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Use of chitosan for the removal of metal ion contaminants and proteins from water

Ashoka Gamage, Fereidoon Shahidi *

Environmental Science Program, Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3X9

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Abstract

Naturally abundant biosorbants such as chitin and chitosan are recognized as excellent metal ligands, forming stable complexes with many metal ions, and serving as effective protein coagulating agents. Chitin isolated from crab processing discards was deacetylated over different time periods in order to obtain three types of chitosans [Type 1 (20 h), Type 2 (10 h) and Type3 (4 h)]. Chitosans so prepared were evaluated for their capacity to chelate metal ions in samples of water obtained from a zinc mining site (Buchans, Newfoundland). Metal chelation capacity of chitosan for wastewater was determined by inductively coupled plasma-mass spectrometry (ICP-MS) at three different pH (5, 6 and 7). Chitosan served as an effective coagulating agent in removing proteins from wastewater as well as for the removal of metal ions [Hg(II), Fe(II), Ni(II), Pb(II), Cu(II) and Zn(II)] from industrial wastewater, especially at pH 7, as measured by ICP-MS. Mercury was best chelated by all three types of chitosan under all pH conditions tested. In the protein flocculation study, Type 1 chitosan showed the best flocculation ability followed by Type 2 and Type 3 chitosans.
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Keywords: Chitosan; ICP-MS; Metal chelation; Protein flocculation

1. Introduction

The processing discards of shellfish pose major technological problems, mainly due to their insolubility in water and resistance to biodegradation (Shahidi & Synowiecki, 1991). Direct use of shellfish processing discards is generally discouraged due to the obnoxious odour of putrefying shells, therefore value-added utilization of the discards is of paramount importance. These shellfish discards contain mainly compounds that can be processed to yield chitin (Selmer-Olsen, Ratnaweera, & Pehrson, 1996) which has a myriad of industrial and other applications.

Chitin (poly[β-(1-4)-2-acetoamido-2-deoxy-D-glucopyranose]), a polymer of *N*-acetyl-D-glucosamine, is widely distributed in nature, especially in the exoskeletons of marine invertebrates such as prawn, crab and losbter (Muzzarelli, 1977). Its derivative, chitosan (poly[β-(1-4)-2-amino-2-deoxy-D-glucopyranose]), a polymer of D-glucosamine, has reactive amino groups which are responsible for complex formation between metal ions and the polymer chain (Fig. 1). Chitosan is a heteropolymer made of D-glucosamine and a small fraction of *N*-acetyl-D-glucosamine residues (Roberts, 1992). Therefore, the adsorption ability of chitosan is found to be much higher than that of chitin, which has relatively fewer amino groups. Chitosan also possesses many other useful features, including hydrophilicity, biocompatibility, biodegradability, antibacterial properties, and a remarkable affinity for many proteins (Bassi, Prasher, & Simpson, 2000). One of the important applications of chitosan is the removal of proteinaceous matter in the food industry. Chitosan, with its positive charge, can be used for coagulation and recovery of proteinaceous materials present in such food processing operations (Knorr, 1991). Removal of proteinaceous matter from wastewater generated by food industry prior to discharge to municipal sewer system is

* Corresponding author. Tel.: +1 709 737 8552; fax: +1 709 737 4000.
E-mail address: fshahidi@mun.ca (F. Shahidi).

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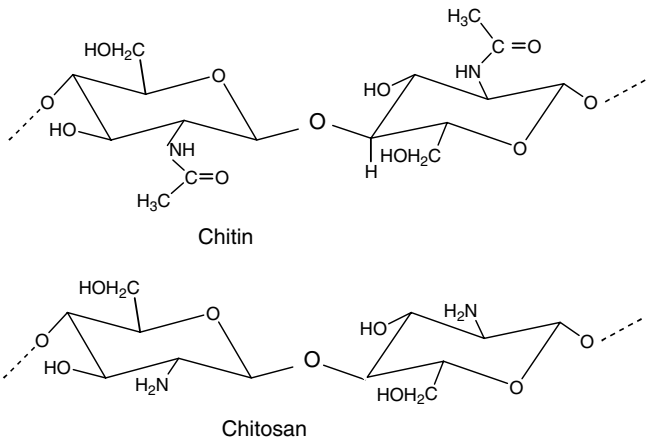

Fig. 1. Chemical structures of chitin and chitosan.

becoming mandatory in many countries. Furthermore, chitosan is largely used as a non-toxic flocculent in the treatment of organic polluted wastewater and as a chelating agent for the removal of toxic (heavy and reactive) metals from industrial wastewater (An, Park, & Kim, 2001).

Most previous studies have examined the use of chitosans for adsorption of metal ions and coagulation of proteinaceous materials from industrial wastewater. However, little attempt has been made to understand the metal adsorbing characteristics of chitosans of different molecular weights obtained by selecting different deacetylation times. Hence, the objectives of the present study were to prepare chitosans under different deacytelation conditions from locally available crab discards and to determine their potential for chelating metal ions present in industrial wastewater. In addition, protein flocculation ability of chitosans so prepared was investigated.

