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Contents

Editorial

Dr. Nisha T. P.
Assistant Editor

Transition to a Green Hydrogen Fuel Economy: Prospects and Pitfalls

In light of the emission of thirty seven Gigatons of carbon dioxide annually and with the dire consequences of climate change becoming increasingly evident, the worldwide pursuit of sustainable and clean energy sources is an imperative undertaking. If otherwise, by the end of twenty first century, Earth could become a mere habitat for sustaining life. This should steer up the courses toward a greener, more sustainable future. The momentum behind the hydrogen revolution has been building for some time. Long heralded as the 'fuel of the future,' hydrogen is now transitioning from a mere concept to a tangible reality. It offers a multitude of advantages, particularly in its potential to reduce carbon emissions and foster energy independence.

Global interests are to transform its heavy reliance on hydrogen fuel as an energy source in various sectors to achieve net-zero carbon technologies. Transportation, a sector historically riddled with carbon emissions, is a focal point in this journey. Hydrogen fuel cell vehicles have emerged as a viable alternative to traditional gasoline and diesel-powered cars. Hydrogen-powered buses and trucks are making their mark, paving the way for cleaner and more efficient public transportation. Further, the influence of hydrogen extends beyond transportation. Industries such as steel, cement, and chemicals have long been reliant on fossil fuels in their manufacturing processes. Hydrogen's role as a clean source of heat in these processes can drastically reduce emissions, helping industries meet ambitious climate targets.

The shift from 'grey hydrogen,' produced using fossil fuels, to 'green hydrogen' is imperative. Moreover, hydrogen utilizes fuel cells to generate electricity, releasing only heated

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air and water vapor as byproducts. This is paving the way for significant growth in stationary energy and transportation industries. The primary methods of green hydrogen production include electrolysis, which splits water into hydrogen and oxygen using renewable electricity, along with biomass gasification, and microbial electrolysis, which are environmentally friendly and sustainable. These processes are entirely sustainable and emissions-free.

India, as part of its National Green Hydrogen Mission (NGHM), is resolutely dedicated to curbing carbon emissions by 5 million tonnes by 2030, with a strong commitment to transitioning from fossil fuel-based transportation to renewable energy sources, notably hydrogen fuel, within the next five years. India also has set an ambitious goal of establishing India as a leading hub for the production, adoption, and global export of green hydrogen and its byproducts. In response, numerous Indian companies (including GAIL, NTPC, Reliance, JSW steel, etc.) are already at the forefront with electrolyzers manufacturing large-scale hydrogen in their plants. However, to meet the demands of massive hydrogen production from water, it is crucial to develop scalable methods for using renewable resources, emphasizing their further. The research community nowadays dwells on utilizing seawater for hydrogen production by alkaline electrolysis. In that context, fostering hydrogen research and bridging the gap between laboratory research and industrial applications is essential in achieving India's objectives of achieving hydrogen fuel economy.

Furthermore, the global community has a role to play in this transformation to a green hydrogen economy. Collaboration among nations is vital for achieving economies of scale and accelerating the development and deployment of hydrogen technologies. Several countries, especially European countries, have already made substantial investments in hydrogen research and infrastructure. By working together to harmonize standards, share research, and establish a robust international hydrogen market, we can hasten the transition to a hydrogen-powered future.

The transition to a green hydrogen fuel economy is a complex proposition, presenting both security and risk to the global community. On the one hand, it offers the protection of sustainable, low-carbon energy, reducing greenhouse gas emissions, improving energy independence, and stimulating economic growth. Yet, the path is not without risks, as it entails substantial upfront costs, relies on the intermittency of renewable energy sources, and necessitates complex infrastructure development, which may pose challenges. Cost remains a significant barrier, with green hydrogen production currently more expensive than hydrogen from fossil fuels. Infrastructure development, including establishing refueling stations for hydrogen vehicles and pipelines for transportation, presents a significant

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challenge. Policy and regulatory support are essential to drive investment and innovation in the hydrogen sector.

Additionally, the transition could divert resources from other pressing global issues. The key to a successful transition lies in striking a careful balance, with smart investments, technological innovation, international collaboration, and sound policies to maximize the security benefits while mitigating the inherent risks for the well-being of humanity and the planet. On this context, public perception is another critical factor in the success of hydrogen as a fuel source. Building public awareness and trust in hydrogen technology is imperative. Safety concerns have been raised, but these can be addressed through stringent safety regulations and proper education. Communicating the potential of hydrogen in reducing air pollution and greenhouse gas emissions will be essential in gaining public support.

To summarize, the path toward a more sustainable and environmentally responsible future presents formidable hurdles, particularly for a country like India. Nevertheless, hydrogen emerges as a pivotal catalyst for transformation. It's potential in reducing carbon emissions, revolutionizing transportation, improving industrial processes, and offering energy storage solutions is undeniable. As we invest in green hydrogen and embrace its potential, we move closer to a world where our energy needs are met without compromising the health of our planet. The hydrogen revolution is well underway in the country and across the geographies, and its success will be a defining factor in our journey toward a cleaner, more sustainable fuel economy.

Biological Activity of Nickel(II)-R-Aniline Complexes

R. Subashini* and Dr. A. S. Ganeshraja**

Abstract

Ni(II) chloride complexes of substituted aniline derivatives were prepared. The chemical forms of the complexes are [Ni (*Aniline*)$_4$ (*Chloride*)$_2$], [Ni(4 – *Methoxyaniline*)$_4$ (*Chloride*)$_2$] and [Ni (4 – *Fluoroaniline*)$_4$ (*Chloride*)$_2$]. All the complexes are six-coordinated, filling the coordination sphere with a combination of chloride ions, aniline based ligands and / or solvent molecules. Transition metal complexes of N-donor ligands are of interest due to their applications in biology, pharmacology, magnetism, and so forth. N-donor ligand based Ni(II) complexes have been found to be active in biological systems. These metal complexes were able to be characterized by various spectroscopic methods. The photoluminescence properties of the complexes were studied by fluorescence techniques. Antibacterial effects of these compounds were evaluated by disc diffusion and micro broth dilution method. These complexes could reasonably be used for the treatment of some common diseases caused by *Escherichia Coli*, *Vibri ocholerae* and *Staphylococcus*. A typical complex is also tested for antioxidant and anti-inflammatory activity.

Keywords: *Ni complexes, Luminescence, Aniline derivatives, Biological activity, and Antioxidant.*

*R. Subashini, M.Sc., Student, Department of Chemistry, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirapalli–620001, Tamil Nadu, India, Email: subashiniramanathan21@gmail.com

**Dr. A. S. Ganeshraja, Assistant Professor, Department of Chemistry, SRM Institute of Science and Technology, (Deemed to be University), Ramapuram Campus, Chennai–600089, Tamil Nadu, India, Email: asgchem84@gmail.com

Transition metal complexes of N-donor ligands are of interest due to their applications in biology, pharmacology, magnetism, and so forth [1]. N-donor ligands such as aniline and substituted aniline derivatives based metal like Ni(II), Cd(II), Hg(II) and Pt(II) complexes have been found to be active in biological systems (C.V. Sastri, et al. 138-145 and K. Dhaveethu, et al. 712-720). Recent publications with nickel (II) complexes and aniline ligands include many chelating ligands and are overwhelmingly six-coordinate. There has been no interest in understanding how structure and coordination of different complexes affects magneto-structural correlations, specifically in mixed organic and inorganic coordination complexes (K. G. Cavigiolio, et al. 61-68, Y. Zhang, et al. 3393–405 and I. Syed Akbar Basha, et al. 1625–31). In the case of nickel **(**II), interest also arises due to its specific properties (D. Jing, et al. 74-78). Further, nickel (II) is the prototypic metal ion. There has also been a focus around N-donating ligands, particularly because of their Lewis base and Schiff base properties. Aniline and its derivatives have been among those well studied ligands and their transition metal complexes have been reported over the past several decades including biological activity. Nowadays, bioinorganic chemists target heterocyclic ligands and their metal complexes to study their pharmacology as the main focus of research (Desguin, et al. 1–12). A wide range of biological activities such as antibacterial, antifungal, antitumor and antiviral activities are exhibited by the nitrogen-containing organic compounds and their metal complexes. In this article, complexes of the type [Ni (R – $Aniline$)$_4$ (Cl)$_2$] (R = H, -OCH_3 or F) were readily obtained by precipitation with ether from ethanolic solutions. Attempts to prepare complexes containing a ligand to ratio of less than 6:1 were not successful by different reported methods although aniline readily forms complexes of the type [Ni]. The absorbance spectra of all the [Ni] complexes are very similar and typical of octahedral nickel(II) compounds. Emission properties of prepared complexes were investigated by photoluminescence spectroscopy. The antibacterial activities of our complexes were examined and results discussed in this report.

Experimental Section

Materials and Methods

$NiCl_2.6H_2O$, aniline, 4-methoxyaniline, and 4-fluoroaniline were purchased from Himedia and SD Fine Chemicals (India). Ethanol was purified by repeated distillations over CaO. All other solvents were distilled before use and water was triple distilled over alkaline potassium permanganate in an all glass apparatus. The FTIR spectra of the complexes were recorded on a Thermo Nicolet - 6700 FT-IR instrument (KBr pellet technique). The UV-Visible spectra were undertaken in aqueous medium at room temperature on Lambda 35 model of Perkin Elmer UV-Visible range 190 nm to 1100 nm. The fluorescence spectra were undertaken on model LS 45 of Perkin Elmer ranging from 200 nm to 900 nm.

Preparation of [Ni(aniline)$_4$Cl$_2$] complex:

A solution of Ni $Cl._2.6H_2O$ (0.692g, 2.91 mmol) in 9 mL of ethanol and 1 ml of ether were added to a solution of aniline (0.634g, 0.589 mmol) with stirring. A light green precipitate began to form within minutes. After stirring, a fine green precipitate was isolated by filtration, washed with cold ethanol and allowed to dry to yield 0.863 g. The complex was filtered using Buchner funnel and the yield was collected and allowed to dry.

Preparation of [Ni (4-methoxyaniline)$_4$ Cl$_2$] complex:

A solution of Ni Cl.$_2$.6H$_2$O (0.692g, 2.91 mmol) in 9 mL of ethanol and 1 ml ether were added to a solution of 4-methoxyaniline (0.634 g, 0.589 mmol) with stirring. A light green precipitate began to form within minutes. After stirring, a fine green precipitate was isolated by filtration, washed with cold ethanol and allowed to dry to yield 0.844 g. The complex was filtered using Buchner funnel and the yield was collected and allowed to dry.

Preparation of [Ni(4-fluoroaniline)$_4$ Cl$_2$] complex:

A solution of Ni Cl.$_2$.6H$_2$O (0.692g, 2.91 mmol) in 9 mL of ethanol and 1 ml of ether were added to a solution of 4-fluoroaniline (0.634 g, 0.589 mmol) with stirring. A light green precipitate began to form within minutes. After stirring, a fine green precipitate was isolated by filtration, washed with cold ethanol and allowed to dry to yield 0.826 g. The complex was filtered using Buchner funnel and the yield was washed with ethanol and allowed to dry.

Biological Studies

The antimicrobial activity of as prepared nickel(II) complexes are tested on three different types of pathogenic bacteria such as *Escherichia coli* which were cultured on agar plates supplemented with different concentration of nickel(II) complex by Agar well method. The plates were incubated for 24 h at 37°C. Detailed procedures are given in supplementary materials.

Result and Discussion

UV-visible spectra were recorded. Aniline is a benzenoid compound. The NH_2 group attached to the benzene ring means that there is a lone pair of electrons that can enter into conjugation with the benzene ring resulting in delocalization in the aniline. Aniline absorbs in the K (220-250 nm) bands exhibited by benzenoid compounds. The K and B bands arise from π-π transition as a result of the group containing multiple bonds being attached to the benzene ring. When dissolved in ethanol, λ_{max} for aniline is 230-232 nm, but in dilute aqueous acid λ_{max} is 203 nm. In the latter case the anilinium cation is formed and the lone pair is no longer available for conjugation with the benzene ring. Consequently, the absorption of the molecules shifts to the lower λ_{max} value and behaves like benzene. It shows blue shift, when absorption intensity of a compound is increased and it is known as hyperchromic shift. Nickel(II)-R-aniline shows very weak absorption bands at 700 nm is attributed to d–d transition in Figures 1-3. It exhibits a bathochromic shift. The blue shift was attributed due to the presence of a fluoro substituted group which increased the torsional angle between phenyl rings. It also shows peaks at 227 nm and 298 nm. This may be attributed to the presence of an electron withdrawing group. A blue shift is observed in a 4-methoxy aniline complex. The wavelength for the absorption peak is due to the steric effect of -OCH_3. This make the space conformation to be tortuosity, which weighs against increasing the conjugation length. It can help to improve the electronic transition energies and contributes to the blue shift phenomenon. It also shows peaks at 223 nm and 293 nm.

The infrared spectra of [Ni(aniline)$_4$]Cl$_2$], [Ni(4-methoxyaniline)$_4$Cl$_2$] and [Ni(4-fluoroaniline)$_4$Cl$_2$] are shown in Figures: 4-6. In nickel(II)-aniline complexes, the peaks are 568 – 605 cm^{-1} (an in-plane ring deformation). It shows N-H stretching bands in the range 1600 cm^{-1} to 1585 cm^{-1}. This shows that it is an aromatic compound and the peak at 1500–1400 cm^{-1} is due to

carbon-carbon stretching vibration. In nickel(II)-4-fluoroaniline, the peaks at 1487 cm^{-1} and 1316 cm^{-1} show C=C and C-N bonds. The peak at 1594 cm^{-1} shows primary amine. In nickel(II)-4-methoxyaniline shows a peak at 1035 cm^{-1} which is due to C-O-C stretching. The peak at 1242 cm^{-1} shows asymmetric C-O-C stretching. The peaks at 2928-2854 cm^{-1} show-CH_3 of C-H stretching. The peak 1030 cm^{-1} shows C-O stretching vibration in Ni(II)-methoxyaniline complex as shown in Figure: 6. FT-IR spectral analysis confirm that aniline, 4-methoxyanile and 4-fluoroaniline are coordinated with nickel(II) central metal ion to form monomeric [Ni(4-R-aniline)$_4Cl_2$] (R = H, F, OCH_3) complexes.

The fluorescence spectra of all the synthesized compounds are shown in Figure: 7-9. In [Ni(aniline)$_4Cl_2$], the emission peak is seen at 600-800 nm. In [Ni(4-fluoroaniline)$_4Cl_2$], the emission peak shows at 550-880 nm. In [Ni(4-methoxyaniline)$_4Cl_2$], the emission peak shows at 500-600 nm. Quenching of emission was observed in all the three complexes. The synthesized nickel(II) complexes show emission properties.

The chemistry of metal complexes derived from heterocyclic compounds has attracted considerable interest due to the broad range of pharmacological activities of such compounds. The important pathogens such as *Vibrio Cholera* and *Strephylococcus Aureus* have wildly caused many diseases. The prepared nickel complexes were evaluated for their antibacterial activity against *Strephylococcus Aureus* and *Vibrio Cholera*. The photographs of the same are presented in Figure 10. The results are listed below in Tables: 1-2. Inhibition zone of complexes shows that they have moderate to high antibacterial activity. The invitro antibacterial activities demonstrated that the complexes have higher antibacterial activity. However, stable Ni(II) complexes have also been reported herein. Aniline ligands with N donor atoms in the coordination sphere of nickel are the most common ligands which is used to stabilize Ni^{2+} ion in an aqueous solution. Reports on the antibacterial properties of nickel(II) complex frequently emphasize the increased effectiveness of nickel(II) ion coordination to a particular aniline and aniline derivative ligand. These complexes showed antibacterial activity against the species. The difference in antibacterial activity of the complexes for *Strephylococcus aureus* and V*ibrio cholera* were [Ni(aniline)$_4Cl_2$] > [Ni(4-fluoroaniline)$_4Cl_2$] > [Ni(4-methoxyaniline)$_4Cl_2$].

Antioxidant activity of the synthesized Ni(II)-aniline complex was tested by DPPH assay, in which there was a decrease in DPPH free radical with increasing concentration of complex. The IC_{50} value is a parameter widely used to measure the antioxidant activity of the test samples (Figures: 13-14). It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, lower the IC50 value, higher the antioxidant activity. It was evaluated for its ability to quench the DPPH, which is one of the few stable organic nitrogen radicals and bears a purple colour. This assay is based on the measurement of the loss of DPPH* after reaction with samples. It is considered as the prior mechanism involved in the electron transfer. The concentration of the sample at 20 mg/ml shows 15.4% of inhibition Ni (Table 3) . The concentration of the sample at 40 mg/ml shows 30.6% inhibition. The concentration of the sample at 60 mg/ml shows 45.1% of inhibition. The concentration of the sample at 80 mg/ml shows 60.2% of inhibition. The IC_{50} value of Ni(II)-aniline complex is 66.3% which is a higher IC_{50} value (54.3%) than as compared with ascorbic acid (X. Totta, 79–93). Hence, Ni(II)-aniline complex is more antioxidant activity and can be used as a biological active compound.

Anti-inflammatory efficacy of the sample was evaluated as a fair anti-inflammatory agent in a concentration-dependent fashion (P. T. Chiver, 590–595). The ability of the sample to inhibit the albumin denaturation was found in the range of IC_{50} value. The sample at the concentration 20 mg/ml shows 25% of inhibition in Table-4. The sample at the concentration 40 mg/ml shows 35% of inhibition. The sample at the concentration 60 mg/ml shows 45% of inhibition. The sample at the concentration 80 mg/ml shows 55% of inhibition. The IC_{50} value of the sample is 70% compared to the IC_{50} value of the ascorbic acid is 60%.

Conclusion

We have successively synthesized [Ni (aniline)$_4$Cl$_2$], [Ni(4-fluoroaniline)$_4$Cl$_2$]and [Ni (4-methoxyaniline)$_4$Cl$_2$] complexes. These nickel(II) complexes were able to be characterized by UV-Vis, FT-IR and fluorescence spectral techniques. Aniline ligands tend to coordinate through the amino nitrogen atom, although that can change dramatically depending upon the substituents. The donor capabilities vary with the substituent's electron-donating or electron-withdrawing capabilities. In the ligands used in this work, for example, the methoxy group in 4-methoxyaniline (p-anisidine) is a strong electron donating group, causing the nitrogen to be more electron rich, which can encourage coordination between the nitrogen and nickel. Similarly, the methyl group in 4-methylaniline (p-toluidine) is also an electron donating group, but only inductively and thus less so than the methoxy group. Finally fluorine substituent in 4-fluoroaniline serves as an electron withdrawing group, causing the nitrogen to be less electron rich and therefore making coordination between the nitrogen and nickel to be weaker. Antibacterial effect of these compounds was evaluated by disc diffusion method. Among the tested compounds the most effective compound was the [Ni(aniline)$_4$]Cl$_2$ >[Ni (4-fluoroaniline)$_4$]Cl$_2$ > [Ni(4-methoxyaniline)$_4$]Cl$_2$. The antibacterial activities were screened for all the compounds by disc diffusion methods. Besides, metal complexes have been shown to have antibacterial activities against Vibrio Cholerae and Strephylococcus Aureus. These complexes could reasonably be used for the treatment of some common diseases caused by E. Coli. The complex was also tested for anti-oxidant and anti-inflammatory tests.

Figures

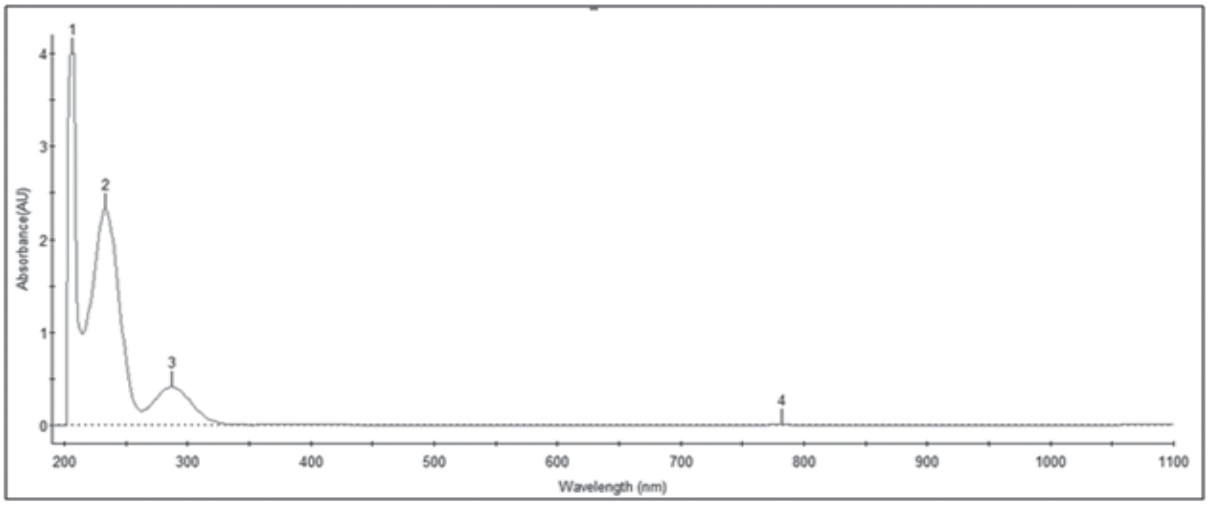

Figure-1: UV-visible spectrum of [Ni(aniline)$_4$]Cl$_2$] complex in ethanol

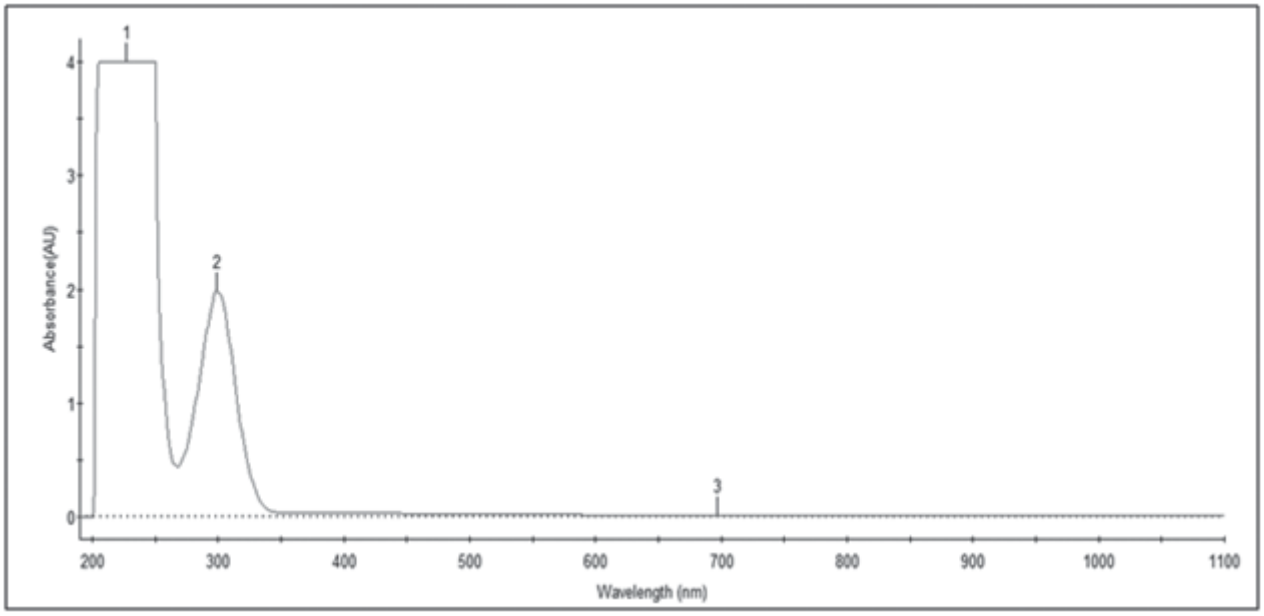

Figure-2: UV-visible spectrum of [Ni(4-fluoroaniline)$_4$Cl$_2$]complex in ethanol

