BDL	BDL	0.05	17.22	BDL	0.02	BDL	0.17	19.2	2.0
GW12	BDL	BDL	0.02	15.26	BDL	0.06	BDL	0.20	40.1	7.8
GW13	BDL	BDL	0.00	6.80	0.01	0.05	BDL	0.20	12.8	BDL
GW14	BDL	BDL	0.01	11.13	BDL	0.01	BDL	0.13	32.1	4.9
MIN	BDL	BDL	0.00	6.09	BDL	BDL	BDL	0.11	BDL	BDL
MAX	0.01	0.26	0.05	27.56	0.75	0.13	BDL	0.28	40.1	7.8

MEAN	0.00	0.03	0.01	11.84	0.15	0.04	0.00	0.18	17.6	2.8
STDEV	0.00	0.08	0.01	5.64	0.23	0.04	0.00	0.05	11.7	2.4
WHO (2011)	0.03	0.05	2.00	0.30	0.40	0.07	0.01	–		

Conclusion

The pH was seen to be in the neutral range. Fluoride contamination is not seen and the groundwater in the region is soft. From the study of the area it was found that Mn, Cd, Cr, Cu, Ni and Pb are within the permissible limit. While very high concentration of Fe was found (mean11.84 mg/L).Nitrate and phosphate is high since it is basically an agricultural area.

Acknowledgement

The work is supported by the Rajib Gandhi National Fellowship (UGC).

References

1. Jalali, M., Application of Multivariate Analysis to Study Water Chemistry of Groundwater in a Semi-arid Aquifer, Malayer, Western Iran, *Desalination and Water Treatment,* 2010, **19**, 307–317.
2. Moussa, A.B., Zouari, K., Valles, V. and Jlassi, F., Hydrogeochemical Analysis of Groundwater Pollution in an Irrigated Land in Cap Bon Peninsula, North-Eastern Tunisia. Arid Land Research and Management, 2012, **26**, 1–14.
3. Balderacchi, M., Benoit, P., Cambier, P., Eklo , O.M., Gargini , A., Gemitzi ,A., Gurel , M., Klove, B., Nakic, Z., Predaa, E., Ruzicic, S., Wachniew, P. and Trevisan, M., Groundwater Pollution and Quality Monitoring Approaches at the European Level, *Critical Reviews in Environmental Science and Technology,* 2013, **43**, 323–408.
4. Bilal Butt, S. and Riaz, M., Determination of Cations and Anions in Environmental Samples by HPLC: Review, *Journal of Liquid Chromatography & Related Technologies*, 2009, **32**, 1045–1064.
5. Singh, P.K., Panigrahy, B.P., Tiwari, A.K., Kumar, B. and Verma, P., A Statistical Evaluation for the Groundwater Quality of Jharia Coalfield, India, *Int. J. ChemTech Res*, 2014-2015, 7, Part 4, 1880-1888.
6. Deshmukh, K. K., The Chemistry of Groundwater in Sangamner Area with regard to their Suitability for Drinking Purposes, *Rasayan J. Chem,* 2011, **4, No.4**, 770-779.
7. Singh. K.P., Malik, A., Sinha, S., Mohan, D. and Singh, V. K., Exploring Groundwater Hydrochemistry of Alluvial Aquifers using Multi-way Modeling, *Analytica Chimica Acta*, 2007, **596**, 171–182.
8. Singh, S., Raju, N.J. and Ramakrishna, C., Evaluation of Groundwater Quality

and Its Suitability for Domestic and Irrigation Use in Parts of the Chandauli-Varanasi Region, Uttar Pradesh, India, Journal of Water Resource and Protection, 2015, **7**, 572-587.

9. Thorbjornsen, K. and Myers, J., Identifying Metals Contamination in Groundwater Using Geochemical Correlation Evaluation, *Environmental Forensics*, 2007, **8**, 25–35.
10. Raju, N.J., Ram, P. and Dey, S., Groundwater Quality in the Lower Varuna River Basin, Varanasi District, Uttar Pradesh, *Journal Geological Society of India*, 2009, **73**, 178-192.
11. Khound, N.J., Phukon, P. and Bhattacharyyaa, K. G, Physico-chemical Studies on Surface Water Quality in the Jia-Bharali River Basin, North Brahmaputra Plain, India, *Archives of Applied Science Research*, 2012, **4**, Part 2, 1169-1174.
12. Kotoky, P., Tamuli, U., Borah, G C., Baruah, M. K., Sarmah, B. K., Paul, A. B. and Bhattacharyya, K. G, A Fluoride Zonation Map of the Karbi Anglong District, Assam, India, *Research Report Fluoride,* 2010, **43**, Part 2, 157–159.
13. Zakir, H. M., Rahman, M. M., Rahman, A., Ahmed, I. and Hossain, M. A., Heavy Metals and Major Ionic Pollution Assessment in Waters of Midstream of the River Karatoa in Bangladesh, *J. Environ. Sci. & Natural Resources,* 2012, **5**, Part2, 149-160.
14. World Health Organization. Guidelines for Drinking-Water Quality. 2004, 3rd edn.
15. Musa, O. K., Kudamnya, E. A., Omali, A. O. and Akuh, T. I., Physico-chemical Characteristics of Surface and Groundwater in Obajana and its Environs in Kogi State, Central Nigeria, *Afri. J. Environ. Sci. Technol,* 2014, **8**, Part 9, 521-531.
16. Deshmukh, K. K., Impact of Human Activities on the Quality of Groundwater from Sangamner Area, Ahmednagar District, Maharashtra, India, *International Research Journal of Environment Sciences*, 2013, **2**, Part 8, 66-74.

