

Preparation and characterization of seaweed encapsulated into chitosan beads

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Abstract

Current case in modern agriculture industry has shown that application of agrochemicals has led to high cost of production and serious environmental pollution. In this study, a natural organism known as seaweed, a renewable bio-resources originated from the sea has been chosen to be encapsulated in chitosan hydrogel. The rationale to use seaweed is because it is common in agriculture and horticulture, that the application of seaweed could promote growth, prevent pests and diseases. The seaweed was encapsulated at different concentrations (w/v%) of 5%-20% in chitosan solution using sodium tripolyphosphate as a crosslinker. The encapsulated seaweed in chitosan has been made into beads to suit the application as fertilizer beads. The analysis of FTIR for all beads confirmed the encapsulation took place when both functional groups of the host, chitosan and the guest, seaweed existed together in all the FTIR spectra of the chitosan-seaweed beads. CHNS further confirmed quantitatively that addition of seaweed at various concentrations into the chitosan beads showed increased percentage of carbon, hydrogen, nitrogen, and sulphur content accordingly. The swelling test showed that when seaweed was encapsulated into the chitosan beads, the swelling percentage decreased accordingly with the increase in concentration of seaweed. This is due to the seaweed filling up the voids in the chitosan thus limiting the uptake of water into the system. The morphology study by SEM supported the results of swelling test, agglomeration of large particles surface out accordingly to the concentration of seaweed when the void spaces has been fulfilled. It could be perceived that the encapsulated seaweed chitosan beads is a promising material to the agriculture sector; promoting plant growth which could enhance the income of the farmers and yet safe to the human and environment.

Keywords : Seaweed, chitosan, beads, hydrogel.

INTRODUCTION

The macro-algae inhabiting the intertidal zones of estuaries, lagoons and in the seas across the world play an important role in the marine ecosystems. The saline nature of the environment favours the growth of certain macro-algae known as the seaweeds. These organisms are important renewable bio-resources of the seas and most are for food, preparation of several industrial products [1, 2] and as raw material for fertilizer in the crop fields as liquid fertilizer or compost for increasing productivity [3, 4, 5]. The major uses of seaweeds are in the production of phyto-chemicals such as carrageen and alginate [6, 7]. In biological agriculture and horticulture, application of seaweed is to promote growth, prevent pests and diseases and improve the quality of the products. The efficacy of the seaweed is due to the plant hormones (mainly cytokinins) and trace nutrients present in the extracts. Seaweed contains all of the mineral components and plant development hormones required by plants [8, 9]

Reports on the seaweed compost revealed that it is rich in potassium, however poor in nitrogen and phosphorus than the homestead fertilizer [10, 11]. Seaweed components such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances could affect cellular metabolism in treated plants leading to enhance growth and crop yield [12]. Recently, extensive reports in peer-reviewed scientific publications related to plant bio stimulant literature explains the scientific benefits of applying seaweed extracts in agriculture [13,14, 15, 16]. However, despite the growing evidence for unique, highly specific and complex functionality of diverse molecules in seaweed extracts, their complex modes of action remain elusive. Seaweed products exhibit growth-stimulating activities, and the use of seaweed formulations as bio stimulants in crop production is well established. Bio stimulants are defined as “materials, other than fertilizers, that promote plant growth when applied in small quantities” and are also referred to as “metabolic enhancers” [17, 18].

Chitosan, a copolymer that is primarily composed of β (1,4) linked 2-amino-2-deoxy-D glucopyranose units, and residual 2-acetamido-2-deoxy-D-glucopyranose units, is a chemical derivative obtained by alkaline deacetylation of chitin and it is also found naturally in some fungal cell walls. Chitin, poly (1,4)-2-acetamido-2-deoxy- β -D glucan is a value-added biopolymer extracted commercially from the shells of crustaceans such as prawns, crabs, krill, insects and shrimps, and the second most abundant biopolymer next to cellulose [19, 20, 21, 22]. Chitosan appears to be more useful as compared to chitin, since it has both amine and hydroxyl groups that can serve as chelating sites and can be chemically modified [20, 23]. Since it is harmless to humans and presents excellent biological properties such as biodegradation in the human body, immunological, antibacterial, and wound healing activity. Chitosan has been widely used in food and pharmaceutical processes and in medical and agricultural drugs [24, 25, 26]. Other useful features of chitosan include its abundance, non-toxicity, hydro-philicity, biocompatibility, biodegradability and anti-bacterial property [24, 27].

