

Preparation, characterization, bioactive and metal uptake studies of alginate/phosphorylated chitin blend films

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ABSTRACT

Alginate/phosphorylated chitin (P-chitin) blend films were prepared by mixing of 2% of alginate and P-chitin in water and then cross-linked with 4% CaCl₂ solution. The blended films were characterized by FT-IR. Then, the bioactivity of blend films was studied by biomimetic method in simulated body fluid solution (SBF) for 7, 14 and 21 days. After 7, 14 and 21 days and films were characterized by FT-IR and SEM studies. The SEM and FT-IR studies showed that the hydroxyapatite was formed on the surface of the blend films after 7, 14 and 21 days in the SBF solution. These studies confirmed that the alginate/P-chitin blend films are bioactive. Furthermore, the adsorption of Ni²⁺, Zn²⁺ and Cu²⁺ onto alginate/P-chitin blend films has been investigated. The parameters studied include the pH, contact time, and initial metal ion concentrations. The maximum adsorption capacity of alginate/P-chitin blend films for Ni²⁺, Zn²⁺ and Cu²⁺ at pH 5.0 was found to be 5.67, 2.85 and 11.7 mg/g, respectively. These results suggest that alginate/P-chitin blend films-based technologies may be developed for water purification and metal ions separation and enrichment.

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1. Introduction

Alginate and chitin are among the most versatile marine biopolymers. Alginates are a family of linear polysaccharides, which contain varying amounts of 1, 4-linked β-D-mannuronic acid and α-L-glucuronic acid residues. Alginate possesses mucoadhesive properties with low toxicity and low immunogenicity, which enabled as a matrix for the entrapment and delivery of a variety of proteins and cells [1,2]. Chitin, a natural abundant polysaccharide, is known to be the β-1,4-glycan of N-acetyl-D-glucosamine. Chitin is also known to be biodegradable polymer in nature and in the animal body [3,4] and to be a low toxicity. Chitin and its derivatives have great potential application in the areas of biotechnology, biomedicine, food ingredients, and cosmetics. Chitin and its derivatives are also capable of adsorbing a number of metal ions. Due to their high nitrogen content and porosity, chitin-based sorbents have exhibited relatively high sorption capacities and kinetics foremost heavy metals [5–7].

Chitin and chitosan have tremendous applications in the biomedical field due to their good biocompatibility, biodegradability, and capacity to form membranes, beads, fibers, scaffold and gels [8–12]. Phosphorylated chitin (P-chitin) is a water-soluble derivative of chitin and it has been reported as good biomedical material [8]. P-chitin is biocompatible, bioabsorbable and osteoconductive material and good be a bone repair material [8]. Both alginate and P-chitin are anionic polymers and these polymers can easily cross-link with Ca²⁺. In this paper, we report the preparation, characterization, bioactivity studies and the metal uptake (Ni²⁺, Zn²⁺ and Cu²⁺) behavior of the P-chitin/alginate blends in details.

2. Materials and methods

2.1. Materials

Sodium alginate (Ducalgin NSPH2: 1000 ± 100 cp) was provided by Kibun Food Chemipha. α-Chitin was received from KYOWA TECNOS Co. Ltd. All other materials used were of analytical grade.

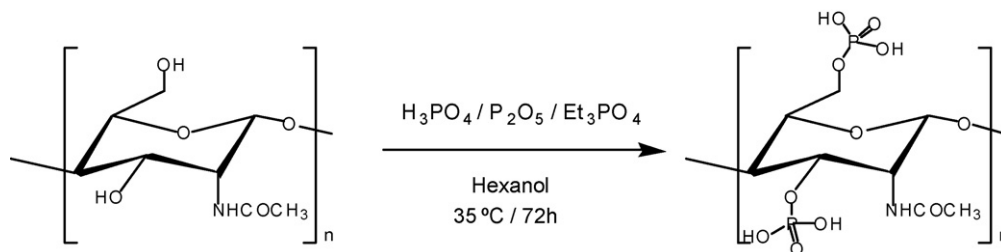
2.2. Synthesis of P-chitin

P-chitin was prepared by the following method [13–15]. In briefly, 5.0 g of chitin was added to the P₂O₅ (62.5 g), H₃PO₄ (20 ml), Et₃PO₄ (20 ml) and hexanol (25 ml) mixture in a 100 ml flask and