24

Role of Selenium Dioxide as a Reagent in Organic Chemistry

Badaker M. Laloo

Abstract

Selenium is used as a catalyst for many organic reactions. Selenium dioxide finds vast utility in industrial and laboratory syntheses, especially in organoselenium chemistry. Selenium and its compounds have found wide commercial use in catalytic liquid phase oxidations and isomerisation of unsaturated oils. In many reactions particularly liquid phase reactions, elemental selenium or its compounds such as the dioxide, halides, or metal selenides may be used directly in unsupported form. Selenium is also used as a dietary supplement and has been found to be beneficial for treating a number of human health problems such as Acquired immune Deficiency Syndrome (AIDS), Alzheimer's disease, arthritis, asthma, cancer, cardiovascular diseases, pancreatic, reproduction, thyroid function and viral infections. Organoselenium compounds have also proven to be attractive intermediates in organic chemistry. They display high levels of selectivity and can be used as ionic liquids in asymmetric synthesis.

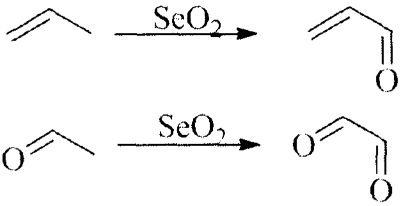
Keywords: *Selenium Dioxide, Glyoxals, Organoselenium Compounds*

Introduction

Selenium is used as a catalyst for many organic reactions. Selenium dioxide finds vast utility in industrial and laboratory syntheses, especially in organoselenium chemistry. Selenium and its compounds have found wide commercial use in catalytic liquid phase oxidations and isomerisation of unsaturated oils. Their utility can be broadened due to several properties that these compounds exhibit. They have been found to be resistant to poisons and their utility is enhanced by their ease of application. In many reactions particularly liquid phase reactions, elemental selenium or its compounds such as the dioxide, halides, or metal selenides may be used directly in unsupported form. Elemental selenium or selenium dioxide may also be vapourized into reactant gas streams as vapour phase catalysts. In some cases, traces of selenium are used to promote solid silver, nickel, copper or other catalysts.

Applications and Reactions

Selenium dioxide functions as a mild oxidizing agent over a wide temperature range, and oxidations of organic compounds with this substance have been likened to auto-oxidation or per-oxidation. While the reduction of selenium dioxide and selenites to selenium by various organic compounds have been studied by investigators before 1900, the specific oxidizing action of selenium was first noted be Riley in 1930-1932. Riley discovered that selenium dioxide exhibits a specific oxidizing action on methyl and methylene radicals adjacent to carbonyl groups in aldehydes and ketones. Riley oxidation was used to prepare many previously inaccessible glyoxals and α, β- diketones, and triketone derivatives from aliphatic, alicyclic, aromatic, and terpene aldehydes and ketones. The ease with which the oxidation products polymerize under normal conditions resulted in very good yield of the products [1].



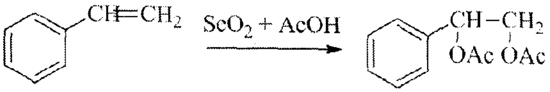
Riley Oxidation

Guillemonat proposed the path of selenium dioxide mediated olefin oxidation based on a set of rules [2]:

1. The oxidation always occurs á to the most substituted end of the double bond.
2. Whenever a double bond is in a ring, wherever possible, oxidation occurs within the ring.
3. The order for preference of oxidation is CH_2> CH_3>CH.

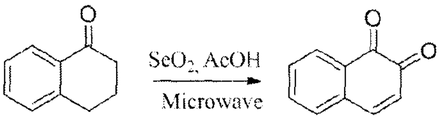
Tsutsumi *et. al.* in 1968 performed the oxidation of styrene with selenium dioxide in presence of glacial acetic acid. The reaction was carried out at 150-

160°C in an autoclave to produce styrene glycol diacetate in good yields. The other products were phenyl glyoxal and styrene glycol monoacetate [3].



1, 2-dicarbonyl compounds have also been synthesized by selenium dioxide oxidation by microwave irradiation and was found to be greatly accelerated by closed vessel microwave irradiation [4].

Under CVMW assisted selenium dioxide oxidation, 1-tetralones can be readily converted to 1, 2-naphthoquinones. The reaction was carried out in the presence of glacial acetic acid as solvent medium [5].



Besides metallic selenium, metal selenites and selenides are also used. Selenides of antimony, cobalt, lead or silver are preferably used as these compounds decompose at the reaction temperature to form metallic selenium.

Selenium is also used as a promoter of catalysts by merely adding it to the reaction mixture. More often however, it is already directly incorporated into the catalyst by co-precipitation. Silver catalysts containing selenium may be prepared by co-precipitating silver carbonate and silver selenate and then reducing the mixture to the catalyst sponge. Generally, supported catalysts containing elemental selenium alone or in combination with other metals may be prepared from any selenium compound that is converted to elemental selenium by heating or reduction. The activity of catalysts incorporating this element will decrease in reactions where selenium is lost from the composition on account of high volatility. In such circumstances, the reaction feeds are passed over molten selenium.