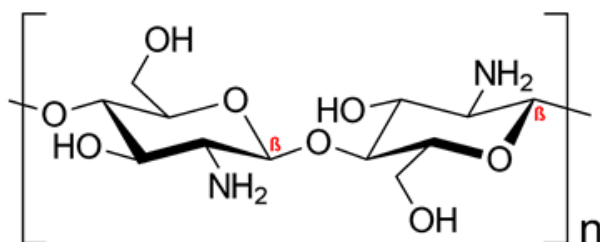


Figure 1: Structure of chitosan

It has been widely used as materials for controlled release, which have been applied in food, drug, biochemical and agricultural areas [28,29]. Chitosan (CS) is a naturally occurring nontoxic, biocompatible, biodegradable, cationic polysaccharide [30, 31]. This hydrophilic polymer can easily cross-link with counter poly anions like TPP to control the release of drugs [32]. Chitosan is a mucoadhesive polymer [16, 33] with permeation enhancing properties, which facilitate opening of the epithelial tight junctions. The aim of this work is to study the encapsulation of seaweed into the chitosan-beads and its physicochemical characterization.

MATERIALS AND METHODS

Chemicals

Chitosan, (1,4)-2-amino-2-deoxy-beta-D-glucan, ($M_w=100,000\text{--}300,000$ g/mol) and tripolyphosphate ($\text{Na}_5\text{O}_{10}\text{P}_3$, with 85.0% purity) were purchased from Friedman Schmidt. Acetic acid (CH_3COOH with 99.9% purity) and sodium hydroxide (NaOH with 95.0%) were purchased from Sigma Aldrich. Seaweed was provided by Chemmieconnex company. All chemicals and reagents were of analytical grade and used without further purification. The deionized water was used in the preparation of all aqueous solution.

Preparation of chitosan-seaweed beads fertilizer

0.5 g of chitosan was added into a beaker containing 15 ml of 5% v/v acetic acid. The chitosan is a strong base with the presence of primary amino groups and become polyelectrolyte when the amino groups get protonated. Thereby, acetic acid a mild acidic solution is suitable to solubilize chitosan. The mixture was stirred and filtered through a borosilicate filter series, (Robu Glasflter-Geräte GmbH) (porosity ranging from 1 to 4). Several concentrations of seaweed solution were prepared at 5%, 10%, 15% and 20% w/v with deionized water in 100 ml of volumetric flask. Then, 15 ml of the seaweed solutions was transferred into the beaker containing the chitosan and the acetic acid. The mixture was stirred and heated until the mixture become homogeneous. The homogeneous solution was drop into 0.5 M NaOH solution by using syringe. The beads formed were left overnight then the wet beads were filtered and wash by using distilled water and placed in the cross-linker solution (sodium tripolyphosphate) and left for an hour. The wet beads were dried in the oven for 72 hours at 35 °C. The dried beads were kept in a sample bottle and ready for further use.

Characterization

The infrared absorption spectrum was obtained by using a Perkin-elmer spectrophotometer model 1725X and the wavenumber range of 400-4000 cm^{-1} . Fourier transform infrared spectroscopy (FTIR) was used to identify the presence of functional groups in the chitosan beads (C-B) and chitosan-seaweed beads (CS-B) at various concentrations of seaweed.

The percentage of carbon, hydrogen, nitrogen and sulphur (CHNS) in the the C-B and CS-B at various concentrations of seaweed were analysed using CHNS-932 LECO. The analysis was carried out to qualify and quantify the encapsulation of seaweed in the chitosan beads.