2. Materials and methods

2.1. Materials

Fresh samples of crab processing discards, comprised of intact cephalothoraxes and abdominal exoskeletons were collected from a local source in Newfoundland. The crab processing discards were ground in an electric grinder (Black and Decker Canada Inc., Oakville, ON) for 15 min, packed in plastic bags and stored at 4 °C until used. Reagents, sodium hydroxide, formaldehyde, formic acid, acetic acid, hydrochloric acid, sulphuric acid, acetone, nitric acid, chloroform, methanol, sodium hypochlorite/sodium oxochlorate, cadmium nitrate tetrahydrate, copper(II) sulphate, and bovine serum albumin, were obtained from Fisher Scientific Co. (Nepean, ON). Boric acid, picric acid, nickel(II) sulphate hexahydrate, silver nitrate, cobalt(II) chloride hexahydrate and ehtylenediaminetetraacetic acid were obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON). ICP-MS standards were obtained from SCP Science (St. Laurent, QC). Wastewater samples were obtained from Asarco Inc (Buchans, NL, Canada).

2.2. Methods

2.2.1. Proximate composition of crab processing discards and chitosan prepared

2.2.1.1. Moisture content. Ground crab processing samples or chitosan samples (2–3 g) were dried in a pre-weighed aluminium pan (Fisher) and placed in a forced-air convection oven (Fisher Isotemp 300). Samples were maintained at 105 ± 1 °C until a constant mass was obtained. The moisture content was then calculated as percent ratio of the weight difference of the sample before and after drying to that of the original material (AOAC, 1990).

2.2.1.2. Ash content. Approximately 3–5 g of ground crab processing discards or chitosan samples were accurately weighed into clean, dry, pre-weighed porcelain crucibles and charred over a Bunsen burner. The charred samples were heated in a muffle furnace (Blue M. Electro Co., Blue Island, IL) and maintained at 550 ± 1 °C until a gray ash was obtained. Crucibles were subsequently cooled in a desiccator and weighed. Ash content was calculated as percent ratio of the mass of the ash obtained after ignition to that of the original material (AOAC, 1990).

2.2.1.3. Nitrogen and crude protein contents. Approximately 0.3–0.4 g of ground crab processing discards or chitosan samples were weighed onto nitrogen-free weighing paper and transferred into a Büchi digestion tube (Büchi 321, Büchi Laboratories, Fawil, Switzerland). The sample was digested with 20 mL of concentrated H_2SO_4 and two Kjeltab tablets (Profamo Analytical Service Inc., Dorval, QC) for 45 min to obtain a clear solution. The digested samples were diluted with 50 mL of distilled water followed by the addition of 150 mL of a 25% (w/v) sodium hydroxide solution. The samples were steam distilled (Büchi 321, Büchi Laboratories, Fawil, Switzerland) to release the nitrogen in the form of ammonia, which was trapped in a 50 mL solution of 4% (w/v) boric acid containing N-Point indicator (EM Science, Gibbstown, NJ) in a receiving flask. Steam distillation was continued for 6 min and the contents in the receiving flask were titrated against a 0.1 N standard solution of sulphuric acid to determine the content of nitrogen (AOAC, 1990). The crude protein content of samples was calculated from nitrogen content data using a conversion factor of 6.25.

$$\text{Percentage } N = \frac{(V_{\text{sample}} - V_{\text{blank}}) \times N \times 14.0067 \times 100}{W}$$

where V_{sample} is the volume of titrate for sample (mL); V_{blank}, the volume of titrant for blank (mL); N, the normality of the H_2SO_4 solution used in the titration and W is the weight of the sample (mg).

2.2.1.4. Lipid content. Total lipid content of samples was determined using the procedure described by Bligh and Dyer (1959). Briefly, approximately 25 g of ground crab processing discards were accurately weighed and then extracted over a 3 min period with a mixture of 25 mL of chloroform and methanol followed by homogenization using a Polytron homogenizer (Model PT-3000, Brinkmann Instruments, Canada Ltd., Rexdale, ON). Approximately 25 mL of distilled water were added and the mixture was then filtered through a Büchner funnel under suction. The filtrate was allowed to separate overnight in a separatory funnel. Ten milliliters aliquots were transferred into a pre-weighed round bottom flask and the solvent was removed using a Büchi Rotorvapor (461, Büchi Laboratories, Fawil, Switzerland). The flask was then placed in the a forced-air convection oven at 80 °C for 1 h. After cooling in a desiccator the round bottom flask containing the lipids was weighed and the total lipid content determined gravimetrically.