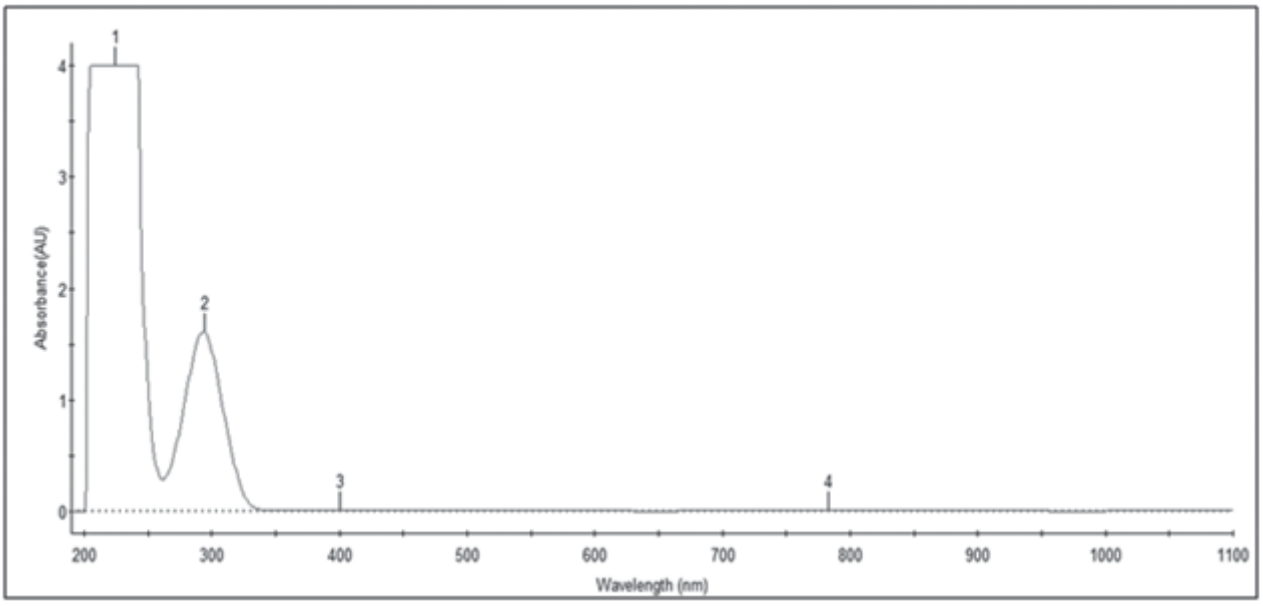

Figure-3: UV–visible spectrum of [Ni(4-methoxyaniline)$_4$Cl$_2$] complex in ethanol

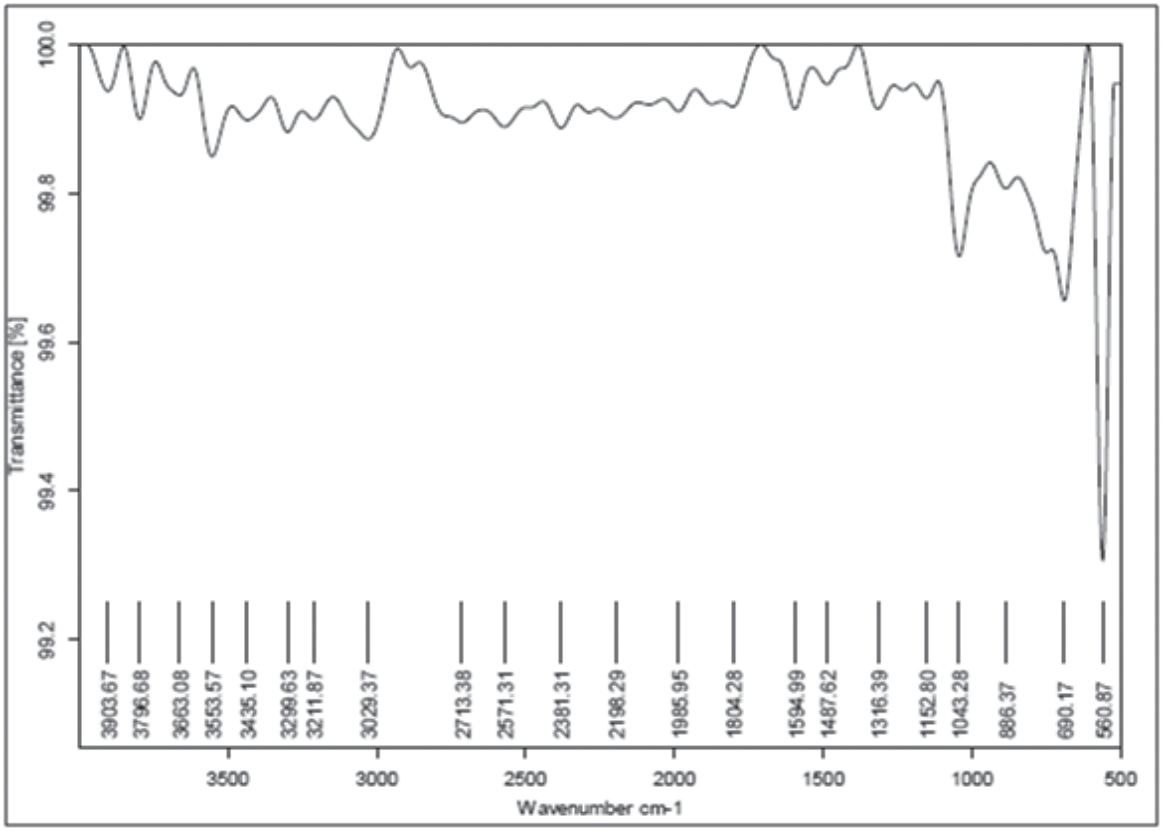

Figure-4: FTIR spectrum of $[Ni(aniline)_4Cl_2]$ complex

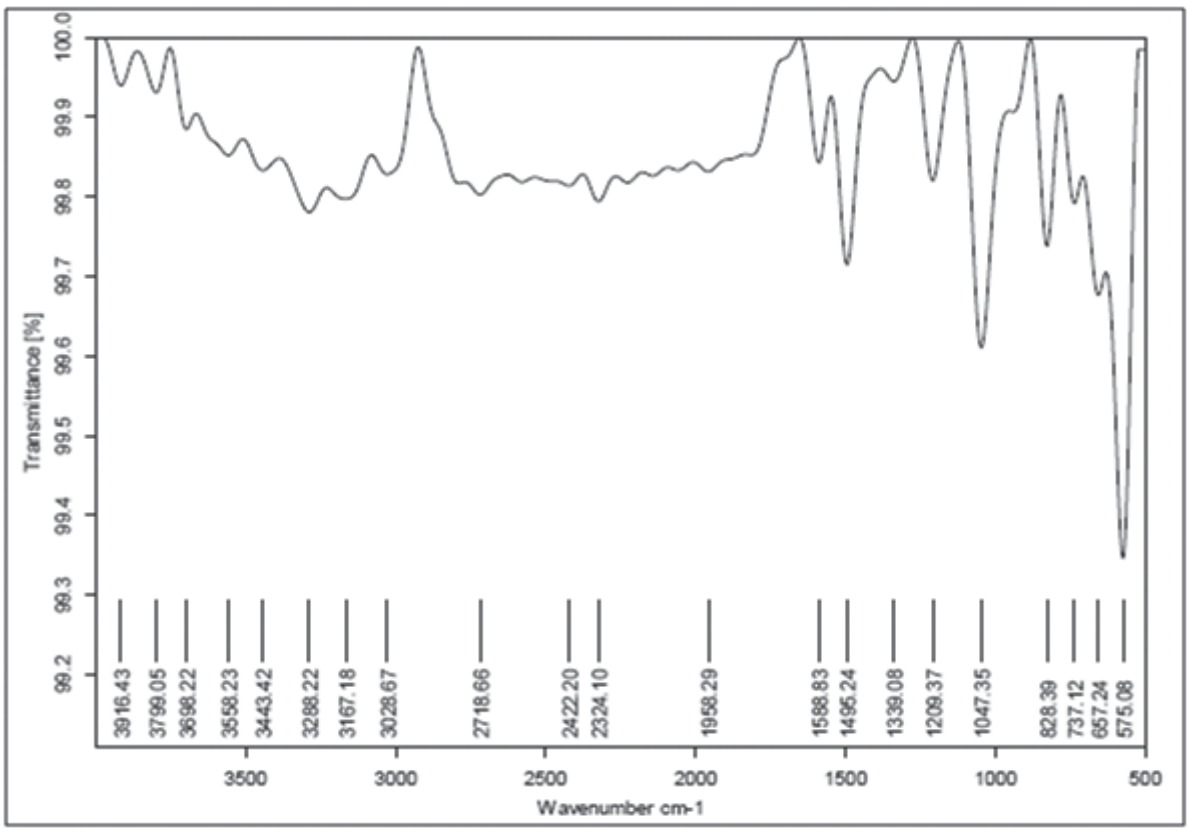

Figure-5: FTIR spectrum of $[Ni(4\text{-fluoroaniline})_4Cl_2]$ complex

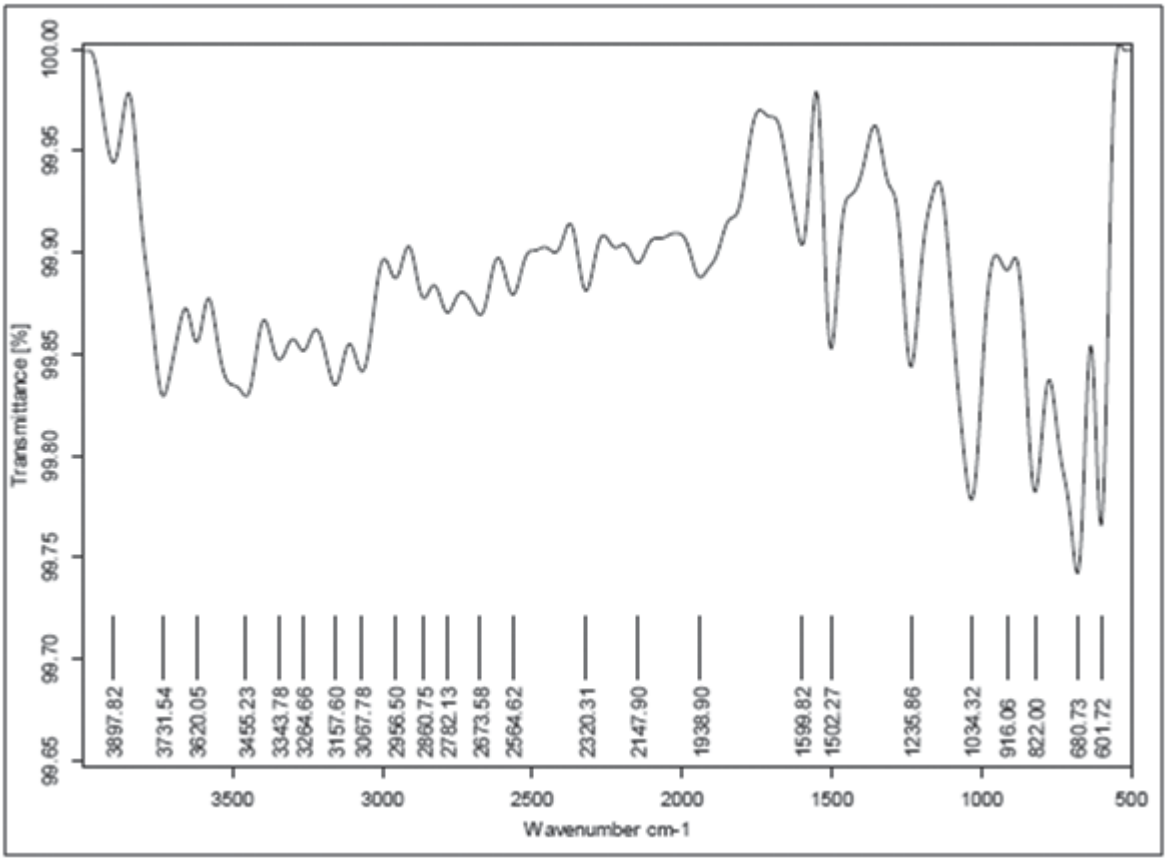

Figure-6: FTIR spectrum of [Ni(4-methoxyaniline)$_4$Cl$_2$] complex

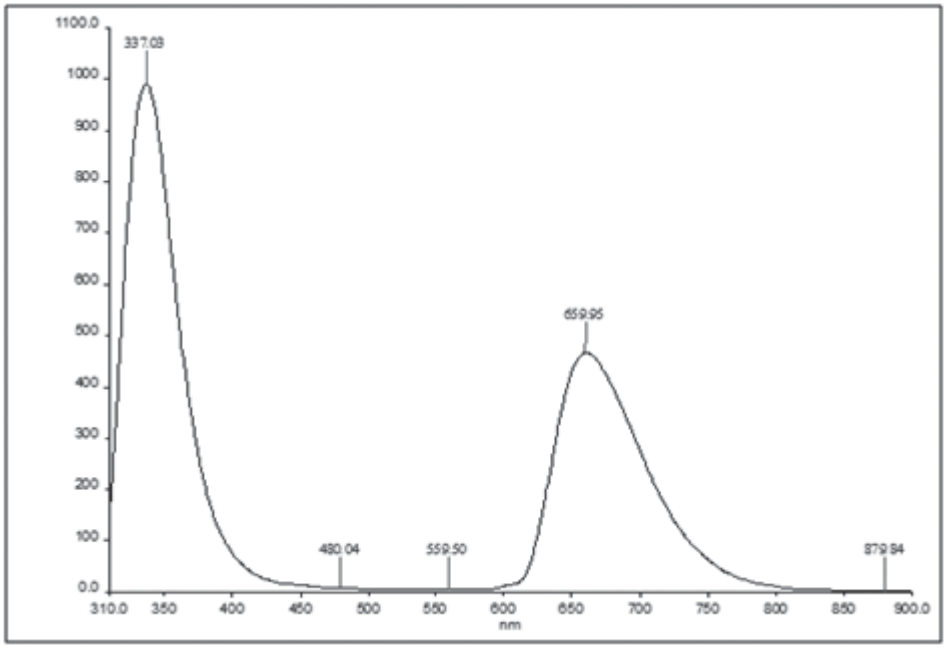

Figure-7: Fluorescence spectrum of [Ni(aniline)$_4$Cl$_2$] complex

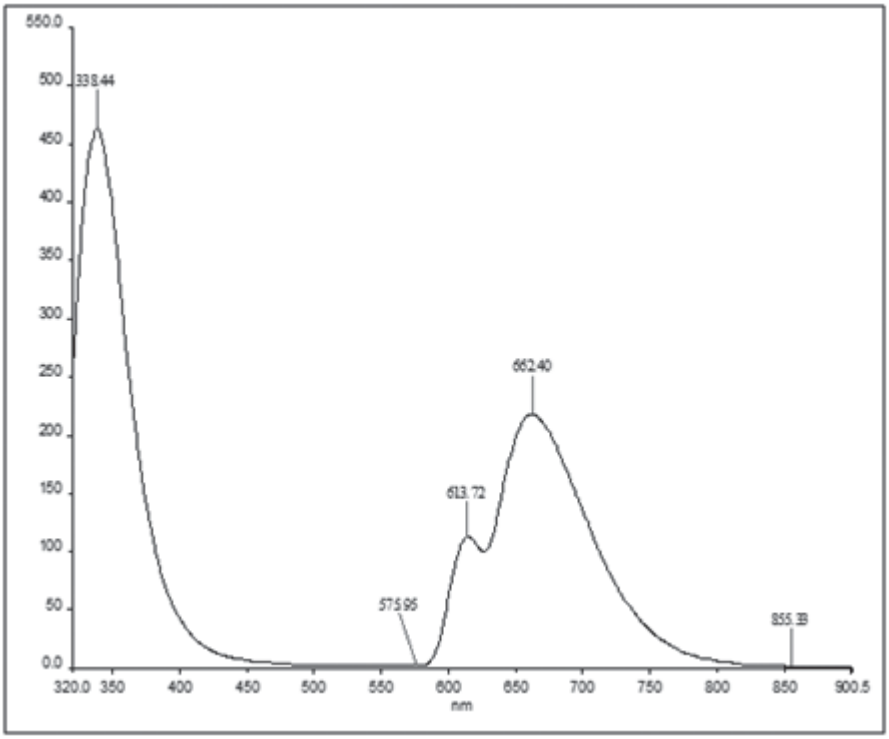

Figure-8: Fluorescence spectrum of [Ni(4-fluoroaniline)$_4$Cl$_2$] complex

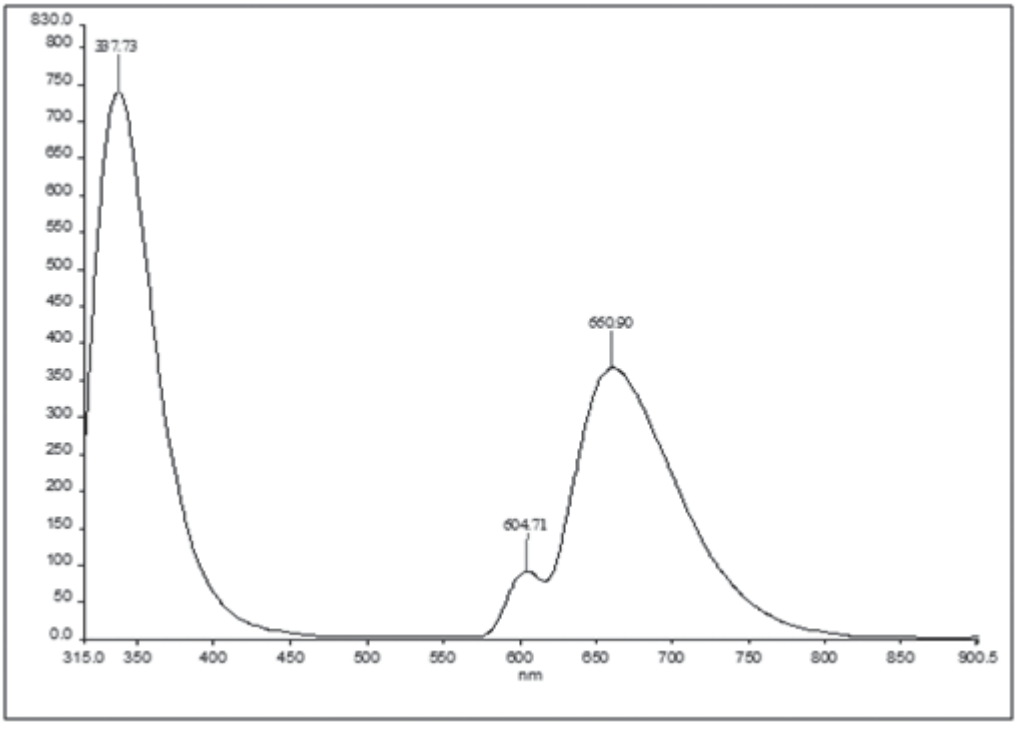

Figure-9: Fluorescence spectrum of [Ni(4-methoxyaniline)$_4$Cl$_2$]

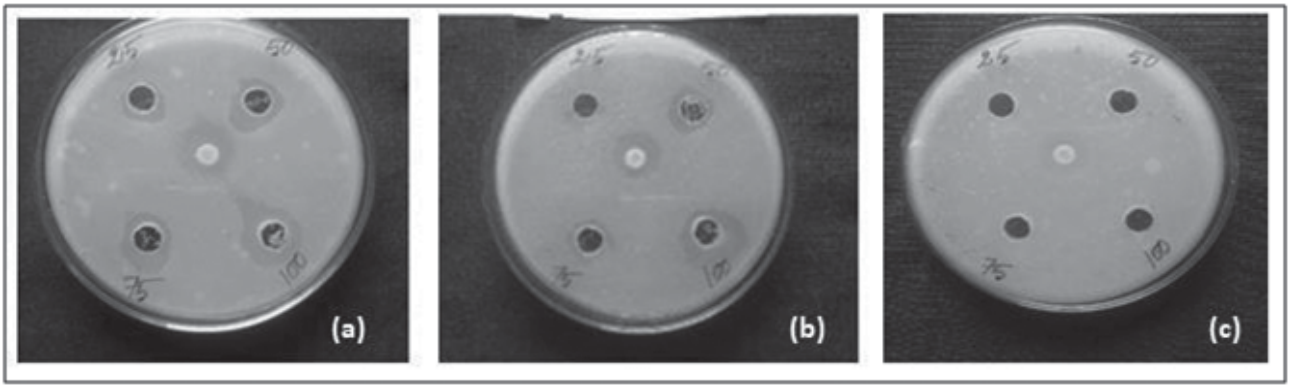

Figure-10: Antibacterial activity of samples (a) [Ni(aniline)$_4$Cl$_2$], (b) [Ni(4-fluoroaniline)$_4$ Cl$_2$] and (c) [Ni(4-methoxyaniline)$_4$Cl$_2$] complexes against common pathogens

