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Green synthesis and characterization of alginate nanoparticles and its role as a biosorbent for Cr(VI) ions

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ABSTRACT

Green synthesis of nanoparticles has attained considerable attention in recent years because of its myriad of applications including drug delivery, tissue engineering and water purification. In the present study, alginate nanoparticles stabilized by honey were prepared by cross-linking aqueous solution of alginate with calcium ions. Honey mediated synthesis has been reported earlier for the production of metal nanoparticles. However no literature is available on the use of this technique for polymeric nanoparticles. Highly stable nanoparticles of 10–100 nm size were generated by this technique. The synthesised nanoparticles were characterized by transmission electron microscopy, scanning electron microscopy, atomic force microscopy, dynamic light scattering and Fourier transform infrared spectroscopic techniques. Potential of using these nanoparticles for heavy metal removal was studied by using Cr(VI) from aqueous solution, where a maximum removal efficiency of 93.5% was obtained. This method was also successfully employed for the production of other polymeric nanoparticles like casein, chitosan and albumin.

1. Introduction

Advances in nanoscience and nanotechnology provide many synthetic protocols for the production of metal and polymeric nanoparticles having dimensions less than 100 nm. Polymeric nanoparticles have attained considerable attention in many applications due to the versatility of its properties [1,2]. The large surface area to volume ratio of the nanoparticles attributes to remarkable physical and chemical properties compared to their macro scaled counterparts [3]. Most of the conventional methods use organic stabilizers to prevent *in situ* aggregation of nanoparticles by capping their surfaces [4]. Complete removal of these organic reagents is difficult and may cause toxicity in bio-related applications [5]. Synthetic strategies for the production of non-aggregatory nanoparticles of high stability are of general importance in various applications. The nanoparticles of Au, Ag and Au–Ag alloy were synthesised using non-toxic reagents like glucose and starch as reducing/protecting agent [6]. Green synthesis of platinum nanoparticles using plant extract has also been reported [7]. Studies have also been carried out on the synthesis of metal nanoparticles using honey as the capping agent [8–10]. Polymers like chitosan, polyethylene glycol, etc. have been employed for the synthesis of silver nanoparticles [11–13].

Nanoparticles of natural polysaccharide, alginate has also been prepared using different techniques. Alginate particles of well-defined sizes (250–850 nm) were produced by the controlled gellification of sodium alginate solution with calcium chloride using poly-L-lysine as the polyelectrolyte complexing agent [14]. The same principle of controlled cation induced gellification of sodium alginate solution in presence of chitosan was employed for the production of drug induced alginate nanoparticles [15]. Alginate particles of 350 nm sizes were prepared by the drop-wise addition

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of a micro emulsion prepared from aqueous $CaCl_2$, dioctyl sodium sulfosuccinate (DOSS) surfactant and isopropyl myristate (IPM) oil to the microemulsion prepared from aqueous sodium alginate, DOSS and IPM [16]. Spherical alginic acid nanoparticles of 100 nm diameter were synthesised by non-solvent aided counter ion complexation between alginic acid and 2,2′-(ethylenedioxy)diethylamine followed by cross-linking with Ca^{2+} [17]. Iron cross-linked alginate nanoparticles of 18–38 nm size were prepared by cross-linking with ferric chloride in presence of paraffin oil [18].

In the present work, a simple and safe technique for the synthesis of highly stable alginate nanoparticles using natural honey as the stabilizing agent is studied. Honey produced from bees consists of carbohydrates, aminoacids, phenolic compounds and vitamins [19,20]. The polysaccharide, alginate, present in the cell wall of the brown algae is a biodegradable, hydrophilic and non-toxic polymer composed of β-D-Mannuronic acid (M) and α-L-Guluronic acid (G), bound by β-1, 4 and α-1, 4 glycosidic linkages. The surface of this natural polysaccharide molecule consists of carboxyl and hydroxyl moieties as binding sites [21]. The carboxylate groups of the polymer interact with bivalent or multivalent cations, forming insoluble three dimensional network structures. In this study, highly stable non-aggregatory calcium cross-linked alginate nanoparticles were synthesised using a very low concentration of honey in aqueous medium. Potential of using these particles for heavy metal removal was studied using Cr(VI) ions. The synthesised alginate nanoparticles were found to be highly effective in removing heavy metal ions from aqueous solutions. Removal of more than 93% Cr(VI) ions was achieved from aqueous solution in batch technique using this biosorbent.