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Scheme 1. Synthesis of P-chitin.

the mixture was stirred continuously for 72 h at 35 °C. After 72 h the reaction mixture was poured into excess methanol for precipitation. The precipitate was filtered and washed with methanol and then dried in vacuum oven at 37 °C for 24 h. The yield of the product was 70%. The degree of substitution of phosphorous was found to be 1.87. The synthesis of P-chitin was shown in Scheme 1.

2.3. Preparation of alginate/P-chitin blend films

0.2 g alginate and 0.2 g of P-chitin was dissolved in 20 of water and then cross-linked with 4% CaCl₂ solution. The prepared blend films were washed with methanol to remove the excess of CaCl₂ solution. Then, the prepared films were dried at room temperature. Fig. 1 shows the cross-linking mechanism of alginate and P-chitin.

2.4. Preparation of SBF solution

The SBF solution was prepared by the following method [16], and contained 15 ml of each of the following: 2.74 mol l⁻¹ NaCl, 0.06 mol l⁻¹ KCl, 0.05 mol l⁻¹ CaCl₂, 0.03 mol l⁻¹ MgCl₂, 0.0895 mol l⁻¹ NaHCO₃, 0.02 mol l⁻¹ K₂HPO₄ and 0.01 mol l⁻¹ Na₂SO₄. These were added 200 ml volumetric flask along with 25 ml of each of 0.4 mol l⁻¹ Tris hydroxy methyl methane amine and 0.36 mol l⁻¹ of HCl. The pH of the solution was adjusted to 7.4 by adding a few drops of HCl with the remainder of the volume being distilled water.

2.5. Bioactivity studies of alginate/P-chitin blend films

The bioactivity studies of alginate/P-chitin blend films were carried out by biomimetic method with 1.5× SBF solutions. The blend films were vertically suspended in plastic jars with cotton threads and 30 ml of 1.5× SBF solutions was added. The SBF solution was replaced each day. Samples were retrieved after 7, 14 and 21 days of soaking at 37 °C. The retrieved samples were thoroughly rinsed with distilled water and dried at 65 °C before performing various characterization studies.

2.6. Metal adsorption experiments

Batch adsorption experiments were conducted by placing a 50 mg of alginate/P-chitin blend films in 50 ml reagent bottles containing 25 ml of aqueous solution of metal ions Ni²⁺, Zn²⁺ and Cu²⁺ (30 mg/l) with different initial pH value. The sample pH was adjusted to the desired value with hydrochloric acid (or nitric acid) or sodium hydroxide aqueous solution. The bottles were agitated at 200 rpm using a mechanical shaker for 8 h. After filtration, the metal ions concentration in the filtrate and initial concentration were determined by atomic adsorption spectrophotometer and the adsorption capacities were calculated as follows:

$$q = \frac{(C_0 - C)V}{m}$$

where q is the adsorption capacities of chitosan derivatives (mg metal ion/g adsorbent), V is the volume of metal ion solution (l), C_0 is the concentration of metal ion before adsorption (mg l⁻¹), C is the concentration of metal ion after adsorption (mg l⁻¹), and m is the weight of chitosan derivatives (g).

The effect of initial concentration of the metal ion on the uptake by alginate/P-chitin blend films was carried out by placing 50 mg of alginate/P-chitin blend films in a series of flasks containing 25 ml of aqueous solution of metal ions at definite concentrations and pH 5.0. The contents of the flasks were equilibrated on the shaker at 200 rpm for 8 h. After the equilibration, the metal ions concentration in the filtrate was determined by atomic adsorption spectrophotometer.