2.2.2. Preparation of chitin and chitosan and evaluation of their characteristics

Chitin was isolated from crab processing discards using a modified version of the methods explained by Mima, Miya, Iwamoto, and Yoshikawa (1983) and Shahidi and Synowiecki (1991). Deproteinization and demineralization steps were carried out using 20 volumes of 4% (w/v) NaOH at 60 °C for 3 h and 10 volumes of 10% (w/v) HCl at 25 °C for 2 h, respectively. The alkali and acid treatments were repeated twice. Chitin residue, firmly complexed with the carotenoid pigments, was extracted with 20 volumes of acetone and dried for 2 h at ambient temperature, followed by bleaching with 0.32% (v/v) sodium hypochlorite solution containing 5.25% available chlorine for 5 min with a solid to solvent ratio of 1:10 (w/v). Chitosan was prepared by alkali treatment of chitin using 10 volumes of 50% (w/v) NaOH in distilled water at 100 °C for 4, 10 and 20 h under a nitrogen atmosphere (Mima et al., 1983). The reactants were immediately filtered under vacuum after alkali treatment, washed with hot-deionized water to neutral pH and lyophilized for 72 h at −49 °C and 62×10^{-3} mbar (Freezone 6, Model 77530, Labconco, Kansas City, MO). According to the deacytelation times, three types of chitosan were obtained (Type 1 (20 h), Type 2 (10 h) and Type 3 (4 h)). Particle size of chitosan types were determined by passing them through a series of sieves of 250, 350, 500 and1000 μm (250, 350 and 500 microns-Gilson Company Inc., Worthinton, OH; 1000 microns-Tyler Co. of Canada Ltd., St. Catherines, ON) (Table 1). The degree of acetylation of chitosan was measured according to the picric acid method explained by Neugebauer, Neugebauer, and Brzeinski (1989). Molecular weight of chitosan was expressed as the viscosity-average molecular weight using a ViscoTek model Y-500 relative viscometer (Viscotek Co., Houston, TX).

2.2.3. Determination of heavy metals in wastewater samples by inductively coupled plasma mass spectrometry (ICP-MS)

Samples of chitosan (0.1 g) of Types 1, 2 and 3 were mixed with 50 mL of industrial wastewater (obtained from a zinc mining site at Buchans, NL) in 50 mL centrifuge tubes. Industrial wastewater samples were adjusted at different pH levels (5, 6 and 7) using pH meter (Model 805 MP, Fisher Accument®, Nepean, ON) calibrated using buffers of pH 4 and 10. The original wastewater sample was used as the control. Industrial wastewater sample-chitosan mixtures were kept for 3 h at room temperature (22 °C). The contents were then centrifuged for 5 min at 4000*g* (ICE Centra M5, International Equipment Co., Needham Heights, MA) and supernatants were collected. Supernatents were subsequently filtered through a 0.45 μm micro filter (Millipore Corporation, Bedford, MA). The metal ions, namely Co(II), Ni(II), Mn(II), Fe(II), Cu(II), Zn(II), Pb(II), Hg(II) and Mo(II) were analyzed for residual metal concentrations using ICP-MS (HP 4500, Agilent, Palo Alto, CA). The concentration of the above metal ions in the original industrial wastewater was separately analyzed using ICP-MS. Before the samples were introduced to ICP-MS, supernatants were acidified with HNO_3. Nitric acid was diluted with ultrapure water to give adequate acid concentrations. Nitric acid was considered as the best choice as other common acids are not suitable for the use with ICP-MS because they contain elements which form polyatomic species.

ICP-MS (HP 4500, Agilent, Palo Alto, CA) was used to acquire raw data from unknown water samples, known reference materials and calibration solutions. Water samples were diluted ten times with 0.2 M HNO_3. Samples were introduced to ICP-MS via 10 mL test tubes arranged in a CETEC autosampler (Agilent, Palo Alto, CA). This enabled automated runs of up to 30 h in length. Data were acquired for 39 elements, namely Li, Be, Mg, Al, Si, P, S,

Table 1
Characteristics of three different types of chitosans (Types 1–3) prepared from crab shells[a]

Properties	Type 1	Type 2	Type 3
Deacetylation time[b] (h)	20	10	4
Moisture (%)	3.75 ± 0.21	3.95 ± 0.34	4.50 ± 0.30
Nitrogen (%)	7.70 ± 0.19	7.63 ± 0.08	7.55 ± 0.10
Ash (%)	0.30 ± 0.00	0.25 ± 0.02	0.30 ± 0.03
Colour	Cream white	Cream white	Cream white
Particle size (μm)	350–500	350–1000	500–1000
Apparent viscosity (cP)	14.00 ± 0.34	57.00 ± 0.96	360.00 ± 0.53
Degree of deacetylation (%)	91.30 ± 1.3	89.30 ± 1.2	86.40 ± 2.1
$\overline{M}_v$[c] (Dalton)	6.60×10^5	9.60×10^5	1.80×10^6

[a] Results are expressed as mean value of three determinations ± standard deviation.