2. Experimental

2.1. Materials

Sodium alginate powder (Sigma Aldrich, London), calcium chloride dihydrate and potassium dichromate (Merck, Darmstadt, Germany) were of analytical grade and were used as such without any further purification. Natural honey used in this study was procured from Kerala Agriculture University and its physico–chemical parameters are shown in Table 1.

Table 1
The physico–chemical parameters of natural honey.

Density	1.362
Viscosity	10 P
Glucose (%)	73
Iron Content (%)	1.5
Calcium Content (%)	20
Magnesium Content (%)	0.63

2.2. Methods

The pH measurements were carried out using a digital microprocessor pH metre (Systronics model µ362). Accurate weight of chemicals and biosorbent used in the study was taken in an electronic balance, Citizen CX 220, Germany. A JEOL JSM-6390LA Analytical Scanning Electron Microscope (SEM) along with Energy Dispersive Spectrum (EDS) analyser was used to study the surface properties of the nanoparticles by coating the samples with a thin layer of gold and applying an electron voltage of 20–30 KV. Transmission Electron Microscopic (TEM) analysis was conducted using JEOL model 1200EX instrument operated at an accelerating voltage at 80 kV after ultrasonicating the colloidal solution in triple distilled water for 15 min. Atomic Force Microscopic (AFM) images were recorded under ambient conditions using NTEGRA (NT-MDT) operating with a use tapping mode regime. Micro-fabricated TiN cantilever tips (NSG10) with a resonance frequency of 299 kHz and a spring constant of 20–80 Nm^{-1} were used. Samples for the imaging were prepared by drop casting the zinc oxide solution on cover slip. A computer controlled particle size analyser (Delsa™ Nano Beckman Coulter) was used to study the average particle size of alginate nanoparticles. Zeta potential measurement was also carried out using the same instrument. The functional groups of the biosorbent were identified by Fourier Transform Infra Red (FTIR) Instrument Model: (SPECTRUM 400) within the range 400–4000 cm^{-1}, using KBr as background material. The residual concentration of the metal ion was determined by Atomic Absorption Spectrophotometry (AAS) using Perkin–Elmer Atomic Absorption Spectrophotometer PinAAcle900 H. All the glass wares used in the study were of A-type borosil glass.

2.3. Synthesis of calcium alginate nanoparticles (CANPs) by ionotropic gelation method

Sodium alginate solution was prepared by the dissolution of sodium alginate powder (1 gm) in 100 ml deionised water containing 1% honey. Calcium chloride solution (1%, 20 ml) was then added slowly and the solution was magnetically stirred for 4 h. The resulting nanoparticles were collected by centrifugation, washed with plenty of water and dried under vacuum. The spherical nanoparticles produced were in the size range 10–100 nm compared to previously reported particles of larger dimensions.

2.4. Preparation of adsorbate

A stock solution of 1000 mgL^{-1} of Cr(VI) was prepared by dissolving 2.83 g of $K_2Cr_2O_7$ in 1000 ml of deionised water. This solution was diluted as required to obtain the standard solutions containing 10–100 $mgL^{-1}$of Cr(VI). pH adjustment was carried out by using 0.1 N HCl and 0.1 N NaOH solutions.

2.5. Metal ion removal from solutions

The effect of pH, contact time, initial concentration and adsorbent dose on the biosorption of Cr(VI) ions onto CANPs was investigated by equilibrating a definite amount of the biosorbent in 100 ml chromium solution with initial concentrations ranging from 10 mgL^{-1} to 100 mgL^{-1}. The experiments were carried out in the pH range of 2–7, by agitating the mixture for varying contact time from 10 to 240 min. The resultant solution was then filtered and the residual concentration of chromium ions in the solution was determined by AAS.

