Effect of biopolymers composition on release profile of iron(II) fumarate from chitosan-alginate microparticles

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Abstract. Microencapsulation using biopolymers is considered as a proper method for protecting the iron from oxidation reaction, inhibitors (fitat, tannin) and competitors (divalent metal). Chitosan and alginate are carbohydrate biopolymers commonly used in food and drug application. In order to have a carrier that can send iron to the small intestine which has a high pH, but previously had to survive through the stomach which has gastric acid, the composition of chitosan-alginate as a carrier must be investigated. The objectives in this work were to investigate the effect of both chitosan and alginate concentrations on release profile of iron(II) fumarate in simulated gastric acid and intestinal fluids and its physicochemical property. The microparticles were prepared by dropping chitosan-iron solution into sodium tripolyphosphate solution as crosslinking agent. The electrostatic complexation was formed when the chitosan microparticles were mixed with alginate solution and followed with calcium chloride solution for ionotropic gelation to form chitosan-alginate-iron microparticles. Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were employed to describe polyelectrolyte properties of microparticles. The results showed that suitable compositions of chitosan-alginate have successfully improved the pH-sensitivity of the microparticles, thus the iron release was sustained in simulated gastric fluid (pH 1.2), while the release in simulated intestinal fluid (pH 7.4) extended. The release profile of iron from the chitosan-alginate microparticle indicated the effect of alginate on the release of iron. The existence of the polyelectrolyte complex of chitosan-alginate was proven by the appearance of certain functional groups at FTIR spectrum and surface images of iron loaded microparticles from SEM.

Keywords: alginate, chitosan, extended release, microparticles, iron(II) fumarate

1. Introduction

Microencapsulation technology has been utilized for several applications, particularly in food industry. Some food component have been successfully encapsulated, such as antioxidant [1], enzyme [2], vitamin [3, 4], and mineral [5-7]. Nowadays, iron microencapsulation is considered as appropriate method for supporting anaemia alleviation programs, especially for food-based approaches [8]. The method could preserve the iron from inhibitors and competitors, reduce their reactivity for oxidation reaction, mask the unfavourable flavour and taste, and control iron release [5, 9-11]. Furthermore, the encapsulating agents play important roles for encapsulation efficiency and microparticles stability. Carbohydrate biopolymers are, such as chitosan and alginate, commonly used in food application.

Chitosan, polycationic polymer, has some interesting biological properties, such as biodegradability, biocompatibility, non-toxicity, and mucoadhesive properties [3, 12]. In addition, chitosan microparticles are easily prepared by crosslinking method using tripolyphosphate (TPP). Ionic crosslinking is formed...
by interaction between positive charge of chitosan and negative charge of TPP [3]. However, the main weakness of chitosan is the easy dissolution of chitosan in acidic pH of gastric fluid, whereas iron is absorbed in intestine [12]. Given of this limitation, a modification method is needed for making it into suitable matrix for gastric-intestinal track.

Alginate, polyanionic polymer, also has the same appealing biological properties with chitosan. In addition, alginate microparticles are formed through gelation mechanism with divalent cations such as Ca$^{2+}$. Alginate shrinks and changes into a porous polymer and does not dissolve at low pH in the gastric tract, so that the encapsulated components do not released [12]. Hereafter, a soluble viscous layer would be created at the higher pH in intestinal. However, the dissolution of alginate, in the higher pH, might create a burst release of main component, thus it would lead negative effects [3, 12]. In view of chitosan and alginate limitations, polyelectrolyte complexation is considered as a proper technique to solve this problem. The alginate networks can prevent chitosan from dissolving at low pH in the gastric tract. The rapid dissolution of alginate at the higher pH could be preserved by chitosan. Some previous studies were conducted to investigate microencapsulation of some bioactive compound utilizing chitosan-alginate polymers [13, 14]. However, there is a lack of information about iron microencapsulation using this composite material.