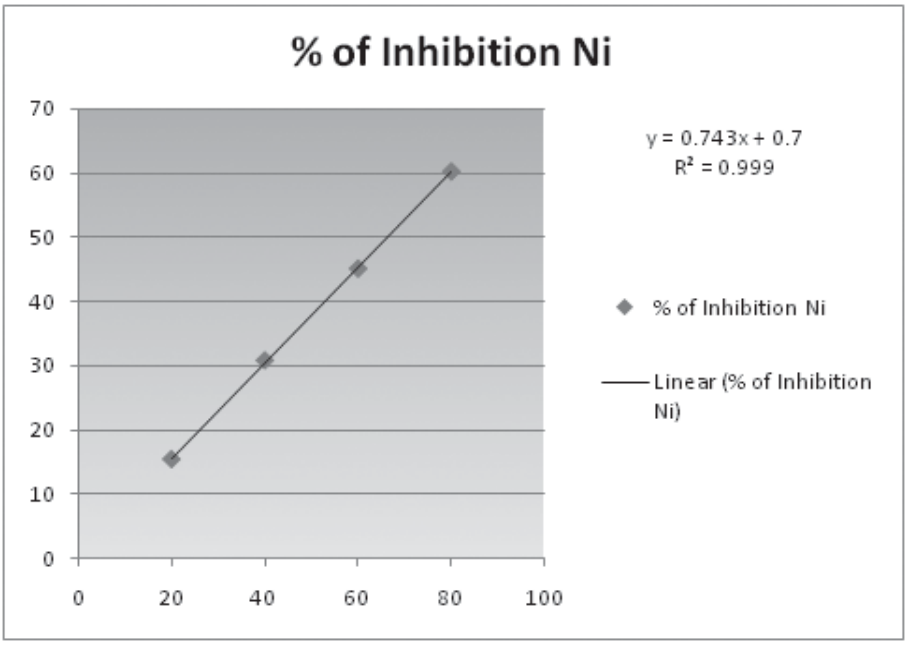

Figure-11: Graph % of inhibition of Ni from the [Ni(aniline)$_4$Cl$_2$] complex

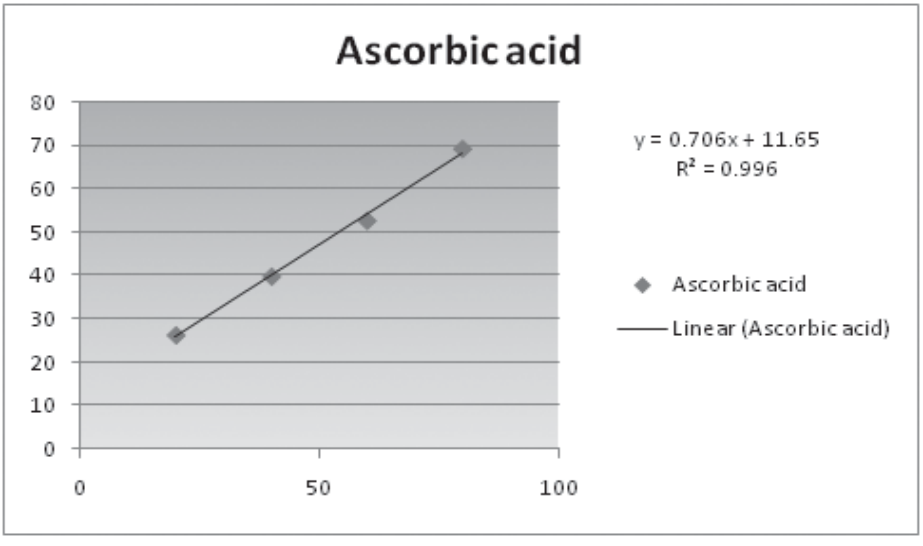

Figure-12: Standard ascorbic acid graph with the [Ni(aniline)$_4$Cl$_2$] complex

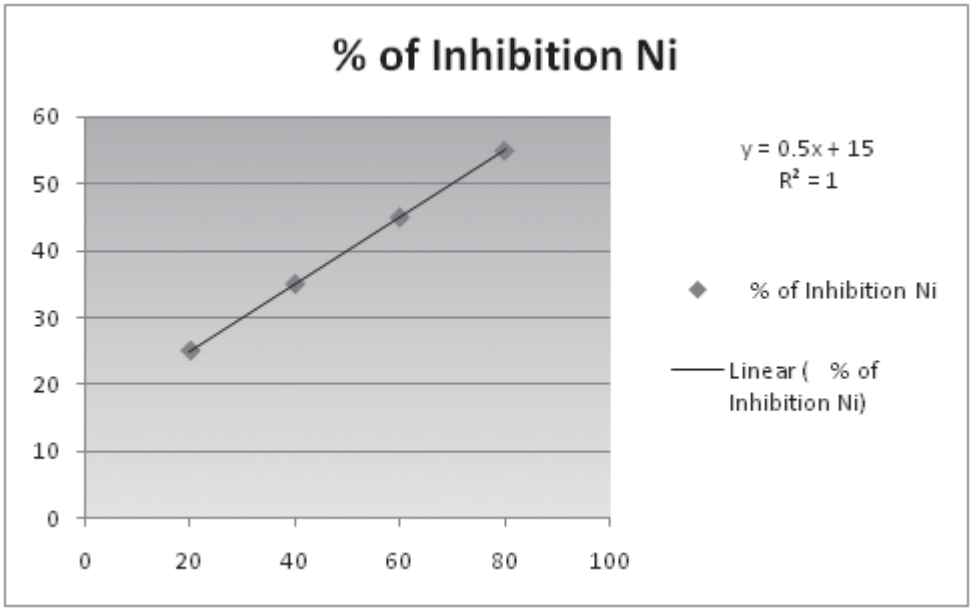

Figure-13: Graph % of inhibition of Ni from $[Ni(aniline)_4Cl_2]$ complex

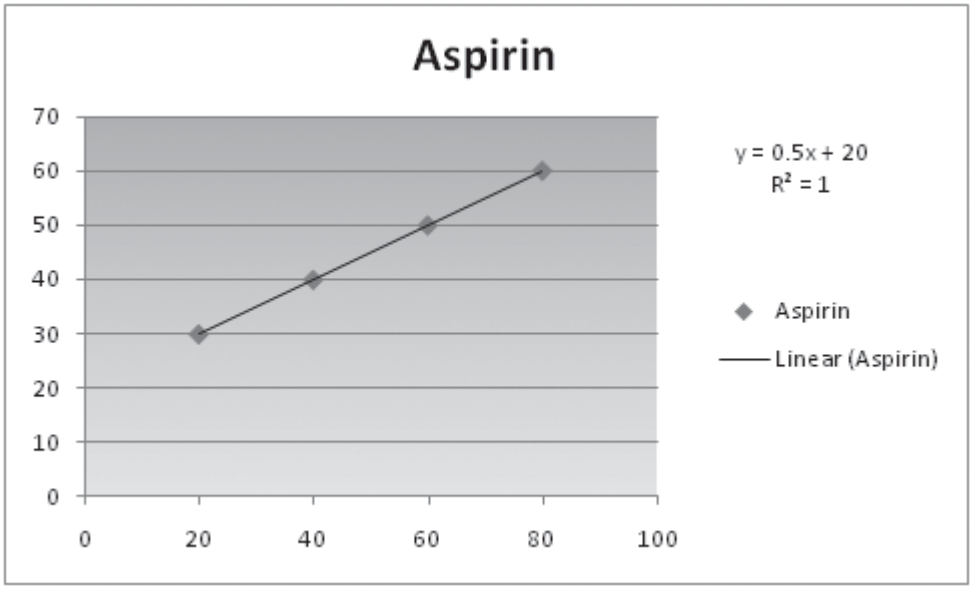

Figure-14: Graph of standard aspirin with the $[Ni(aniline)_4Cl_2]$ complex

Table-1: Antibacterial activity of our prepared complexes against common pathogens (E.Coli)

Sample	Zone of inhibition (mm/ml)				Control
	25 µl	50 µl	75 µl	100 µl	
$[Ni(An)_4Cl_2]$	05	06	12	18	20
$[Ni\ (4\text{-}FAn)_4Cl_2]$	06	14	16	22	18
$[Ni(4\text{-}MeOAn)_4Cl_2]$	04	6	12	14	16

Table-2: Antibacterial activity of sample $[Ni(4\text{-}MeOAn)_4Cl_2]$ complex against common pathogens

Sl. No.	Organism	Zone of Inhibition (mm/ml)				Positive Control
		25 µl	50 µl	75 µl	100 µl	
1.	E.coli	4	6	12	14	16
2.	Vibrio chlorea	16	19	23	28	24

Table-3: Anti-oxidant Activity of $[Ni(An)_4Cl_2]$ complex

Test	Concentration of the Sample(mg/ml)	% of Inhibition *Ni*	Ascorbic acid
DPPH Assay	20	15.4	26.3
	40	30.8	39.8
	60	45.1	52.6
	80	60.2	69.1
IC_{50} value		66.3	54.3

Table-4: Anti-inflammatory activity of $[Ni(An)_4Cl_2]$ complex

Test	Concentration of the sample (mg/ml)	% of Inhibition Ni	Aspirin
Albumin denaturation assay	20	25	30
	40	35	40
	60	45	50
	80	55	60
IC_{50} Value		70	60

Supplementary Materials

Test Organisms

The pure cultures of bacteria maintained in the microbiology Laboratory were used for the microbiological work. The test organisms were maintained on Nutrient agar medium. The test organism used for the work is Escherichia coli.

Preparation of Inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of microorganism from the stock cultures to test tubes of Nutrient broth and incubated for 24 h at 37°C. The cultures were diluted with fresh Nutrient broth.

Preparation of Media

The medium was prepared by dissolving the different ingredients in water and autoclaved at 121°C for 15 minutes. This was used for preliminary antibacterial studies.

Microorganisms and Culture Media

Bacterial cultures such as Escherichia coli were obtained from doctor diagnostic center, Trichy. Bacterial strains were maintained on nutrient agar broth, (Himedia) at 4°C.

Nutrient Broth Medium

Bacterial cultures were sub cultured in liquid, medium (Nutrient broth) at 32°C for 24-48 hours and used for experiments. The nutrient broth medium consisted of the following composition.

Peptone	-	59
Beef extract	-	3g
Sodium chloride	-	5g
Yeast extract	-	1.5g
Distilled water	-	1000 ml

After adding all the ingredients into the distilled water, it was boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs for 15 minutes

2.5.6 Inoculum Preparation

Bacteria cultured in liquid medium (nutrient broth) at 37°C for 8 hours and further used for the test. These suspensions were prepared immediately before the test was carried out.

Preparation of Culture Media

Nutrient Agar Medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes.

Ingredients: grams/liter

Peptone	:	5 gm
Beef extract	:	3 gm
Agar	:	15 gm
Sodium chloride	:	5 gm
Yeast extract	:	1.5 gm
Distilled water	:	1000 mL
pH	:	7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressures (121°C) for 15 minutes.

Agar Well Diffusion Method

Agar well method is also known as the whole plate diffusion method or cup diffusion method.

Principle

It is an important method for studying the inhibitory effect of any compound (antibiotics) on the growth and multiplication of a particular

bacteria or fungus. Here well or cups are made using a sterilized cork borer on the seeded nutrient agar or potato dextrose agar in a Petri dish to which the test compound is added. The treated Petri dishes are incubated at 37°C for 24 hrs for bacteria and 48 h for fungus. The inhibition zone formed around each well indicates the antimicrobial activity.

Procedure:

Nutrient agar or potato dextrose agar was used as the culture medium for this assay. The molten nutrient agar or potato dextrose agar was dispensed in pre-sterilized Petri dishes (25 ml) and allows cooling. These agar plates were homogenously incubated with the test bacterium previously suspended in distilled water. The plates were allowed to solidify. After solidification holes/wells of 6 mm diameter were punched into the agar with the help of flamed cork borer. Three wells were prepared for each plate. One hole was filled with 100% concentrated of our suspended solution extract, another well was filled with 50% concentration. The last hole was filled with the solvent as a control. The Petri dishes were incubated at 37°C for 24 hours for bacteria. After the incubation, the plates were observed for the zone of inhibition.

Antioxidant Activity:

Antioxidant capacity is an overall ability of organism or food to catch free radicals and prevent their harmful effect. Generally, the amount of reactive oxygen species (ROS) and antioxidants produced in the body are balanced, but somehow under some unavoidable circumstances, antioxidant defense mechanism proves to be insufficient to compensate the highly enhanced ROS which is harmful for the body. Likewise , various toxicants act through the ROS like superoxide anions (O-), hydroxyl radicals (HO.) and non-free radical species such as H_2O_2, singled oxygen (O) and nitric oxide (NO) that play a major role in initiation of degenerative processes such as cellular damage that may be related to many body ailments viz., heart diseases, cancer, and aging. Antioxidants are intimately involved in prevention of cellular damage. Catalase, superoxide dismutase and glutathione peroxidase are some of the natural antioxidants found in body. They neutralize free radicals as the natural by-product of normal cell processes.

Procedure:

2, 2-diphenyl-1-picrylhydrazyl (DPPH) was taken. Stock solution of DPPH in buffered methanol solution (violet-purple colour). The test tube was wrapped in aluminum foil and kept at 30c for 30 minutes in dark. Absorbance is measured at 517 nm.

Assay for Invitro DPPH-Free Radical Scavenging Activity (DPPH Assay):

DPPH radical gives strong absorbance at 517 nm (deep violet colour) due to its unpaired electron. When this radical pairs off in presence of a free radical scavenger, the absorption vanishes and the resulting discoloration is stoichiometric with respect to the number of electrons taken up.

DPPH – free radical scavenging activity assay of nickel (ll) aniline was carried out using reported method with suitable modifications.

Reagent/solutions: DPPH solution - 0.3 mM in methanol (freshly prepared), Standard Ascorbic acid solution – 1 mg/ml in methanol.

Sample preparation: 1 ml of PG was dried on mild heat in a water bath; the residue was taken with methanol to make 1 mg/ml (PGE1) and used for the test.

Different volumes (equivalent to 5-300 μg) of PGE1/standard were taken in a set of test tubes and methanol was added to make the volume to 3 ml. To this, 1 ml of DPPH reagent was added, mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature. 1 ml of DPPH reagent diluted to 4 ml with methanol was taken as reagent blank.

% Scavenging = Ao-A1/Ao × 100

Anti-Inflammatory Activity:

A drug or substance is that reduces inflammation in the body. Anti-inflammatory agents block certain substances in the body that cause inflammation in the body that cause inflammation. They are used to treat many different conditions. Some anti-inflammatory agents are being studied in the prevention and treatment of cancer. Inflammation is a defense mechanism that enables the body to protect itself against infection, burn, toxic chemical allergens, or any other harmful stimuli. Inflammation is a substantial reaction to damage, disease or destruction portrayed by heat, redness, pain, swelling, and disturbed physiological functions.

Principle of Albumin Denaturation Assay

Protein denaturation is defined as a process where due to external factors such as heat, strong acid or strong base; an organic solvent or a concentrated inorganic salt causes the protein to denature that means the protein's tertiary structure and secondary structure is disoriented. [1, 2] enzymes lose their activity since the substrates are able to no longer attach to the active sites. Non-steroidal anti-inflammatory drugs (NSAIDS) are the commonly prescribed medications in the world because of their verified effectiveness in reducing pain and inflammation. NSAIDs have accounted for prevention of the protein denaturation, which act as antigens and prompt autoimmune diseases. These drugs contain several adverse effects, particularly gastric irritation prompting the development of gastric ulcers.

Procedure:

It was performed by taking aspirin (0.1 mg/ml) and sample solution at a range of concentrations. The assay mixtures were incubated for 20 minutes at 37°C followed by heating of the sample for 30 minutes at 60°C. The samples were then cooled back to room temperature and turbidity recorded. The percentage inhibition of protein denaturation was calculated as follows:

% Inhibition = [(Acontrol – Asample) / Acontrol] x 100

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Selective Sorption of Divalent Manganese Ion from Real Water Samples Using Mn(II) - Ion Imprinted Sorbent

Rohith P.* and Dr. Girija P.**

Abstract

Manganese, a ubiquitous and essential element, has taken center stage as a double-edged sword. While it plays a crucial role in various industrial processes and biological systems, its unchecked presence in our environment has sparked an urgent need for investigation. From contaminated soils to polluted water sources, the insidious rise of manganese pollution poses a grave threat to the delicate balance of our ecosystems and human health. This article embarks on the selective biosorption of Mn(II) ion which was carried out using a novel ion-imprinted biosorbent, prepared from alginic acid and NNMBA-crosslinked polyacrylamide by the metal ion imprinting technique. The prepared sorbent showed higher sorption capacity and selectivity compared to the non-imprinted sorbent. The efficient sorption capacity of Mn(II)-ion imprinted sorbent showed wide application prospects in Mn(II) ion removal from environmental samples.

Keywords: *Alginic acid, Biosorption, Ion imprinting, Manganese, Sorbent, and Selectivity.*

*Rohith P., P.G. Department of Chemistry and Research Centre, Sanatana Dharma College, Kanyakumari-Panvel Road, Sanathanapuram P.O., Alappuzha-688003, Kerala, India, Email: rohithpm2000@gmail.com

**Dr. Girija P., Associate Professor, P.G. Department of Chemistry and Research Centre, Sanatana Dharma College, Alappuzha-688003, Kerala, India, Email: girijakallelil@gmail.com

1. Introduction

Ion-imprinted polymers are polymeric receptor systems that can selectively recognize and bind target metal ions from complex mixtures. The binding sites of ion imprinted polymers are tailored in such a fashion to fit the target species which ensures that the sites possess complementarity to the target specie's size, shape, and charge distribution. This property is extensively studied in various potential applications such as in sensors (Dahaghin Zohreh, et al. 125374), solid phase extraction (Otero-Romani, et al. 1-9), and membrane separation (Zhai Yunhui, et al. 284-91).

Even though manganese ion is an essential trace element required for various physiological functions in living systems, it possesses toxicity in a number of ways. It is found to be exerting direct or indirect generation of reactive oxygen species (Brouillet E.P., et al. 89-94), (Ali Syed F., et al. 329-34), the oxidation of biomolecules (Archibald, Frederick S., and Curtis Tyree 638-50), and the disruption of cellular calcium (Gavin Claire E., et al. 329-34) and cellular iron homeostasis (Kwik-Uribe, et al. 1-15). An elevated Mn concentration leads to the inhibition of complexes within the mitochondrial electron transport chain (Brown, Simon and Nicolas L. Taylor 49-57), (Zheng, Wei and Qiuqu Zhao 175-79), and (Galvani, et al. 377-83). Due to its toxic be-haviour, it is a necessity for the well-being of the environment to remove it from water bodies.

In the present work Mn(II) ion imprinted N, N' Methylene-bis-acrylamide (NNMBA) crosslinked polyacrylamide was synthesized from aqueous medium. The newly prepared polymer showed high sorption capacity and remarkable selectivity in Mn(II) ion separation from a mixture of metal ions. The method of preparation is simple, rapid, economical, and eco-friendly due to the use of the aqueous medium. Characterization of polymer networks, rebinding studies, and investigation of the effect of various parameters on rebinding was done. Selectivity study of the ion imprinted polymer was also done.

2. Experimental

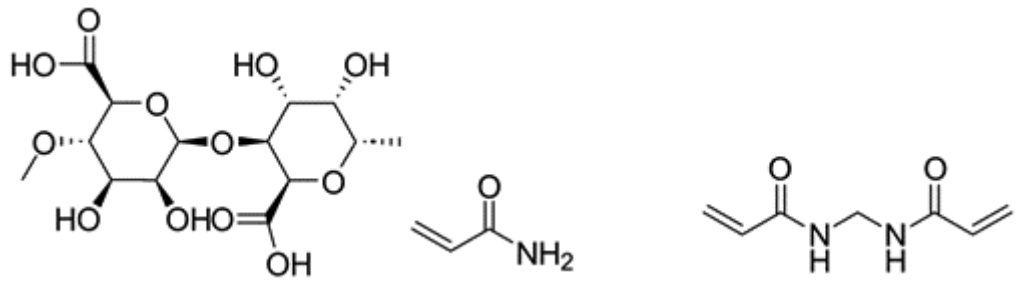
Reagents of analytical and spectral grade were used for all experiments. Fourier Transform Infrared (FTIR) spectra of the Mn(II) ion-imprinted, non-imprinted, and the Mn(II) ion bound polymers were recorded between 4000-400 cm^{-1}, using a Perkin Elmer 400 FTIR spectrophotometer. UV- Visible (UV- Vis) spectrophotometric measurements were carried out using Shimadzu UV-Vis spectrophotometer. Scanning Electron Micrographs (SEM) were taken on JEOL-JSM-840 Scanning Electron Microscope. Sorption capacity of metal ion was determined before and after binding, using Perkin Elmer Atomic Absorption Analyzer 300.

2.1. Preparation of Mn(II) Ion Imprinted and Non-imprinted Polymers

Alginic acid (7.5 g) was mixed with (0.86 g) of manganese sulphate in aqueous medium. This mixture was added to acrylamide (10.66 g), N, N' Methylene-bis- acrylamide (NNMBA) (7.71g) and kept at 70°C in a water bath with stirring, using potassium persulphate as initiator. The polymer obtained was washed with water to remove unreacted monomer and with 2N HCl to remove Mn (II) ions. The bulk polymer obtained was dried, sieved and weighed. Non imprinted polymer (NIP) network was also prepared using the same procedure without using metal ion.

2.2. Metal Ion Sorption

To analyse specific binding capacity, Mn(II) ion-imprinted and non-imprinted polymeric sorbents were investigated towards solutions of Mn



Alginic acid **acrylamide** **N,N′-Methylenebisacrylamide**

Scheme 1. Structures of acrylamide, alginic acid and NNMBA

(II), Cd(II), Cu(II), and Fe(II) ions by batch equilibration method. Metal ion concentration before and after binding was determined by Atomic Absorption Spectroscopy (AAS).

2.3. Optimization of Mn(II) ion Resorption Conditions

Conditions of Mn(II) ion rebinding by the developed polymeric sorbent was investigated by varying factors such as concentration, time, pH etc.

2.4. Effect of Concentration

The batch wise metal ion binding experiments were carried out to evaluate the imprinting efficiency. Similar rebinding studies at various concentrations of Mn(II) ion (1 ppm - 5 ppm) were carried out and analyzed by AAS.

2.5. Time Dependence on Sorption

The time required for maximum sorption was determined by batch equilibration method using Mn(II) ion solution**.** 100 mg of Mn(II) ion-imprinted and nonimprinted polymeric sorbent was added to 10 ml of Mn(II) ion solution, sorption of Mn(II) ion was determined at regular intervals of time.

2.6. Influence of pH of Solution on Mn(II) Ion Sorption

About 50 mg of Mn(II) ion-imprinted and non-imprinted polymeric sorbent was subjected to sorption studies using Mn (II) ion solution at different pH. The amount of Mn (II) ion sorbed at different pH was determined as described earlier.

2.7. Selectivity Studies

Selective sorption studies were carried out by column experiment. 1g of NNMBA-cross linked Mn(II) ion-imprinted or non-imprinted polymer was made into a slurry with De-mineralized Water (DMW) and then poured into a pyrex glass column, plucked with small portion of glass wool at the bottom. The column was pre-conditioned by passing DMW and then mixture of metal ion solution (1×10^{-3} M, 10 ml) was passed through the column at a flow rate of ~ 0.5 ml min^{-1}. The eluted solution was collected and the amount of metal ion sorbed was determined by AAS.

3. Results and Discussion

3.1. Synthesis of Mn(II) Ion-Imprinted and Non-imprinted Polymer Networks

The Mn(II) ion-imprinted polymer networks were synthesized by free radical polymerization of acrylamide and NNMBA in presence of alginic acid. Potassium persulphate was used as the initiator and the polymerization was carried out at 70ºC. The bulk polymer obtained was washed with water to remove unreacted monomers and with dil. HCl to remove Mn(II) ions.

The polymer was dried, crushed, and sieved. Non-imprinted polymer networks were also prepared without using the template metal ion.

3.2. FTIR Characterization

Mn(II) ion-imprinted polymer network showed absorption at 1647 cm^{-1} assigned to -COOH group of alginic acid which is shifted to 1642 cm^{-1} in Mn(II) bound polymer network. The absorptions band at 1455 cm^{-1} shifted to 1442 cm^{-1} indicating that -COOH group of alginate participated in the sorption process. The imprinted polymer showed bands at 2919 cm^{-1} and the non-imprinted polymer showed bands at 2922 cm^{-1} due to C-H stretching vibrations. Certain peaks are observed below 400 cm-1 and are attributed to metal-oxygen bond stretching.

3.3. UV-Vis. Spectra

Mn(II) ion-imprinted polymer showed bands at 603 nm and 626 nm and these are shifted to 616 nm and 684 nm on Mn (II) ion complexation (Figure-1). These shifts indicate the successful sorption of Mn(II) by the polymer.

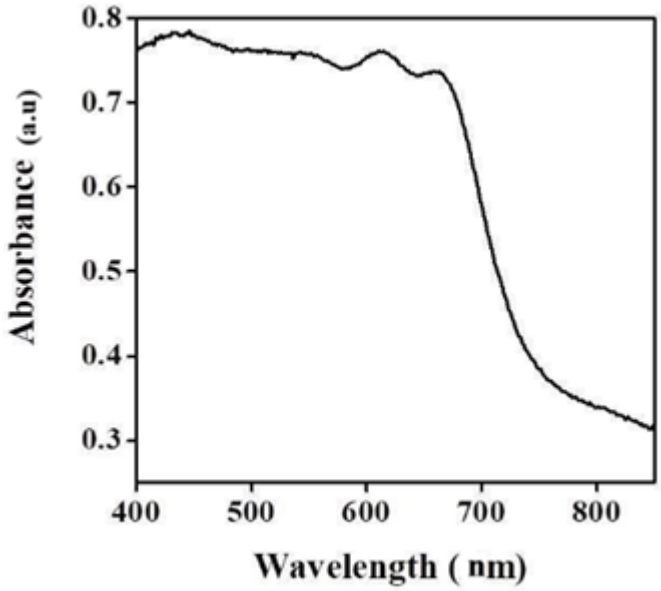


Figure-1: UV-Vis. spectrum of NNMBA-crosslinked Mn(II) ion-imprinted complex

3.4. Scanning Electron Microscopy

Scanning electron microscopy was applied to analyse the surface properties of Mn(II) ion-imprinted and non-imprinted polymeric sorbents, and Mn (II) complexes. SEM images show the difference in surface morphology (Figure-2).