The percentage removal of chromium and the uptake capacity of the biosorbent were calculated using the following equations.

$$\text{Removal of Cr(VI) from solution (\%)} = \frac{C_0 - C_e}{C_0} \times 100 \tag{1}$$

Adsorption of Cr(VI) on nanoparticles at equilibrium q_e (mgg^{-1})

$$q_e = \frac{C_0 - C_e}{w} \times V \tag{2}$$

where C_o and C_e are the initial and equilibrium concentrations of Cr ions (mgL^{-1}) respectively, V is the volume in litre of the solution and w (g) is the mass of dry sorbent used.

3. Results and discussion

3.1. Characterisation of the biosorbent

3.1.1. Microscopic analysis

Fig. 1(a)–(d) shows the TEM images of the nanoparticles. TEM image shows the formation of well-defined spherical alginate nanoparticles of 10–100 nm size.

SEM image shown in Fig. 2(a) also confirm the formation of spherical alginate nanoparticles with an average size of 102 nm as determined by measuring the size of fifty nanoparticles. Presence of calcium ions in the nanoparticle is confirmed by the EDS spectrum shown in Fig. 2(b). Fig. 2(c) is a clear indication for the presence of chromium after treatment with aqueous chromium solution. The negatively charged carboxylate group of the polymer interacts ionically with the Ca^{2+} ions, forming cross-links in nanoalginate.

In the absence of honey the prepared nanoparticles aggregate together as shown by the AFM image in Fig. 3(a) while the presence of honey facilitates the formation of stable nanoparticles with reduced size which is evident from the AFM image shown in Fig. 3(b).

DLS and surface charge density of the synthesised nanoparticles are shown in Fig. 4(a) and (b) respectively.

The size of particles synthesised using honey was found to be 142.4 nm with a polydispersity index of 0.480. Since DLS measurements give the hydrodynamic diameter rather than the actual diameter of solid hydrophilic nanoparticles, the particle size obtained was slightly higher than that observed by microscopic techniques. This may also be due to the swelling of the nanoparticles during dispersion in aqueous medium. Zeta potential measurement was carried out using the same instrument and was found to have a value of −4.60 mV, indicating the presence of negatively charged groups on the surface. This is due to the presence of uncross-linked carboxylate ions of the polymeric chain in the nanoparticles [22].

3.1.2. Fourier transform infra red spectroscopy (FTIR)

FTIR was taken to identify the biomolecules in honey responsible for the stabilization of nanoparticles and the nature of interaction between them. Fig. 5 shows the FTIR spectra of nanocalcium alginate prepared with and without honey.

Major broad band at 3349 cm^{-1}, due to the stretching vibrations of the characteristic hydroxyl group of the polysaccharide appears in both the spectra, indicating that honey is not chemically bound to nanoalginate [23]. If there exist a chemical interaction between the hydroxyl group of alginate and honey, there would have been a shift in the vibrational frequency at 3349 cm^{-1}. No change in peak position is observed, indicating the absence of chemical interaction between alginate and honey, which can be attributed to the intermolecular hydrogen bonding between different alginate molecules. The C=O stretching mode of monomeric carboxylic acid group at 1726 cm^{-1} is shifted to 1650 cm^{-1} in the case of alginates [24]. This red shift is due to intramolecular hydrogen bonding between carbonyl oxygen and hydroxyl group of the same alginate molecule [25]. In presence of honey, the C=O stretching frequency of alginate is further reduced to 1612 cm^{-1} due to resonance stabilization. The possible resonating structures of pure alginate, honey and alginate–honey conjugate (nanoalginate) are shown in Scheme 1(a)–(c) respectively.

In nanoalginate, weak dipole–dipole interaction between the intermittent positive charge on nitrogen of the amide group and the oxygen atom of the carbonyl group reduces the C=O frequency and hence causes a red shift. The resonance stabilization of amide group shown above stabilizes the ground state causing a reduction in transition probability and a corresponding decrease in the C=O band intensity.