In 2003, it was found that the global consumption of selenium was about 2000t/ year. Selenium and its compounds have found vast utility in industries such as glass, chemical and pigments, electronics, metallurgy as well as other uses. In glass manufacturing, selenium has been found to decolourize the green tint caused by iron impurities present in glass containers and other soda-lime silica glass. It is also used in art and other glass, such as that used in traffic a ruby red colour and in architectural plate glass, to reduce solar heat transmission through the glass. More than one-half of the metallurgical selenium is used as an additive to cast iron, copper, lead and steel alloys. Small quantities of selenium (0.02%) when added to low antimony lead alloys improves the casting and mechanical properties of the alloy. Agricultural industries also employ about 9% of selenium market [6].

Selenium has been found to exhibit antioxidant and other curative properties which have facilitated research for alleviating a number of health problems. Selenium is also used as a dietary supplement and has been found to be beneficial for treating a number of human health problems such as Acquired immune Deficiency Syndrome (AIDS), Alzheimer's disease, arthritis, asthma, cancer, cardiovascular diseases, pancreatic, reproduction, thyroid function and viral infections. Ongoing research on the role of selenium in reducing the risk of skin cancer from prolonged exposure to ultra-violet light is underway.

Conclusion

Selenium and organoselenium compounds find increasing importance in carrying out versatile synthetic transformations. Organoselenium compounds have also proven to be attractive intermediates in organic chemistry. They display high levels of selectivity and can be used as ionic liquids in asymmetric synthesis. They exhibit fluorescent properties and are often linked to important biological properties.

References

1. Riley, H.L, Morley, J. F, and Friend, N. A. C., *J. Chem. Soc.*, **1932**, 1875.
2. Gulllemonat, A., *Compt. rend.*, 1935, **200**, 1416.
3. Javaid, A., Khan, Sonoda, Noburu, Tsutsumi, and Shigeru., *Bulletin of the Chemical Society of Japan.*, 1969, **42**, 2056.
4. Goswami, S.; Adak, A. K., *Synth. Commun.*, 2003, **33**, 475.
5. Gerlman, Danny M, and Perlmutter, Patrick, *Tetrahedron Letters*, 2009, **50**, 39.
6. George, W. M., *U.S. Geological Survey Minerals Yearbook*, **2003**, 65.

25

Microwave Assisted Michael Addition of Nitroalkane to Nitroolefin in Aqueous Medium at Neutral pH

Porag Bora
Ghanashyam Bez

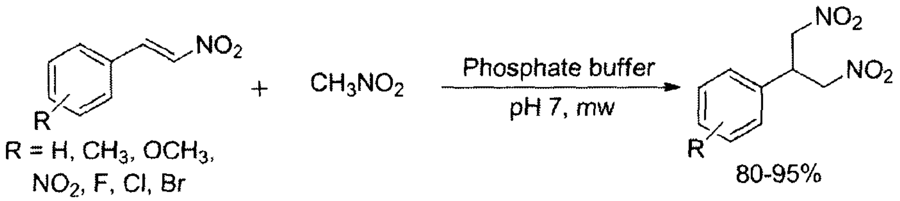
Abstract

An operationally simple method has been developed for the conjugate addition of nitroalkanes to nitroolefin in aqueous phosphate buffer at pH 7.0 under microwave irradiation without using any additional base. The reaction affords good yields of 1,3-dinitro adducts at 60°c.

Keywords: *Base-free, Michael reaction, High Temperature, Microwave Irradiation, Nitroolefin, Nitroalkane, Phosphate Buffer, pH 7.0.*

Introduction

Michael addition which leads to the formation of 1,3-dinitro compounds is one of the most important C"C bond forming reaction in organic synthesis. 1,3-Dinitro compounds are versatile intermediates for a variety of 1,3-difunctionalized molecules, heterocycles, carbohydrate derivatives and potentially active energetic material [1]. Though, nitroolefins are widely used as Michael acceptors for the C-C bond formation reactions, because of its high reactivity it is difficult to control the addition of nitronate anion to nitroolefins and generates a mixture of oligomerized product [2]. In continuation to our interest [3] in the development of green synthetic protocols we have developed an efficient method for Michael addition of nitroalkane to nitroolefin in aqueous medium at neutral pH (SchemeI). The reaction affords very good yield within a short period of time.



Scheme I

Since the pioneering experiments by Gedye *et al.* [4] and Giguere *et al.* [5], the use of microwave irradiation as an energy-efficient heat source for organic reactions has seen widespread applications [6]. Microwave assisted reactions often benefit from short reaction times, higher yields, and improved selectivities. So, it is not surprising that microwave assisted reactions have been found advantageous for conductive heating for accelerating chemical reactions [7]. Recently, we observed that the use of aqueous phosphate buffer can yield the nitroalcohol from the reaction of aldehyde and nitroalkane without any trace of nitroalkene side product [3]. Based on that observation we carried out a Michael reaction of nitroalkane to nitro olefin at neutral pH at room temperature. But due to prolong reaction time at room temperature development of an alternative procedure was important. Given the fact that use of microwave irradiation is known to reduce the reaction time drastically, we wanted to explore if the reaction can be carried out at elevated temperature to reduce the reaction time. we report here an extremely efficient method for the synthesis of nitroalcohols in aqueous phosphate buffer under microwave irradiation at 60 °C.