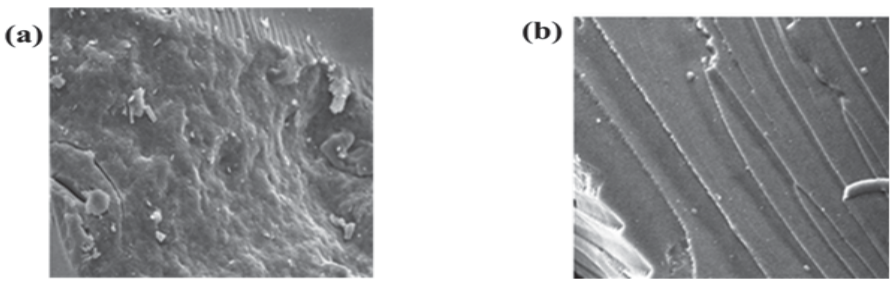


Figure-2: SEM images (a) Mn(II) ion-imprinted and (b) Mn(II) bound MIP

3.5. X-ray Diffraction (XRD) Patterns

XRD patterns of Mn(II) ion-imprinted and Mn (II) bound polymer are shown in Figure-3. As there is no strong reflection peaks observed in XRD patterns of both Mn(II) ion-imprinted and Mn(II) bound MIP, it depicts the amorphous nature of the polymer. The XRD curve of Mn (II) bound polymer showed the characteristic peaks corresponding to manganese which is not seen in the XRD curve of Mn(II) ion-imprinted polymer. This result indicates the binding of Mn(II) ion in manganese ion-imprinted sorbent.

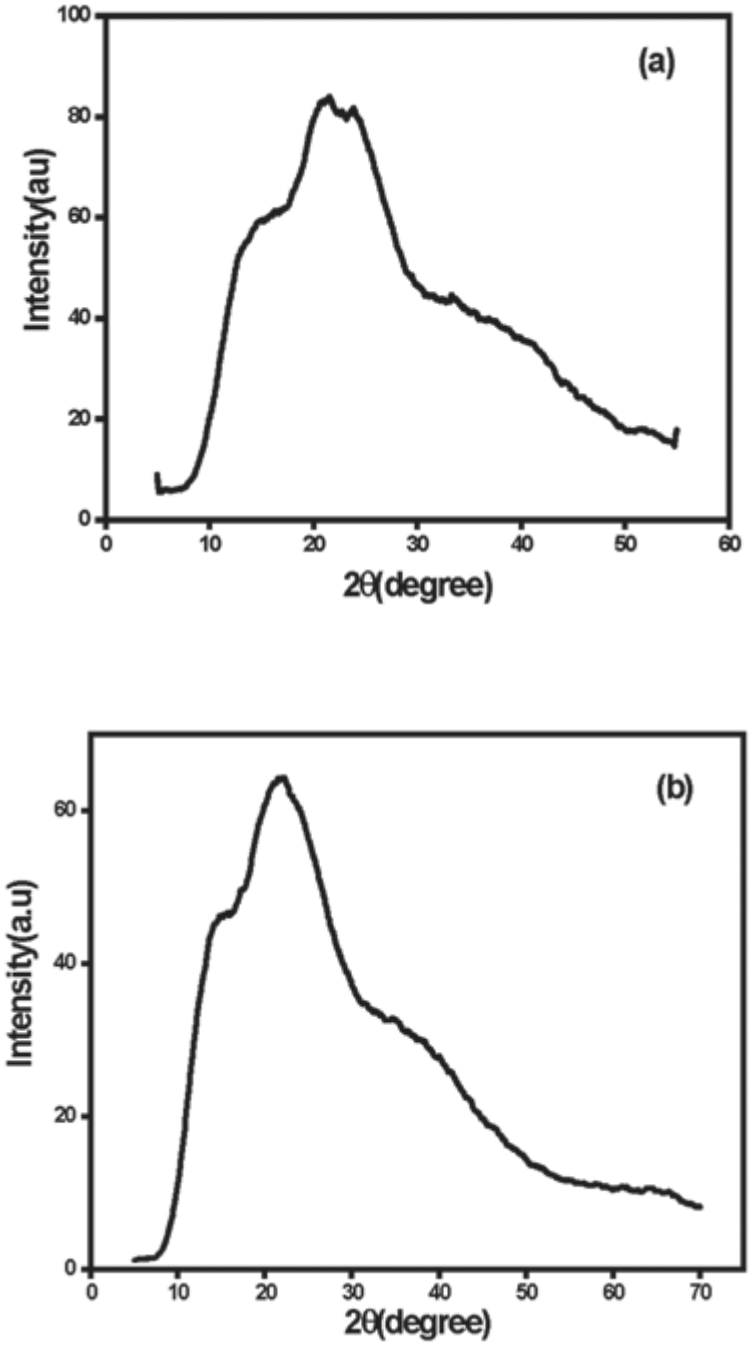


Figure-3: X-ray diffraction patterns of (a) Mn(II) ion-imprinted and (b) Mn(II) bound MIP

3.6. Specificity Studies

To find out the specific binding of Mn(II) ion, both Mn(II) ion-imprinted and NIP were equilibrated with metal ion solutions like Mn(II), Co(II) and Ni(II) ions. The results indicated that the ion-imprinted sorbent showed specificity towards Mn(II) ion (Figure-4). This is in accordance with the fact that coordination geometry was not disturbed during rebinding, so the polymeric system specifically binds the desorbed metal ion.

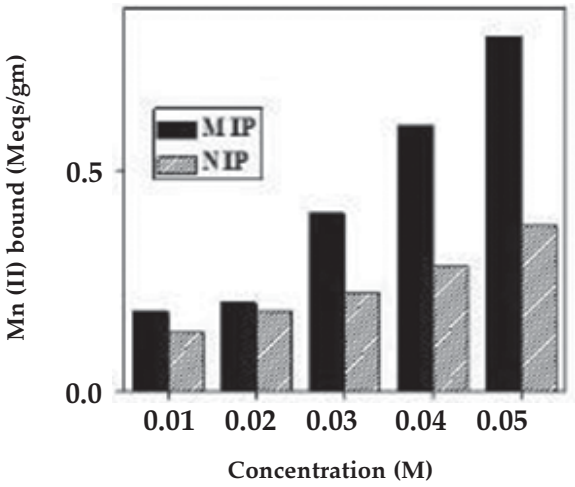


Figure-4: Specific rebinding of Mn(II) ions by Mn(II) ions imprinted and non imprinted polymers

3.7. Surface Area Analysis

The specific surface area of the particles was measured by Brunauer-Emmett-Teller (BET) model using single point analysis. The Mn(II) imprinted sorbent showed a value for specific surface area as 18.4 m^2g^{-1}, and that for non imprinted sorbent was 0.41 m^2g^{-1}. In Mn(II) ion-imprinted sorbent, the cavities are created after the removal of the Mn(II) ion and hence the porosity seems to be responsible for higher surface area than the polymer without imprinted Mn(II) ion.

3.8. Metal Ion Binding Studies

3.8.1. Effect of Concentration of Metal Ion Solution

The dependence of initial concentration of Mn(II) ion solution on sorption was investigated

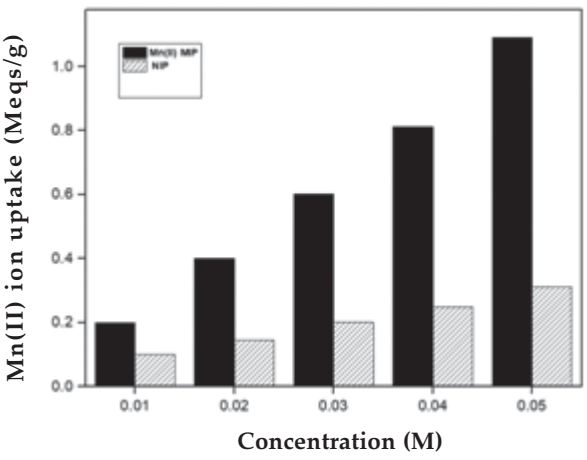


Figure-5: Effect of concentration on NNMBA-crosslinked Mn(II) ion-imprinted and non-imprinted polymers

by varying the initial concentration and determining the metal ion uptake. The studies revealed that as the concentration increases, sorption of metal ion increases (Figure-5). This result could be explained on the basis of a high driving force for mass transfer, where the increase in concentration of metal ion increases the competition to occupy all the available coordination sites in the adsorbent.

3.8.2. Dependance of Time on Mn(II) Ion Binding

Manganese ion sorption capacities were determined as a function of time. To determine the optimum contact time for the adsorption of Mn(II) ion, batch equilibration method was carried out. An equilibrium time period of 90 min was required for complete binding of Mn(II) ion by both polymers (Table-1).

Table-1: Effect of time on Mn(II) ion uptake by NNMBA-crosslinked Mn(II) ion-imprinted and non-imprinted polymers

Time	Mn(II) ion uptake by MIP(mg/g)	Mn(II) ion uptake by NIP(mg/g)
15	0.25	0.15
30	0.38	0.26
45	0.49	0.34
60	0.65	0.42
75	0.81	0.51
90	0.98	0.59

3.8.3. pH Dependance on Metal Ion Binding

pH of the medium plays a vital role in controlling the sorption of metal ions by sorbent. The sorption of Mn (II) ion uptake at different pH was analysed. The acidity of the medium influence metal adsorption. Protons can protonate the binding sites in acidic medium, but when the medium is basic, hydroxide ion may precipitate many metals. The pH of the solution was adjusted using 1M NaOH and 1M HCl. Adsorption of Mn(II) ion increases with increase in pH of the medium and then decreases (Table-2). The optimum pH for ion sorption is attained at pH 4.5.

Table-2: Effect of pH on Mn(II) ion uptake by NNMBA-crosslinked Mn(II) ion-imprinted and non-imprinted polymers

pH	Mn(II) ion uptake in MIP (mg/g)	Mn(II) ion uptake in NIP(mg/g)
3	0.25	0.20
3.5	0.36	0.29
4	0.65	0.46
4.5	0.76	0.64
5	0.65	0.40

3.8.4. Selectivity Studies

To investigate the Mn (II) ion selectivity of the imprinted particles, competitive adsorptions of Co(II), Ni(II), Zn(II), Cd(II), Mn(II) ions were conducted by column experiment, in which 1 g of the MIP or NIP micro particles were treated with 10 ml of a solution of these metal ions (0.001 M). After adsorption equilibrium was reached, the concentration of metal ions in the eluted solution was measured by AAS. The results indicate that the functional host molecules on the MIP bead surface are immobilized with the strict configuration suitable for manganese ions, and that the ionic recognition is influenced by the nature of metal ion, ionic radius and charge (Figure-6).

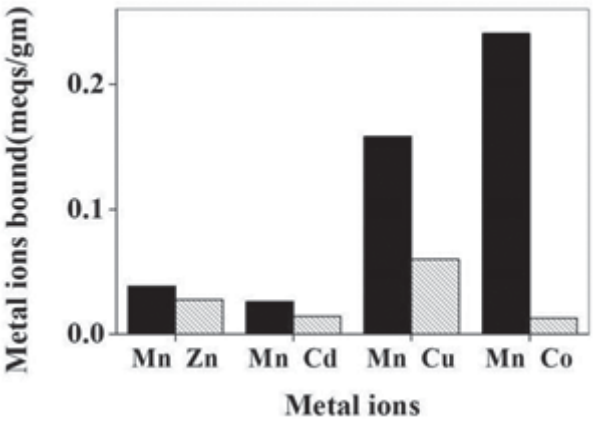


Figure-6: Selectivity studies of NNMBA-crosslinked Mn (II) ion-imprinted polymers towards mixture of competing metal ions

Table-3: Analysis of Environmental Water Samples

Source	Amount of Mn(II) ion Found(mg/L)	Removed (mg/L)	Percent recovery
Lake water	0.05	0.02	95
Waste water	0.08	0.07	95
Canal water	0.04	0.03	95

3.8.5. Application of the Method

Mn(II) ion-imprinted polymeric sorbent was applied for the selective removal of Mn(II) ions from water samples collected from various sources and analysed. The obtained values describe the suitability of the developed Mn(II) ion-imprinted polymers for the removal of Mn(II) ions from different samples. The results are listed in Table-3.

4. Conclusion

The Mn(II) ion-imprinted sorbent was synthesized and characterized. The developed polymeric sorbent seems to be very efficient and economical for removing Mn(II) ion from an aqueous medium. The binding studies revealed that as the concentration increases metal ion binding increases. The Mn(II) ion binding increased with an increase in pH and a saturation value was

obtained at pH 4.6. The optimum time required for maximum binding for Mn (II) was 90 min. The selectivity experiments proved that Mn(II) ion-imprinted polymeric sorbent showed higher sorption efficiency in selectivity than non-imprinted polymeric sorbent. The prepared sorbents are stable, possess fast resorption capacity, and can be successfully applied for the removal of corresponding metal ions from various water samples collected from the environment. Based on all the above experimental results, it can be concluded that the synthesized polymeric sorbent finds excellent applications in the field of Mn(II) ion recovery from environmental samples.

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Surface Electron Donating Properties of Neodymium - Zirconium Mixed Oxides

Dr. Binsy Varghese V.*, Dr. Deena Antony C., Vandhana P. V.***, and Elsa C. Babu******

Abstract

The electron donating properties of Neodymium - Zirconium mixed oxides at various compositions have been reported at three activation temperatures- 300°C, 500°C and 800°C. The strength and distribution of electron donor sites are determined from the studies on the adsorption of electron acceptors (EA) of various electron affinities in acetonitrile, a solvent with low basicity. Surface area was measured by BET and adsorbed EA was studied by ESR.

Keywords: *Electron donating, Electron acceptors, Electron affinity, TCNQ, Chloranil, PDNB, MDNB, Neodymium, Zirconium, and Mixed oxides.*

*Dr. Binsy Varghese V., Associate Professor, Department of Chemistry, St. Joseph's College (Autonomous), Irinjalakuda-680121, Thrissur District, Kerala, India, Email: binsyvarghese@yahoo.com

**Dr. Deena Antony C., Associate Professor and HOD, Department of Chemistry, St. Joseph's College (Autonomous), Irinjalakuda-680121, Email: deenarajan96@gmail.com

***Vandana P. Viswanathan, Assistant Professor, Department of Chemistry, MES Ponnani College, Palakkad-Ponnani Road, Ponnani-679577, Malapuram, Kerala, India, Email: vandanapv791@gmail.com

**** Elsa C. Babu, Third Semester M.Sc Chemistry Student, St. Joseph's College Irinjalalakuda, Irinjalakuda-680121, Email: elsacbabu@gmail.com

1. Introduction

Zirconia is reported as an excellent catalyst, and a very good supporting material (Sugunan and Binsy 1998) for many reactions. It shows both acidic and basic properties. The addition of basic oxides (La) suppresses the acidic behaviour whereas addition of an acidic oxide promotes acidic property (Scotti, et al. 2020). Sulfated Zirconia is known as a super acid and it is shown (Delarmelina, et al. 2021) to be a better acid catalyst than Amberlyst-15. Rare earth oxides are basic and can act as effective catalysts for a number of reactions (Sugunan and Binsy 1997). So far, no attempt has been made to study the effect of mixing of ZrO_2 with Nd_2O_3 in their surface electron donating properties. In this paper, the surface electron donor properties of Neodymium - Zirconium mixed oxides at various compositions (viz., 20, 40, 60, and 80 weight % of the rare earth oxide) and three activation temperatures were reported and the data were compared with those for pure oxides.

2. Experimental

The mixed oxides were prepared through the hydroxide route. The mixed oxides with various compositions, viz., 20, 40, 60, and 80% (abbreviated as 20 Nd, 40 Nd, 60 Nd, and 80 Nd) of the rare earth oxide were prepared. Pure oxides were also prepared in the same fashion and incorporated in the study for comparison. All the oxides were heat treated at a particular temperature viz., 300°C, 500°C, and 800°C, for two hours prior to each experiment. All the reagents were purified by standard methods before use. The adsorption studies were carried out by a procedure reported earlier (Sugunan and Binsy 1996). The amount of electron acceptors adsorbed were determined by noting the concentrations of the electron acceptors before and after adsorptionby means of a UV-Visible spectrophotometer at the λ_{max} of the EA in acetonitrile, ie., at 393.5, 288, 262, and 237 nm for TCNQ, Chloranil, PDNB and MDNB, respectively.

Surface area of the oxides was determined by the BET method using Carlo Erba Strumentazione Sorptomatic Series 1800. The ESR spectra of the adsorbed samples were measured at room temperature using a Varian E-112 X/Q band ESR spectrophotometer.

3. Results and Discussion

The strength and distribution of electron donor sites are determined from the studies on the adsorption of electron acceptors (EA) of various electron affinities in acetonitrile, a solvent with low basicity (Meguro and Esumi, 1977). The following electron acceptors were used: (electron affinity values in eV are given in brackets) 7, 7, 8, 8 Tetracyanoquinodimethane (TCNQ) {2.84}, 2,3,5,6 Tetrachloro-p-benzoquinone (Chloranil) {2.4}, 1, 4 Dinitrobenzene (PDNB) {1.77} and 1, 3 - Dinitrobenzene (MDNB) {1.26}.

The adsorption of EA on the oxides was of Langmuir type. From the Langmuir plots, the limiting amount of EA adsorbed was determined. In the case of PDNB and MDNB, adsorption was so negligible that the amount could not be detected by spectrophotometric method. When EA were adsorbed, the surface of the oxides showed characteristic coloration owing to the interaction between the EA adsorbed and the oxide surface. The ESR spectrum of these coloured samples gave unresolved spectral lines with g values of 2.003 and 2.011 for TCNQ and Chloranil, respectively. The electronic spectrum of the adsorbed samples gave bands near 400, 600 and 700 nm corresponding to physically adsorbed state of neutral TCNQ (Meguro and Esumi 1990), dimeric TCNQ radical (Boyd and Philips 1965), and to Chloranil anion radical (Foster and Thomson 1962), respectively. The data are given in Table-1.

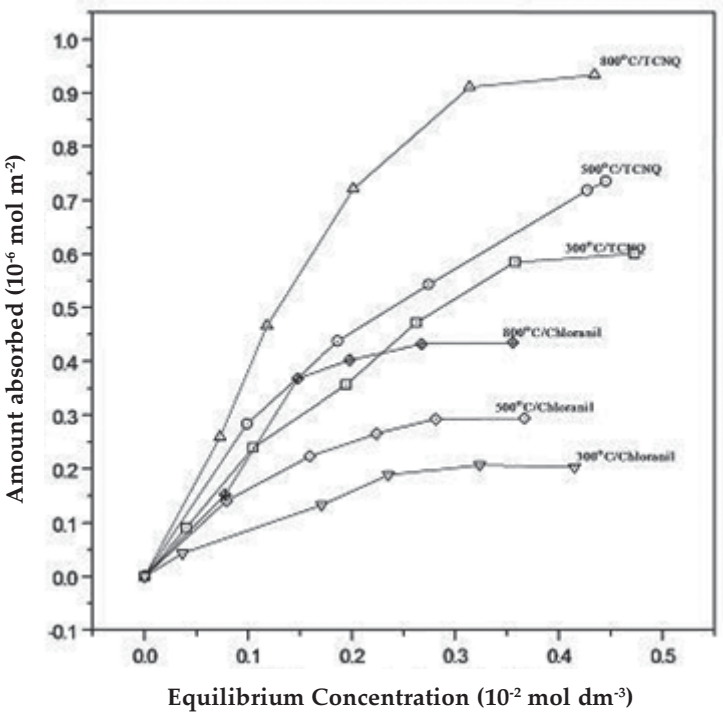

Figure-1: Langmuir type Adsorption Isotherms on Neodymia at different activation temperatures

Table-1: Limiting Amounts of Electron Acceptors Adsorbed on Neodymium-Zirconium Mixed Oxides Activated at Different Temperatures

Weight % of Nd_2O_3	Activation Temp. (°C)	Limiting amount Adsorbed 10^{-6} mol / sq.m	
		TCNQ	Chloranil
0	300	0.9656	0.6933
20	300	1.3733	0.9036
40	300	1.1653	0.9517
60	300	1.1498	0.9978
80	300	1.6089	1.8045
100	300	0.6008	0.203
0	500	2.0814	1.0613
20	500	1.884	1.3391
40	500	1.2585	0.9421
60	500	1.6319	1.2429
80	500	2.2291	1.8995
100	500	0.7358	0.294
0	800	2.2064	0.6835
20	800	1.5241	0.6878
40	800	4.4756	0.3608
60	800	2.5526	1.6708
80	800	1.8163	1.2484
100	800	0.9338	0.4348

The limiting amount of TCNQ adsorbed is a measure of the total number of electron donor sites on the surface. Two possible electron sources exist on the oxide surface capable of electron transfer, namely electrons trapped in intrinsic defects and surface hydroxyl ions. It has been reported at higher activation temperatures that the donor sites consist of a coordinatively unsaturated O^{2-} associated with a nearby OH^- group and the concentration of these sites is related to the base strength on the surface (Choudhary and Rane 1991). The more basic the surface, the higher is the number of O^{2-} which can transfer electrons to the EA.

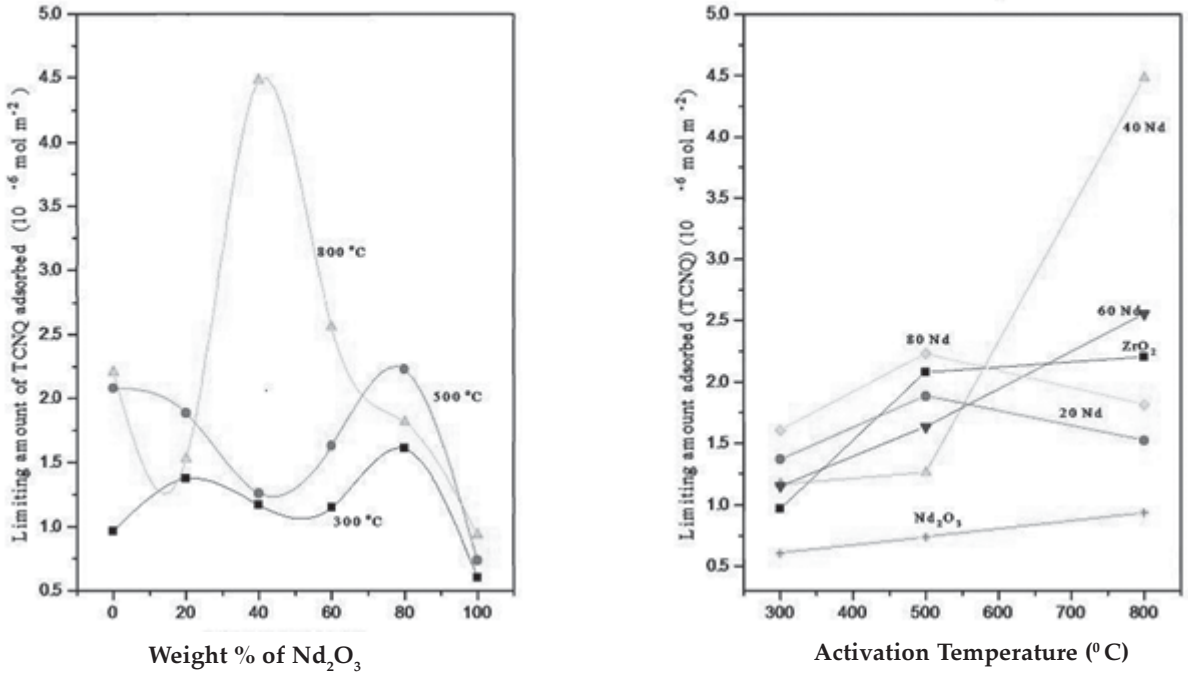

Figure-2: Limiting Amount of TCNQ adsorbed as a function of composition and as a function of activation temperature

The limiting amount of electron acceptor adsorbed (Figure-2) depends on the electron affinity of the electron acceptor. For ZrO_2, the limiting amount of TCNQ adsorbed increased with activation temperature having its maximum value for the sample activated at 800°C. For pure Nd_2O_3, the limiting amount adsorbed is less than that on ZrO_2 having the same trend as for ZrO_2 with the activation temperature, having its maximum value at 800°C. At 300°C, all the mixed oxides have larger values for limiting the amount adsorbed than the component oxides. At 500°C, the limiting amount of TCNQ adsorbed on the mixed oxides is intermediate between those of the component oxides, except in the case of 80 Nd which is having a higher value than the component oxides. At 800°C, the limiting amount of TCNQ adsorbed on 20 Nd and 80 Nd is intermediate between those at 300°C and 500°C. i.e., for these two mixed oxides, the limiting amount of TCNQ adsorbed first increases with activation temperature and then decreases. For 40 Nd and 60 Nd, the limiting amount of TCNQ adsorbed increased with activation temperature as in the case of pure oxides. For 40 Nd, the increase is very steep from 500 to 800°C.