The amide I and II bands of proteins in honey are expected to occur around 1660 and 1535 cm^{-1} respectively [26]. The band observed at 1612 cm^{-1} in the honey mediated alginate has a prominent decrease in intensity due to the merging of the amide band of honey with the C=O stretching band of alginate. The

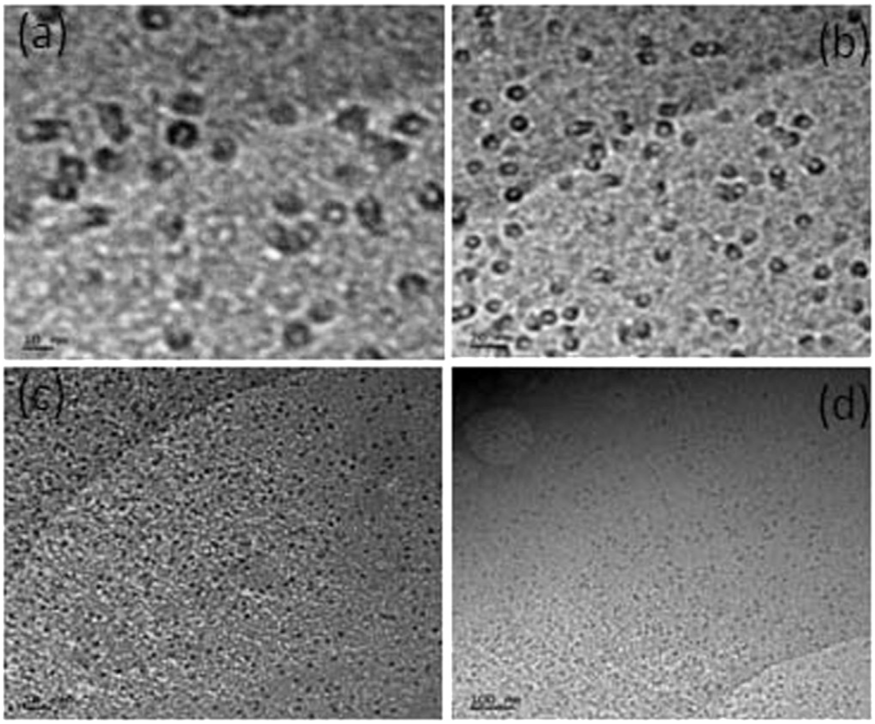


Fig. 1. TEM images of synthesised alginate nanoparticles.

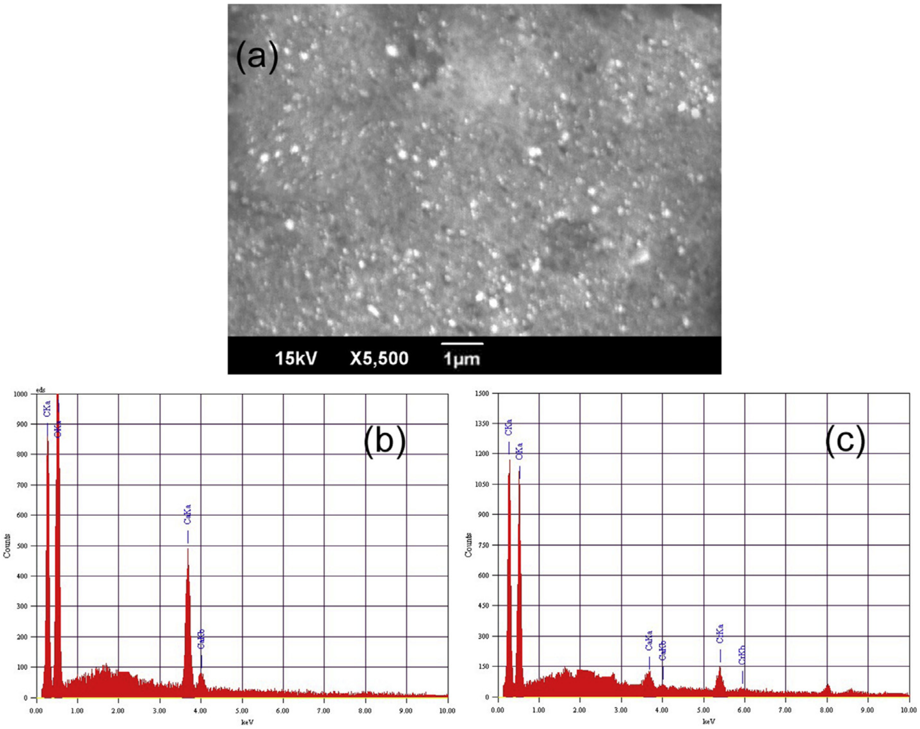


Fig. 2. (a) SEM of nanoalginate; (b) EDS of nanoalginate and (c) EDS after Cr biosorption.