The effect of equilibration time on the uptake of Ni²⁺, Zn²⁺ and Cu²⁺ by alginate/P-chitin blend films was investigated using a batch method. A 50 mg of alginate/P-chitin blend films was placed in a flask containing 25 ml of aqueous solution of metal ions (30 mg/l) at pH 5.0. The contents of the flask were equilibrated on a shaker at 200 rpm for various time intervals. After the equilibration time, the metal ions concentration of the solution was detected.

2.7. Measurements

The IR spectra of the films were recorded in a PerkinElmer FT-IR 2000 series spectrophotometer at room temperature. The surface

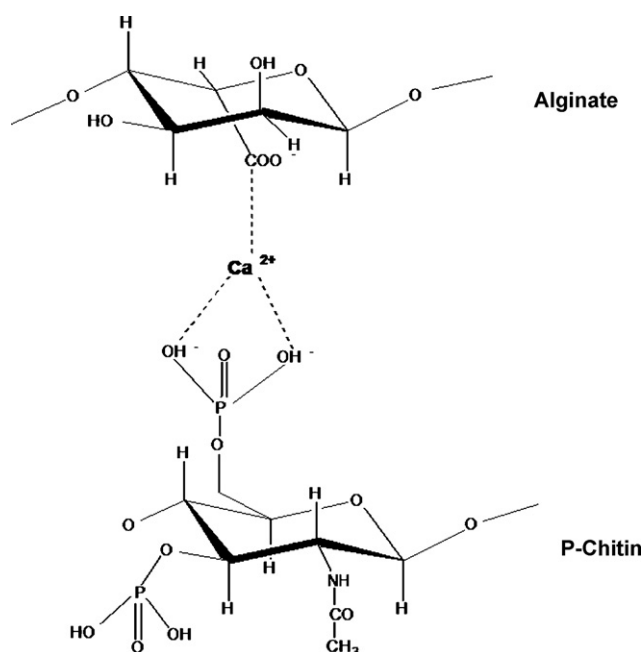


Fig. 1. Cross-linking mechanism of alginate/P-chitin with Ca²⁺.

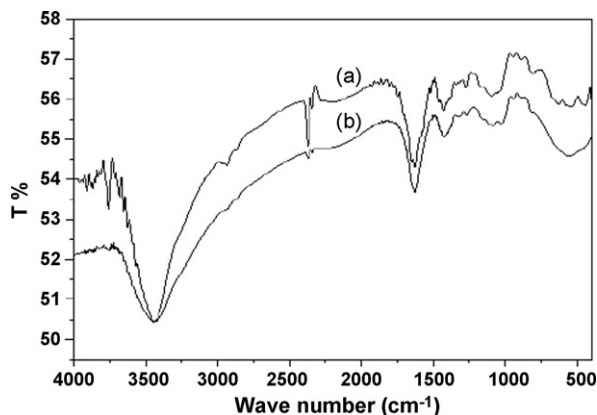


Fig. 2. FT-IR Spectra of (a) alginate and (b) alginate/P-chitin blend film.

morphology of films was analyzed by scanning electron microscopy (SEM) by using JEOL JSM-6700 microscope.

3. Results and discussion

3.1. FT-IR spectra of alginate/P-chitin blend films

Fig. 2 shows the FT-IR spectra of the pure and blend films in the wavelength ranges of 4000–400 cm^{-1} . The characteristic peak of P-chitin was observed at 3445 and 570 cm^{-1} are due to the P-OH group. On the other hand, the bands of alginate was appeared at 3500 cm^{-1} for the hydroxyl groups and at 1624 and 1433 cm^{-1} for the asymmetric $-\text{COO}^-$ stretching vibration and symmetric $-\text{COO}^-$ stretching vibration, respectively. The FT-IR results indicated that the alginate and P-chitin are blended well.

