Oxidation Models of Encapsulated Iron Using Alginate by Gelation Method

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Abstract. Alginate had been used as an additive on many food materials due to its biodegradability and non-toxic properties. As the alginate was extracted from natural sources, it had various properties depending on its source and purification method. In general, alginate has high gelation which hinder its use in high concentration in specific applications. This work aimed to study the effect of alginate modification in protecting oxidation of iron. The modification was conducted using ultrasound. The degraded alginate was applied for encapsulating iron using gelation method. The oxidation profile of the iron was modelled. The result showed that the lower alginate concentration and longer ultrasonication process decreased the alginate ability on protecting the oxidation. After 15 days, the lowest oxidation (8.47%) was able to be suppressed by ultrasonication of 5 % alginate for 30 min. The Kirby model (R2>0.76) was more suitable in describing the oxidation rate from the iron encapsulated by the degraded alginate.

1. Introduction
Iron has an important role in the human body for formation of hemoglobin in red blood cells, which serves to bind and circulates oxygen throughout the body. Lack of iron in the human body causes health problems, such as anemia, which becomes health problems worldwide. Iron was easily degraded by inhibitors and was easily oxidized from Fe2+ to Fe3+, which prevented iron to be absorbed [1]. The iron interaction with the oxidizer needed to be limited to prevent the sensory changes and nutritional value reduction [2]. Encapsulation provided protection from the factors that cause iron damage [3]. Alginate was qualified as a matrix encapsulant for various active compounds [4].

Alginate is a polysaccharide that consists of 1,4-linked β-δ-manuronic acid (M) and α-L-guluronic acid (G). The copolymer consists of sequences of M residue (block M) and G residue (block G) that alternate with MG sequences (MG blocks) [5]. Due to alginate’s non-toxicity and biodegradability, it is widely used as food substitutes and additives. However, some applications require alginate to be modified to meet the properties. Sodium alginate with a viscosity ≤ 20cps at 20⁰C [6] and a molecular weight between 80,000-120,000 g/mol were used for Lactococcus lactis encapsulation purposes [7]. The alginate medium viscosity, ~217 cps [8] and molecular weight ranging from 100,000 to 150,000 g/mol [9] were used for film making. Furthermore, the combination of high molecular weight (250,000 g/mol) and low molecular weight (50,000 g/mol) of alginate was used for injectable alginate study [10].
Modification of alginate could be conducted using degradation method. This process modified its molecular weight and viscosity properties, hence it can be used for various applications, such as iron encapsulation. Degradation process through ultrasonic method can be an effectively way to degrade and reduce the viscosity of alginate [11].

The objective of this works was to study the effect of alginate modification in protection iron oxidation. Alginate concentration and degradation time were selected as variables of ultrasonic degradation. The degraded alginate was subsequently applied to encapsulate iron using gelation method. Furthermore, the oxidation profile of the iron was modelled.

2. Materials and Methods

2.1. Materials
Food grade sodium alginate was bought from a local store in Semarang, Central Java, Indonesia. The weight average molecular weight was 579,700 g/mol using Mark-Houwink determination. Ethanol (96%) was obtained from PT. Brataco Chemika (Semarang, Central Java, Indonesia), while the other materials were in analytical grade (Merck Chemical Co., Darmstadt, Hesse, Germany).

2.2. Ultrasonic Degradation of Sodium Alginate
The sodium alginate degradation was conducted using 2.8 L Krisbow Ultrasonic Cleaner (PT. Krisbow Indonesia, West Jakarta, Jakarta, Indonesia) at a frequency of 40 kHz. The duration and alginate concentration was varied in the range of 30-150 min and 5-10%, respectively. The resulted alginate was filtered and dried in a vacuum desiccator. The molecular weight of degraded alginate was determined using a Cannon Fenske Capillary Viscometer size 100 (Schott AG, Mainz, Rhineland-Palatinate, Germany). The average molecular weight of the alginate was calculated using the viscosity value and the Mark-Houwink equation (Equation 1).

\[ [\eta] = 2.0 \times 10^{-5} \frac{M_w}{\eta} \]

where \( [\eta] \) = intrinsic viscosity and \( M_w \) = the weigh average molecular weight [12].

2.3. Iron Encapsulation
The iron-alginate solution was made by adding FeSO\(_4\) \( \cdot \) 7H\(_2\)O solution (0.1 g in 10 mL distilled water) to the degraded alginate solution (1.5% w/v, 40 mL). The solution was subsequently dropped in CaCl\(_2\) solution (1.5 N, 100 mL) at a constant height to form the iron bead. The beads were collected after 30 min immersion process. The access liquid on the beads was removed using cloth.

2.4. Iron Oxidation
The iron bead (0.5 g) were exposed in ambient condition (25-27°C) for 15 days. The iron determination was conducted every 3 days by dissolving 0.1 g of the beads in 20 mL of sodium citrate solution. The solution was added by 1,10-phenanthroline (1 g/l, 10 mL), sodium acetate buffer (1.2 M, 8 mL), hydroxylamine hydrochloride (100 g/L, 1 mL). The absorbance of the mixed solution was measured using visible spectrophotometer at 508 nm.

The oxidation rates of iron were mathematically modelled using Kirby model [13] and Wang model [14]. The experimental data were fitted into the models using linear regression.

Kirby model followed the Pseudo-First-Order Law for Fe\(^{2+}\) oxidation as described in Equation 2.

\[ \ln \left( \frac{(Fe^{2+})_t}{(Fe^{2+})_0} \right) = -k_k t \]

where \( \ln \left( \frac{(Fe^{2+})_t}{(Fe^{2+})_0} \right) \) is the Fe\(^{2+}\) fraction which remained unchanged at time \( t \), while \( k_k \) is rate constant.