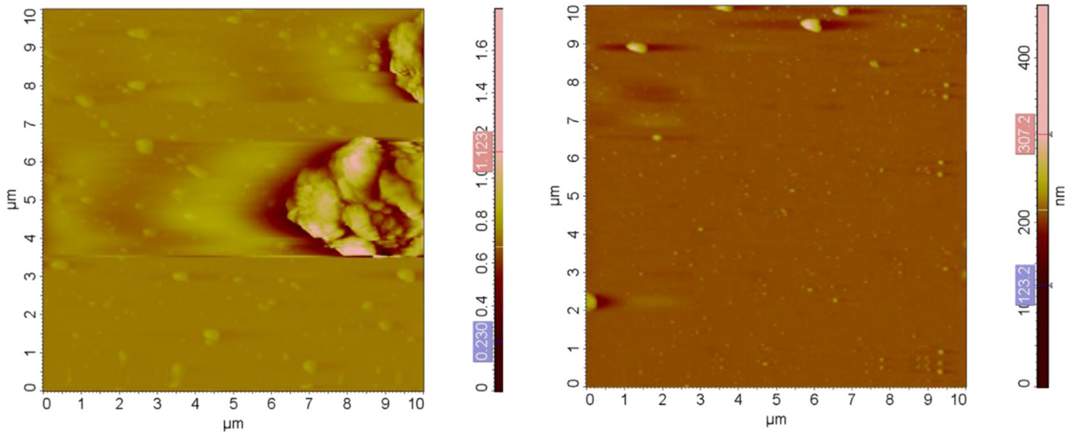


Fig. 3. AFM of nanoalginate synthesised (a) in the absence of honey and (b) in the presence of honey.

merging of bands causes broadening of the spectrum and a decrease of intensity. The decrease in intensity of the C═O stretching band at 1423 cm^{-1} may be due to resonance stabilization in honey mediated alginate. The resonance stabilization in honey creates a partial positive charge on its N-atom causing a weak dipole–dipole attraction between alginate and honey. This intermittent attraction between carbonyl group of alginate and amide group of honey decrease the probability of transition and hence a reduction in intensity of IR band.

The bands around 1000–1050 cm^{-1} due to C–O–C stretching present in both the spectra are attributed to the saccharide structure of the alginate [18]. This band may also be due to the C–O–C symmetric stretching and the C–O–H bending vibration of protein in honey [7]. The resemblance of the major peak values indicated that the basic polymeric structure of the parent compound was retained. From the FTIR data, it is clear that only weak intermolecular force of attraction exists between alginate and honey, proving that honey serves only as a stabilizer in nanoalginate formation.

3.2. *Application in heavy metal removal*

3.2.1. *Adsorption of Cr(VI) from aqueous solutions*

3.2.1.1. Effect of initial pH. Initial pH of the solution has a marked influence on the biosorption of metal ions as it determines the net charge on the biosorbent and its ability to sequester the metal ion [27]. The adsorption studies were conducted in the pH range 2–7 and the percentage removal increased gradually from pH 2, reaching a maximum at 4 and thereafter decreasing (Fig. 6(a)). This is in agreement with previous reports using alginate beads [28,29]. At higher pH, metal ions get precipitated as hydroxide, lowering its concentration in the medium, which in turn causes a reduction in adsorption on the surface of biosorbent [30,31].