3.2. Bioactivity studies of alginate/P-chitin blend films

3.2.1. Characterization by SEM

Fig. 3 shows the FE-SEM of the alginate/P-chitin blend films after 7, 14 and 21 days. The membranes formed calcium phosphate apatite layer on the surface after immersion in the SBF, and these deposits grew in size with increasing soaking time. This indicates that these deposits are from crystalline apatite. These SEM results showed that alginate/P-chitin blend films can induce apatite formation in the body environment. Thus the films are having the bioactivity properties and hence they can be used for tissue engineering applications.

3.2.2. Characterization by FT-IR

FTIR spectrum reveals (Fig. 4) the apatite nature of the mineral layer previously observed by SEM. The intense bands at 1036, 636, 624 and 540, 535 cm^{-1} are due to the PO_4 vibrations [17–20]. In addition, peaks at 1425 and 825 cm^{-1} were also observed. These peaks are referring the formation of apatite [16–20]. Thus, these results provide strong evidence that HAP crystals were formed on the alginate/P-chitin blend films.

3.3. Metal adsorption studies

The pH values selected in the experiments were prior to the precipitation limit of each metal ion (pH 7, 6 and 7 for Ni^{2+} , Cu^{2+} and Zn^{2+} , respectively). As shown in Fig. 5, the higher uptake capacity was achieved at higher pH values. The observed lower uptake in an acidic medium may be attributed to the partial protonation of the active groups and the competition of H^+ with metal ions for adsorption sites on the alginate/P-chitin blend films. At $\text{pH} < 2$, no appreciable uptake was detected for Ni^{2+} , Zn^{2+} and Cu^{2+} ions.