Wang model followed the Second-Order Law for Fe\(^{2+}\) oxidation as described in Equation 3.

\[ \frac{1}{q_e - q_t} = \frac{1}{q_e} + k_w t \]
where $q_s$ = total iron concentration in solution, $q_t$ = ion concentration at time $t$ in solution, and $k_w$ is the rate constant.

3. Results and Discussion
This study aims to determine the effect of alginate modification to protect iron oxidation. Alginate degradation using ultrasonic radiation aims to cleave the alginate polymer chain into smaller molecules. The weight average molecular weight of alginate decreased after degradation. Ultrasonic degradation is a non-random chain-breaking process [15]. The polymer chain-breaking process occurs in the middle part [15-16]. The degradation process modified the molecular weight and viscosity of the alginate.

The degraded alginate is used for the iron encapsulation using gelation method. Encapsulation is expected to protect iron from interacting with other materials, preventing the oxidation of Fe$^{2+}$ to Fe$^{3+}$ under environmental conditions and reducing the possibility of unexpected side effects [17-18]. The gel-forming ability of alginate was formed when a divalent crosslinker, such as CaCl$_2$ was added [19]. The calcium alginate was produced as the divalent ion (Ca$^{2+}$) of the crosslinker replaced the sodium ion of alginate and entrapped the iron in the alginate gel. The CaCl$_2$ concentration of 1.5 N was high enough for encapsulating the iron, as the gel-forming ability of alginate was affected by the crosslinker concentration [20].

The iron oxidation was observed by exposing the iron beads at ambient condition for 15 days. The iron content was then mathematically modelled using the model of Kirby et al [13] and Wang et al [14]. Figure 1a shows the iron oxidation development using degraded alginate in various degradation periods. Longer ultrasonic duration decreased alginate ability on protecting oxidation as the degradation lowered the viscosity of alginate and formed weaker crosslinking bonds [21]. Oxidation increased sharply in encapsulation using alginate with longer degradation period. Less degradation results in more intact beads, hence performed better oxidation protection. Meanwhile, research conducted by Yujian et al on the use of PVA complexes and sodium alginate cross-linked with Ca(NO$_3$)$_2$ for immobilization of Acidithiobacillus ferrooxidans cells indicated that the concentration of Fe$^{2+}$ decreased slowly in the first 24 hours, and after that, it started to decline rapidly [22]. Another study conducted by Long et al found that the maximum oxidation rate of iron was 6.7 g/l/hour [23].

![Figure 1. Oxidation development of iron encapsulation by degraded alginate in various (a) ultrasonic periods and (b) alginate concentrations.](image-url)
Figure 1b showed the oxidation level iron encapsulated by alginate treated using ultrasonication in different alginate concentrations. Application of ultrasound was less effective in degrading higher alginate concentration. This higher concentration tended to form higher viscosity solution which limit molecule movements and ultrasonic penetration. As a result, this less degraded alginate was better in protecting iron. After 15 days, the lowest oxidation (8.47%) was able to suppressed by ultrasonication of 5% alginate for 30 min.

Fitting of the mathematical models on the oxidation profile is presented in Figure 2 and 3 for various period degradations and alginate concentrations, respectively, while their constants are presented in Table 1 and 2. In general, the Kirby model was more suitable than the Wang model for describing the oxidation profile of the iron using modified alginate in both variations. The less fit ($R^2=0.760$) of the Kirby was shown by the encapsulation using the less degradation alginate which performed the best iron protection.

Figure 2. Fitting of the Kirby dan the Wang model to oxidation profiles of iron encapsulated by ultrasonicated alginate for (a) 30, (b) 90, (c) 120, and (d) 150 min.
Table 1. The constants and regression value of oxidation model by the variation of ultrasonication duration of alginate

| Degradation duration (min) | Kirby Model   | Wang Model   |
|----------------------------|---------------|--------------|
|                            | $k_K$ | $R^2$ | $k_W$ | $R^2$ |
| 30                         | 0.0042 | 0.760 | 0.0043 | 0.7496 |
| 90                         | 0.0060 | 0.912 | 0.0062 | 0.9396 |
| 120                        | 0.0085 | **0.959** | 0.0090 | 0.9529 |
| 150                        | 0.0153 | **0.954** | 0.0169 | 0.9521 |

Figure 3. Fitting of the Kirby dan the Wang model to oxidation profiles of iron encapsulated by ultrasonicated (a) 5, (b) 7.5, and (c) 10 % alginate concentration.
Table 2. The constants and regression value of oxidation model of iron encapsulated by ultrasonicated alginate in various alginate concentration.

| Concentration of Alginate in Ultrasonication (%) | Kirby Model | Wang Model |
|-------------------------------------------------|-------------|------------|
|                                                 | $k_k$       | $k_w$      | $R^2$      |
| 5 %                                             | 0.0165      | 0.0187     | 0.8495     |
| 7.5 %                                           | 0.0151      | 0.0170     | 0.7928     |
| 10 %                                            | 0.0139      | 0.0155     | 0.8328     |

4. Conclusion
Alginate modification had been successfully conducted using ultrasonic degradation, which led to change in alginate properties. The degraded alginate was used to encapsulate iron as a protection against the oxidation. The lower the alginate concentration and the length of time for degradation, the lower the alginate ability to protect the iron. After 15 days, the lowest oxidation (8.47\%) was able to suppressed by ultrasonication of 5\% alginate for 30 min. The Kirby model ($R^2$>0.76) was more suitable in describing the oxidation rate from the iron encapsulated by the degraded alginate.

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