Experimental

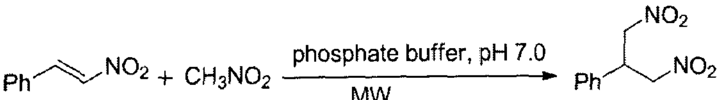
Buffer Tablet pH 7.0 (product no. 43155, batch no. 0198/Mfg/6385/81) was purchased from S.D. Fine Chemicals Ltd., India. The reaction was carried out in a CEM Discover benchmate microwave synthesizer at 60°C under closed vessel conditions. Infra-red (IR) spectra were recorded on a Shimadzu IR-460 spectrometer. CEM Discovery Benchmate (operating frequency 2450 MHz) was used to carry out the reactions under microwave irradiation. Nuclear Magnetic Resonance (NMR) such as ^{1}H NMR spectra were recorded at 400 and ^{13}C NMR spectra at 100 and on Bruker AC-400 MHz using deuterochloroform ($CDCl_3$) as solvent and tetramethylsilane (TMS) as internal standard.

Microwave synthesis of(1,3-dinitropropan-2-yl)benzene as a Representative Procedure: The aqueous phosphate buffer solution (pH 7.0, 0.5 mL) was added to a microwave tube containing a mixture of *trans*-β-nitrostyrene (0.25mmol) and nitromethane (15.25 mg, 0.75 mmol) and the tube was introduced into the microwave system (CEM-Discover) and initially irradiated at 150W and 60 °C for 20 min. The reaction mixture was extracted with ethyl acetate (3×15 mL), The solvent of the combined organic phases was dried with anhydrous sodium sulfate, evaporated under reduced pressure, and the resulting reaction crude was purified by column chromatography (ethyl acetate/hexane) to obtain the pure product.

Results

We started our investigation by using the reaction between *trans*-β-nitrostyrene and nitromethane as a model reaction in phosphate buffer at pH 7, applying only two equivalents of nitromethane. To start with, we carried out the pilot reaction in aqueous phosphate buffer by vigorously stirring the mixture under microwave irradiation for 30 minute in a closed vessel CEM Discovery Benchmate microwave synthesizer at 100 °C. The starting nitroolefin were found to be exhausted, but the isolated yield was 65% due to the formation of other unidentified products. When the irradiation was carried out at 60 °C keeping other parameters intact, the reaction completed within 30 min to give the product in 82% isolated yield. By increasing the amount of nitromethane to 3 equivalents of the aldehyde led to a further decrease in reaction time (20 min) for the pilot reaction. But the reduction of temperature to 40 °C reduced the reaction yield to 68% in 0.5 h.

Table 25.1: Optimization of Reaction Condition



1a-j

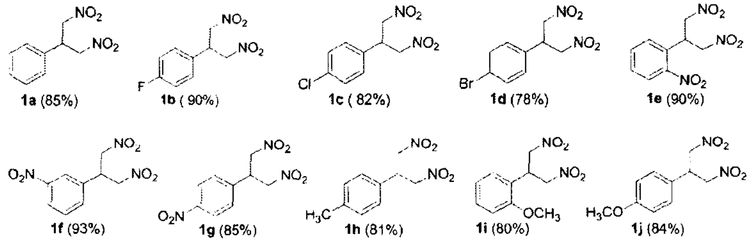
Entry	Reaction Conditions[a]	% Yield[b]
1	100 pC, 30 min	65
2	60 p C, 30 min	82
3	40 pC, 30 min	68
4	60 pC, 20 min	85[c]

[a] 0.25mmol of *trans*-β-nitrostyrene, 0.5mmol of nitromethane

[b] isolated yields

[c] 0.75mmol of nitro methane used

Table 25.2: Synthesis of 1,3-dinitropropanes[a, b]



[a]General reaction conditions: 60°C, phosphate buffer (0.5mL), β-nitrostyrene (0.25 mmol), nitroalkane (0.75 mmol)

Conclusion

In summary, we have developed a practical route for rapid synthesis of 1,3-dinitropropanes by Michael reaction in aqueous phosphate buffer in the absence of any added base catalyst. The reaction time could be reduced drastically by elevating the temperature to 60°C under microwave irradiation without affecting the yield.

References

1. Ballini, R., Palmieri, A., and Righi, P., *Tetrahedron*, 2007, **63**, 12099–12121.
2. Rabalakos, C., and Wulff, W. D., *Synlett.*, 2008, **18**, 2826–2830.
3. Bora, P. P., and Bez, G., *Eur. J. Org. Chem.*, 2013, 2922–2929.
4. Gedye, R., Smith, F., Westaway, K., Ali, H., Baldisera, L., Laberge, L., and Rousell, J., *Tetrahedron Lett.*, 1986, **27**, 279-282.
5. Giguere, R.J., Bray, T.L., Duncan, S.M., and Majetich, G., *Tetrahedron Lett.*, 1986, **27**, 4945-4946.
6. Kappe, C.O., *Angew. Chem. Int. Ed.*, 2004, **43**, 6250-6284.
7. Adam, D., *Nature*, 2003, **421**, 571-672.