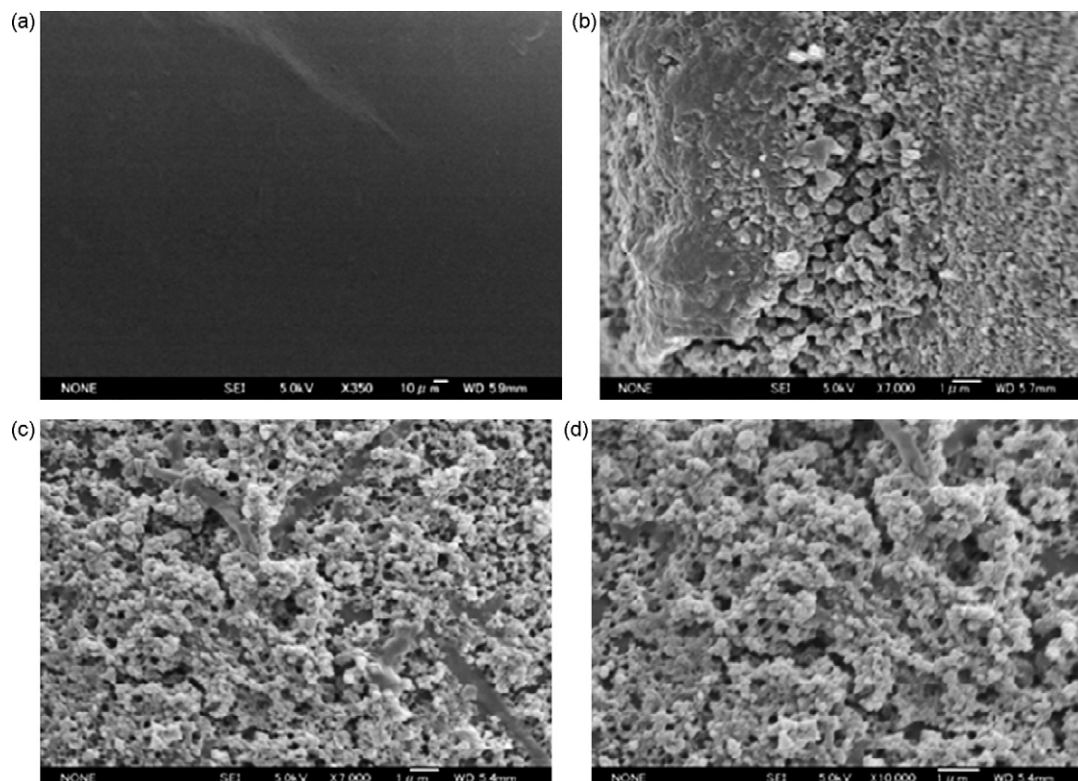


Fig. 3. SEM images of alginate/P-chitin blend (a) 0 control, (b) 7 (c) 14 and (d) 21 days after immersing in SBF.

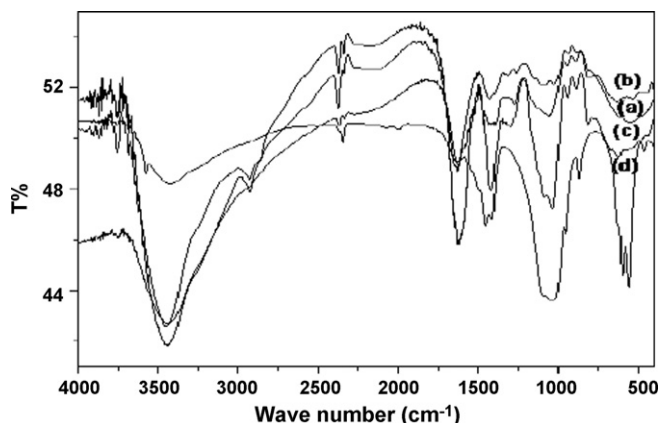


Fig. 4. FT-IR spectra of (a) alginate/P-chitin films after 14 days in SBF, (b) alginate/P-chitin blend film, (c) alginate/P-chitin films after 21 days in SBF and (d) hydroxyapatite.

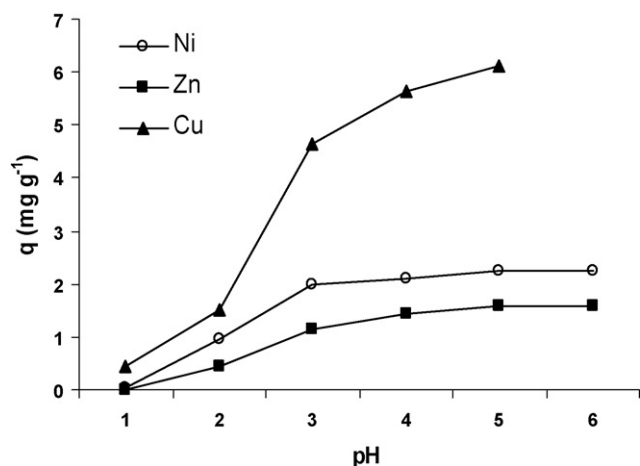


Fig. 5. Effect of pH on the uptake of Ni²⁺, Zn²⁺ and Cu²⁺ by alginate/P-chitin blend films (metal ion concentration: 30 mg/l, adsorbent dose: 2 g/l).

3.3.1. Effect of initial concentration of metal ions

Fig. 6 shows the uptake of Ni²⁺, Zn²⁺ and Cu²⁺ on alginate/P-chitin blend films at the natural pH. The uptake of three metal ions on alginate/P-chitin blend films increases as the initial concentration increases until they reached a plateau. At the plateau region,

