Biopolymeric Chitosan Ligand Produced from Snail Shell as the Ligand Molecule for Chelation of Zinc (II) Metal Ion in Waste Water

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Abstract: A new biological chitin was extracted from snail shell and employed for the production of high purity and porous chitosan. The chitin and chitosan were analyzed using IR spectroscopy and element analysis to show the efficiency of N-deacetylation. The prepared chitosan was investigated as a chelating material for zinc (II) metal ion from ZnO waste water by equilibrium method. Atomic absorption spectrophotometer (AAS) was used to determine the milligram of chelated Zn (II) ion per gram of ligand molecule. At pH 4.5, the maximum chelation capacity was 6.5×10^6 mg of zinc per gram of chitosan over 24hrs of the experimental duration.

Keywords: Biopolymeric ligand, chelation, porous chitosan, Zn^{2+} metal ion, high purity, system performance, chitin, synthetic polymer, chelation capacity and ligand molecules.

1. Introduction

The study of methods of controlling the level of heavy metals in the environment is important because of the toxic effects that these pollutants can cause, especially in organisms at the top of the marine food chain, although some metals are essential for living organisms, other may become highly toxic when present in high concentration. Carson [1]. When heavy metals are present in marine and aquatic environments, they could enter the food chains, becomes bio-accumulated in aquatic organisms including fish and later becomes bio-transferred to man, the ultimate consumer of these organisms. Metals cannot be degraded bio-chemically in nature and the stability of these metals therefore allows them to be transport for considerable distance by water. As a result of the above mention process the levels of heavy metals in the upper members of a food chain can reach a value sufficiently high to become health hazards when such organisms are used as food.

Nigeria, my country where fish is one of the cheapest sources of protein, contamination of marine environment deserves serious attention. The annual demand for fish in 1.5 million tons according to the Federal department of fisheries. Unfortunately the country produces less than 0.4 million tones leaving a deficit of 1.1 million tons annually. To fill the gap, Nigeria has to import fish, but has been doing that recklessly. Currently, Nigeria is the largest importer of frozen fish in the world. Osohobo [2]. Large scale fishing is often carried out in high seas.

High sea is defined in Article 86 of the United Nations Convention, on the law of the sea 1982, "as all parts of the sea that are not included in the exclusive economic zone, in the territorial sea, or in internal waters of a state, or in the archipelagic waters of an archipelagic state". Hence, the high seas are open to all states, whether coastal or land locked. Apart from being open to all nations, the high seas could be used by both coastal and land locked states for the purpose of navigation, over flight, Laying submarine cables and pipelines, constructing artificial Islands and other installations permitted under international law, fishing and scientific research. Wren *et al* [3]

The pollution of marine and aquatic environment is commonly results from deliberate dumping or merely carelessness, the outcome is the same. Dumping is the deliberate disposal into the sea from ship or air

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craft of waste loaded on board for this purpose are used to be a legitimate means of disposal because of the low cost. Deliberately, factories and sewage works are built to discharge into seas and rivers. Spoliation has also been inadvertently and many of Scandinavian lakes has now been sterilized by rain made acid by the exhausts of motor cars and factories, hundreds of Kilometers away. Johnson [4]

The increasing sea borne transport of oil and other hazardous substance constitute a growing pollution risk to the seas and oceans. The most common contaminant from ship is oil. It also has the highest public profile. Whether by accident or design, oil pollution will exist as long as oil is moved by sea. The word oil refers to a wide range of materials. It includes petroleum, bunker fuels, petrol, and lubricants Vessel source pollution includes pollution originating from vessels involved in navigation and transportation and is distinct from dumping. However, mankind as a whole treats the oceans, rivers and lake as a dumping ground for sewage, noxious chemicals, radioactive waste and generalized junk. Such effluents often accelerate the degradation of the water quality and increase the mortality of aquatic organism living in the water. Johnson [4]