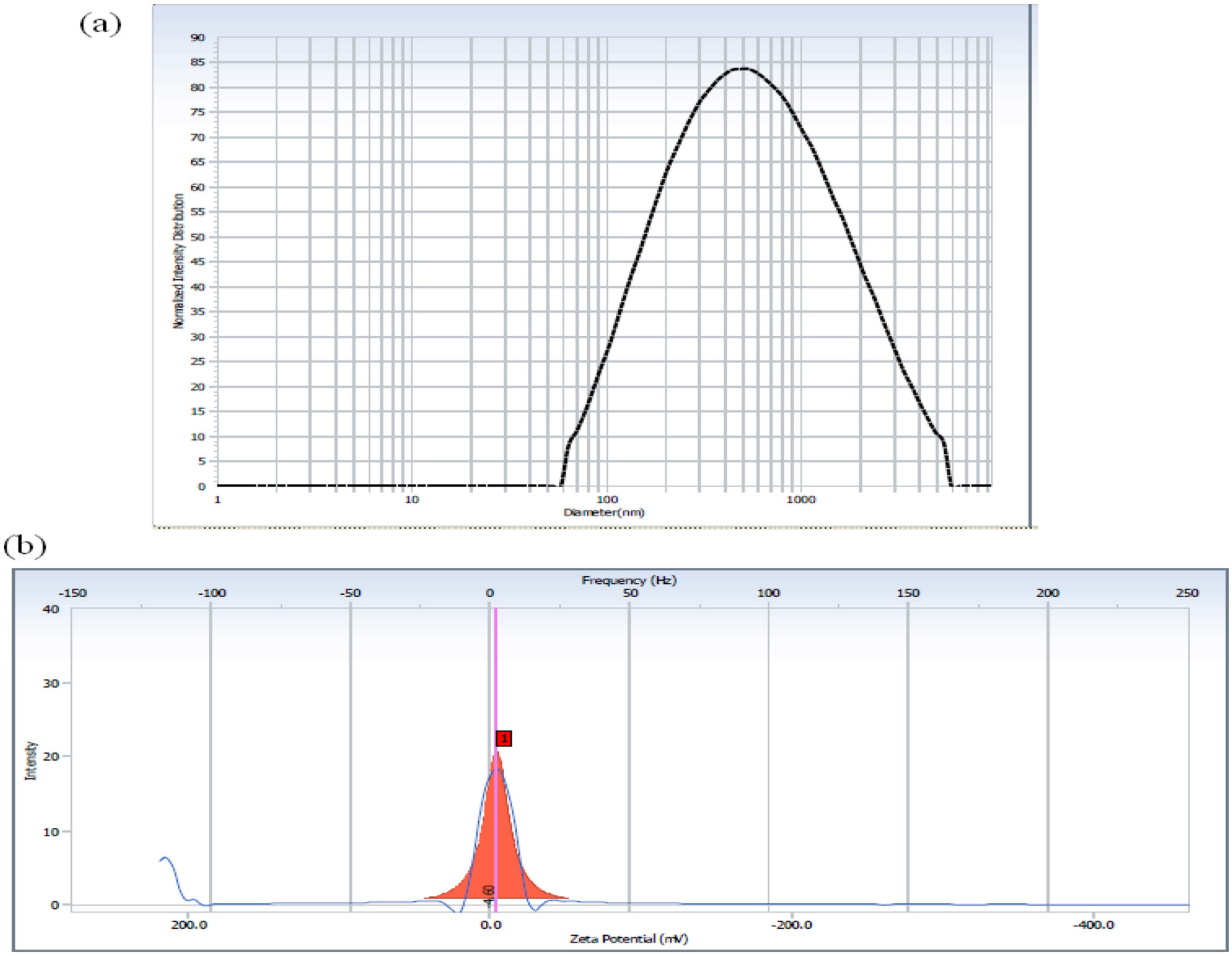


Fig. 4. DLS and surface charge density of the synthesised nanoparticles.