[b] Deacetylation for preparation of chitosans Types 1, 2 and 3 was achieved using a 50% (w/v) NaOH solution at 100 °C for 20, 10 and 4 h, respectively. Apparent viscosity (cp) – 1% (w/v) chitosan level in a 1% (v/v) acetic acid solution at 25 C.

[c] Viscosity-average molecular weight.

Cl, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Mo, Ag, Cd, Sn, Sb, I, Cs, Ba, La, Ce, Hg, Ti, Pb, Bi and U. The data for elements used as internal standards were also acquired. Five calibration standards and one blank were used. Data obtained were exported from ICP-MS to a spreadsheet application, where metal ion concentration was calculated. All standards were prepared using ICP standard solutions (SCP Science, St-Laurent, QE) for elements of interest. High purity distilled water was used to dilute these standards to required concentrations. All limits of detection (LOD) for elements of interest were quoted at ppb (μg/kg) levels. The LOD of ICP-MS elements were Ag, 0.09; As, 2.00; Cd, 0.04; Co, 0.03; Cr, 0.08; Cu, 0.02; Fe, 0.03; Hg, 1.00; Mn, 0.02; Mo, 0.03, Ni, 0.10; Pb, 0.30 and Zn, 1.00. For ICP-MS, Ar was used as the plasma, carrier and auxiliary gas with sample uptake rate of 1 mL/min. Sample acquisition time was 579.79 s, rinse time, 180 s (0.5 M HNO_3) and integration time, 10 s.

2.2.4. Metal chelation capacity and recovery of metals from aqueous solutions using small-sized columns containing chitosan by ICP-MS

Removal and recovery of metal ions from aqueous solutions were done according to a modified version of the method explained by Chui, Mok, Ng, Luong, and Ma (1996). Ten grams of chitosan (Type 1) were taken into a 500 mL beaker containing 200 mL of deionized water (pH 7). The mixture was stirred for 24 h at ambient temperature. The pre-soaked chitosans were packed into five glass columns (15 cm × 0.9 cm). Samples of 50 and 100 ppm of Ni(II), Co(II), Cd(II), Cu(II) and Ag(I) prepared by dissolution in pH 7 deionized water from the corresponding salt. Ten milliliters of metal ion solutions were eluted from each separate column at neutral pH at a rate of 2 mL/min, at room temperature. Twenty milliliters of deionized water (pH 7) were passed through each column to wash the remaining metal ions, and the eluate was collected. The combined eluates were used for ICP-MS analysis. Ten milliliters of a 0.1 M solution of ehtylenediaminetetraacetic acid (EDTA) were used to desorb each metal ion from the column and the eluate was collected again. Twenty milliliters of deionized water were passed through the column to wash the remaining EDTA, and the eluate was collected. The amount of metal ions recovered in the eluate was also determined by ICP-MS using the procedure explained in Section 2.2.3 Metal chelation capacity and recovery values were then calculated.

2.2.5. Determination of protein flocculation capacity by chitosans

Protein flocculation efficiency of chitosan were determined by passing known concentrations of a standard protein [Bovine serum albumin (BSA)] through chitosans packed in glass columns and subsequently quantifying the protein concentration in the eluates. Ten grams of chitosan (Types 1–3) were weighed into a 500 mL beakers containing 200 mL of deionized water (pH 7) separately and the mixtures were stirred for 24 h at ambient temperature. The pre-soaked chitosans were packed into three glass columns (4 cm × 0.9 cm).

Bovine serum albumin (BSA) was dissolved in deionized water to obtain different concentrations (2–8 mg/mL). BSA solutions were passed through the columns containing three chitosan types at a flow rate of 12 mL/min and subsequently 5 mL of eluted solutions were collected in three glass tubes. The content of protein in each tube was measured using the method explained by Lowry, Rosebrough, Farr, and Randall (1951).

2.3. Statistical analysis

All experiments were carried out in triplicates and results were reported as mean ± standard deviation. The significance of differences among the values was determined at $p < 0.05$ using analysis of variance (ANOVA) followed by Tukey's multiple range test (Snedecor & Cochran, 1980).

3. Results and discussion

3.1. Proximate analysis of crab wastes and isolation of chitin

Moisture content of crab processing discards was 6.96%. The chemical composition of crab (*C. opilio*) processing discards was as follows; crude protein, 21.5 ± 0.53%; ash, 43.7 ± 0.1%; lipid, 0.35 ± 0.1% and chitin 35.3 ± 1.9% (all measured and reported on a dry weight basis). Crab processing discards comprise a composite of dried waste of shell, cephalothoraxes, legs and claws. Non-chitinous protein was the main component which should be removed effectively to obtain a high quality chitin (Austin, Brine, Castle, & Zikakis, 1981). For isolation of chitin, a deproteinization step was included followed by demineralization to remove inorganic matter. For demineralization, it is important that the amount of acid be stoichiometrically equal or greater than all minerals present in the shells to ensure complete reaction with inorganic salts (Shahidi & Synowiecki, 1991). Acid and alkali treatments may produce a coloured chitin product because of the carotenoids remaining in the preparations. When a bleached chitinous product is desired, pigments can be removed using acetone followed by bleaching with sodium hypochlorite.