The limiting amount of Chloranil (Figure-3) adsorbed on ZrO_2 increased from 300°C to 500°C and then decreased. In the case of Neodymia, the limiting amount increased with activation temperature. Neodymia has a very low EA adsorption compared to Zirconia. At 300°C the mixed oxides have higher value for the limiting amount of Chloranil adsorbed than pure component oxides. At 500°C, all the mixed oxides except 40 Nd are having higher values.

For most mixed oxides, the limiting amount of Chloranil adsorbed is greater than the component oxides. For 40 Nd activated at 500°C, the limiting amount adsorbed is intermediate between those of component oxides. 80 Nd is having the highest value for samples activated at 300°C and 500°C. At 800°C, 40 Nd is having a lower value than the component oxides; all others have higher values than the component oxides. 60 Nd has the highest value at 800°C.

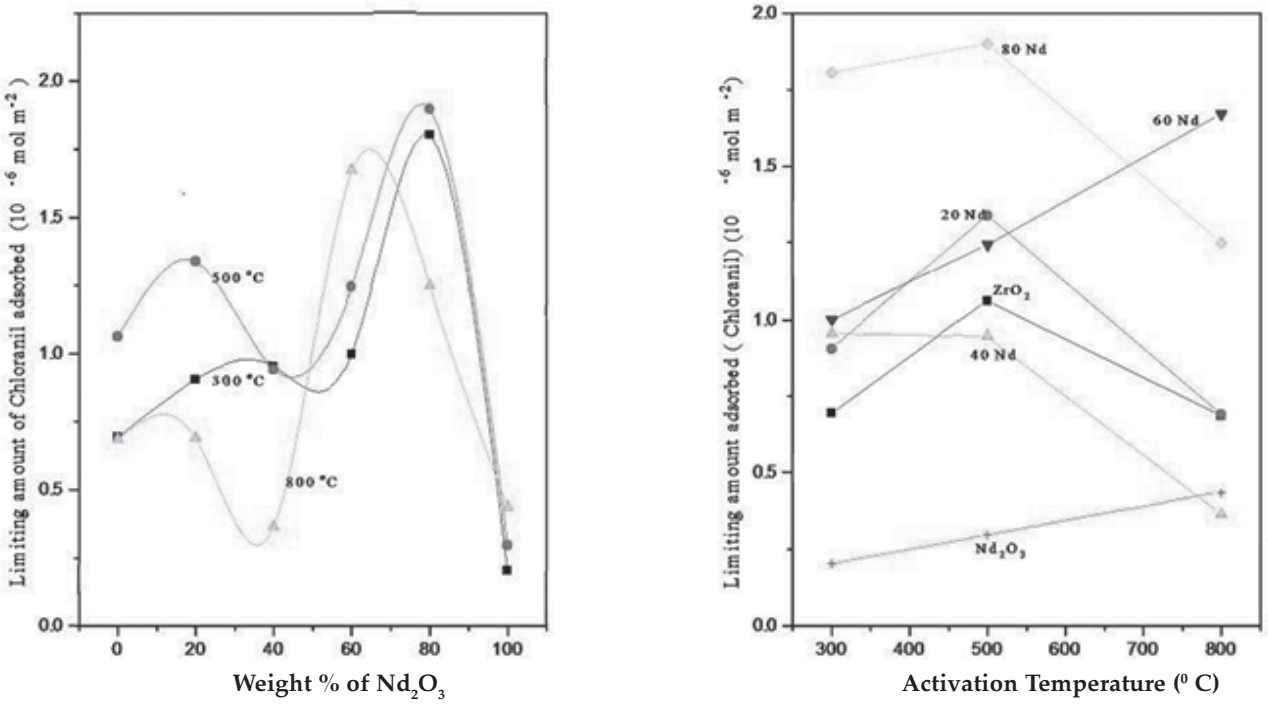

Figure-3: Limiting amount of Chloranil adsorbed as a function of composition and activation temperature

Fluorite structure is the structure of the high temperature form of ZrO_2 and of the so called stabilised Zirconias. At room temperature, ZrO_2 has a deformed structure, derived from the fluorite structure. Pure ZrO_2, when cooled from high temperatures, undergoes several phase transitions which are accompanied by relatively large volume changes. Due to the volume changes caused by the phase transitions, sintered compacts of pure ZrO_2 are pulverised on cooling. By doping ZrO_2 with lower valent ions, the cubic structure is stabilised and remains viable down to room temperature, even though it is metstable at that temperature.

In the stabilised Zirconias, the oxygen vacancies, which are present as the ionic majority defects, are randomly distributed over the crystallographic oxygen positions. In particular, ordering effects may occur at high dopant concentrations, depending on the ratio of the radii of the Zr^{4+} to dopant ions. When an equimolar

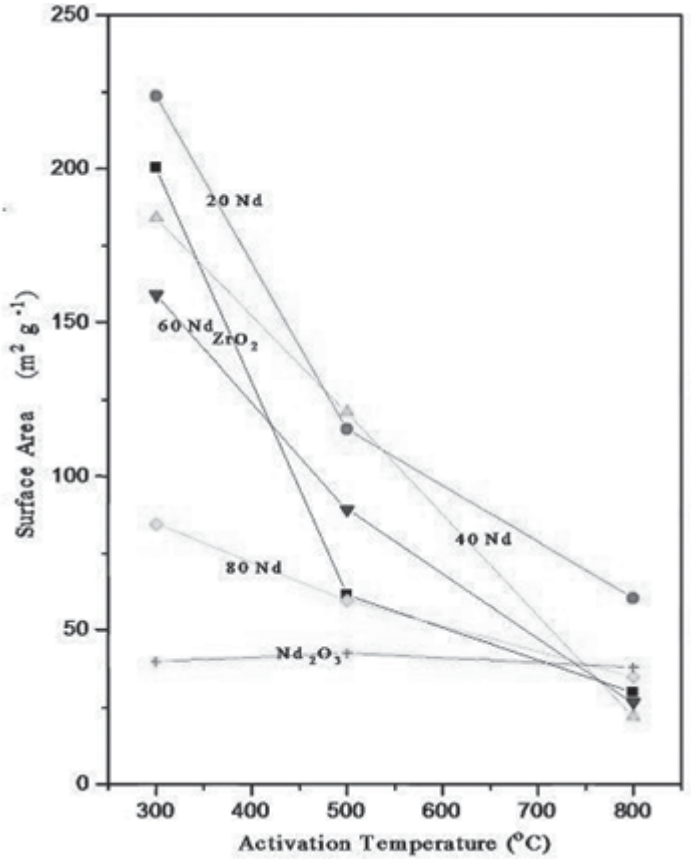

Figure-4: Surface areas of Nd-Zr mixed oxides as a function of activation temperatures

amount of trivalent ions has been added, the formula of the mixed oxide is $A_2B_2O_7$ and, depending on the type of A and B ion, an ordered superstructure of the Fluorite structure, the pyrochlore structure is formed. Pyrochlore has a unit cell, consisting of 8 fluorite cells with an ordered arrangement of oxygen vacancies. Six of the remaining oxygen are of the same crystallographic type and the seventh has a different crystallographic type. There is also cation ordering and the lattice is distorted in such a way that the smaller of the cations is surrounded by a distorted octahedron of oxygen, the larger by a distorted cube of oxygens.

When comparing the phase diagrams of the different ZrO_2-Ln_2O_3 systems, it is clear that the pyrochlore structure becomes less stable as the radius ratio $\frac{r_{Ln^{3+}}}{r_{Zr^{4+}}}$ decreases. As a consequence of the lanthanide contraction, from Gadolinium onwards, the pyrochlore structure is no longer formed. Around the corresponding composition, the stable phase is then the cubic fluorite structure with a random distribution of the metal cations over the accessible sites in the structure (Gellings and Bouwmeester, 1992).

ZrO_2 has a very high surface area. When activated at 300°C, surface area decreases with activation temperature and the decrease is steep (Table-2 and Figure-4). In the case of Nd_2O_3, the surface area is less compared to ZrO_2. The surface area of Nd_2O_3 increases from 300°C up to 500°C and then decreases. At 300°C and 500°C, surface areas of all mixed oxides are greater than that of Neodymia. At all activation temperatures, 20 Nd have larger surface areas than Zirconia. At 800°C, surface areas of 60 Nd and 40 Nd were less than the component oxides. However, surface areas can be considered to be improved by the mixing of oxides, especially at lower activation temperatures.

Table-2: Surface Areas of Neodymium-Zirconium Mixed Oxides

Weight % of Rare Earth Oxide	Surface areas (m^2 g^{-1}) of Nd-Zr Mixed Oxides Activated at		
	300°C	500°C	800°C
0	200.3	61.496	29.75
20	223.5519	115.2785	60.29195
40	183.7774	120.568	21.45165
60	158.8979	89.098	26.334
80	84.3458	59.594	34.884
100	39.7	42.4	37.9

Conclusions

The amount of electron acceptor adsorbed depends on the composition of the mixed oxide, activation temperature and electron affinity of the acceptor. Electron donor power increases with the basicity of the oxide. The limit of electron transfer is between 1.77- 2.40 eV for all oxides under study.

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Carbon fiber reinforced composites with interleaved nanofibers

Abdul Ahad Mokhtar*, Sarath Sasidharan*, C.G. Jayalakshmi, Avinash S. Hood**, and Anoop Anand****

Abstract

Benefits of nanotechnology reside mostly in laboratory scale studies. Nanofiber interleaved composites is one such area of research, wherein structural applications are restricted due to the inability to incorporate electrospun nanofibers in a large amount uniformly within the composite. In this study, we aim to tackle this problem by effectively incorporating commercial nanofiber veils as interleaves in composites through an out of autoclave composites manufacturing process named Resin Film Infusion (RFI). Carbon fiber/ epoxy composites interleaved with commercially available electrospun nylon 6, 6 nanofiber veils are realized through RFI process. Composite laminates are subjected to non-destructive/ultrasonic testing. Specimens from these laminates are characterized for their properties and compared with their respective control specimens made from laminates that are realized under identical conditions but without interleaved veils. Remarkable improvement in mechanical properties has been resulted in for interleaved composites, when compared to control specimens.

Keywords: *Fiber reinforced composites, Nanofiber veils, Mechanical properties, and Resin film infusion.*

*Abdul Ahad Mokhtar and *Sarath Sasidharan, Composites Research Center, Research and Development Establishment (Engineers), DRDO, Ministry of Defence, Alandi Road, Pune–411015, Maharashtra, India. School of Materials Science and Engineering, National Institute of Technology, Calicut–673601, Kerala, India, Email:

**C.G. Jayalakshmi, **Avinash S. Hood, and **Anoop Anand, Composites Research Center, Research and Development Establishment (Engineers), DRDO, Ministry of Defence, Alandi Road, Pune–411015, Maharashtra, India, Email: aanand.rde@gov.in,
ORCID iD: https://orcid.org/0000-0002-9064-6803

Fiber reinforced polymer (FRP) composite materials (Schwartz 1977) are being successfully used over their metallic counterparts in several industries for weight critical structural components. These include several applications in aviation (Bachmann, J., et al. 1301-17), (Nakada, M. and Miyano Y. 805-13), construction (Hollaway, L.C. 2419-45) and (Zaman, A., et al. 1966-88), military engineering (Jayalakshmi, C.G., et al. 47241), etc. Glass fiber (GFRP) and carbon fiber reinforced polymer composites (CFRP) are the most popular and are being used in several primary load bearing applications (Benzarti, K. and Colin, X. 2013). Especially, carbon fiber composites are preferred for high performance applications due to better strength and modulus over other fiber reinforced composites. These composites are generally being manufactured either in an autoclave or using out of autoclave (OoA) processing techniques. Though the former is considered as the gold standard for aerospace quality composites, out of autoclave composite manufacturing techniques (Harshe, R. 207-20) such as Resin Transfer Molding (RTM), Vacuum Assisted Resin Transfer Molding (VARTM), Resin Film Infusion (RFI) etc., are being preferred over conventional autoclave processing techniques, mostly due to the higher capital and running costs of production and less flexibility with reference to the size and shape of products, in autoclave molding. In particular, RFI is renowned for its ability to process high viscous resin matrices and filler modified resins to result in composites with high fiber volume content. It is an open mold vacuum based curing technique, which ensures consolidated laminates of near zero void fractions.

One of the most popular thermosetting matrix materials for making structural composites is epoxy. Nevertheless, epoxy is known for its inherent brittleness and relatively poor toughness, which makes its composites unsuitable in bearing impact loads, for example bird strike, tool drop during maintenance, etc. (Liu, Y., et al. 108-13). Hence there is a necessity for toughening epoxy matrix-based composites. Toughening of a matrix can usually be achieved by the incorporation of toughening additives. Incorporation of thermoplastic toughening phase in a thermosetting composite has been gaining popularity and has been proven to be effective (Nash, N.H., et al. 582-97) and (Inamdar, A., et al. 4479-504). Common techniques for toughening thermosetting composites using thermoplastic additives can be categorized into two: Bulk matrix toughening as well as interlaminar toughening. Bulk matrix toughening method involves the use of toughening fillers such as rubber particles, hyper branched polymers, etc. But this method can lead to increase in viscosity of the matrix, which causes difficulties for processing and in turn can result in voids within the composite due to incomplete wet-out and poor resin flow during manufacturing. Interlaminar toughening can be carried out by the use of commingled fibers (Patou, J., et al. 260-75), thermoplastic films (Hojo, M., et al. 665-75), non-woven veils Kuwata, M. and Hogg, P.J. 1551-59) etc. This is expected to improve the weak interlaminar properties and reduce delamination tendencies of composite laminates (Beckermann, G.W. and Pickering, K.L. 11-21), (Hogg, P.J. 97-103), and (Beckermann G.W. 289-93). Non-woven veils are being used as a cost-effective toughening method, as more and more industrial firms are coming forward with the production of such veils commercially. In this method of inter-laminar toughening, a low areal density fibrous veil is placed at the interlaminar regions of the composite laminate. In general, this act of insertion of an additional layer (interleave) within the composite laminate to impart property modifications can be termed as 'interleaving.'

In the present study, an attempt has been made to use polyamide 6, 6 nanofiber veils. Polya-

mide 6, 6 or nylon 6, 6 is a thermoplastic well known for its high mechanical strength, rigidity, good stability under heat and/or chemical resistance. High melting point of nylon 6, 6 (264°C) ensures that these nanofibers remain unaffected during and after the curing process of the host composite laminates. Nylon contains monomers of amides joined by peptide bonds. Strong interaction between epoxy and nylon is possible due to covalent bond formation between the reactive amide hydrogens and epoxide groups. Another mechanism for good adhesion between the epoxy and nylon are hydrogen bond formation between amide hydrogen in polyamide (hydrogen bond donor) and oxygen (hydrogen bond acceptor) in epoxy resin (Deng, S., et al. 121-32), (Zhong, Z. and Guo, Q. 3451-58), and (Gorton, B.S. 1287-95).

Peijs, et al. (1122-24) in 1991, for the first time used the method of interleaving to investigate the impact properties of carbon/epoxy composite using high performance polyethylene interleaves. They concluded that the hybrid effect caused a tendency to lower the initiation energy and considerably increase the propagation energy. Beckerman and Pickering (11-21) in their experiment concluded that nylon 6, 6 interleaves of 4.5 gsm in carbon/epoxy prepregs exhibited remarkable interlaminar fracture toughness properties (mode I and mode II) compared to many other thermoplastics. In an earlier attempt, our group has (Anand, A., et al. 1-8) manufactured glass/epoxy structural composites through RFI technique wherein one side of each glass fabric was electrospun (Sajeev, U.S., et al. 343-51) with nylon 6, 6 nanofibers at a very low areal density. It was observed that the compressive strength and interlaminar shear strength (ILSS) had a substantial enhancement in comparison to the control composites. Quan, et al. studied the fracture behaviour of carbon-epoxy composites interleaved with multiwall carbon nanotube (MWCNT) and graphene nanoplatelet (GNP) doped polyphenylene sulphide (PPS) veils (235). They observed that presence of MWCNT enhanced the adhesion between the PPS fiber and matrix, whereas GNP reduced the adhesion. Recently, some interesting studies related to this field are also reported (Shin, Y.C., et al. 236).

ILSS is an important property especially for high end structural applications. This value depends on the fiber - matrix interaction within the composite. Various test methods categorized as micro and macro mechanical tests are developed in order to determine the interfacial strength between fiber and matrix. Tests such as single fiber pull out, single fiber push out belong to the category of micro-mechanical testing methods, which are generally used in model composites with single or a few fibers. Macro-mechanical testing methods include short beam test, four-point flexural test, etc. Generally, short beam shear test is considered as the standard test for ILSS of fiber reinforced composites, hence selected in this work. In addition to interlaminar shear, other mechanical properties such as compressive and flexural characteristics of the composite have also been evaluated as per ASTM.

As per the authors' literature survey, limited efforts have been reported on the use of RFI technique for interleaved composites. None of them investigated the laminate defects that might have crept in due to the interleaving process. This systematic way of manufacturing can help in realizing the use of interleaved composites in commercial industries practically feasible. In this experiment, composite laminates of bi-directionally woven carbon/epoxy, interleaved with nylon 6, 6 nanofiber veils have been fabricated. Specimens from these laminates are then mechanically characterized for their properties and are compared with that of control specimens. Outstanding enhancements in structural proper-

ties are obtained through this method of composite manufacturing.

Experimental methodology

Materials

Fiber reinforced polymer composite laminates with carbon fiber reinforcements (CFRP) are prepared with and without (control) interleaves. Reinforcement used for fabricating CFRP composite is a bidirectional carbon fabric (T300) with a ply thickness of 0.20 mm, areal density of 200 g/m^2 (material density = 1.78 g/cm^3), from Urja Products Private Ltd., India. A commercially available nanofiber veil with the trade name Xantu.Layr^{TM}, from Revolution fibres, New Zealand, has been used as interleaving veils between each ply during layup. It consists of continuous nylon 6, 6 nanofibers, which are electrospun onto a silicone release film using sonic electrospinning technology. The veils have an areal density of 1.5 g/m^2. All materials for the subject study were used as received. An in-house developed proprietary epoxy formulation has been used as the matrix polymer in the RFI process.

Composite fabrication

Composite laminates with and without interleaves were manufactured through resin film infusion (RFI) technique (Cherukattu Gopinathapanicker, J., et al. 7502-11), (Anand, A., et al. 161824), and (Jayalakshmi, C.G., et al. 2217-22). The number of plies required for laminate fabrication is determined depending on the thickness of the final test specimens required. Ply dimensions are chosen such that a minimum set of 8 specimens can be ensured for each mechanical test (ILSS (American Society 2006), compression, flexural). The nanofiber interleaves are cut (Composite Strengthening 2020) to required dimensions and are placed between each fabric plies during layup. The incorporation of these interleaves has to be done carefully as they tend to get shifted from its original position or may get torn or wrinkled, which can affect the final properties of the composite. The number of plies (n) is determined using the values of individual ply thicknesses and the required thickness of test specimens. Accordingly, n-1 interleaves are incorporated between each plies for CFRP composite layup.

In the RFI process (Anand, A., et al. 1618-24), (Panse, P., et al. 1216-22), and (Anand, A., et al. 2937-43), epoxy resin is cast to film form and sandwiched between fabric plies along with the interleaves. This stack of fabric plies, interleaves and resin films are then vacuum bagged along with the consumables required for RFI. The whole setup is placed in an oven and is subjected to high temperatures, which causes the resin films to melt and infuse into the plies and interleaves, followed by the curing of laminate. Uniform resin distribution within the laminate is achieved through RFI technique. This is one of the reasons why this method is employed in producing large structures (Lionetto, F., et al. 2020). In addition, good compaction and local flow of the matrix resin ensures near zero void fractions.

Once the laminates are prepared, they are subjected to ultrasonic scanning. Further the required number of specimens for each test is cut out of the laminates using a specimen cutting tool. The specimens are polished using emery paper and are subjected to subsequent testing according to relevant ASTM. Extreme caution to avoid human errors, instrumental errors as well as experimental inefficiencies has been undertaken. The delicate structure of the veils should be handled carefully especially during layup since, even a wrinkle in the veil can drastically influence the properties of the composite.

Characterization

Morphology of the nanofiber veil/interleave is analyzed with high-resolution field-emission scanning electron microscope (Hitachi SU-6600) at 5 kV accelerating voltage. Ultrasonic A-scan is carried out on the composite laminates to determine the laminate quality using a high voltage pulser - receiver (Olympus Panametrics Model 5058PR) in pulse - echo mode.

A universal testing machine (BISS, load cell: 100 kN) is used in this investigation to record the mechanical properties of composites according to ASTM D2344 (American Society 2006) (ILSS), ASTM D3410 (175-186) (compressive strength), and ASTM D 7264 (2015) (flexural strength). A minimum of 8 specimens from each laminate is tested for consistent values.

Results and discussion

Mechanical characteristics of CFRP composites, fabricated through RFI technique, with and without interleaved nylon 6, 6 nanofiber veils are reported in this section. Prior to making laminates, scanning electron microscopy (SEM) of the nanofiber veils was carried out in order to understand the structure of the electrospun nanofibers. The SEM images at different magnifications are shown in Figure-1. It is understood from these images that the nanofibers are randomly oriented and can be considered direction independent. It is seen that this interleave possess a web like structure consisting of continuous nanofibers and have multiple layers of these webs in Z-axis direction. Diameter of the nylon nanofibers is observed to be varying in the range 241 nm - 338 nm. As evident from the SEM images, these interleaves possess a porous structure, thus contributing to high surface area of interaction with its surrounding matrix, which can positively affect the mechanical properties of the composite.

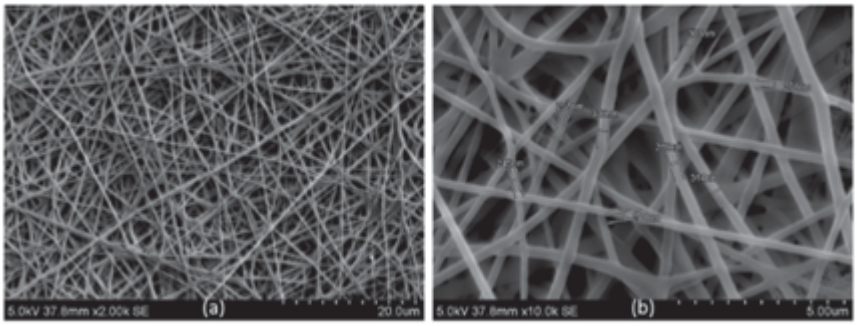

Figure-1: SEM image of nylon 6, 6 nanofibers interleave at (a) 2k magnification (b) 10k magnification

Non-destructive testing of laminates

Manufactured laminates were inspected non-destructively using ultrasonic A-scan method. In this technique, an ultrasonic impulse at a particular frequency is produced by a transducer, which propagates through the thickness of laminate and returns back to the transducer. The amplitude of this resultant signal can help determine if the laminate has any flaws or defects such as air bubbles, cracks etc. Variations in the amplitude peaks represent the presence of defects in the laminate. Figure-2 represents the amplitude plot of the two sets of laminates that are manufactured in this experiment, each tested at a random location of the laminate. From the Figure, it can be concluded that laminates are showing consistent peaks, which is an indication

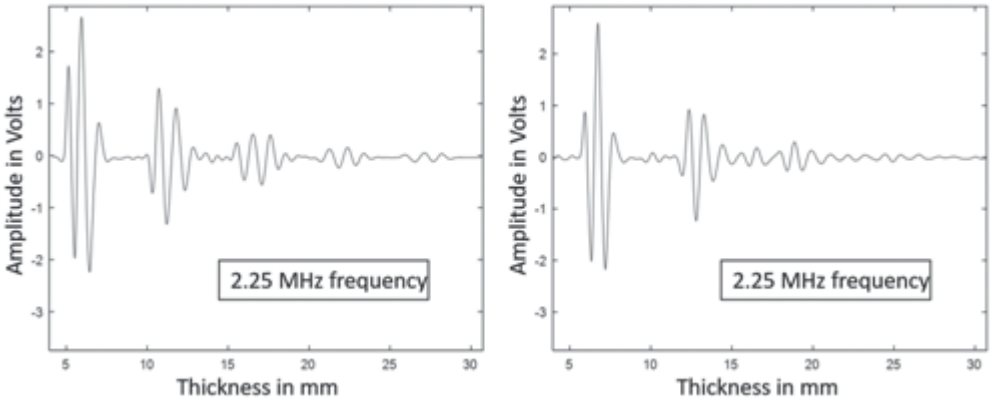

Figure-2: Amplitude vs thickness plots of control CFRP (left) and interleaved CFRP (right) obtained through ultrasonic A-scan, conducted at random locations of each laminate

that the laminates are defect free. And no detectable air bubbles or cracks are present in these laminates. However, there is a limit to the size of defects that can be detected using this technique. The minimum size that can be detected is generally considered as one-half wavelength of the sound wave propagating through the laminate. This can be calculated using the following equation:

$$Wavelength\ (m) = \frac{Speed\ of\ sound\ wave\ in\ the\ laminate\ \ (\frac{m}{s})}{Frequency\ (Hz)} \quad (1)$$

where, speed of sound wave in CFRP is 3070 m/s (Material Sound Velocities 2020). The waves were produced at a frequency of 2.25 MHz for the CFRP laminates. Hence the half wavelength (minimum detectable size of flaws/ bubbles) was found to be 0.682 mm for CFRP laminates. The thickness build-up in the laminate due to the presence of interleaves can be inferred from the slight rightward shift (towards increasing thickness) of the peaks compared to control laminates.