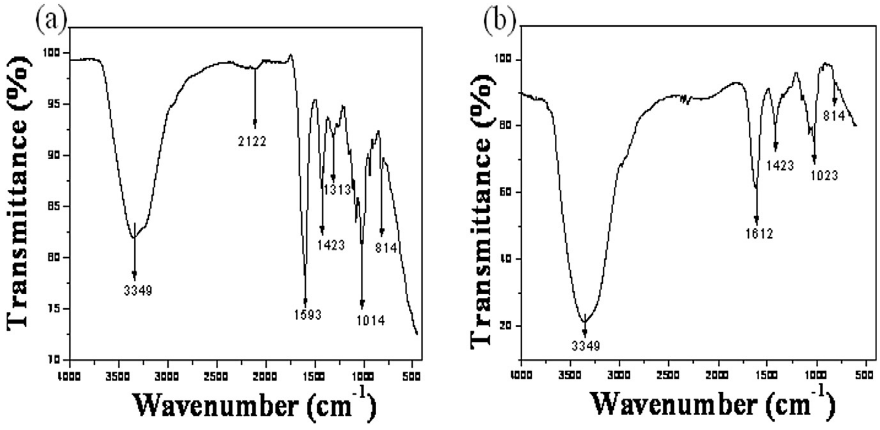
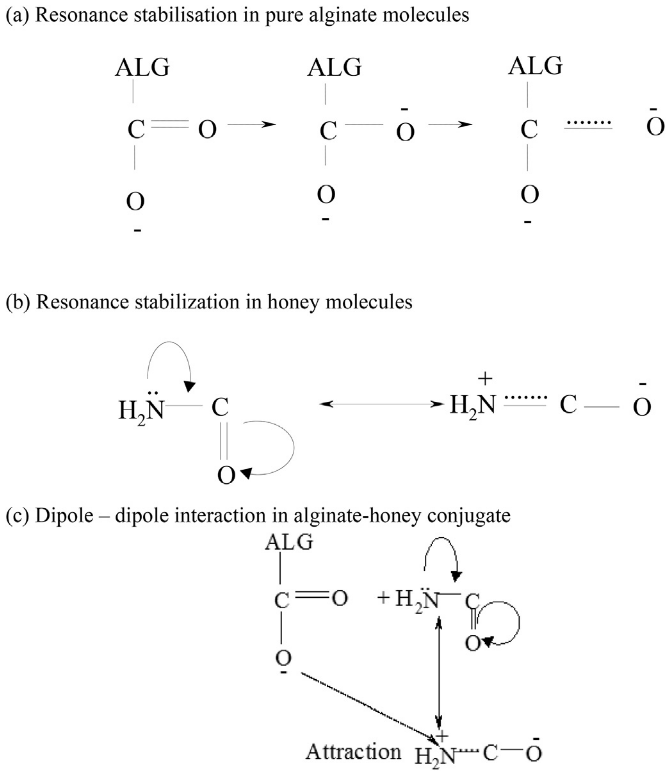


Fig. 5. FTIR spectra of nanocalcium alginate prepared (a) without and (b) with honey.

3.2.1.2. Effect of initial metal ion concentration and contact time. Results of adsorption studies at various initial concentrations of metal ions (10, 20, 50 and 100 mgL^{-1}) revealed that the percentage of biosorption decreased with increase in initial metal ion concentration due to the saturation of adsorption sites on the surface of the adsorbent as shown in Fig. 6(b). However, actual amount of metal ions adsorbed per unit mass of the adsorbent (qmgg^{-1}) was found to increase with increase in initial concentration. Effect of contact time on adsorption of Cr(VI) on the biosorbent is shown in Fig. 6(c). Equilibrium was found to establish in 180 min for low



Scheme 1. Resonance structures of alginate, honey and alginate–honey conjugate.

concentration, i.e. 10 mgL^{-1} solution. At low concentrations, the adsorbent sites take up the available metal ions more quickly, while at higher metal ion concentrations, metal ions compete for the fewer number of available biosorbent sites, which results in a decrease in the percentage removal [32,33].

3.2.1.3. Effect of adsorbent dosage. Adsorbent dose has a strong effect on the adsorption of the metal ions at a particular initial concentration. Fig. 6(d) shows that an increase in biosorbent amount from 0.02 to 0.08 mgL^{-1} resulted in an increase of percentage removal from 90.26 to 93.52% (uptake capacity 43.82–11.66 mgg^{-1}). This is due to the increase in the number of active sorption sites at the adsorbent surface. From this it is evident that the synthesised alginate nanoparticles effectively remove 93.52% of Cr(VI) ions from aqueous solution, at pH 4 within 180 min contact time.