Many organisms have a natural ability to extract and concentrate minerals from their surroundings even if these are present only in very low concentrations. Thus tunicates (creatures commonly known as sea squirts and distantly related to vertebrate) naturally accumulate vanadium or titanium which are present in seas water only in a few parts per billion. Tunicates accumulate those rare metals for physiological purpose. Virtually all creature, though are capable of scavenging rare materials from the environment and accumulating then effectively by "mistake". Thus all animals living in the wild used to accumulate DDT in their fat stores, in the days when DDT was a common pesticide. All of us pick up and accumulate lead (Pb) distributed around the environment from the car exhaust. Allision [5]

Heavy metals in metallic forms are not particularly dangerous, but many of the organic compounds that can be made from them are extremely toxic, some of the heavy metals were already in organic forms at the time of discharge and the compounds undergo further bio-transformation inside the fish, which rendered them extremely dangerous. The compounds of these heavy metals have ghastly effect on the human organs including nervous system, causing convulsion, blindness and death. Hakason [6]

Extraneous impurities enter from the surrounding air and from the machine on which the lubricant is being used. These are: dust, dirt, moisture, metallic particle, carbonaceous particles, and unburned fuel. These contaminants affect the life and efficiency of the device being lubricated as well as the life of the lubricant. Dirt and metal parts worn out from the surfaces are deposited into the lubricating oils. With increased time of usage, the lubricating oil loses its lubricating properties as a result of over-reduction of desired properties, and thus must be evacuated and a fresh one replaced. Large and increasing volumes of lubricating oil are produced each year that after use, are considered hazardous waste. This is so because waste oils typically consist of a mixture of undegraded base oil and additives with high concentration of metals, varnish, gum and other asphaltic compounds coming from overlay on bearing surfaces and degradation of the fresh lubricant components. With the large amount of lubricating oils used the disposal of used lubricating oils has now become a major in management problem.

Many countries are now addressing the problem of environmental pollution posed by used lubricating oils. This has led industries and governments to find environmentally friendly solutions that will reduce the contribution of used lubricating oil to pollution and also recover these valuable hydrocarbon resources. Cooke [7]. In disposing used lubricating oil, many used it as a dust cure; that is for dust prevention Bennet et al [8]. This method of disposal is in many ways unsatisfactory as the lead-bearing dust and run-off constitute air and water pollution. Incineration is another method by which used lubricating oil is disposed. This method is another unsatisfactory used of such a valuable product, and the resultant emission of probably carcinogenous products contributes to environmental pollution Georgel and La Tour [9]. Both of these practices lead to non-end environmental problems, whether through the smoke of furnaces, the content of soot, toxic metals or contamination of surface water source and underground.

In extreme cases, these contaminants damage furnaces, thus leading to increase environmental pollution Kajdas [10]. Emission of zinc (Zn) can be as high as 600 times and copper (Cu) can be 2100 times if waste oil is burnt instead or re-refining Boughton and Horvath [11]. It is a fact that four litres of oil is able to

contaminate four million litres of drinking water and can form a thin layer oil on the surface of the water which prevents oxygen from being dissolve in water, which hampers all kinds of aquatic life and the processes of photosynthesis as indicated in Rahman et al [12].