The objectives of this work are to produce composite chitosan-alginate microparticles loaded with iron. Microparticles are investigated on encapsulation efficiency, iron loading, morphological appearance, and stability properties (in simulated gastrointestinal fluid). FTIR analysis from composite microparticles is also discussed in this paper.

### 2. Method

#### 2.1. Material
Ferrous fumarate pharmacy grade, as iron source, was supplied from Jost Chemical, Co (Lackland, USA). Ferrous ammonium sulfate, 1,10 phenanthroline, chloride, sodium acetate, hydroxylamine hydrochloride, sodium hydroxide, acetic acid, sodium tripolyphosphate (TPP), hydrochloric acid, calcium chloride potassium and monopotassium phosphate were obtained by Merck (Darmstadt, Germany). Chitosan (medium molecular weight) and alginate, as wall materials, were purchased from CV. Chimultiguna (Cirebon-Indonesia).

#### 2.2. Preparation of iron microparticles using composite polymers
Iron microparticles were formulated using composite polymers, chitosan as the first layer and alginate as the second layer. Chitosan microparticles loaded with iron were prepared according to the method described by Handayani et al. [11]. Dried microparticles were added into 25 mL of sodium alginate solution and homogenized under constant stirring at 1000 rpm for 30 minutes. Afterwards, the solution was extruded through a pipette into 6% (w/v) of calcium chloride solution to form beads. Composite beads were allowed to harden for an hour. The collected beads were rinsed and pre-cooled to 0°C prior to freeze drying process. Furthermore, dried beads were shredded into micro form using mortar and pestle. The composition of chitosan and alginate is showed in table 1.

| Formulation code | Iron: Chitosan: Alginate | Formulation code | Iron: Chitosan: Alginate |
|------------------|-------------------------|------------------|-------------------------|
| F 01             | 1:10:0                  | F 05             | 1:12.5:5                |
| F 02             | 1:5:5                   | F 06             | 1:10:2.5                |
| F 03             | 1:7.5:5                 | F 07             | 1:10:3.75               |
| F 04             | 1:10:5                  |                  |                         |
Note: Effect of alginate in microparticles could be seen on formulation F01, F06, F07 and F04, where the mass ratio of alginate increased from 0 to 5. Effect of chitosan in microparticles would be investigated by comparing the performance of microparticles F02, F03, F04, and F05.

2.3. Total iron measurement
Phenanthroline method was applied to total iron determination as represented by Handayani et al. [11]. Hydroxylamine hydrochloride, sodium acetate, and 1,10 phenanthroline were used as reducing agent, pH buffer, and complexing agent, respectively. The ferrous-phenanthroline complex solutions were measured using UV-VIS Spectrophotometer at 510 nm. The intensity of red-orange complex is independent of pH in the range 2 to 9. Therefore, the buffer solution should be added into the sample solution.

2.4. Encapsulation efficiency (EE) and iron loading measurements
Encapsulation efficiency (EE) and iron loading were determined using Equations (1) and (2), respectively. The total iron was quantified using UV-VIS Spectrophotometer at 510 nm. The value of mass of total iron is defined as ferrous-phenenthroline complex.

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\text{Encapsulation efficiency (EE)} = \frac{\text{mass of total iron in microparticles (mg)}}{\text{mass of total iron used (mg)}} \times 100\% \tag{1}
\]

\[
\text{Iron loading} = \frac{\text{mass of total iron in microparticles (mg)}}{\text{total mass of microparticles (mg)}} \times 100\% \tag{2}
\]

2.5. Iron release study in simulated gastrointestinal fluid
Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared using a method explained by Handayani et al. [11]. Hydrochloride acid and potassium chloride were used to prepare SGF at pH 1.2, while monopotassium phosphate and sodium hydroxide were formulated to create SIF at pH 7.4. Iron release profile was determined by direct method. The sample was taken using a syringe which has been equipped with filter (Nylon syringe filter 0.45 µm 25 mm). Fresh SGF and SIF would be returned into solution using the same syringe. Each collected sample was analysed utilizing UV-VIS Spectrophotometer to obtain iron release concentration.