To study the water absorption capacity of C-B and CS-B beads at various concentrations of seaweed, the swelling studies was conducted by using the solution of sodium chloride at pH 7.0 ± 0.5 . An accurate mass of dried beads were immersed in a petri dish containing 10 mL of sodium chloride solution at room temperature for duration of 7 days. Each day at the same set time, the beads were gently taken from the solution, both mass of wet and dried beads were measured. The swelling data for chitosan beads and chitosan-seaweed beads were recorded. The formula used to calculate the swelling percentage is as follows:

$$\text{Swelling percentage (\%)} = (M_w - M_d) / M_d \times 100 \dots \dots \dots (\text{Eq 1})$$

Where: M_w = weight of swollen beads
 M_d = weight of dried bead

The micrograph of C-B and CS-B at various concentrations were obtained using the variable pressure scanning electron microscopy (VPSEM) model LEO 1455 VPSEM. Prior to the analysis, the bead samples were gold coated. The surface morphology analysis was done to study the difference on the surface of the chitosan beads as encapsulation of seaweed took place.

RESULTS AND DISCUSSION

Fourier Transforms Infrared Spectroscopy

Figure 2 represents the FTIR spectra of solely chitosan beads, seaweed and chitosan-seaweed beads at different concentrations of seaweed at 5%, 10%, 15% and 20%. The absorption band in the range of 3200-3600 cm^{-1} is observed in all spectrum of chitosan-seaweed beads, which indicates the -OH stretching vibrations of the hydroxyl group contained in chitosan compound. Blank chitosan showed two strong peaks at 1655 cm^{-1} , assigned as amide I, and at 1597 cm^{-1} , assigned as symmetrical and

asymmetrical bending of amine and amide II [34, 35]. As the concentration of the seaweed, increases in the chitosan-seaweed beads both of the bands shifted to lower wave number and the intensities of two peaks became much stronger [36]. In addition, the stretching of the C–N could be observed at 1372 cm^{-1} . The absorption bands at around 1560 and 1388 cm^{-1} were assigned to the symmetric and antisymmetric O–C–O stretching modes of $-\text{COO}-$ groups of the chitosan, respectively. However, both bands were found to be widened after binding of the seaweed to the chitosan took place.

An important observation on the presence of new functional group absorption is shown in the spectra of chitosan-seaweed beads that give strong peak in the range of $1600\text{--}1500\text{ cm}^{-1}$. This peak comes from the benzene ring originated from plant hormones in the seaweed such as cytokinin, auxin, vitamin etc [37]. From the FTIR spectra obtained, as the encapsulation of seaweed took place in the chitosan, both functional groups of chitosan and seaweed existed in the spectra of chitosan-seaweed beads, which confirmed the encapsulation episode.

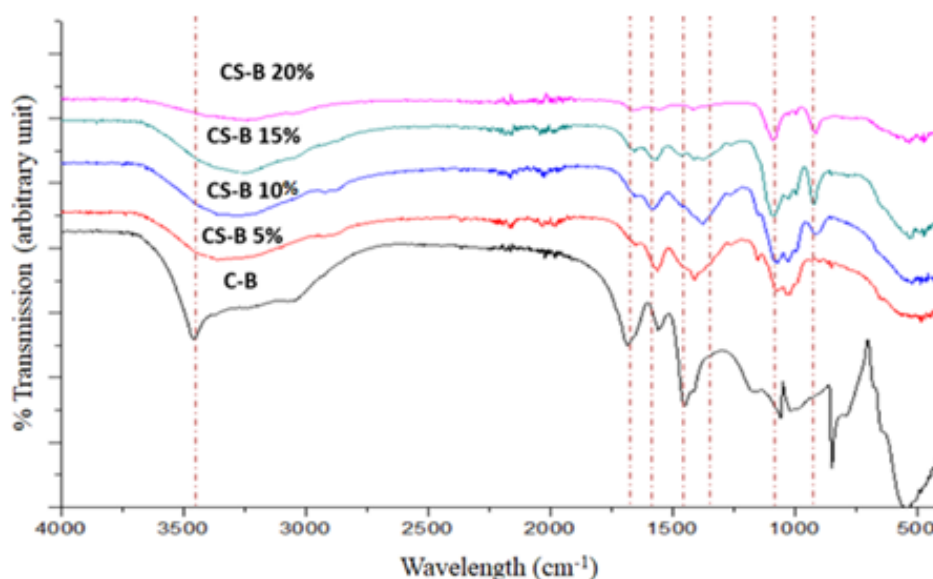


Figure 2: FTIR spectrum for chitosan-seaweed beads for blank chitosan beads C-B, CS-B 5% at 5% w/v of seaweed, CS-B 10% at 10% w/v of seaweed, CS-B 15% at 15% w/v of seaweed and CS-B 20% at 20% w/v of seaweed.