3.2. Production and characterization of chitosan extracted from crab processing discards

Chitosan was prepared by alkali treatment of chitin using a 1:10 (w/v) solid to solvent ratio of 50% sodium hydroxide in distilled water at 100 °C for 20, 10 and 4 h under a nitrogen atmosphere (Mima et al., 1983). According to the length of deacetylation time, three types of chitosan were obtained (Types 1–3). The degree of deacetylation was 91.3%, 89.3%, and 86.4% for the chitosans Types 1–3, respectively. The large positive charge density due to the

high degree of deacetylation (86.4–91.3%) makes crab chitosans unique for industrial applications as their properties are highly dependent on charge density. Li, Dunn, Grandmaison, and Goosen (1992) and Muzzarelli (1985) reported that the term chitosan could only be used when the degree of deacetylation is above 70% and nitrogen content in the product is higher than 7% by weight. Characteristics of chitosans prepared with different deacetylation times are listed in Table 1.

3.3. Heavy metals in wastewater samples

Wastewater samples obtained from the zinc mining site (Buchans, NL) contained excessive amounts of Mn(II), Co(II), Cd(II), Hg(II), Pb(II), Cu(II) and Zn(II) based on the Canadian Water Quality Guidelines for protection of fresh water (1999) (Table 2). Out of the three pH conditions examined, pH 7 served best for chelation of multiple metal ions. This could be due to the greater availability of amino groups at higher pH values (Bassi et al., 2000). Considering the three types of chitosan; Type 1 showed significantly ($p < 0.05$) higher metal chelation activity compared to Types 2 and 3. Chitosan Type 1 was best for metal chelation followed by Types 2 and 3. Chitosans Type 2 and 3 showed the same chelation pattern. Of the metal ions tested, Hg(II), was best chelated under different pH conditions by all three types of chitosan. Fe(II) was best chelated at pH 5.

Zinc is an essential element mediating a variety of metal-containing enzymes and the biosynthesis of nucleic acids and polypeptides. Toxicity symptoms of zinc include nausea, vomiting and diarrhea, and in several cases with blood, and abdominal cramps (Elinder & Piscator, 1979). It was possible to achieve 50% reduction of Zn in wastewater using chitosan Type 1. However, the final concentration achieved was inadequate to meet the Canadian water quality guide-

Table 2
Effectiveness of chitosan in metal ion chelation from industrial wastewater