26

Synthesis of Symmetrical α, α'-Dicarbonyl Selenides from Aryl Methyl Ketones in presence of Selenium Dioxide and *p*-Toluenesulfonic Acid Monohydrate

O. Risuklang Shangpliang
Bekington Myrboh

Abstract

A simple route to α, α'-dicarbonyl selenides has been developed. A coupling reaction between aryl methyl ketones and selenium dioxide takes place in the presence of *p*-TsOH.H_2O, leading to C–Se bond formation.

Keywords: *Selenium Dioxide/C-Se coupling/p-TsOH.*H_2O

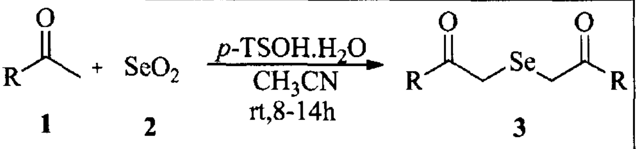
Introduction

Selenium is an important trace element involved in different physiological functions of the human body. Organoselenium compounds have substantially greater bioavailability than that of inorganic selenium [1]. More importantly, organic selenium is usually found to be less toxic than inorganic forms 2-5]. Lowig, in 1836 prepared the first organoselenium compound, diethyl selenide [6]. Organoselenium compounds have attracted much attention in recent decades due to their important biological effects [7] and their application as chiral catalysts [8], anticancer, antitumor, antiviral, antimicrobial, and antioxidant properties [9].

Traditional methods require the use of strong reducing agents such as Na or NaH, and harsh reaction conditions, such as high reaction temperatures, UV light, and the use of toxic solvents like HMPA [10]. To overcome these drawback, an alternative method have emerged for the synthesis of selenides by using transition metal catalyst like Palladium [11], nickel [12], lanthanum [13], iron [14], indium [15], and copper-based [16]. However, most of these reactions take a long time to complete. To avoid the contamination of the product selenides with transition metals, the development of synthetic methods in the absence of transition-metal catalysts is desirable. Moreover, one of the two commonly used selenium sources, aryl selenol, is foul-smelling, and the other, diaryl diselenide, is not always commercially available and requires extra preparation steps. Therefore, it is very desirable to explore reaction conditions with an easily available selenium source. Here, we report the synthesis of α, α'-dicarbonyl selenides from aryl methyl ketones and easily available selenium dioxide in the presence of *p*-toluenesulfonic acid monohydrate.

Results and Discussion

Herein we report the synthesis of α,α – dicarbonyl selenides from aryl methyl ketones in the presence of selenium dioxide and *p*-toluenesulfonic acid monohydrate as depicted in **Scheme I**.



Scheme 1

Thus, treatment of *p*-methylacetophenone **1a** (1 equiv) with selenium dioxide **2** (0.5 equiv) in the presence of *p*-toluenesulfonic acid monohydrate (0.5 equiv) and acetonitrile as a solvent (3 mL) at room temperature for 8 hours afforded 2,2'-selenobis(1-(p-tolyl)ethanone) **3a** in good yield (58%) (**entry 1, Table 26.1**).

It may be mentioned here that *p*-toluenesulfonic acid monohydrate appears to be specific for these reactions since the use of other Lewis acids ($AlCl_3$, $SnCl_4$) either gave no reaction or resulted in the formation of multiple products that are difficult to isolate. Similarly, the reaction of **1b, 1c, 1d** and **1e** with selenium dioxide **2** and *p*-toluenesulfonic acid monohydrate in acetonitrile at room temperature for 8-14 hours proceeded smoothly to give the α,α –dicarbonyl selenides **3b, 3c, 3d** and **3e** respectively in moderate to good yields (entries **2, 3, 4** and **5, Table I**).

Table 26.1: Reaction of Aryl Methyl Ketones with SeO_2 in the presence *p*-$TsOH.H_2O$

Entry	Substrate 1 (R)	Product 3	Yield (%)
1	4-$CH_3C_6H_4$**1a**	**3a**	58
2	C_6H_5**1b**	**3b**	55
3	4-ClC_6H_4**1c**	**3c**	60
4	4-BrC_6H_4**1d**	**3d**	62
5	4-$CH_3OC_6H_4$**1e**	**3e**	61

Conclusion

We have developed a simple and efficient method for the synthesis of α,α'–dicarbonyl selenides from aryl methyl ketones in the presence of selenium dioxide and *p*-toluenesulfonic acid monohydrate. The reaction proceeds efficiently at ambient temperature and ordinary reaction conditions. The attractiveness of the method is further augmented by the fact that the starting materials used are quite common and inexpensive.

Acknowledgments

The authors thank the DST-PURSE for financial assistance under project number (No. F. 1/NEHU/DST-FIST/PURSE/2011-515), SAIF NEHU, CDRI Lucknow and IIT Bombay for spectral analysis.