Thickness, when measured under pulse echo mode is the product of velocity of the wave within the material and one half the transit time of the wave. Echo peaks are observed at thickness values around 12 mm, 18 mm, 24 mm (multiples of 6mm, the original thickness of the laminate) and is diminishing in nature since the strength of the sound waves also diminishes with each round trip due to attenuation.

Mechanical properties

Mechanical properties of CFRP such as ILSS, flexural and compressive strengths were measured, with and without interleaving Nylon 6, 6 nanofibers. Photograph of a representative ILSS test specimen with Nylon 6, 6 nanofiber interleaves is shown in Figure-3. Interleaved composites manufactured though RFI process exhibited a remarkable ILSS improvement of 19.15%. Compressive strength was enhanced by 9% and flexural strength showed an improvement of 12%. These improvements in mechanical properties could be attributed to the presence of nanofiber deposits that act as a barrier to crack initiation

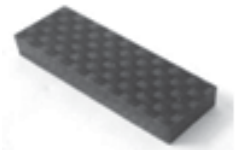

Figure-3: Representative CFRP specimen (36 mm × 12 mm × 6 mm) with Nylon 6, 6 nanofiber interleaves for testing of interlaminar shear strength

and propagation within the matrix and also resisting micro buckling of fibers within the structure. In addition, a mechanical interlocking effect between the adjacent fiber laminas is formed by the interleaves that contribute to the improvement in interlaminar properties. Indeed, previous studies reported improvement in several properties by adding nanomaterials in the matrix or sizing (Sasidharan, S. and Anand, A. 12617-31), (Sreekumar, P., et al. 198-204), and (Anand, A. and Joshi M. 233-47). For example, Zhang, et al. (1543-52) in 2012 incorporated 5 wt% graphene oxide in the sizing of carbon fabric/ epoxy composites and observed an enhancement in ILSS by 12%. Similarly, Safi et al. (314-21) obtained 35% improvement in impact strength by using nanosilica dispersed epoxy matrix for a GFRP composite. However, matrix / sizing modification through nanoparticle incorporation face several difficulties in practical large-scale productions due to lack of proper mixing and dispersion of nanoparticles in the system. But through this method of interleaving, enhancement in properties can be achieved with better efficiencies with regard to manufacture time, cost and ease of fabrication. Figure-4, Figure-5, and Figure-6 represent the plot of average ILSS, flexural strength and compressive strengths of interleaved and non-interleaved composite specimens.

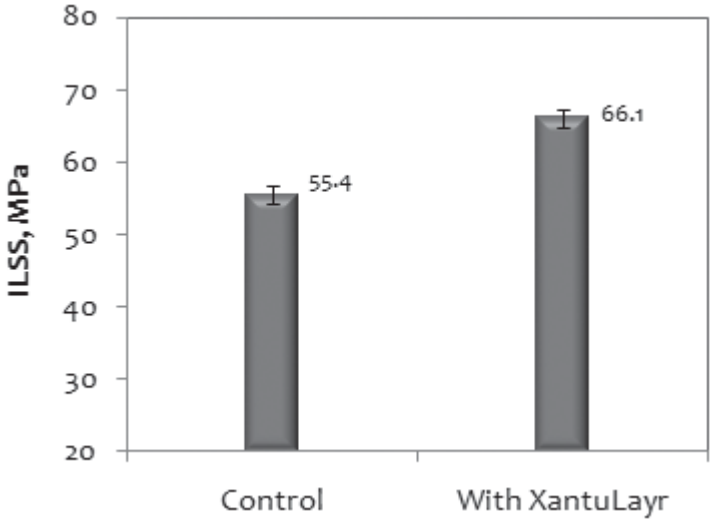

Figure-4: Average interlaminar shear strength (in MPa) of composite specimens with and without interleaves tested as per ASTM D2344

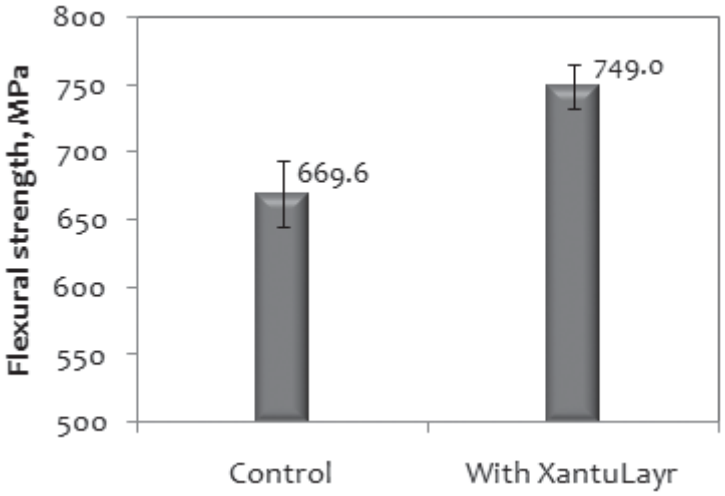

Figure-5: Average flexural strength (in MPa) of composite specimens with and without interleaves tested as per ASTM D7264

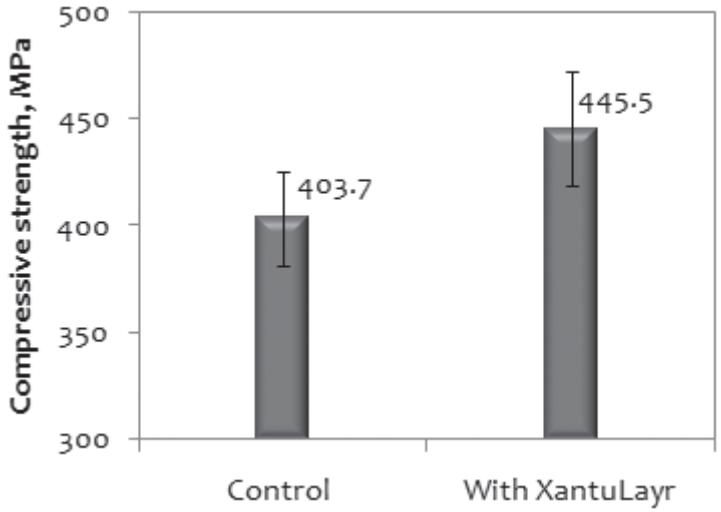

Figure-6: Average compressive strength (in MPa) of composite specimens with and without interleaves tested as per ASTM D3410

Conclusions

Carbon fabric reinforced epoxy composite laminates were manufactured using Resin Film Infusion (RFI) process with and without electrospun nylon 6, 6 interleaves. Interleaves were placed between fabric plies along with epoxy resin film during layup and were vacuum cured. The nylon 6, 6 interleaves were made of porous randomly oriented mat of nanofibers of approximate diameter 240-340 nm. Ultrasonic A-scan was used to ensure that the laminates with interleaving had not undergone any quality deterioration due to the nanofibers at the interface and no cracks or air entrapments were found in them. Interlaminar shear strength of interleaved composites exhibited a remarkable improvement of 19.15% compared to their respective control specimens. Similarly, compression strength and flexural strength of the interleaved specimens were observed to be increased in comparison to their respective control specimens.

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Sources and Means of Derivations of Chitin and Chitosan

Alena Mariam Aji,* Manya S. Nair,* Feba Anna John, and Ajith James Jose****

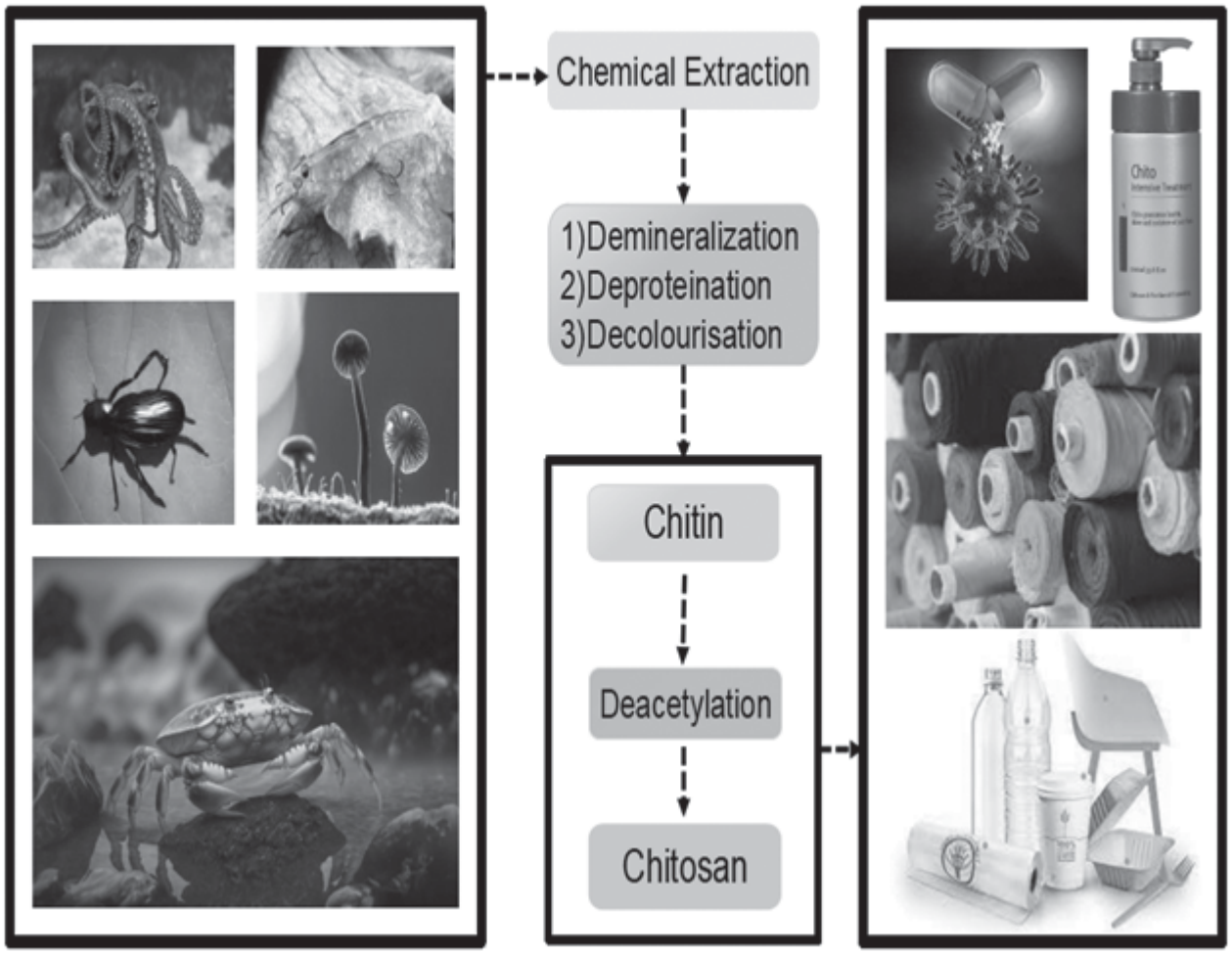
Abstract

In recent years, the use of complex molecules based on natural biopolymers seems to be under intense progress in various research fields. Chitin and its deacetylated derivative chitosan are biodegradable natural polymers which possess properties such as biocompatibility, mucoadhesion, nontoxicity, haemostatic, anti-microbial, anti-inflammatory, antioxidant, analgesic, anti-hypertensive, anti-cholesterolemic, anti-cancer and anti-diabetic. Among the different biopolymers which are of marine origin, chitin and chitosan are of great interest due to their unique biological and technological properties. Despite its biodegradability, it has many reactive aminoside groups, which offer possibilities of useful derivatives that are commercially available or can be made available via graft reactions and ionic interactions. Chitin and chitosan are commercially manufactured from biowastes obtained from aquatic organisms. This biopolymer materials have tremendous potential to be used as drug delivery systems, tissue engineering scaffolds, wound dressing materials, antimicrobial agents and biosensors. The present review investigates the structure, properties, sources, applications and synthesis of chitin and chitosan.

Keywords: *Chitin, Chitosan, Anti-microbial, Antioxidant, Biopolymer, and Deacetylation.*

*Alena Mariam Aji and *Manya S. Nair, St. Thomas College, Pazhavangadi P. O., Ranni, Pathanamthitta-689673, Kerala, India.

**Feba Anna John and **Ajith James Jose, St. Berchmans (Autonomous) College, Changanacherry-686101, Kerala, India. Corresponding Author: Ajith James Jose, Email: ajithjamesjose@gmail.com



Nowadays, the significance of environment-friendly technology has stimulated interest in biopolymers and bio-based polymers, due to their outstanding physical and biological properties. Chitin, and its derivative chitosan are considerably versatile and promising biomaterials. They can be found as supporting materials in many aquatic organisms, terrestrial organisms, and some microorganisms and also present in vertebrates (zebrafish gut and epithelial cells of fish scales). Competing with cellulose and starch for being the most abundant natural organic compound, chitin and chitosan (CS) are considered to be materials of great futuristic potential with immense possibilities for structural modifications to impart desired properties and functions. Research and development work on chitin and chitosan have reached a status of intense activities in many parts of the world (Sultankulov, Bolat, et al. 2019). The positive attributes of excellent biocompatibility, admirable biodegradability with ecological safety, low toxicity, antimicrobial activity and low immunogenicity have provided ample opportunities for further development. Moreover, the physicochemical characteristics of chitin and chitosan vary from batch to batch due to the seasonality of raw materials, quality of the shell, species present, climate, and difficulties in process control. This has led to expanding interest in biotechnology research regarding the production of chitin and chitosan from different sources such as the exoskeleton of insects and cell walls of fungi, etc., (Azuma,

Kazuo, et al. 2015 and Jollès, Pierre, and Riccardo A. A. Muzzarelli 1999).

Despite its huge annual production and easy availability, chitin still remains as an under-utilized resource primarily because of its intractable molecular structure (Younes, Islem and Marguerite Rinaudo 2015). Traditional methods for commercial preparation of chitin and chitosan from their resources present some drawbacks, since it is expensive, have inconsistent molecular weight (MW), degree of acetylation (DA), and are environmentally unfriendly. The insolubility of chitin in almost all common solvents has been a stumbling block in its appropriate utilization. However, biotechnology fermentation processes, i.e., deproteination and demineralization by protease and organic acid bacteria and deacetylation by chitin deacetylase were a suitable and economical method to extract chitin and their derivatives with recovery of bioactive compounds; these processes can offer new perspectives for the production of highly viscous chitosan, with a promising potential for innumerable application (Kafetzopoulos, et al. 1993, Sreerag Gopi, et al. 2020, and A. Zamani, and M. Taherzaheh, 2010).

There have been a number of earlier attempts at reviewing the area on chitin and chitosan fibers covering certain aspects of their importance, properties and applications. Rathke and Hudson (Kumari, Suneeta and Rupak Kishor, 2020) pointed out that chitin's microfibrillar structure indicated its potential as fiber and film-former, but as chitin was found to be insoluble in common organic solvents, the N-deacetylated derivative of chitin, chitosan, was developed. After Rinaudo and coworkers (Raval, Ritu 2013), who described the production of chitin and chitosan fibers by wet spinning method and Rajendran and Anand (Pagnoncelli, Maria Giovana Binder, et al. 2010), discussed briefly the properties of Chitin and chitosan fibers. There have been no serious attempts at reviewing the production, properties, and applications of chitin and chitosan fibers. Considering the potential applications of chitin and chitosan fibers, it appears that a consolidation of the data relating to the chemistry, properties, synthesis, and applications of chitin and chitosan polymers is required. The aim of this review is to concentrate on the preparation, structure, and properties of chitin and chitosan following various applications of both polymers and emphasizing the effects of the polymers' characteristics on these applications (Sinha, Sujata, et al. 2014 and Xia, Wenshui, et al. 2008).

2. Structure and Properties of Chitin and Chitosan

2.1 Chemical Structure of Chitin and Chitosan

Chitin is a white-coloured hard, and inelastic nitrogenous polysaccharide, occurs in nature as semi crystalline to crystalline microfibrils. It is a linear copolymer of β- (1, 4)-linked 2 amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan. Structurally, chitin is composed of β- (1, 4)-linked D-glucosamine monomers (N- GlcNAc) as shown in Figure-2. Unlike cellulose (Figure-1), the hydroxyl group of cellulose is replaced by N-acetyl group (-$NHCOCH_3$) (H.K. Nu, et al, 2003 and S. Hajji, et al. 2014). Chitin occurs in different polymeric forms such as α, β, and γ, depending upon the bonding between the chains. The α-chitin form is most abundant, followed by the β and γ forms (K.L.B. Chang, et al. 1997). α-Chitin comprises two antiparallel molecules per unit cell with intermolecular hydrogen bonds, whereas only one molecule is present in the parallel arrangement in β-chitin, and γ-chitin consists of mixed parallel and antiparallel orientations.

Chitosan is an N-deacetylated derivative of chitin obtained by transforming the acetamide groups into primary amino groups. It consists of N-acetyl-2-amino-2-deoxy-D-glucopyranose (acetylated unit) and 2-amino 2-deoxy-D-glucopyranose (deacetylated unit), where the repeating units are linked by β- (1, 4)- glycosidic bonds as shown in Figure-3. It is a polycationic polymer with more than 5000 D-glucosamine units. Chitosan is a chitin derivative in which the attached acetamide groups are transformed into primary amino groups (H. K. Nu, et al. 2003, S. Hajji, et al. 2014, KLB Chang, et al. 1997, and T. Sannan, et al. 1976).

In chitin and chitosan structure, the hydroxyl group C-2 position of cellulose has been replaced by an acetamidegroup (J. Bossaer 2011). Unlike cellulose, chitin and chitosan contain 5-8% nitrogen, which in chitin is in the form of acetylated amine groups and chitosan in form of primary aliphatic amine groups, which makes chitin and chitosan suitable for typical reactions of amines (C. J. Jiong and M.Q. Xu 2000). However, chitosan is chemically more active than chitin due to the presence of primary and secondary hydroxyl groups on each repeat unit and the amine group on each deacetylated unit. These reactive groups are readily subject to chemical modification to alter the mechanical and physical properties of chitosan.

2.2 Properties of Chitin and Chitosan

Chitin and chitosan have some special properties that make them suitable for versatile applications. The use of chitin and chitosan in the medical and pharmaceutical sectors has grown rapidly and currently received a great deal of interest from researchers throughout the globe due to their interesting properties such as strong antibacterial effect, biodegradability, non-toxicity and high humidity absorption (T. Sannan, et al. 1976).

2.2.1 Solubility

Like cellulose, chitin as a biopolymer is a highly crystalline material having specific solvent behaviour. The solubility and reactivity of chitin is highly influenced by $-NH_2$ and -OH functional groups. Being a typical natural polymer obtained from different sources, chitin possesses excessive hydrogen bonding which in turn degrades it before melting and thus making it essential to be dissolved in a suitable solvent. Chitin is generally hydrophobic in nature and hence insoluble in water and organic solvents at room temperature, but soluble in hexafluoro isopropanol, chloroalcohols, and hexafluoro acetone in association with mineral acid aqueous solution and dimethyl acetamide having 5% lithium chloride (R. K. Mohammed 2009). In other words, water is a thermodynamically poor solvent for uncharged chitin units due to strong interactions. Thus, the charge on the polymeric chain can make the chitin soluble by releasing attractive forces. It has also been reported that a small amount of chitin can be dissolved in a 1% acidic solution at 121°C for 20 min and on reacting it with HCl and NaOH, decrement in crystallinity index (Khong, Thang Trung 2013 and Ahmed S. and Ikram, S. 2015). In case of α, β, and γ-chitin, both α and β conformations maintain a strong network dominated by inter-chain hydrogen bonds between the groups of C=O—NH and C=O—OH within a distance of 0.47 nm. In α-chitin conformation, additional inter-chain hydrogen bonds bind the hydroxymethyl groups while this type of interaction is not observed in β- conformation. Thus the β- chitin is more reactive than the α- form and is more susceptible to dissolving in solvents than other forms. γ- chitin is a mixture of both α and β- forms (Zamani, A. Edebo 2007, Szymańska, E., Winnicka, K. 2015, and Kumar, M.N.V.R. 2000).

Chitosan is generally soluble in some organic as well as inorganic acids having a pH less than 6.0 in the solution and forms a non-newtonian, thin shearing fluid. Usually, the organic acids display solubility including methanoic acid, acetic acid, hydrochloric acid, dilute nitric acid, lactic acid etc. Out of this 0.2-100%, aqueous methanoic acid is considered the best solvent to dissolve chitosan. But the most frequently used solvent is 1% acetic acid at pH 4.0. Chitosan is usually insoluble in phosphoric and sulphuric acid but at an elevated temperature around 95-121°C, both low as well as medium molecular weight chitosan can be dissolved. Chitosan can form the water-soluble salt such as pyruvate, malate, lactate, malonate, ascorbate, acetate, tartrate, glyoxylate, and glycolate. Solubility of chitosan in solution mainly depends on the degree of deacetylation, method of extraction, time, temperature, concentration and molecular weight (Philippova, O. E. and Korchagina, E. V. 2012, Pillai, C.K. et al. 2009, and Kim, S. K. 2010).

2.2.2 Viscosity

Generally, the viscosity of the chitin and chitosan solution is affected by many factors such as temperature, pH, concentration, molecular weight, degree of deacetylation and method of extraction. If the temperature of chitosan solution increases, its viscosity will decrease. The reverse is observed in the case of concentration where there is an increase in viscosity as the concentration increases. In an acid medium, chitosan acts as an excellent viscosity enhancer.

The hydrolysis of chitin with concentrated acids under drastic conditions produces relatively pure D-Glucosamine. Additionally, the intrinsic viscosity of chitin can be considered a function of demineralisation time. Temperature is also an important criterion for purity of chitin which is carried out at high temperature and produces better results as compared to low temperature and obtains stable viscosities with time as well (Zamani, A. Edebo 2007, Szymańska, E., Winnicka, K. 2015, and Kumar, M.N.V.R. 2000).

2.2.3 Reactivity

Nitrogen content available in chitin depends on the extent of degree of deacetylation and is found to be 5-8%. However, the nitrogen content available in chitosan is present in the form of aliphatic amino groups and hence undergoes important amine N-acylation Schiff reactions (Khong, Thang Trung 2013).

Chitosan is a linear chain biopolymer having $–NH_2$ and -OH functional groups as a reactive site of its backbone. More the protonated $–NH_2$ group available in the polymeric chain, more is its reactivity and hence the ability to bind with toxic metal ions and also forms chelates with transition metal ions. This chelating ability of chitosan exemplifies its importance in water treatment and air purification. Chitosan can react with aldehydes and ketones to yield aldimines and ketimines, respectively to room temperature. The cationic nature of chitosan affects the reactivity, solubility, absorption and biodegradability of chitosan (R. K. Mohammed 2009).