Heavy metals	Original concentration (ppb)	Types of chitosan	Chelation capacity (%)		
			pH 5	pH 6	pH 7
Fe(II)	161.5 ± 12.0	Type 1	94.1 ± 0.00^{b}	93.8 ± 0.45^{b}	92.2 ± 6.85^{b}
		Type 2	14.6 ± 0.00^{a}	15.2 ± 0.07^{a}	17.5 ± 0.24^{a}
		Type 3	15.4 ± 2.89^{a}	14.4 ± 0.00^{a}	15.3 ± 0.00^{a}
Mn(II)	158.5 ± 0.91	Type 1	2.11 ± 0.59^{a}	8.92 ± 0.29^{a}	11.2 ± 0.37^{a}
		Type 2	21.2 ± 1.03^{c}	23.4 ± 1.27^{b}	22.9 ± 3.42^{b}
		Type 3	18.5 ± 0.82^{b}	21.9 ± 0.85^{b}	21.9 ± 0.83^{b}
Co(II)	0.47 ± 0.00	Type 1	22.1 ± 1.81^{c}	24.2 ± 1.85^{b}	26.3 ± 1.83^{b}
		Type 2	2.47 ± 0.50^{b}	4.20 ± 0.06^{a}	4.20 ± 0.06^{a}
		Type 3	2.10 ± 0.00^{a}	2.10 ± 0.00^{a}	2.10 ± 0.00^{a}
Ni(II)	1.15 ± 0.13	Type 1	53.2 ± 6.15^{b}	54.9 ± 6.36^{b}	62.8 ± 7.29^{b}
		Type 2	11.3 ± 1.31^{a}	12.6 ± 0.84^{a}	20.8 ± 6.76^{a}
		Type 3	9.04 ± 0.78^{a}	9.51 ± 0.12^{a}	22.3 ± 0.09^{a}
Cu(II)	15.4 ± 0.47	Type 1	58.3 ± 1.14^{c}	51.8 ± 0.74^{b}	64.9 ± 1.56^{b}
		Type 2	19.3 ± 2.07^{b}	29.9 ± 8.08^{a}	41.9 ± 8.21^{a}
		Type 3	12.0 ± 1.51^{a}	35.3 ± 2.60^{a}	38.0 ± 3.05^{a}
Zn(II)	1331 ± 27.8	Type 1	48.9 ± 1.01^{b}	49.5 ± 0.96^{b}	50.3 ± 0.88^{b}
		Type 2	34.1 ± 1.28^{a}	34.2 ± 3.34^{a}	37.0 ± 0.98^{a}
		Type 3	30.9 ± 1.55^{a}	34.5 ± 1.43^{a}	35.9 ± 1.17^{a}
As(II)	0.43 ± 0.02	Type 1	15.6 ± 0.00^{b}	13.3 ± 0.00^{b}	13.3 ± 0.00^{b}
		Type 2	4.44 ± 0.00^{a}	4.44 ± 0.00^{a}	4.44 ± 0.00^{a}
		Type 3	4.44 ± 0.00^{a}	4.44 ± 0.00^{a}	4.44 ± 0.00^{a}
Mo(II)	1.40 ± 0.05	Type 1	12.9 ± 0.51^{b}	15.7 ± 1.38^{b}	19.9 ± 2.22^{c}
		Type 2	2.77 ± 0.01^{a}	4.47 ± 0.43^{a}	9.96 ± 0.03^{b}
		Type 3	2.64 ± 0.19^{a}	2.70 ± 0.05^{a}	2.78 ± 0.00^{a}
Cd(II)	4.99 ± 0.08	Type 1	26.9 ± 5.56^{a}	27.5 ± 4.50^{a}	39.1 ± 0.09^{c}
		Type 2	20.4 ± 1.07^{a}	24.3 ± 1.14^{a}	30.6 ± 0.52^{a}
		Type 3	25.1 ± 2.69^{a}	26.0 ± 1.53^{a}	35.0 ± 1.38^{b}
Hg(II)	0.59 ± 0.02	Type 1	95.8 ± 0.99^{b}	95.8 ± 0.99^{b}	97.5 ± 1.07^{b}
		Type 2	90.7 ± 0.75^{a}	91.5 ± 0.41^{a}	93.2 ± 2.80^{a}
		Type 3	89.8 ± 1.91^{a}	90.7 ± 0.75^{a}	91.5 ± 0.41^{a}
Pb(II)	32.9 ± 1.15	Type 1	41.1 ± 1.43^{c}	57.9 ± 3.24^{b}	91.0 ± 2.33^{c}
		Type 2	8.75 ± 0.03^{b}	14.2 ± 0.15^{a}	16.9 ± 0.57^{b}
		Type 3	5.94 ± 1.07^{a}	7.26 ± 0.34^{a}	7.91 ± 0.97^{a}

Means mentioned against the same metal ion under each of the three pH levels are not significantly ($p > 0.05$) different.

Table 3
Summary of guidelines for protection of aquatic life and dinking water

Metal ions	Canadian water quality guideline for protection of aquatic life[a]	Drinking water MCL[b]
Cr(III)	1	50
Fe(II)	300	200
Mn(II)	–	50
Co(II)	–	1000
Ni(II)	25–150	20
Cu(II)	–	1000
Zn(II)	30	5000
As(II)	5	50
Mo(II)	73	40
Ag(I)	0.1	–
Cd(II)	0.017	3
Hg(II)	0.1	1
Pb(II)	1–7	1.5

Values are given in ppb or μg/L.
[a] Adapted from Canadian water quality guideline (1999).
[b] MCL – maximum contamination levels for potentially toxic metals in dinking water; Adapted from Siegel (2002).

lines for protection of aquatic life (1999) (Table 3). Therefore, multiple passes through chitosan might be necessary to achieved the desired water quality standards.

Research has been done on the use of chitosan for the removal of some heavy metal ions from industrial wastewater. The use of commercially available chitosan for potable water purification has been approved by the United States environmental protection agency (US EPA) up to a maximum level of 10 mg/L (Knorr, 1984). It not only removes toxic metal ions such as Cd and Pb when present in the environment, but also prevents accidental contamination by radioactive isotopes such as cobalt-60. Chitosan also eliminates undesirable, though harmless, metal ions such as Fe(II), which are known to possess unpleasant organoleptic properties in the drinking water (Muzzzarelli, Weckx, & Filippini, 1989).

Under alkaline conditions, chitosan exhibits a strong cation exchange behaviour to remove metal ions from industrial wastewater while under acidic conditions, chitosan causes anion exchange sites to be made available to bind the anion. Therefore, exchange columns with pH adjustment may be developed to replace the conventional process for industrial wastewater treatment. Huang, Chung, and Liou (1996) reported that number of adsorption sites on chitosan increased with decreasing particle size.