Natural biopolymers are industrially attractive because of their capability to lowering transition metals ions concentration to parts per billion concentrations Aydemir [13] and Xiao [19]. Treating waste water using "greener" methods has become an ecological necessity. Integration of natural polymer made of crustacean residue into an existing system has achieved a twofold purpose. First, it improves the effectives of water treatment while reducing or even eliminating synthetic chemical products and synthetic chemical products and synthetic polymer. Secondly, it potentially reduces the use of alum by up to 60% and eliminates 100% of the polymer from the treated water thereby improving the system performance (suspended solid and chemical oxygen demand) and significantly reducing odor Clermount [14]. Chitosan, (2 – acetamiclo – 2 – deoxy – b – D – glucose – N -acetylglucosamine) due to its natural origin and being biodegradable, had proven to be a most interesting alternative from several points of view Clermount [14] and Saifuddin [15]. It is a partially N-deacetlated polymer of chitin and is usually prepared from chitin by N-deacetylation with a strong alkaline solution Taboada [17]. Although zinc (II) ion is an essential nutrient required for a healthy body, too much of zinc can be harmful. Excessive absorption of zinc can suppress copper and iron absorption Valko [18]. Recently, Aydemir [13] group showed killing of 93% of all daphnia in water by just micromolar amounts. There is evidence of induced copper deficiency at lower intakes of 100-300mg Zn/dose, even lower levels closer to 15mg Zn/dose may interfere with the utilization of copper and iron Xiao [19]. This study therefore, generally aimed to establish chelation of Zn^{2+} metal ion from waste water using the biopolymeric chitosan produced from snail shell as the ligand molecule.

2. Materials and Methods

2.1 Sampling

The snail shell sample was obtained from towns in Idemili North of Anambra State. The sample was made powdery with diameter ranging between 0.5mm and 0.35mm particle size. Sample of zinc oxide waste water was collected from the discharged end point at A-Z Industry Nig. Limited, Nnewi North, Anambra State. It was thereafter stored in a plastic container in a dark room.

2.2 Procedure

The powdered sample was deproteinated and demineralized using the method of Seiichi et al., [10]. Chitosan was thereafter produced by N-deacetylation of the chitin [11].

The chitosan-zinc chelation was performed with 20mL of 100 and 400 mg/L standard solution of zinc (II) chloride pentahydrate ($ZnCl_2.5H_2O$) respectively placed in contact with 100mg chitosan in a glass stopper flask. The mixtures were agitated at 300 rpm for 24h using orbital shaker at room temperature and filtered at equilibrium.

The concentrations of the filtrate were determined by atomic absorption spectrophotometric method. Using same procedure, Zn^{2+} metal ion was chelated from the Zinc (II) oxide (ZnO) waste water effluent.

FT-IR spectra of chitin, chitosan and chitosan – Zn metal chelate were measured using IR spectra as KBr pellets on a Perkin-Elmer 2000 FT-IR spectrometer.

3. Results and Discussion

Fig. 1 shows the chelation behavior on a chitosan surface of a standard solution of Zn (II) (Zn^{2+} = 100mg/L and 400 mg/L) and ZnO effluent (38.12mg/L). The calculation of milligrams of bioligand per gram of chelated metal ion were determined using the difference between the mass of initial Zn^{2+} and the mass of Zn^{2+} in solution after contact between bioligand and the effluent. The total chelation of Zn^{2+} ions in solution at pH 4.5 occurred within 1h of experimental duration. Considering the Zn^{2+} standard solution of 100mg/L, an average amount of the metal ion had been chelated from the solution after 30min of agitation.
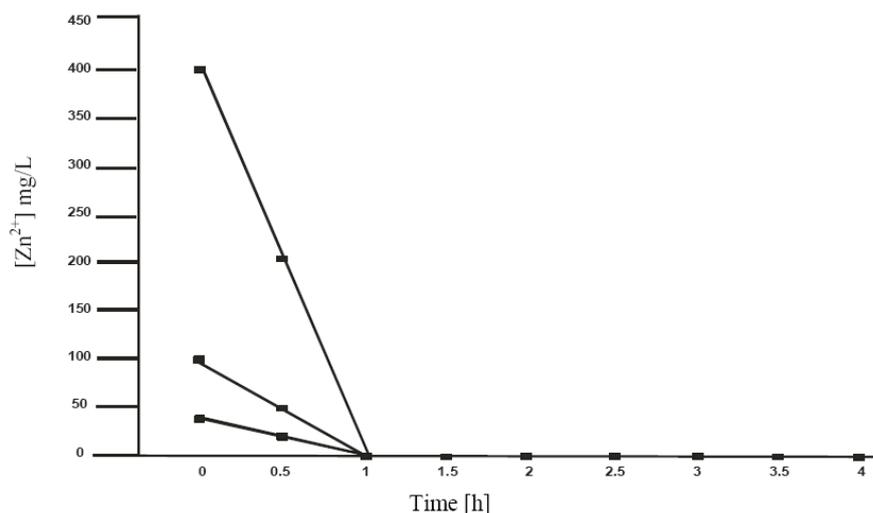
Figure 1: Complexation of Zn^{2+} on chitosan surface: ◆Zn^{2+} standard solution (100mg/L), ▢- Zn^{2+} Standard solution 9400mg/L). ▲Zinc Oxide efficient (38.12mg/L) (pH = 4.5, particle size, 0.35mm – 0.5mm, T = RT