2.2.4 Molecular Weight

Both chitin and chitosan are considered as high molecular weight biopolymers. Their molecular weight varies with the variation of source material and the preparation and extraction methods. The molecular weight determines the suitable application of the biopolymers. Molecular weight of chitin is as high as many Daltons.

The molecular weight of chitosan ranges from 100 to 1500 kDa. Generally, molecular weight of chitosan deal with the average of all the molecules present in the sample and can be

evaluated with the help of advanced techniques such as light scattering, osmometry, NMR, viscometry, gel permeation chromatography and size exclusion chromatography. Low and high molecular weight chitosan shows different effect on their physicochemical properties such as viscosity, hydrophilicity, moisture content, thermal properties and stability. The low molecular weight chitosan has ability to penetrate inside the bacterial cell thereby inhibits the RNA transcription leading to the death of the cell. Also high molecular weight chitosan is considered as more stable. Various factors viz., thermal stability, temperature, pH, and mechanical shearing of chitosan affect the molecular weight of chitosan and are responsible for the variation in polydispersity index (Kim, S. K. and Chitin, Chitosan 2010 and P. A. Sandford and A. Steinnes 1991).

2.2.5 Degree of Deacetylation

The degree of deacetylation is one of the most important parameters that affect the physical and chemical characteristics of chitin and chitosan. It is the ratio of 2-acetamido-2-deoxy-D-glucopyranose to 2-amino-2-deoxy-D-glucopyranose structural units. This ratio has a noticeable effect on chitin solubility and solution properties. Various methods like Infrared spectroscopy, ^{13}C solid-state NMR, High-pressure liquid chromatography (HPLC) are explored for the determination of chitin and chitosan deacetylation (H. E. Knidri 2018, W. G. Birolli 2016, and J. A. S. Moreno, et al. 2018).

3. Sources and Extraction of Chitin and Chitosan

Chitin is generally produced from natural sources, terrestrial organisms, marine organisms, and microorganisms like fungi and enzymatically from crustacean shell. Chitosan is obtained from the deacetylation of chitin. Now a days, chitin and chitosan are commonly manufactured from biowaste obtained from aquatic organisms. But due to the seasonal and capricious availability of raw materials, terrestrial crustaceans and mushrooms are the alternative source for their production (A. J. Al-Manhel 2018).

3.1 Sources and Extraction of Chitin

Chitin is usually isolated from the exoskeletons of arthropods, crustaceans, molluscs, insects and certain fungi. It is a biological nanocomposite material which is strictly hierarchically organized which reveals various structural levels. At the molecular level is the polysaccharide chitin itself. The next structural level is the arrangement of such molecules in the form of narrow and long crystalline units, which are wrapped by proteins, forming nanofibrils of about 2-5 nm diameter and about 300 nm length. The next step in the scale consists of the clustering of some of these nano fibrils into long chitin-protein fibres of about 50-300 nm diameter. Chitin mainly occurs in three different polymeric forms- α, β, and γ as shown in Figure-5.

Chains are arranged in stacks of sheets in α- chitin and adjacent sheets along the c-axis have the same direction in a parallel arrangement. The α- chitin occurs in the exoskeleton of crustaceans (eg: crabs, lobsters and prawns). In the case of β-chitin, the adjacent sheets along Chitin can be extracted from insect cuticle, tracheae and peritrophic matrix, shellfish waste such as shrimps, crabs, krill, fishes and microorganisms such as fungi or mushroom mycelia and some bacteria (Sreerag Gopi 2020). γ-chitin is a mixture of α- chitin and β-chitins with an orientation of parallel and antiparallel chains and found in mushrooms (Kumar, M. N. V. R. 2000). Generally, extraction of chitin involves the following steps:

i. **Demineralization**

ii. **Deproteination**

iii. **Decolourisation**

i. Demineralization

It can be performed by using strong acids such as hydrochloric acid, sulphuric acid and weak acids such as acetic acid and formic acid, but generally hydrochloric acid seems to be the best one. Initially the concentration of hydrochloric acid was about 1 N or 2 N, but from further studies it is found that only 0.25 M - 2 M concentration of hydrochloric acid for 1-36 or 48 hour is sufficient at temperature 0-100°C for just 15 minute. This step is particularly performed to remove the minerals, especially calcium carbonate (J. Bonilla, et al. 2014).

ii. Deproteination

It is generally performed under an alkaline medium of 0.75 N-2.5 N or 1 M sodium hydroxide aqueous solution for 2-42 or 1-72 hour at temperature 40 or 65-100°C in crustaceans and marine shrimp shells, respectively. Sodium hydroxide, potassium hydroxide, sodium bicarbonate, potassium carbonate, calcium hydroxide, sodium sulphate are the reagent that can be used for deproteination of the crustaceans. Due to the adverse effect of these perilous chemicals on environment nowadays, proteolytic enzymes such as Trypsin and Pepsin have been used to raise the efficiency of deproteination (V. K. Mourya and N. N. Inamdar 2008 and Cord-Landwehr 2021).

Enzymatic Deproteination: Proteolytic enzymes can be extracted from various sources such as Bacillus mojavensis A21, B. subtilis A26, B. licheniformis NH1, B. licheniformis MP1, Vibrio metschnikovii, Goby and Grey Triggerfish. One unit of protease activity can be defined as the amount of enzyme required to liberate 1 μg of Tyrosine per minute. The degree of proteination is calculated as follows:

$$\% \text{ DDP} = \frac{(Pi \times S) - (Pf \times R) \times 100}{Pi \times S}$$

where Pi and Pf are the concentrations of protein at initial and final hydrolysis, while S and R are the mass of the original sample and hydrolysed residue, respectively (Amorim, R. V. S. 2006).

iii. Decolourisation

In order to remove pigmentation and melanin, the mixture has been treated either with hydrogen peroxide or potassium permanganate solution. Mohammed et al., prepared approximately 35% of chitin (dry weight) from prawn shells after deproteination, decolourisation, and demineralisation. Chitin can be extracted either using biotechnological method or chemical method (Kumari, S. and Rath, P. K. 2014).

3.2 Sources and Extraction of Chitosan

Chitosan is found in similar sources as chitin. Similarly, terrestrial organisms include silkworm, insects, honeybee, arthropod and nematodes. The exoskeleton of crustaceans, shrimp shells, crab, krill, lobster, etc., has been successfully utilized in the extraction of chitosan. On the other hand mushrooms; cell wall of fungi, mycelia and septa of Zygomycetes and Ascomycetes are good sources of chitosan besides Basidiomycetes. Generally, chitosan is extracted from the deacetylation of the chitin. Chitin can be extracted from different sources, mainly crustacean and shellfish waste via demineralisation, deproteination and decolourisation. The deacetylation is a chemical process and can be achieved either by chemical or enzymatic method (Zuber, M. 2019 and S. Thomas 2013).

4. Preparation Methods of Chitin and Chitosan

4.1 Preparation of Chitin

Chitin which is normally isolated is not a single unique substance since it will contain varying amino units of residual protein and will be partially deacetylated to an extent dependent on the source and the conditions of purification. In view of this, Kurita, et al. (Percot, A., et al. 2003) attempted to prepare pure chitin poly- [β- (1, 4)-2-acetamido-2-deoxy-D-glucose] by N-acetylation of water soluble chitosan. Conditions for N-acetylation that were examined include:

(a) Acetic anhydride-pyridine

(b) Acetic acid-dicyclohexylcarbodiimide (DCC)

The acetic anhydride-pyridine system reacted rapidly with the gel formed on pouring an aqueous solution of the polymer into pyridine. Reaction times as short as 3 minutes at room temperature gave a product whose IR spectrum showed the presence of ester groups, in addition to the increase in intensity of the amide groups absorption bands. Hydrolysis of the ester groups was carried out by treatment with saturated aqueous sodium bicarbonate at 20°C for 141 hours. Treatment of water soluble chitosan with acetic acid – DCC, using a 20-fold excess of the reagents in 60 vol.% aqueous DMF, gave a highly swollen gel whose IR spectrum after isolation, showed no evidence of ester formation. Analysis of the sample prepared in this way gives values of 96%, 97% and 100% N-acetylation. Hirano, et al. (Younes, I., et al. 2014) have developed a process for N-acetylation of chitosan in aqueous acetic acid – methanol mixtures using acetic anhydride. Some esterification of hydroxyl groups also occurs and these are removed by treatment for 16 hours at room temperature in 0.5 M alcoholic KOH. This is claimed not to hydrolyse any of the N-acetyl groups but it has been demonstrated qualitatively that chitin shows an increased absorption of anionic dye from solution in 0.1 M aqueous acetic acid after treatment in 0.5 M alcoholic potassium hydroxide. This indicates that the alkaline treatment does in fact cause limited hydrolysis of the N-acetyl groups in addition to hydrolysing the o-acetyl groups (Crestini, Claudia, et al. 1996).

In view of the margin of error inherent in the analysis for the extent of N-acetylation,it cannot be stated definitely that such products are pure chitin although they are closer to it than chitin prepared directly from crustacean shells. They therefore represent the purest chitin obtained to date with the possible exception of that from diatoms (Percot, A., et al. 2003, Younes, I., 2014 and Fan, Zhaoqian, et al. 2018).

4.2 Chitosan Preparation Methods

Chitosan is prepared by the deacetylation of chitin. Acetyl groups are removed during the deacetylation process and molecular weight changes due to the depolymerisation reaction. There are two processes - the enzymatic process and chemical process as shown in Figure-6. Chitosan is produced by chemical processes. It is preferable for large scale production (Wang, Yun, et al. 2014).

4.2.1 Deacetylation of Chitin to Chitosan

Glycosidic bonds are attracted towards acids and alkalis. Chitin is processed homogeneously or heterogeneously. In the homogeneous method, chitin is defused in concentrated alkyl at 25°C for 3 hours and allowed to disperse in compressed ice at around 0°C (Ibrahim, Sara, et al. 2016). In the heterogeneous process, chitin is treated with hot high concentration alkali and then washed with distilled water until the pH is neutral.

It is difficult to produce higher deacetylated chitosan. The addition of thiophenol as a catalyst during the process would minimise the degradation by trapping oxygen and enhances the effective deacetylation process of chitin to get chitosan, if the alkyl concentration is four times greater than the total amino group in the polysaccharide at temperature around 100°C for a duration of 1 hour. It is recommended to use low concentration alkali and short contact time between alkali and polymer (Lu, Kun-Ying, et al. 2019).

Chemical deacetylation has many disadvantages like high energy consumption and environmental pollution problem (Yang, Chih-Hui, et al. 2016). An alternative method of enzyme deacetylation has been developed to overcome these drawbacks. Chitin deacetylation enzyme act as catalysis to N – acetamide bonds (Abdelrahman, Ehab A. and R. M. Hegazey 2019). This enzyme is extracted from the fungi Mucorrouxii, Absidiacoerulea, Aspergillushidulans and two strains of Celletotrichumlindemuthianum. The enzyme is thermally stable and has a binding affinity towards β-(1, 4)- linked. N-acetyl-D-glucosamine polymers (Kadokawa, Jun-ichi, et al. 2015). Most of the time the enzyme process is carried out in both batch and continuous culture. In the batch process the molecular weight of chitosan is lower with respect to time. Moreover, chitosan of higher molecular weight is obtained in a specific culture even though the yield is comparatively low (Martinez, L., et al. 2004).

4.2.2 Methods of Deacetylation of Chitin to Chitosan

◆ **Chemical Deacetylation**

Deacetylation of chitin involves the transformation of the N-acetyl group in chitin to ($-NH_2$) amino groups. The process is like the production of citric acid from Aspergillusniger, Mucorrouxii and streptomyces and can be achieved by acid or alkali treatment (Figure-6). The use of alkali is the most prevalent means of deacetylation, of chitin because glycosidic bonds are known to be susceptible to acid (Kim, Seon Jeong, et al. 2003 and Yao, Kang De, et al. 1994). It is either performed heterogeneously (Aranaz, Inmaculada, et al. 2009) or homogeneously (Morganti, Pierfrancesco 2013). In heterogeneous deacetylation (Aranaz, Inmaculada, et al. 2009, Zhao, Yong, et al. 2010, and Ladchumananandasivam, Rasiah, et al. 2012) chitin is treated with a hot concentrated NaOH solution and chitosan is produced as an insoluble residue.

In homogeneous deacetylation, alkali chitin is prepared after dispersal of chitin in concentrated NaOH followed by dissolution in crushed ice at around 0°C. Homogeneous deacetylation results in chitosan with acetyl groups uniformly distributed along the chain. Although complete removal of the acetyl group has not been reported (Bajaj, Mini, et al. 2011 and Dhillon, Gurpreet Singh, et al. 2013). The repeated use of NaOH on chitin can yield up to 98% deacetylation. The efficiency of the process is dependent on several factors including NaOH concentration, the reaction temperature and time. Depending on the method and the species used the degree of deacetylation usually ranges between 56% and 99%. However, degree of deacetylation of over 85% is required for solubility of chitosan to be achieved (Kim, Seon Jeong, et al. 2003, Das, Subha Narayan, et al. 2015 and Ghorbel-Bellaaj, Olfa, et al. 2013).

Several attempts have been made to produce chitosan with a very high degree of deacetylation. To obtain chitosan with 98% degree of deacetylation, Dung, et al. (Hayes, Maria 2011) applied a reducing agent (NaBH4) and reported that the reducing agent did not have any significant effect on the degree of acetylation of chitosan but affect the molecular weight. The addition of

sodium borohydride also prevented polymer degradation (HPS, Abdul Khalil, et al. 2016). Younce, et al. (Xu, Wenhua, et al. 2018) also invented the use of sodium borohydride as an oxygen scavenger using statistical methods. With a combination of temperature, time and atmospheric conditions of the study was able to realize a degree of deacetylation of 99%.

Another method of deacetylation of chitin that has been examined is the use of autoclaving conditions (Yoon, Soon-Do, et al. 2017). Deacetylation was successfully achieved by treatment of chitin under elevated temperature and pressure with 45% NaOH for 30 min and a solids/solvent ratio of 1:15. A mixture of glycerol and NaOH has also been used for the deacetylation of chitin using the above figure. The process involved soaking chitin for 4-16 hour at temperature (120^{o}C-180^{o}C) with 10%-40% NaOH solution at a ratio of 1:30-1:60. One percent of water was added to promote the ionization of NaOH and prevent the polymerization of glycerol catalysed by NaOH at higher temperature (Figure-8). The final solution was obtained by centrifugation and washed thoroughly with distilled water to remove the remaining NaOH and glycerol completely (Mendes, J. F., et al. 2016).

The study realized chitosan with a degree of deacetylation up to 85.36% ±1.04%. Mima, et al. (Kim, Seon Jeong, et al. 2003) reported the realization of 100% deacetylation of chitin using a series of treatments according to the scheme in the Figure-8 given below. Other authors also applied water-miscible organic solvent (Sharma, Parul, et al. 2016), dispersing organic liquids and high temperature.

Alsharabasy (Kadir, M. F. Z., 2010) proposed a new method for deacetylaton of chitin by introducing a controlled cooling step. The study realized chitosan with degree of deacetylation of 98% without degradation of the polymer chains. Some authors also used a combination of alkali and acid to arrive at a high degree of deacetylation (Buraidah, M. H., et al. 2016 and Sudhakar, Y. N., and M. Selvakumar 2012). Anwar, et al. (Uranga, J., et al. 2019) investigated different green methods of deacetylation including grinding, maceration and sonication. Yuan, et al. (Wang, Zhen, et al. 2018) used acetic acid for deacetylation of chitosan degree of deacetylation of 99.7% was determined using UV spectroscopy. He, et al. (Liu, Lingxiao, et al. 2018) investigated the application of compressive pressure at (0.11-0.12 MPa) at high temperature. The study reported the production of chitosan with 100% degree of deacetylation using low concentration alkali conditions. Other method that have been applied in the deacetylation of chitosan include the use of ethanol (Prabhu, Subbaiah Muthu, et al. 2014), n-butyl alcohol-sodium hydroxide and amyl alcohol (Pramanik, Sheersha, and Vaishnavi Sali 2021) and dimethyl sulfoxide (Liu, Yujia, et al. 2016). These methods were able to record an increase in degree of deacetylation to about 99.7%.

Microwave assisted deacetylation has also been applied to shorten the time needed for deacetylation (Zeimaran, Ehsan, et al. 2017). Although 20 kGy gamma radiation in 60% concentration alkali in 100^{0}C resulting in a 13% increase in degree of deacetylation, it poses serious health challenges (Gomes, Susana, et al. 2017). A new route of deacetylation involving the use of freeze-pump out-thaws cycles was proposed by Guillaume (Antunes, Bernardo Paiva, et al. 2015). Although high degree of deacetylation has been obtained from chemical deacetylation, the method suffers from high energy consumption, high effluent waste generation and produces a broad range of soluble and insoluble products. The use of ultrasound to improve deacetylation at very low concentrations of NaOH has been investigated by Ngo (Rasool, Atta, et al. 2019).

The use of water-miscible organic solvent was also examined by Batista and Roberts (Lewandowska, Katarzyna, et al. 2016).

◆ **Enzymatic Deacetylation**

The use of chitin deacetylases to prepare chitosan offers the possibility of developing an enzymatic process that overcomes the potential drawbacks of the chemical deacetylation methods. The rationale behind the use of chitin deacetylases is that it catalyses the hydrolysis of the N-acetamido bonds in chitin to produce chitosan (Haghighi, Hossein, et al. 2019).

The mechanism of deacetylation with chitin deacetylases is a multiple attack mechanism on monomeric acetylglucosamine units of chitin producing high quality chitosan (Uranga, J., et al. 2019 and Shankar, Shiv, and Jong-Whan Rhim 2018). Key enzymes that have been studied are those from the fungi M. Rouxii (Huang, Xuejiao, et al. 2018, Liu, Yaowen, et al. 2019, and Chen, Guiyun, et al. 2019), Absidiacoerulea (Indumathi, M. P. and G. R. Rajarajeswari 2019) Aspergillusnidulans (Zhang, Cheng, et al. 2019), and strains of Colletotrichumlindemuthianum (Niu, Xun, et al. 2018 and Zhang, Xiaodong, et al. 2017). Enzymatic deacetylation with chitin deacetylases offers a possibility of producing specific chitosan oligomers and polymers. Tokuyasu, et al. (Niu, Xun, et al. 2018) developed and purified chitin deacetylases from C.lindemthianum. Results showed that the enzyme was active toward glycol chitin partially, N-deacetylated water-soluble chitin and chitin oligomers with more than fourfold degree of polymerization. The enzyme was inactive with N-acetylglucosamine. Zhang, et al. (Revathi, T. and S. Thambidurai 2018) studied the optimization and fermentation conditions of Rhizopus japonicas for the deacetylases and chitosan.

Most of the enzymes that have been used in deacetylation display outstanding thermal stability at their optimal temperatures and exhibit very strong specificity for β-(1, 4)-linked N-acetyl-o-glucosamine polymer. Yang, et al. (Tareq, Foysal Kabir, et al. 2018) investigated the production and purification of protease enzymes from Bacillus subtilis capable of deproteinizing crustacean wastes. Protease produced by Pseudomonas acruginosa has also been investigated for deproteinization of shrimp and crab shell (Tareq, Foysal Kabir, et al. 2018).

In another study, protease producing bacterium has been investigated for the deproteinization of squid to produce β-chitin (Rambabu, K., et al. 2019). Morely, et al. (Hu, Shihao, et al. 2018) showed that acetyl xylan esterase is capable of hydrolysing N-acetyl groups in chitin to form chitosan of varying degree of deacetylation. Cai, et al. (Qiu, Bo, et al. 2019) also investigated enzymatic preparation of chitosan.

Using chitin deacetylases the study recorded degree of deacetylation of 73.6% which was 2% lower than chemical deacetylation. Martinou, et al. (Xie, Haixia, et al. 2018) studied the mode of action of deacetylases extracted from M. Rouxii on partially N-acetylated chitosan. Kohlhoff, et al. (Shavandi, Amin, et al. 2015) also investigated the functionality of chitosanase enzyme on the generation of partially acetylated chitosan.

Jiang, et al. (2018) studied the kinetics of chitin deacetylase in the presence of ionic liquid. Ionic liquids were found to increase the activity of the enzyme (Abdel-rahman, Ehab A. and R. M. Hegazey 2019). Pareek, et al. (Samuel, Melvin S., et al. 2018) investigated the use of chitin deacetylase from Penicilliunoxalicum for bioconversion of chitin to chitosan using a two stage chemical and enzymatic process. Results showed that pre-treatment and optimization of the process variables led to a 3.2 - fold increase in DD of chitosan. Martinou, et al. (Wu, Di, et al. 2019)

developed a monitoring process for deacetylation using chitin deacetylase. Other studies on the use of enzymes for deacetylation of chitin have been reported (Li, Jixiang, et al. 2017). The major disadvantages of this method are that the enzyme is ineffective in deacetylating insoluble chitin substrates. Many researchers report that crystallinity and insolubility of chitin are the major reason to development of the enzymatic deacetylation process (Abdelrahman, Ehab A. and R. M. Hegazey 2019).

4.2.3 Commercial Preparation

The commercial scale production of chitin and chitosan imposes additional constraints on the processing routes that may be used, the most important of these constrains being.

(a) The cost of the reagents and the need to recycle them where possible;

(b) The costs of disposal of as liquid or solids for which no market can be found.

The use of hydrochloric acid as the most commonly used reagent for the demineralisation step on a laboratory scale has a number of disadvantages when used on an industrial scale. It is relatively expensive; its use results in large volumes of $CaCl_2$ solution that must be disposed of and being a strong acid. It is likely to cause some hydrolysis of the chitin chains, thereby reducing the molecular weight of the purified material. The cheaper sulphuric acid, cannot be used since the calcium sulphate produce is too insoluble to be readily removed from the chitin matrix.

In view of these disadvantages the use of sulphurous acid has been proposed. This must be used in excess of the amount of $CaCO_3$ present in the shell since, if stoichiometric amount are used $CaSO_3$, is formed. This has very low aqueous solubility (~0.05g d m-3) so that removal from the chitin is difficult, but if excess H_2SO_3 is used the more soluble $Ca(HSO_3)_2$ is formed and this is readily removed. Half of the SO_2 may be recovered from the $Ca(HSO_3)_2$ solution by steam - stripping and the other half by treating the resultant precipitate of $CaSO_3$ with H_2SO_4. Thus assuming 100% recovery of the SO_2, the overall process is equivalent to demineralisation with H_2SO_4. Another proposed process involves carrying out the deacetylation step without a prior demineralisation treatment, using high NaOH concentrations and temperature of 120-150°C to ensure that the equilibrium reaction,is driven over to the right-hand side.

$$2NaOH + CaCO_3 \rightleftharpoons Ca(OH)_2 + Na_2CO_3$$

The chitosan produced contains substantial concentrations of $Ca(OH)_2$ and after washing to remove the Na_2CO_3 this chitosan – $Ca(OH)_2$ mixture is subjected to counter current extraction with an aqueous solution of sucrose which removes the $Ca(OH)_2$ as the soluble calcium saccharate, from which it can be precipitated as $CaCO_3$ by treatment with carbon dioxide (Li, Jixiang, et al. 2017).

Peniston and Johnson have also reported a deacetylation process for demineralised chitin that involves the use of considerably lower temperatures. The process consists of mixing the chitin with the NaOH solution, heating to a temperature of 40-80°C, expelling any air from the container and letting the mixture stand without stirring, for up to 160 hour. Although Peniston and Johnson claim the use of as little as two parts of sodium hydroxide solution per part of chitin they state that this gives insufficient liquor to wet the particles of chitin thoroughly, leading to limited swelling of the particles, reduced rate of reaction.

The use of higher liquor-to-solids ratios, to ensure thorough wetting of the chitin particles

and provided a continuous liquid phase, is recommended by these workers. Although these processes have been patented it is not known whether they are being used industrially at the present time (Wang, Yun, et al. 2014).