ICP-MS, a powerful analytical tool, can be used to measure metal concentration of wastewater from different effluents, including those from Zn mining sites. Since ICP-MS has a very low detection limit and a wide analytical concentration range, it is often used for elemental analysis and studies on environmental materials.

3.4. Determination of metal chelation capacity using a chitosan column

Out of the metal ions investigated, no significant ($p < 0.05$) difference in metal chelation ability of chitosan was observed for metal ion concentrations between 50 and 100 ppm, except for Co(II). Considering Co(II), a significantly increased adsorption efficiency of chitosan was noticed upon raising the concentrations. Increasing metal ion concentration in the solutions seems to reduce the external diffusion of the adsorbate and enhance intraparticular diffusion (Jansson-Charrier, Guibal, Roussy, Delanghe, & LeCloire, 1996). Chitosan removed over 99% of metal ions from 50 ppm metal solutions, except for Co(II) and over 98% from 100 ppm (Table 4).

The lone pair electrons present on the amino nitrogen can establish dative bonds with transition metal ions. Some hydroxyl groups in chitosan may function as second donors; hence, deprotonated hydroxyl groups can be involved in coordination with metal ions (Micera et al., 1986). Acidic pH favours protonation of the amino sites, hence leading to a low complexing and chelating capacity. On the other hand, metal chelation has been reported to diminish under high pH conditions due to rapid changes in protonated and unprotonated forms of chitosan (Udaybhaskar, Iyengar, & Prabhakara Rao, 1990). Therefore, neutral pH was maintained in the chitosan columns for metal adsorption. The amount of metal adsorbed by chitosan was directly proportional to the increase in metal ion concentration, demonstrating a linear relationship (Chui et al., 1996).

EDTA satisfactorily recovered 52–97% and 56–97% of Ni(II), Co(II), Cd(II) and Cu(II) from solutions containing 50 and 100 ppm of metal ions, respectively. Out of the metal ions investigated, no significant ($p < 0.05$) difference in metal recovery efficiency was observed for metal ion concentrations between 50 and 100 ppm, except for Cu(II). The recovery efficiency for Cu(II) was 97.5% for 50 ppm and 56.5% for 100 ppm. Recovery is an important process that can be performed to collect precious metal ions, and recycling of metal ions from industrial wastewater for reuse, to reduce operational costs.

Chelation formulations based on EDTA are used in a wide variety of applications in agriculture, food processing, and water treatment industries to sequester metal ions (Gonsior, Sorci, Zoellner, & Landenberger, 1997). Concern has been expressed that EDTA, because of its high binding constants with metals, has the potential to solubilize metals

Table 4
Comparison of metal chelation and recovery of chitosan Type 1

Metal ion	Chelation (%)		Recovery (%)	
	50 ppm	100 ppm	50 ppm	100 ppm
Ni(II)	99.6 ± 0.01^{c}	98.9 ± 0.00^{a}	87.0 ± 10.9^{cd}	97.9 ± 1.69^{d}
Co(II)	76.6 ± 0.17^{a}	99.7 ± 0.00^{d}	52.2 ± 0.00^{b}	84.7 ± 3.18^{c}
Cd(II)	99.1 ± 0.01^{b}	99.1 ± 0.00^{b}	79.8 ± 0.21^{c}	74.3 ± 1.56^{b}
Cu(II)	99.9 ± 0.06^{c}	99.5 ± 0.00^{c}	97.5 ± 2.26^{d}	56.2 ± 0.51^{a}
Ag(I)	99.9 ± 0.00^{c}	99.9 ± 0.00^{d}	0.08 ± 0.00^{a}	0.00 ± 0.00

Results reported are mean vales of three determinations ± standard deviation.
Means in each column sharing the same superscript are not significantly ($p > 0.05$) different.

from solid phases (i.e. sediments and colloidal particulates). This process could occur via several possible interrelated mechanisms affecting overall metal ion speciation. The chelant may complex free metal ions in solution, thus shifting precipitation and sorption equilibria toward increased dissolution of metals. Alternatively, EDTA could interact directly with solid phases by complexing metal ions present in, and/or adsorbed to precipitate solids and minerals (Gonsior et al., 1997). Because of wide spread usage, high water solubility, and slow biodegradation rate, EDTA has been detected in various water sources, including waste water treatment plant effluents and river waters in both the USA and Europe.

3.5. Protein flocculation capacity of chitosan

Chitosan, with its positive charge, can effectively function as a polycationic coagulating agent in wastewater treatment (Li, Dunn, Grandmaison, & Goosen, 1997). Chitosan as a coagulating agent for wastewater streams is particularly effective in removing protein from waste; the coagulated proteins could serve as an animal feed ingredient (Bough & Landes, 1976).