References

1. Cantor, A., Scot, M., and Noguch, T., *J. Nutr* ., 1976, **105**, 96.
2. Bendsleve, D.A., Abdulla, M., Jepsrn, A. and Pedeson, E., *Trace. Elem. Med.*, 1988, **5**, 29.
3. Khalil, A.M., *Mutat. Res* ., 1989, **224**, 503.
4. Khalil, A.M., Toxicol. *Environ. Chem* ., 1994, **41**, 147.
5. Saito, Y., Fiji, T., Honda, M., Maeda, A., Seo, H. and Chikuma, M., In; Proceedings of the seventh International Symposium, Selenium in Biology and Medicine, Venenzia, 2000, 1.
6. Lowig, C., *J. Pogg. Ann* ., 1836, **37**, 552.
7. (a) Kumar, B. S.; Kunwar, A.; Singh, B. G; Ahmad, A., and Priyadarsini, K. I., *Biol. Trace Elem. Res.*, 2011, **140**, 127; (b) Terazawa, R.; Garud, D. R.; Hamada, N.; Fujita, Y.; Itoh, T.; Nozawa, Y.; Nakane, K.; Deguchi, T.; Koketsu, M., and Ito, M., *Bioorg. Med. Chem.*, 2010, **18**, 7001; (c) Borges Filho, Carlos; Del Fabbro, Lucian; Boeira, Silvana P.; Furian, Ana Flavia; Savegnago, Lucielli; Soares, Letiere Cabreira; Braga, Antonio Luiz, and Jesse, Cristiano R., *Cell Biochem. Funct.*, 2013, **31**, 152;

(d) Silva, M. H.; Rosa, E. J. F.; Carvalho, N. R.; Dobrachinski, F.; Rocha, J. B. T.; Mauriz, J. L.; Gonzalez-Gallego, J., and Soares, F. A. A., *Neurotox. Res.*, 2012, **21**, 334.

8. (a) Godoi, M.; Paixao, M. W., and Braga, A. L., *Dalton Trans.*, 2011, **40**, 11347; (b) Andrade, L. H.; Silva, A. V.; Milani, P.; Koszelewski, D., and Kroutil, W., *Org. Biomol. Chem.*, 2010, **8**, 2043; (c) Braga, A. L.; Ludtke, D. S., and Vargas, F., *Curr. Org. Chem.*, 2006, **10**, 1921; (d) Braga, A. L.; Ludtke, D. S.; Vargas, F., and Braga, R. C., *Synlett.*, 2006, 1453.
9. (a) Sarma, B. K.; Manna, D.; Minoura, M., and Mugesh, G., *J. Am. Chem. Soc.*, 2010, **132**, 5364; (b) Back, T. G., and Moussa, Z., *J. Am. Chem. Soc.*, 2003, **125**, 13455; (c) Anderson, C.-M.; Hallberg, A., and Hogberg, T., *Adv. Drug Res.*, 1996, **28**, 65.
10. (a) Tiecco, M.; Testaferri, L.; Tingoli, M.; Chianelli, D., and Montanucci, M., *Tetrahedron Lett.*, 1984, **25**, 4975; (b) Tiecco, M.; Testaferri, L.; Tingoli, M.; Chianelli, D., and Montanucci, M., *J. Org. Chem.*, 1983, **48**, 4289; (c) Suzuki, H.; Abe, H., and Osuka, A., *Chem. Lett.*, 1981, **1**, 151; (d) Rossi, R. A., and Penenory, A. B., *J. Org. Chem.*, 1981, **46**, 4580.
11. (a) Ranu, B. C.; Chattopadhyay, K., and Banerjee, S., *J. Org. Chem.*, 2006, **71**, 423; (b) Fukuzawa, S.; Tanihara, D., and Kikuchim, S., *Synlett,*, 2006, 2145.
12. Taniguchi, N., *J. Org. Chem.*, 2004, **69**, 6904.
13. Murthy, S. N.; Madhav, B.; Reddy, V. P., and Nageswar, Y. V. D., *Eur. J. Org. Chem.*, 2009, **34**, 5902.
14. Wang, M.; Ren, K., and Wang, L., *Adv. Synth. Catal.*, 2009, **351**, 1586.
15. Ranu, B. C., and Mandal, T., *J. Org. Chem.*, 2004, **69**, 5793.
16. (a) Li, Y.; Nie, C.; Wang, H.; Li, X.; Verpoort, F., and Duan, C., *Eur. J. Org. Chem.*, 2011, *2011*, 7331; (b) Kumar, S., and Engman, L., *J. Org. Chem.*, 2006, **71**, 5400; (c) Taniguchi, N., and Onami, T., *J. Org. Chem.*, 2004, **69**, 915; (d) Ricordi, V. G., Freitas, C. S., Perin, G., Lenardao, E. J., Jacob, R. G., Savegnago, L., and Alves, D., *Green Chem.*, 2012, **14**, 1030.

27

Synthesis, Structure, Characterization and Properties of Heterobimetallic Complex $[Cu\ Ni(\mu\text{-}OAc)(\mu\text{-}OH)(\mu\text{-}OH_2)](BF_4)_2$ from Bipyridine

Sunshine D. Kurbah
R.A. Lal

Abstract

Hetero-bimetallic complex $[NiCu(\mu\text{-}OH)(\mu\text{-}OH_2)(\mu\text{-}OAc)(bipy)_2](BF_4)_2$ was synthesized in moderate yield through solid state reaction at 150 °C and has been characterized by elemental analysis, molar conductance, mass spectrum, magnetic moment, cyclic voltammetric studies, IR and UV–Vis spectroscopic studies. The structure of the complex has been established by X-ray crystallography. The complex functions as an efficient catalyst in aqueous medium towards oxidation of alcohols to carbonyl compounds mediated by hydrogen peroxide.