Carbon, Hydrogen, Nitrogen and Sulphur Analysis

Table 1 shows the percentage of carbon, hydrogen, nitrogen and sulphur in solely chitosan beads, seaweed and chitosan-seaweed beads at different concentrations of seaweed (w/v%), 5%, 10%, 15% and 20%. Both chitosan and seaweed contained the element of carbon and hydrogen. Element of nitrogen come from the chitosan and the seaweed. From the result of CHNS, the encapsulation of seaweed into chitosan could be confirmed quantitatively, an increase of carbon and hydrogen content in the chitosan-seaweed beads could be observed when compared to the solely chitosan beads. The percentage of carbon, hydrogen, nitrogen and sulphur in the chitosan-seaweed beads increased accordingly to the concentration. This concludes that the seaweed is successfully binding or encapsulated into the chitosan due to increasing percentage number of elements. The CHNS results compliment the encapsulation of seaweed into the chitosan-seaweed beads as shown in the FTIR spectra.

Table 1: Percentage of carbon, hydrogen, nitrogen and sulphur in solely chitosan beads and chitosan-seaweed beads at different concentrations of seaweed.

Concentrations of seaweed (w/v%)	% Carbon	% Hydrogen	% Nitrogen	% Sulphur
0	29.5	5.4	4.2	0.0
5	30.6	5.6	4.6	0.9
10	33.9	6.8	5.4	1.3
15	35.7	7.2	5.8	1.5
20	38.2	7.5	6.2	1.7

Swelling Analysis

The swelling studies of chitosan-seaweed beads were done to determine the swelling behavior of solely chitosan beads and chitosan-seaweed beads. The swelling analysis is done by using the *Eq 1* and data are tabulated in Table 2. Chitosan has three times less swelling properties when compared to other hydrogel [38]. However, the chitosan beads without addition of seaweed has the highest swelling behavior compared to chitosan beads with addition of seaweed. The encapsulation of seaweed into chitosan caused significant reduction of micro/macrosopic pores and cracks observed on the surface, which in turn lead to decreased permeability of the beads. This explains the lower swelling behavior chitosan-seaweed beads have when compared to the solely chitosan beads.