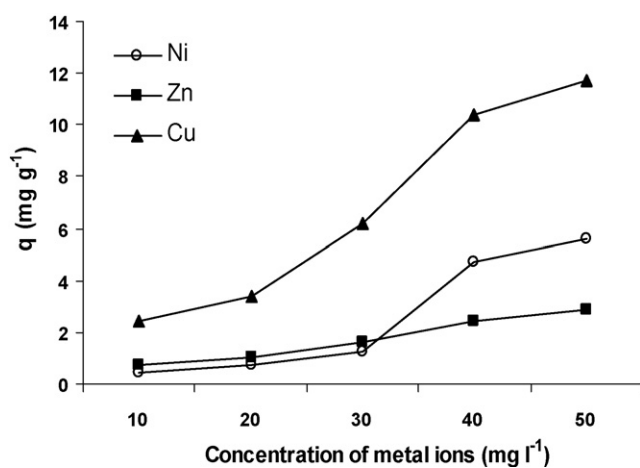


Fig. 6. Effect of initial concentration on the uptake of Ni²⁺, Zn²⁺ and Cu²⁺ by alginate/P-chitin blend films (pH: 5.0, adsorbent dose: 2 g/l).

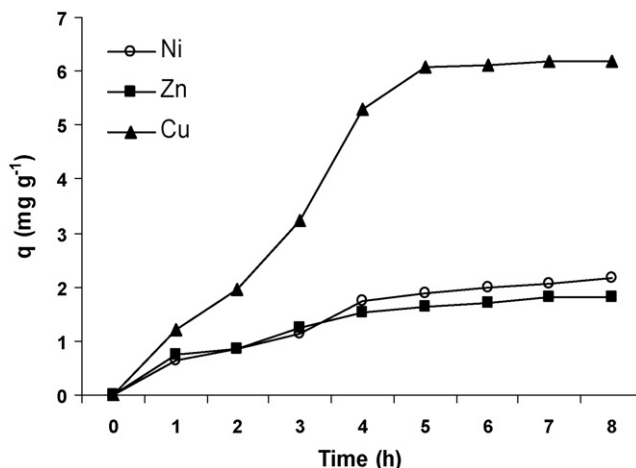


Fig. 7. Influence of contact time on the uptake of Ni²⁺, Zn²⁺ and Cu²⁺ by alginate/P-chitin blend films (metal ion concentration: 30 mg/l, pH 5.0, adsorbent dose: 2 g/l).

the maximum uptake values of Ni²⁺, Zn²⁺ and Cu²⁺ on alginate/P-chitin blend films are 5.67, 2.85 and 11.7 mg/g, respectively.

3.3.2. Adsorption kinetics

Simple batch kinetic experiment of alginate/P-chitin blend films for Ni²⁺, Zn²⁺ and Cu²⁺ ion adsorption was conducted (Fig. 7). It could be seen that the adsorption of alginate/P-chitin blend films for these three metal ions was in high speed. Further, the experimental results suggest that the amount of Ni²⁺, Zn²⁺ and Cu²⁺ adsorbed (mg/g) increased with increasing contact time and reached equilibrium at 5 h for Cu²⁺ and 4 h for Ni²⁺ and Zn²⁺. The heavy metal uptake potential may indicate that most of the active sites of alginate/P-chitin blend films are exposed for interaction with the metal ions.

4. Conclusions

Alginate/P-chitin blend films were prepared by mixing of alginate and P-chitin in water and then cross-linked with CaCl₂ solution. The SEM and FT-IR studies showed that alginate/P-chitin blend films are bioactive. At pH 5.0 the maximum adsorption capacity of alginate/P-chitin blend films for Ni²⁺, Zn²⁺ and Cu²⁺ was found to be 5.67, 2.85 and 11.7, respectively. Further, the amount of Ni²⁺, Zn²⁺ and Cu²⁺ adsorbed (mg/g) increased with increasing contact time and reached equilibrium at 5 h for Cu²⁺ and 4 h for Ni²⁺ and Zn²⁺. We predict that these novel alginate/P-chitin blend films may have potential applications in environmental areas, and for the removal of Ni²⁺, Zn²⁺ and Cu²⁺ from water and wastewater. These results also indicated that these films may be very useful for drug delivery, tissue engineering and other environmental applications.

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