5. Application of Chitin and Chitosan

Chitosan has functional groups like hydroxyl and amino groups, which might be modified by controlled chemical reactions. They are also used to chemically alter its physical and solution properties (Figure-10).

◆ **Biomedical Applications**

Chitin and chitosan biopolymers are useful non-toxic and biocompatible materials to be used in various medical devices to treat, augment, or replace tissues or organs, or functions of the body. Chitosan serves as a potential material for nerve regeneration, wound-healing management products and wound-dressing, burn treatment, etc., (H. K. Nu, et al. 2003). Moreover they have antioxidant, antitumour, antidiabetic and antiulcer activities.

◆ **Waste Water Treatment**

Large scale pollution of water caused by industrial, agricultural and domestic activities generates waste water which contains both inorganic and organic pollutants. Common pollutants include phenols, dyes, detergents, insecticides and heavy metals, there pollutants are often toxic and cause adverse effects on human and animal life if present above certain concentration levels. The use of chitin and chitosan and its derivatives for removing various pollutants from water and wastewater presents many attractive features such as the outstanding adsorption capacity, especially for metal ions and dyes, and the fact that these materials are low cost, non-toxic and biocompatible. However, their potential for other pollutants, eg., phenols, anions, pesticides, humic substances needs extensive research. Although the amount of available literature data for chitin/chitosan application in waste and wastewater treatment is increasing at a tremendous pace.

◆ **Textile Industry**

Chitin and Chitosan are environment friendly and renewable natural polymers, due to their antimicrobial property; they are becoming a standard finish for some textile products as well as for medical, institutional, and hygienic uses. They can be used in sportswear, women's wear and aesthetic clothing to impart anti-odour or biostatic properties.

◆ **Packaging of Food**

They show antimicrobial properties against bacteria, yeasts, mould and fungi. The polycationic nature of chitosan interferes with the bacterial metabolism by electrostatic stacking at the cell surface. Chitosan treatment offer protection against contamination and microbial spoilage. Moreover, they also operate as a chelating agent of some essential minerals. Chitosan has certain solubility in acetic acid and HCl, which can promote the film-forming ability (Li, Jixiang, et al. 2017).

6. Conclusion

With the growing importance of green chemistry and the use of bio-polymers, Chitin and Chitosan is the most abundant and renewable polysaccharides with N-acetyl-D-glucosamine long chain polymeric unit having -(1, 4)-linkage and glycosidic linkage. Chitin and chitosan have gained more attention in the research field due to their various properties such as purity, solubility, pH, viscosity, biocompatibility, hygroscopicity and reactivity, especially the molecular weight and degree of deacetylation. Both chitin and chitosan are extracted from same

sources such as terrestrial, marine, microorganisms and enzymes. Viewing biomedical aspects of chitin as well as chitosan, it has been observed that marine originated more deacetylated products give better results in terms of their properties, as compared to less deacetylated products. This article has reviewed the structure, properties, sources, application and mode of the derivation of chitin and chitosan. The increasing demand of chitosan in the end user industries mainly in waste water treatment and food and beverages industry will proliferate the market growth. An important application is wastewater treatment with chitin or chitosan. For the removal of dyes from industrial waste water (e.g., textile waste waters), as well as other organic pollutants such as organochloride pesticides, organic oxidized or fatty impurities, and oil impurities.

Some specific features of Chitin and Chitosan such as cationic nature, film forming and moisture retaining properties as well as the ability to release bioactive agents, are helpful to introduce new modified biocompatible products ensuring microbiological safety in the future.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. Figures and Tables

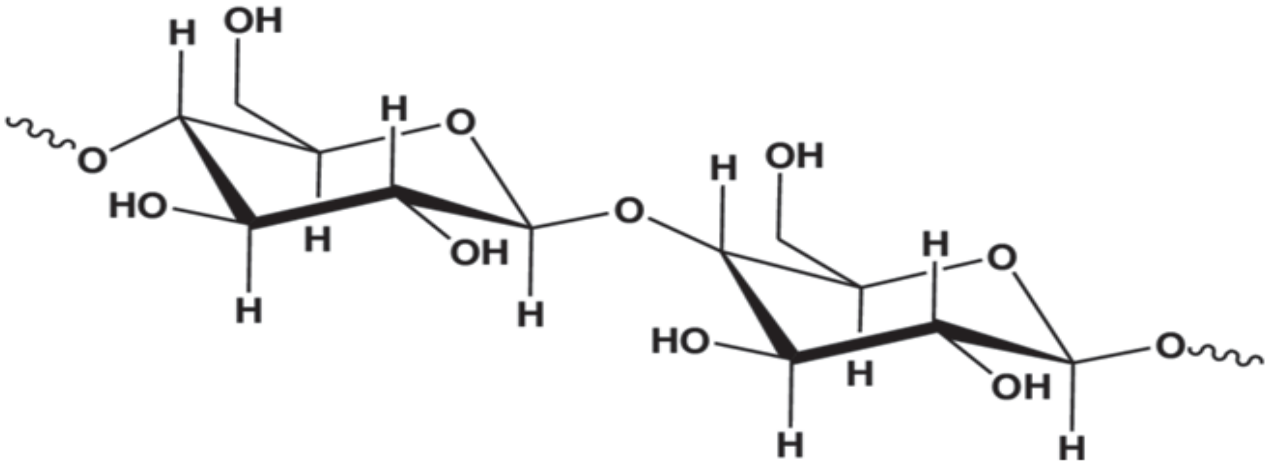


Figure-1: Chemical Structure of Cellulose Polymer

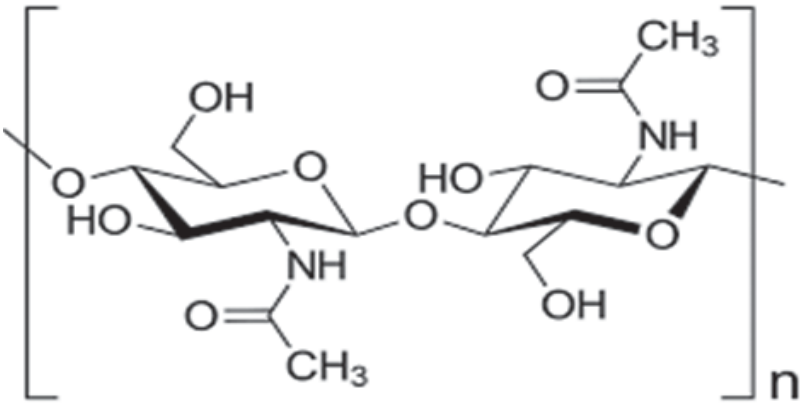


Figure-2: The Structure of Chitin

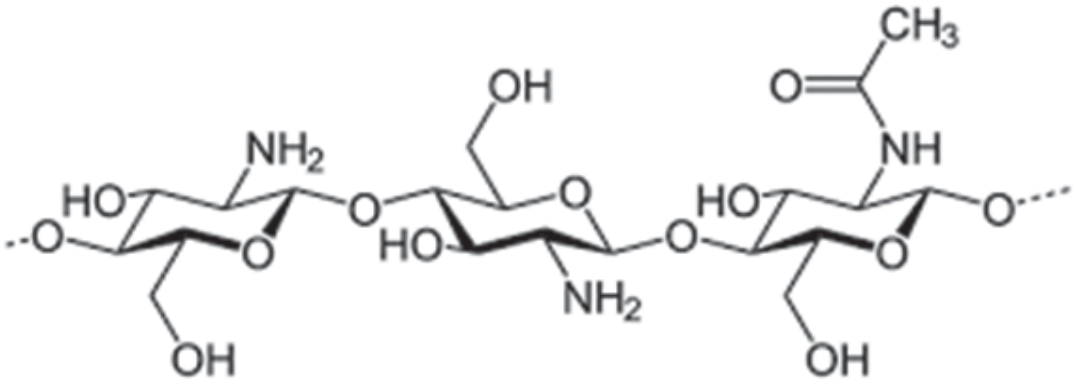


Figure-3: Chemical Structure of Chitosan

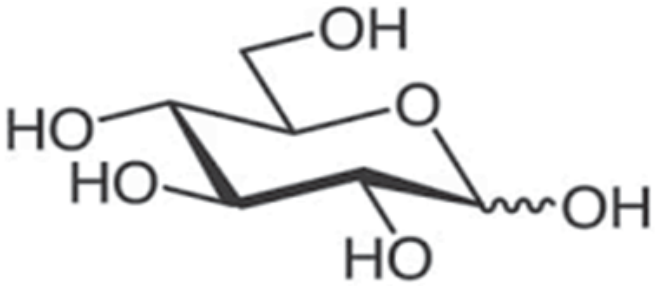


Figure-4: (1, 4)-β-D-Glucan Unit

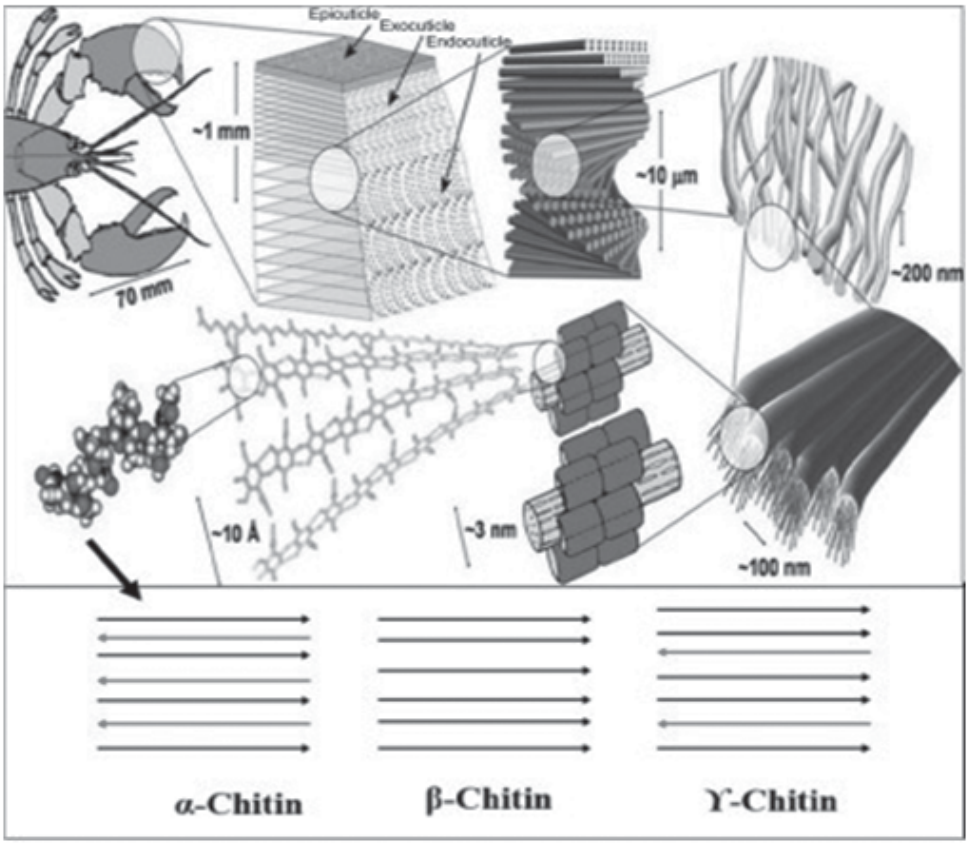


Figure-5: Hierarchy of the Main Structural Levels and Microstructure Elements of Exoskeleton Materials

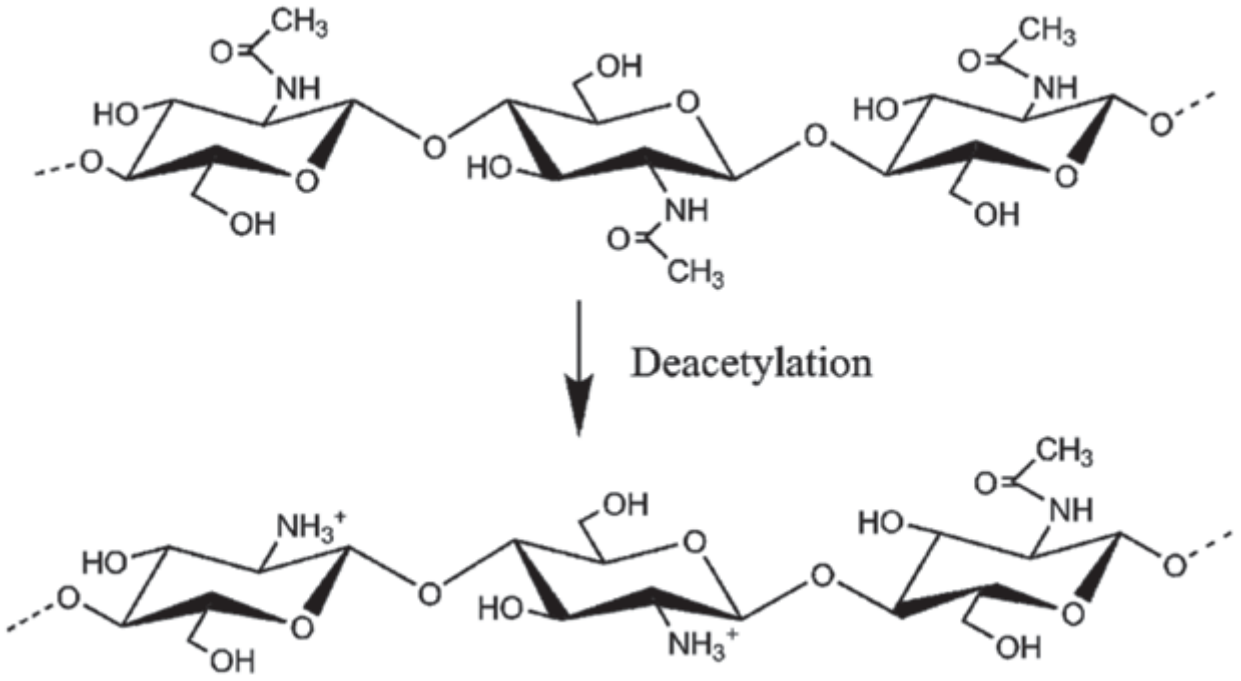


Figure-6: Chemical Deacetylation of Chitin to Chitosan

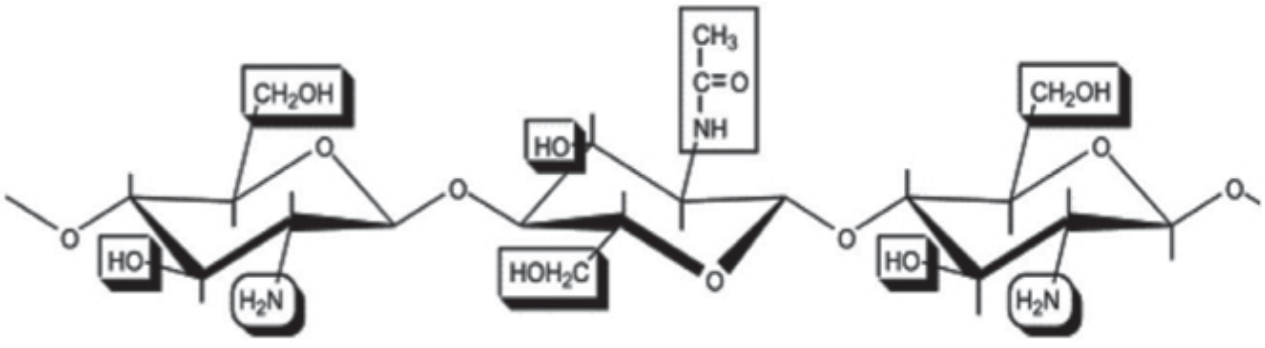


Figure-7: Illustration of the Possible Reaction Site in Chitin and Chitosan [21]

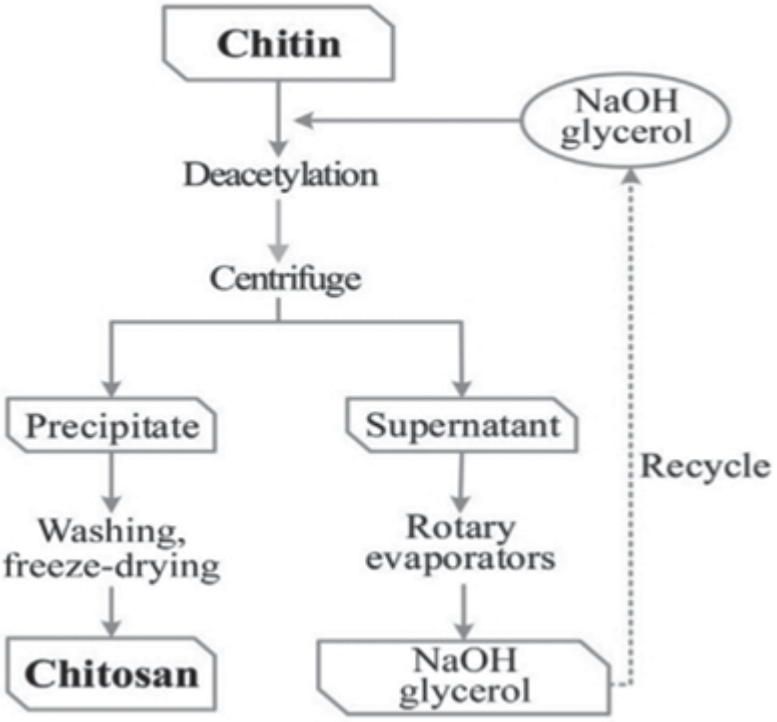


Figure-8: Schematic of Chitosan Preparation by Glycerol as Reaction Solvent

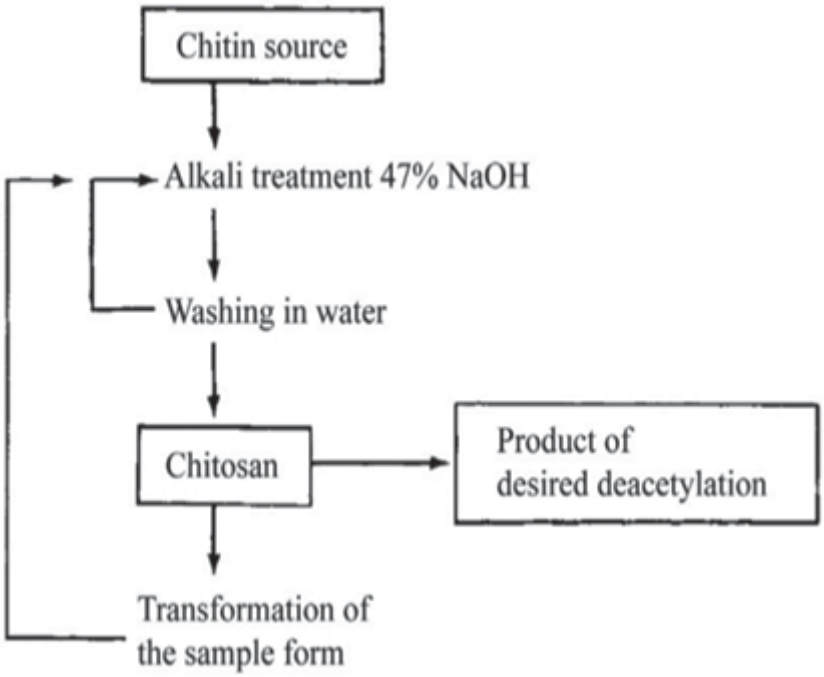


Figure-9: Schematic of Chitosan Preparation by Alkali Treatment

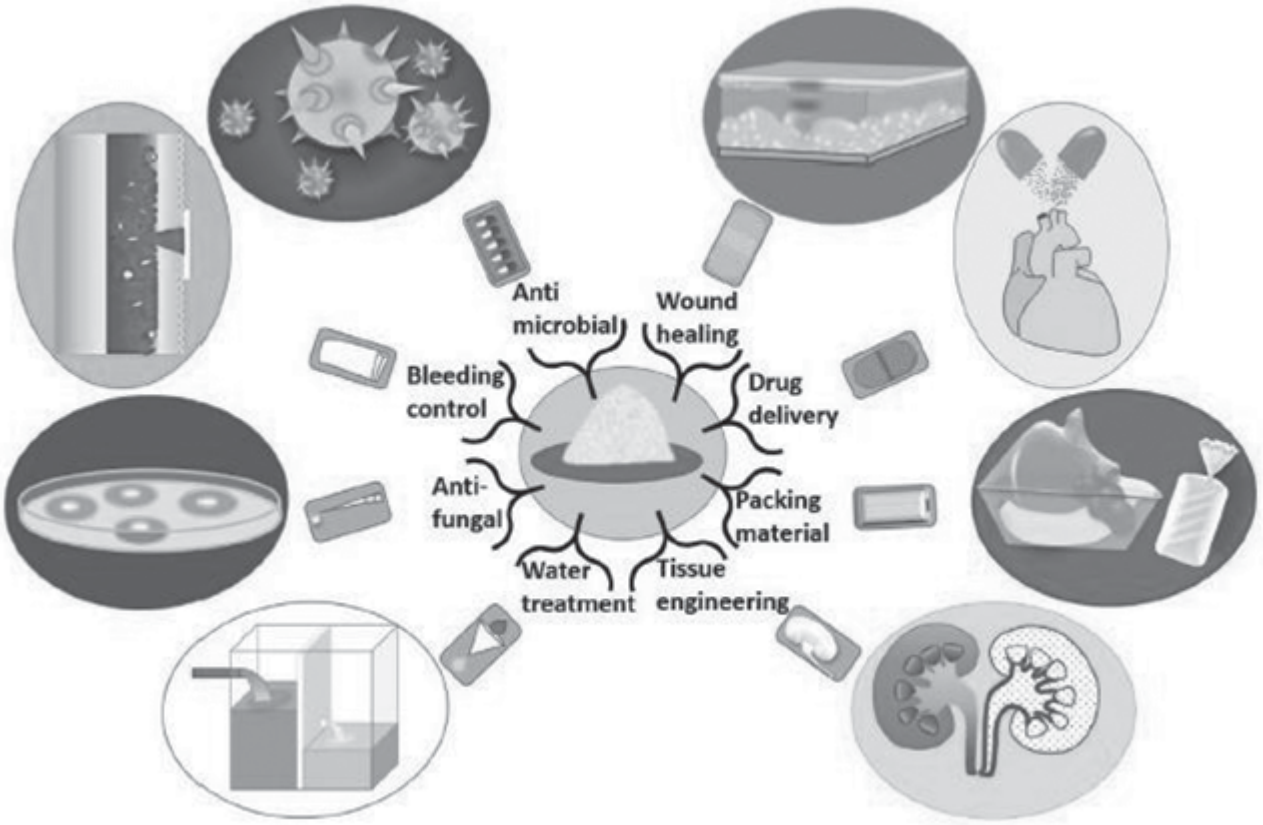


Figure-10: Applications of Chitin and Chitosan

Table-1: Comparison between Chitin and Chitosan

Chitin	Chitosan
Chitin is an amide derivative of glucose.	Chitosan is a linear polysaccharide compound.
Chitin has no free amine group.	Chitosan has free amine group.
Chitin occurs as a primary component of cell walls in fungi, in the exoskeleton of the arthropods, in the radular of molluscus and in the scale of fishes.	Chitosan is produced by the deacetylation of chitin.
Chitin has high molecular weight.	Chitosan has lower molecular weight.
Shows less nitrogen content (7.01%).	Shows higher nitrogen content (7.16%).
Shows high viscosity.	Shows lower viscosity.
Chitin has less water binding capacity.	Chitosan has higher water binding capacity.
The degree of deacetylation (DD) of Chitin is <20%.	The degree of deacetylation (DD) of Chitosan is >80%.

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I, Principal, St. Teresa's College (Autonomous) Ernakulam declare that the particulars given above are true to the best of my knowledge and belief.

Saturday, September 30, 2023.

Principal
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St. Teresa's College (Autonomous) Ernakulam
Park Avenue Road, Cochin-682011, Kerala, India
Tel: 0484-2351870, Fax: 0484-2381312
Email: principal@teresas.ac.in, Website: www.teresas.ac.in

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