Introduction

The multimetallic complexes are the focus of intense research in contemporary inorganic chemistry because such complexes have played significant roles in development of magnetochemistry [1] bioinorganic chemistry [2], synthesis of mixed metal oxides [3] and catalysis [4]. In particular, the heterodinuclear cores are present at the active sites of some enzymes like purple acid phosphatase [FeZn] [5], human calcineurin [FeZn] [6] and human protein phosphatase [MnFe] [7] respectively. This has stimulated interest in functions of heterodinuclear metal complexes. When two or more metals are present in a single molecular system, a synergic effect results [8]. This give rise to unique physico-chemical properties that arise from metal-metal interaction [9]. This ultimately leads to cooperative interaction between metal ions giving rise potential to the system to mediate certain chemical reactions of industrial relevance either more efficiently than an isolated metal centre, monometallic and homobimetallic systems or in a different manner.

Hence, it was imperative to synthesize some new heterometallic complexes from some simple organic molecules by "direct synthesis method" and to characterize them by various physico-chemical studies and to establish their structure by X-ray crystallography. Accordingly, we have chosen some biologically important metal ions such as copper and nickel and isolate their heterometallic complexes from bipyridine to establish their structure by X-ray crystallography. Further, we aimed at studying the catalytic properties of the complex so obtained.

Results and Discussion

The heterobimetallic complex $[NiCu(\mu\text{-}OH)(\mu\text{-}OH_2)(\mu\text{-}OAc)(bipy)_2](BF_4)_2$ was obtained through solid state reaction of $Cu(OAc)_2.H_2O$, $Ni(OAc)_2.4H_2O$, 2,2'-bipyridine and $NaBF_4$ in open air at 150 ^{0}C for 5 h using electronic oven. Single crystal suitable for X-ray analysis was obtained by slow evaporation of the of the solvent at room temperature. The complex consists of dinuclear $[CuNi(\mu\text{-}OH)(\mu\text{-}OH_2)(\mu\text{-}OAc)(bpy)_2]^{2+}$ cation and two well separated tetra fluoroborate anions (Fig. 1). In the cation, Cu^{II} and Ni^{II} are double bridged by the endogenous hydroxido oxygen and aquo oxygen, respectively, with the Cu....Ni separation being 3.0289(2) Å. The Cu^{II} and Ni^{II} are also bridged by an acetato group in the bidentate η^1, η^1, μ-bridging mode, packing diagram of the complex is shown in Fig. 2 along with Hirschfeld analysis. The arrangement of ligand atoms around the metal ions provides distorted five-coordinate geometry for both the metal ions.

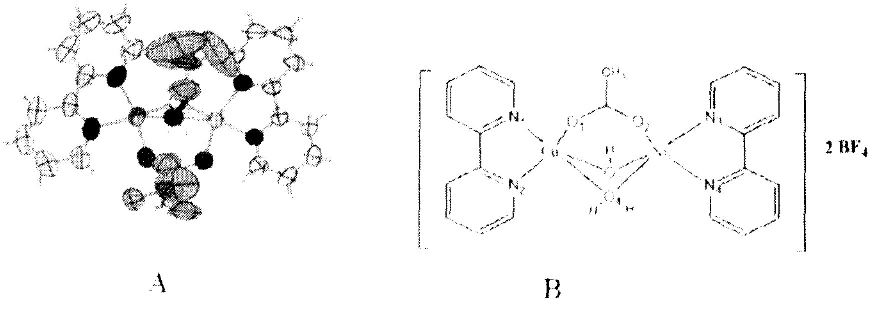


Fig. 27.1: ORTEP Plot of Complex (A) along with the Chemical Structure

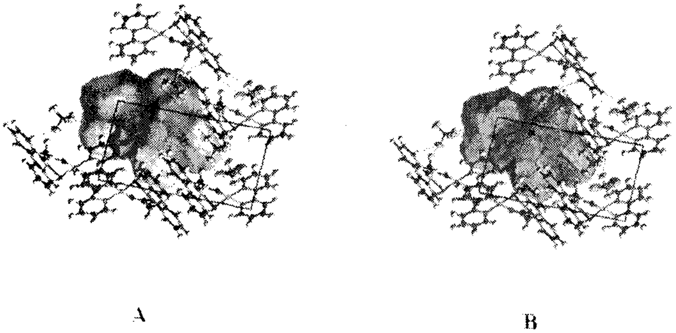
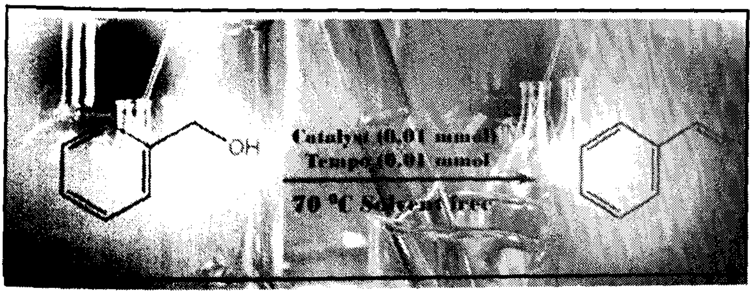


Fig. 27.2: Packing Diagram of the Complex along with Hirschfeld Surface Plot d_n (A) and d_{curve} (B)

In the present study, we have carried out limited study on oxidation of alcohols to aldehydes and ketones in aqueous medium using the newly synthesized heterobimetallic complex $[CuNi(\mu\text{-}OAc)(\mu\text{-}OH)(\mu\text{-}OH_2)(bpy)_2](BF_4)_2$. The study revealed that a concentration of 0.010 mmol of catalyst at temperature of 70 °C oxidized to benzaldehyde (90%) by 15% H_2O_2 with a selectivity of 93% in a time period of 5 h (Scheme 1). This reaction has provided a new environmentally friendly catalyst for the oxidation of primary and secondary benzylic alcohols to the corresponding carbonyl compounds, respectively.