Increasing the contact time to 1h, the amount of chelated metal ion increased to 100mg, implying that all the Zn^{2+} metal ions were completely chelated. However, at higher Zn^{2+} standard solution of 400mg/L, 200mg of the metal ion formed chelated with the bioligand after 30min of agitation which equally reached completion at the end of 1h.

The chelating property of the snail shell based chitosan with Zn ion can therefore be described as efficient. This high chelating ability has been attributed to the free amino groups exposed in chitosan because of the N-deacetyletion [9]. The free electron pair in nitrogen allows the efficient binding of metal cation to amine groups (Fig. 2b). In addition, the OH group on the C3 position of the glucosamine ring also participate in the formation of complexes with transition metals ions [11].

Similar trend was observes with the ZnO effluent (initial concentration of 38.12mg/L) where almost half of the initial metal ion concentration had formed a chelate with chitosan after 30min of contact. Further increase in contact time to 1h also led to complete chelation of Zn^{2+} metal ions in the effluent (Fig. 1).

Beyond this time, the metal chelation with chitosan remained constant over 4h indicating the stability of the metal-ligand chelate. Therefore, the higher the agitation time, the greater is the amount of Zn^{2+} ions chelated by chitosan within 1h, beyond which almost all the metal ions are chelated. Hence, the level of Zn^{2+}-chitosan chelate formation is directly proportional to the increase in the agitation time.
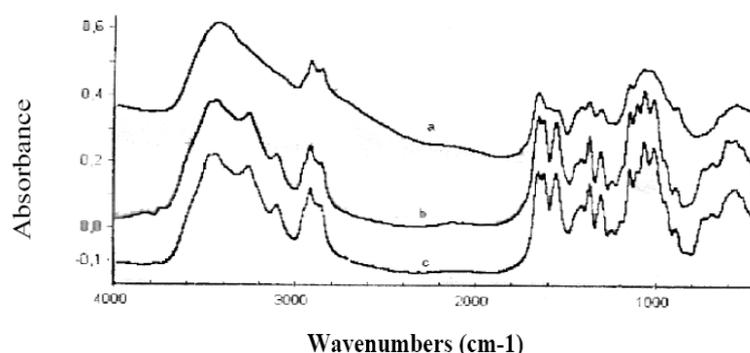

Fig 2a: FT-IR spectra of a: chitin, b: chitosan-Zn^{2+} chelate in standard solution and c: Chitosan –Zn^{2+} chelate in ZnO effluent

The Spectra of chitin and chitosan – Zn chelate are shown in figure 2a. The amide 1 band ($\upsilon c= o$) around 1637cm^{-1} cm stretching frequency, characteristic of chitosan with acetylated units is present in all the spectra. However, in chitosan-metal chelates, a new band around 1625 – 1635 cm^{-1} appears. These bands correspond

to the bending in plane (Scissoring) of N-H which also appear as a shoulder at around 1605cm^{-1}. This observes red shift is due to interaction of Chitosan with the metal ions.