2.6. Fourier transform infrared spectroscopy (FTIR)
The FTIR spectra of chitosan (pure, F 01) and chitosan-alginate microparticles (F02-F08) loaded with iron were analysed with FTIR Thermo Scientific (Diamond Nicolet IS 5). The spectra were observed at 500-4000 cm\(^{-1}\) by 64 scans at a resolution of 32 cm\(^{-1}\).

2.7. Surface morphology of composite microparticles loaded with iron
The surface morphology of composite microparticles was determined by scanning electron microscope (SEM) (JEOL-JSM 6510LA). A thin layer of gold (Au) was applied on specimen surface to enhance the image quality. The SEM micrographs were taken at 15 kV of excitation voltage and a magnification of 250, 1K and 5K and an excitation voltage of 15 kV.

3. Results and Discussions

3.1. EE of microparticles loaded with iron
Composite microparticles loaded with iron were prepared by 3 mechanisms, (i) ionic crosslinking with TPP, (ii) electrostatic complexation by alginate, and (iii) ionotropic gelation with calcium ions. Fig. 1 depicts the value of EE from all formulations. Commonly, iron was successfully encapsulated ranging from 35.59% to 75.69%. The highest of EE was obtained by single chitosan microparticles. However, the percentage of trapped iron decreased in the presence of alginate. This effect might be explained by the interferences of ionic cross links due to complexation process by alginate. The broken links induced
the iron release from its microparticle. The ionically network has non-permanent properties, thus it might be broken and change in a certain condition [3]. In addition, mass increase of chitosan (F02-F05) would enhance the number of ionic cross links and inhibit the iron release from microparticles during complexation process. This formulation managed to upgrade EE values from 35.59% to 62.66%. Moreover, the higher composition of alginate has no significant effect on the obtained EE. The EE values of F06 and F07 were 67.08% and 64.76%, respectively.

![Figure 1](image-url)  
**Figure 1.** EE (%) of chitosan (pure, F 01) and chitosan-alginate (F02-F07) microparticles prepared by freeze drying method.

The result is in agreement with Nnamonu et al. [15] and is contrast with Mulia et al. [13]. Nnamonu et al. [15] encapsulated imazaquin herbicide using chitosan and alginate as encapsulating agents. The EE value of 64% was obtained using this procedure. However, mangosteen extract was highly entrapped around 97% using the same polymers [13]. The physical and chemical properties of each core compound might lead the differences of EE values.

### 3.2. Iron loading of microparticles loaded with iron

The mass of iron entrapped on the mass of total dried microparticles is presented in Fig. 2. Iron was loaded ranging from 1.41% to 3.13% within microparticles. Single chitosan microparticles had been able to load 2.58% of iron. This result was higher than previous study that reported the total iron loading of single chitosan microparticle was 0.8% while using ratio iron to chitosan 1:1 [11]. However, the increase of chitosan and alginate could decrease the iron loading from 1.92% (F03) to 1.41% (F05) and from 3.13% (F06) to 1.55% (F04), respectively. These might be caused by the enhanced of total mass of dried microparticles. However, the higher viscous of alginate solution might burst the microparticles due to the high osmotic pressure [16]. Furthermore, it could reduce the total iron entrapped thus decreasing the value of iron loading.
Figure 2. Total iron content of chitosan (pure, F 01) and chitosan-alginate (F02-F07) microparticles prepared by freeze drying method.

3.3. Iron release profile

The release profile of iron within chitosan and composite microparticle in the simulated gastric intestinal fluid is represented in Fig. 3. A burst release profile of iron from chitosan microparticle without alginate (F01) was occurred in SGF at the first 1 hour with iron release about 90% and about 99.99% of iron was identified in SIF at 6 hours. The chitosan microparticle with alginate (F05) showed about 35% of iron was released at the first 1 hour, thus indicating that the effect of alginate complexation was significant to reduce the release in SGF. At certain amount of alginate in the microparticle, the interaction between alginate and chitosan reduces the porosity of chitosan microparticle, thus decreases the release of encapsulated active compounds [17]. This might be due to low solubility of alginate under low pH condition such as in SGF.