Of the three types of chitosan used Type 1 showed the best flocculation ability followed by Types 2 and 3 (Fig. 2). Protein flocculation capacity of Type 1 was the highest (nearly 100%) at a protein concentration of 2 mg/mL. The flocculation capacity remained high over 2–4 mg/mL and then drastically dropped to 65% at 6 mg/mL and remained almost constant up to 8 mg/mL concentration. The flocculation percentage was reduced with increasing protein concentration afterwards. Type 2 chitosan showed the same flocculation efficiency (nearly 65%) at all protein concentrations employed (2–8 mg/ml). In the present study, pH 7 was used for determination of protein flocculation. Under constant pH, the higher molecular weight of chitosan achieved better turbidity removal (Chung, Li, & Chen, 2005).

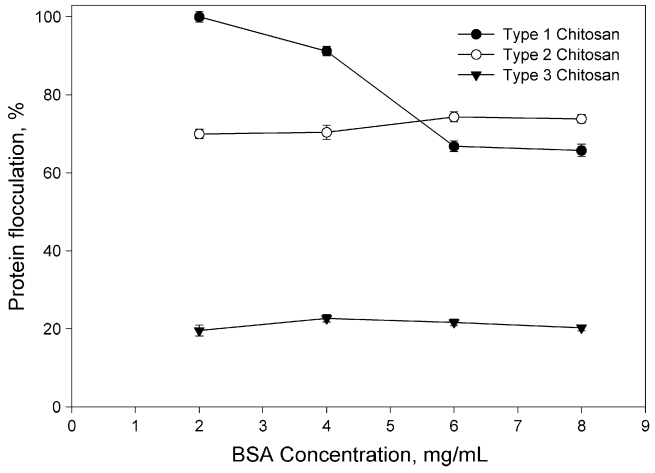

Fig. 2. Protein flocculation capacity of chitosan using different concentrations of bovine serum albumin.

Chitosan is an excellent coagulating agent and a flocculant due to the high density of amino groups on the polymer chain that can possibly interact with negatively charged substances such as proteins, solids and dyes (Li et al., 1997). Wu, Bough, Holmes, and Perkins (1978) investigated the effectiveness of different chitosans for removing proteins from cheese whey. They found that the effectiveness of chitosan in coagulating solids and proteins was inversely proportional to its molecular weight. In the present study, Type 1 chitosan had a lower molecular weight (6.6×10^5 Da) than Type 2 (9.6×10^5 Da) and Type 3 (1.8×10^6 Da). Because chitosan, with its higher deactylation degree, carries more positive charges, it exhibits better performance in removing pollutants by adsorption, coagulation, or acting as bacteriostatic than agents with a lower deacetylation degree (Chung et al., 2005). The present results lend further support to the finding of other workers (Chung et al., 2005; Li et al., 1997; Wu et al., 1978), in that chitosan Type 1 had the highest protein flocculation ability when compared to Types 2 and 3 chitosans.

Protein and fat can be reclaimed from wastewater by a multitude of physical/chemical and biological techniques. Reclamation of protein yields not only economically valuable products, but also the pretreatment of food industry wastewater which is becoming a common requirement prior to discharge to the municipal sewer systems (Selmer-Olsen et al., 1996). The most widely researched application of chitosan is perhaps its use as a coagulant for suspended matter in potable water and in food processing waste treatment. Chitosan has been used to treat waste effluents of a wide range of food process operations, including egg breaking, vegetable, shrimp, cheese, meat, beer and apple juice processing. In these operations, chitosan was demonstrated to be a very good coagulating agent.

4. Conclusions

All chitosans (Types 1–3) used were effective in the removal of metal ions in wastewater samples. Removal of metal ions [Hg(II), Fe(II), Pb(II), Ni(II), Cu(II) and Zn(II)] from industrial waste water was best effective at pH 7 as measured by ICP-MS. Mercury was best chelated under all pH conditions by all three types of chitosan. Chitosan packed in columns removed more than 98% of Ni(II), Cd(II), Cu(II) and Ag(I) from aqueous solutions at both 50 and 100 ppm levels of metal ion concentrations. Out of the metal ions tested, there was no significant difference among metal chelation both at 50 and 100 ppm metal concentrations. EDTA satisfactorily removed 52–97% and 56–97% of Ni(II), Co(II), Cd(II) and Cu(II) when used at 50 and 100 ppm, respectively. In the protein flocculation study, Type 1 chitosan showed the best flocculation ability followed by Types 2 and 3 chitosans. Considering Type 1, protein flocculation was highest between 2 and 4 mg/mL protein. The flocculation percentages were reduced with increasing protein concentration after 4 mg/mL. Type 2 chitosan showed the same flocculation percentages for

2–8 mg/mL protein; chitosan can be used at 0.2 g/L for such a purpose. Based of the above, it is suggested that crustacean shell waste serves best for production of Type 1 chitosan which may in turn be used as an environmentally friendly material for waste water purification.

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