The broad bands in the region of 3000-3600 cm^{-1} could be attributed to the stretching of OH groups of the chitosan and overlapping stretching bands of NH. With the Chitosan – metal chelates, the bands are unfolded and the stretching of O-H is observed between 3418 and 3450cm^{-1} while the stretching of N-H is observed between 3260 and 3266cm^{-1}. The chitosan – Zn chelate formation is therefore proposed (Fig. 2b).
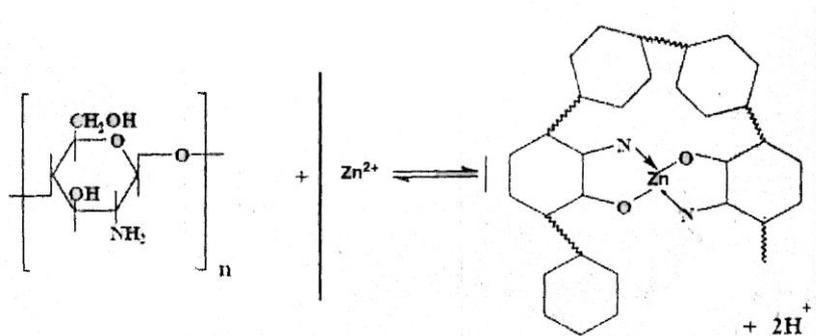

Fig. 2b: Formation of chitosan chelates with zinc (II) ions
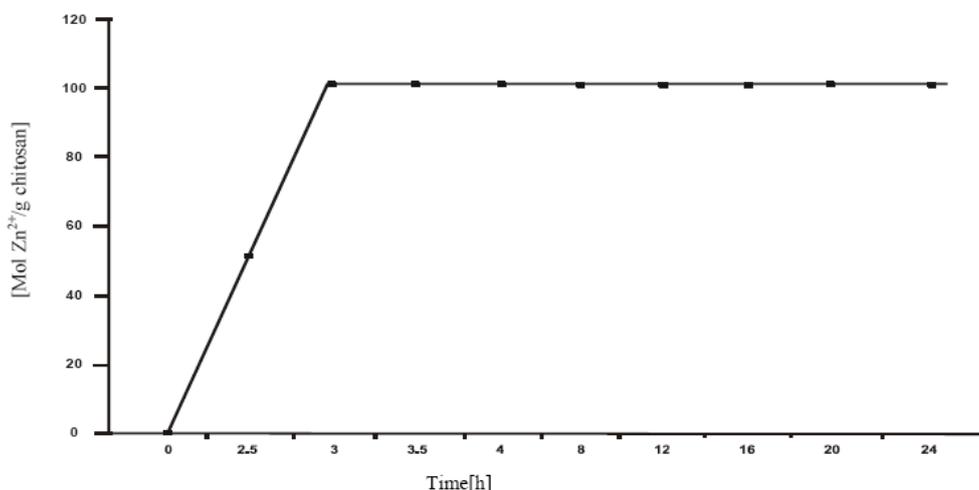

Figure 3: Maximum complexation of Zn^{2+} ion on chitosan extracted from snail shell (Deacetylation time = 5h, pH = 4.5, particle size = 0.35mmm, T = RT)

Fig. 3 shows the maximum complexation capacity of chitosan, described as moles of Zn^{2+} chelated per gram of chitosan. At the end of 3h, the chelating capacity achieved the maximum (100 mol Zn^{2+} per gram of chitosan). Above which the chelating capacity remains constant over 24h. The maximum chitosan chelating capacity with Zn^{2+} at pH 4.5 was determined to be 6.5 x 10^6 mg gram of chitosan.

Conclusion

Chitosan produced from snail shell was employed for the chelation of Zn^{2+} from Zinc oxide waste water and standard solutions. This bioligand demonstrated an efficient chelating capacity with Zn metal ions with maximum capacity of 6.5 x 10^6 mg zinc per gram of chitosan over 24h of the experiment. This is attributed to the free amino groups exposed in chitosan because of the N—deacetyletion and the free OH groups in the ring skeleton. This result confers technical and economic advantage on chitosan over other biosorbent materials.