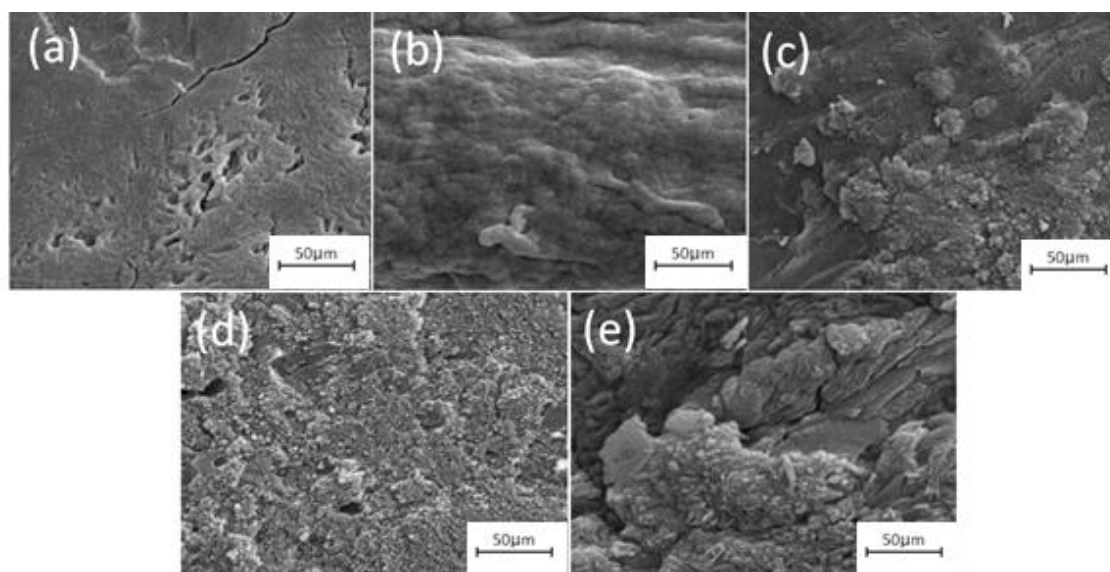
The NaCl soaked beads tend to absorb water (free or bulk water) in order to fill the void regions of the polymer network within the beads that remain dehydrated, until they reach the equilibrium state [39]. The phenomenon was influenced by the relaxation of the polymer network at the presence of osmotic pressure. Swelling of the beads in NaCl lasts for about 6-7 days until the osmotic pressure equals the forces of the crosslinking bonds that maintain the structure of the polymer network stable and the beads tends to de-swell slowly. As the concentration increased, the number of bulk-like structure on the beads tends to reduce the pore of chitosan surface, which prevents the absorption of NaCl solution into the beads. This resulted to chitosan-seaweed beads containing higher concentration of seaweed to de-swell and showed the lowest percentage of swelling when it has reached the highest swelling percentage.

Morphology analysis

Figure 3 (a-b) show the scanning electron micrograph (SEM) taken for solely chitosan beads and chitosan-seaweed beads at different concentrations with the magnification of 5000x to investigate the surface morphologies. Based on the micrograph obtained, the surface morphology of solely chitosan beads still show some void spaces on the surface. However, as the concentrations of seaweed into the chitosan increased, the void spaces have been filled up showing no more void spaces. When 20% w/v of seaweed was encapsulated into the chitosan, the surface of the chitosan-seaweed beads showed strong agglomeration in large distribution of particle size on the surface that could possibly be the excess seaweed that was unable to fill up the fully filled void spaces [40, 41]. The morphology of chitosan beads underwent significant changes when the sample is encapsulated with seaweed.

Table 2: Swelling percentage of solely chitosan beads and chitosan-seaweed beads at different concentrations of seaweed.

Days	Swelling percentage (%)				
	CB	CS-B (5%)	CS-B (10%)	CS-B (15%)	CS-B (20%)
1	66.33	63.58	50.06	46.87	45.66
2	75.25	69.58	62.49	50.11	49.43
3	89.68	74.32	68.89	56.44	53.59
4	87.51	77.29	75.16	63.09	59.68
5	74.55	73.36	71.19	70.66	66.75
6	70.40	68.07	66.99	64.98	60.25
7	65.69	52.22	50.48	52.21	51.36

**Figure 3:** The surface morphology of chitosan-seaweed beads at 5000X magnification; a) solely chitosan beads, b) chitosan-seaweed beads at 5% w/v% of seaweed, c) , d) chitosan-seaweed beads at 15% w/v% of seaweed, e) chitosan-seaweed beads at 20% w/v% of seaweed.

CONCLUSION

From this study, it is proven qualitatively and quantitatively that seaweed has been encapsulated into chitosan beads. Results of FTIR confirmed the encapsulation when both functional groups of the host, chitosan and the guest, seaweed existed together in all of the FTIR spectra of the chitosan-seaweed beads. CHNS further supported the FTIR when the percentage of carbon, hydrogen, nitrogen and sulphur increased accordingly with the increase of seaweed concentrations. However, swelling test showed otherwise that when seaweed was encapsulated into the chitosan beads, the swelling percentage decreased accordingly with the increase in concentration of seaweed, which is due to the seaweed filling up the voids in the chitosan thus limiting the uptake of water into the system. The morphology study by SEM

supported the results of swelling test that agglomeration of large particles surface out accordingly to the concentration of seaweed when the void spaces has been fulfilled

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