Figure 3. Release profile of total iron in the SGF and SIF for 120 min and 300 min, respectively.

However, at high alginate concentration, the highly viscous of alginate solution might retard the Ca$^{2+}$ diffusion into the microparticles, and it might form a weak cross linking and more porous structure [16]. Furthermore, the alginates with weak cross linking were easy to swell, so the SGF solution might enter the pores and degrade the chitosan microparticle, thus a burst release profile was occurred. Fig. 3 also
shows that increased amount of chitosan with the same amount of alginate in the composition do not affect significantly the release profile of active compounds encapsulated.

3.4. FTIR analysis

FTIR spectra of chitosan (pure, F01) and composite microparticles (F02-F07) are depicted in Fig. 4. The overlapping of O-H and N-H bonds was intense at about 3194 cm\(^{-1}\) in single chitosan microparticles. Peaks around 1052 cm\(^{-1}\) were observed in all formulations which indicated the presence of C-O stretching. The changes of some band’s position and appearance of new peaks were observed after complexation. The formation of new –C=O and –OH groups of sodium alginate and –OH and –NH\(_2\) groups in chitosan led the emergence of intense band at about 3240-3336 cm\(^{-1}\). In addition, the symmetric stretching vibration of carboxylate anions (at near 1410 cm\(^{-1}\)) was still visible in polyelectrolyte materials. It might be described by the excess of alginate which did not interact with cationic amino groups of chitosan. These results contrasted with previous study [14]. New peak around 1580 cm\(^{-1}\) was recorded in all composite formulation. However, the other new peak at about 1730 cm\(^{-1}\) was observed in F02, F05, and F07 formulation. It indicated a presence of asymmetric stretching of –COO carbonyl groups, thus polyelectrolyte complex formation was occurred [14].

![Figure 4. FTIR spectra of chitosan (pure, F01) and chitosan-alginate microparticles (F02-F07).](image)

3.5. Surface Morphology of Iron Microparticles

Freeze drying method was utilized to form matrix of iron-chitosan and iron-chitosan-alginate. The surface morphology of chitosan (pure, F01) and composite microparticles (F03 and F07) are described in Fig. 5. Chitosan microparticle surfaces showed homogenous and very smooth. However, the structures of composite materials were more fibrous and irregular due to polyelectrolyte interactions.

In this study, iron was entrapped in the matrix of chitosan as the first encapsulating agent. Furthermore, alginate was added as the second layer for improving the pH-sensitivity and extending the iron release of the microparticles. The amino terminals (NH\(_3^+\)) of chitosan and carboxyl residues (COO\(^-\)) of alginate could form polyelectrolyte interactions [13, 15]. The conceptual of these interactions are shown in Fig. 6. The similar description has been also explained by others authors [14, 18].
Figure 5. Surface morphology of chitosan (F 01) and chitosan-alginate (F03 and F 07) microparticles loaded with iron prepared by freeze drying method in 500 (a) and 1000 (b) magnification.

Figure 6. Polyelectrolyte interaction between carboxyl function of alginate and amino function of chitosan.
4. Conclusions
Chitosan-alginate microparticles loaded with iron were prepared through tripolyphosphate crosslinking, electrostatic complexation by alginate, and also ionotropic gelation with calcium ions. Those procedures have been successfully improved the pH-sensitivity thus could extend the iron release property on simulated gastric (pH 1.2) and intestinal (pH 7.4) fluids. The release profile of iron from chitosan microparticle without alginate indicated a burst release profile, while addition of alginate in the composition of chitosan microparticle reduced the iron release in simulated gastric fluid. The polyelectrolyte complex of chitosan-alginate was proven by the appearance of functional groups and surface images of iron microparticles.

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