Scheme I.

Characterization Data for Benzaldehyde

Colorless liquid; Yield: 93 %; ^1H (400 MHz, $CDCl_3$): δ 9.96 (s, 1H), 7.87-7.41 (m, 5H); 1 3C (100 MHz, $CDCl_3$): δ192.5, 136.2, 134.4, 129.8, 128.9; IR (KBr): 1702 cm^{-1}.

In conclusion, we have synthesized a highly efficient catalytic i.e., heterobimetallic complex for oxidation of alcohols to corresponding aldehydes and ketones. The method employs inexpensive and easily available starting materials which allow the oxidation of a wide variety of primary and secondary alcohols to corresponding aldehydes or ketones in high yield, mild oxidation conditions and easy selectivity make this catalyst system highly practical.

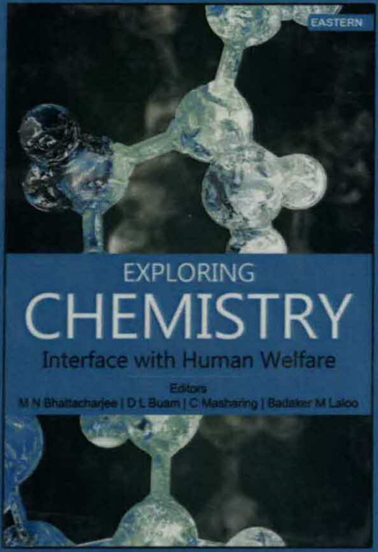
Acknowledgement

Authors would like to thank to the Head, SAIF, North-Eastern Hill University, Shillong-793022, India for recording ^{1}H and ^{13}C NMR spectra. Sunshine D. Kurbah would like to thank North-Eastern Hill University, Shillong-793022, India for award of University fellowship.

Reference

1. Pardo, E., Ruiz-Garcia, R., Lloret, F., Julve, M., Cano, J., Pasan, J., Ruiz-Perez, C., Filali, Y., and Chamoreau. L-M., *Inorg. Chem*, 2007, **46**, 4504-4517.
2. Gheorghe, R., Madalan, A. M., Plostes, J., Wernsdorfar, W., and Andruh, M., *Dalton Trans*, 2010, **39**, 4734.
3. Seisenbaeva, GA., Kessler, V.G, Pazik, R., and Strek, W., *Dalton Trans.*, 2008, **34**, 21.
4. Lassahn, P.-G., Lozan, V., Timco, G A., and Christian, P., *et. al.*, *J. Catal,*, 2004, **222**, 262.
5. Nesterov, D. S., Kokozay, V. N., Jezierska, J., Pavlyuk, O. V., Boca, R., and Pombeiro, A. J. L., *Inorg. Chem.*, 2011, **50**, 4401-4411.
6. Lassahn, P. G., Lozan, V., Timco, G A., Christian, P., Jamiak, C., and Winpenny, R. E. P., *J. Catal*, 2004, **222**, 260.
7. Shilor, A. E., and Shulpin, G. B., *Chem. Rev*, 1997, **97**, 2879.
8. Lin, J., and Ma, S., *Org. Lett.*, 2013, **15**, 5150.
9. Breunfuhrar, A., Neuman, H., and Beller, M., *Angew. Chem Int. Ed.*, 2009, **48**, 4114-4133.

Index



About the Book

This publication contains the papers presented by a number of chemists, academicians and eminent personalities in the National Seminar on "Recent Advances: Exploring Chemistry for Human Welfare", organised by the Department of Chemistry, Shillong College, Shillong and IQAC, Shillong College, Shillong on 30th and 31st July, 2015. Chemistry is used in every field – physics, biology, earth science, material science, medicine, engineering etc. In the 21st century, chemistry has become the largest scientific discipline, producing over half a million publications a year ranging from direct empirical investigations to substantial theoretical work. In this age of convergence and synergy, chemistry and chemical technology finds common ground with biology, and information & communications technology. The papers included in this publication depict some of the emerging areas in Chemistry which are important in public welfare, and progress of civilisation.

About the Editors

Dr. M. N. Bhattacharjee is an Associate Professor and Head of the Department of Chemistry, Shillong College, serving the College since 1985. He is involved in Science popularisation programmes and has number of research publications.

Dr. (Smt.) D. L. Buam is an Associate Professor in the Department of Chemistry, Shillong College, serving the College since 1991. She is proficient in organising Seminars etc. and also has number of research publications.

Dr. C. Masharing is an Assistant Professor in the Department of Chemistry, Shillong College, serving the College since 2006. He has number of research publications in Chemistry and mainly working in the field of Coordination and Organometallic Chemistry.

Dr. (Smt.) Badaker M. Laloo is an Assistant Professor in the Department of Chemistry, Shillong College, serving the College since 2013. She has number of research publications in Chemistry and mainly working in the field of Organic Chemistry.

ISBN: 978 93 83252 65 7
9 789383 252657
2017 $ 55.00 ₹ 1100.00

EBH Publishers (India)
an imprint of Eastern Book House®
136, M.N. Road, Panbazar, Guwahati-781001