4. Conclusions

The method reported in this study affords the synthesis of many different protein and polysaccharide nanoparticles like chitosan, casein and albumin. Since the materials used are all non-toxic and biocompatible, this method can also be used in the synthesis of nanoparticles for biomedical applications. Alginate nanoparticles synthesised using honey was highly stable. Even though it showed slight aggregation on drying, it can be redispersed in water by mild sonication. Various microscopic analyses confirmed the formation of stable nanoparticles. Particles produced were of narrow size range as shown by polydispersity index of 0.480. The zeta potential

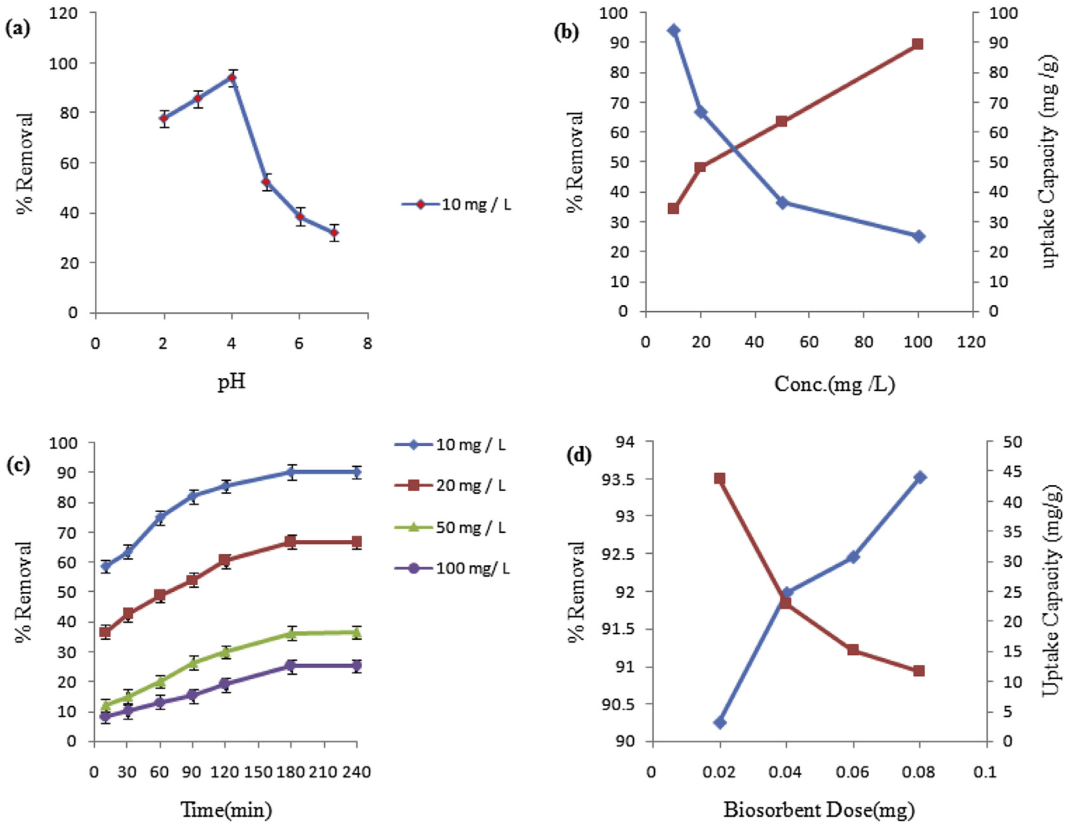


Fig. 6. Effect of operational parameters on biosorption of Cr(VI) using nanoalginate.

value of −4.60 mV indicated negative surface charge of nanoalginate due to the presence of ionised carboxyl groups on the surface. The negative surface charge on nanoparticles facilitated the adsorption of positive metallic ions on its surface, providing a method for metal loading after the particles were formed. This can also be employed for drug delivery in biomedical applications. FTIR spectrum of nanoalginate with and without honey indicated that honey has no chemical interaction and it acts only as a coating agent to stabilize the nanoparticles. Presence of honey prevented the stickiness and aggregation of nanoalginate particles on centrifugation. High adsorption capacity of the synthesised nanoparticles explores its possibility for the removal of toxic metal ions from contaminated water.

Ever since honey is used in ayurvedic formulations and is nontoxic in nature, honey mediated synthesis of polymeric nanoparticles has tremendous potential for use in nanomedicine and drug delivery applications. Calcium alginate nanoparticles are efficient biosorbents for detoxification of water contaminated with toxic metal ions.

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