References

[1]   Carson, B.L; H.V Ellis and J.L McCaun, Toxicology and Biological Monitoring of Metals in Humans, Lewis Publishers Chelsea, M.I. 300-328, 1987.

[2] Osohobo, V.O, Fishing in Troubled Water, The News Magazine Vol. 17 No. (2) 32-33, 2001.

[3] Wren, D.C. Mac Crimmon R.H, Loescher, R.H, Examination of Bio-accumulation and Bio-magnification of metals in a pre Cambrian shield lake, Water, Air, Soil Pollute 19, 277-291, 1983.

[4] Johnson, G.M. *Trace elements loading to sediments of fourteen Ontario lakes and correction with* concentration *in fish*, Canad., J.Fish. Aquat Sci. 44, 3-13, 1987.

[5] Allision, W.R, Maldives G.C; RMN Pilot Local reef monitoring study results and recommendation, Draft reports to Ministry of Fisheries, Agriculture and Marine Resources, Republic of Maldives 20:33-36, 1999.

[6] Hakason, L., *Metals in fish sediments from the river Kolbackson water system Sweden*; archHydrobiol, 101:375-400, 1984.

[7] Cooke, VB., "Gasoline Engine Oils: Performance, Evaluation and Classification," 10th World Petroleum Congress, Moscow. p.112, 2000.

[8] Bennet, AY "An Engine Test for predicting the performance of Engine Lubricants", 1980.

[9] Georgel, WC, La TGG. "Extended Engine Oil Life through New Technology," Seminar paper at the National Petroleum Refiners Association 1997 Annual Meeting. March 27-29, San Francisco California, 1997.

[10] Kajdas, C., "Major Pathways for Waste Oil Disposal and Recycling," Part 1. Tribotest J. 7(1): 61-74, 2000.

[11] Boughton, B, Horvath, A Environmental Assessment of Waste Oil management Methods". Environ. Sci. Technol. 38(2):353-358, 2004.

[12] Rahman MM, Siddquee TA, Samdani S, Effect of operating variables on regenerating of base-Oil from Waste Oil by Conventional Acid –Clay method, chemical Engineering research Bulletin, 12:24-27, 2008.

[13] Aydemir, T.B , Blanchard, R.K. and Cousins, R.J. , Zinc Supplementation of Young Men Alters Metallothionein, Zinc Transporter, and Cytokine Gene Expression in Leucocyte Populations *PNS*, **103(3),** 1699-17-04, 2006.

[14] Clermount, B., Application of Chitin and Chitosan, *Journal of the Chilean Chemical Society*, **51(1)**, 5-10, 2005.

[15] Saifuddin, M.N and Kumaran, P., Removal of heavy metal from industrial waste water using chitosan coated oil palm shell charcoal, *Environmental Biotechnology,* **8(1)**, 2-3. 2005.

[16] Seiichi, M., Masan, M., Reikichi, I. and Susumu ,Y., Highly deacatylated chitosan and its properties, *Journal of Applied Polymer Sciences,* **28**, 1909-1917, 1983.

[17] Taboada, E. , Cabrera, G. and Cavdenas, G., Retention capacity of chitosan for copper and mercury ions, *Chilean chemical journal,* **48(1)**, 1-13,2003.

[18] Valko, M., Morris, H. and Cromin, M.T.D., Metals, Toxicity and Oxidative Stress, *Current Medical Chemistry*, **12**, 1161-1208, 2005.

[19] Xiao, W., Yong, S.C., Won, S.L. and Byung, G.M., Preparation and properties of Chitosan/poly (vinylalcohol) Blend Foams for Copper Adsorption, *Polym Int.,* **55**, 1230-1235, 2006.