

Research Article

Cyclic Voltammetry and Oxidation Rate Studies of Ferrous Gluconate Complex Solutions for Preparation of Chitosan-Tripolyphosphate Microparticles

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Received 26 August 2019; Accepted 27 January 2020; Published 26 February 2020

Academic Editor: Maolin Guo

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A proper understanding of the properties of iron could increase the effectiveness of programmes for alleviating iron deficiency. Recently, encapsulation has been considered an appropriate method for protecting iron from injurious reactions. However, several events may occur during encapsulation processes, including changes in the iron's oxidation state. Oxidation of ferrous iron is not desirable since the intestines can only absorb iron in the ferrous form. In this study, a cyclic voltammetry method was applied to investigate the likelihood of ferrous gluconate oxidation for the preparation of chitosan-tripolyphosphate microparticles. Then, the electrochemical properties of ferrous gluconate were confirmed experimentally. The oxidation rate of ferrous gluconate is also discussed in this paper. All the experimental solutions were formulated in detail to produce conditions similar to those of microparticle production. Cyclic voltammetry analysis was conducted using a configuration of three electrodes connected to an electrochemical analyser. Graphite, platinum wire, and Ag/AgCl were employed as the auxiliary, working, and reference electrodes, respectively. The cyclic voltammetry results show that the observed potential for each anodic peak shifted negatively in the presence of chitosan and sodium tripolyphosphate. Moreover, the rate of ferrous oxidation tended to increase during 75 min of experiments due to the presence of chitosan and sodium tripolyphosphate. These behaviours indicate the transformation of ferrous iron to ferric iron during iron microparticle preparation. Furthermore, these findings suggest that spray drying is a preferable method to minimise the oxidation reaction.

1. Introduction

Currently, iron deficiency (ID) is an important issue in public nutrition, affecting 20% of the world's population [1, 2]. ID can disrupt oxygen transportation and enzyme reactions due to the depletion of erythrocyte and haemoglobin [3–5]. These effects may also interfere with metabolic pathways bringing about negative consequences in terms of growth, cognitive capability, mental development, immune function, and reproductive performance, with children under 5 years old and women of child bearing age being the most affected subjects [6–10]. In women, ID can form a vicious circle, if the amount of iron does not meet the body's needs starting from infancy, through adolescence, and worsening in pregnancy, thereby increasing the risk of

giving birth to infants with low iron sources [1, 4, 5, 11, 12]. The Indonesian government has implemented two programmes for ID prevention through iron supplementation and fortification [9, 10]. Indeed, since the 1970s, women have been advised to consume 90 tablets of iron/folic acid supplements during pregnancy [6]. Moreover, the iron fortification of flour has been implemented since 1998. However, the prevalence of ID in Indonesia remains higher than 15% due to the following factors: (i) low dietary intake of iron (less than 20 mg/day), (ii) poor bioavailability, and (iii) chronic blood loss by infection (in certain regions) [4, 7, 13].

Fortification and supplementation programmes may not be optimal because of the reduced availability of ferrous iron due to the presence of inhibitors (phytic acid, tannins, and

casein), competitors (calcium and zinc), or oxidising compounds [5, 14–19]. The interaction of iron with other dietary compounds can lead to the formation of unavailable iron components in the gastrointestinal tract, thereby decreasing bioavailability [17]. Given these properties, encapsulation is considered a suitable method to protect iron from oxidation and other injurious reactions, by forming an impermeable barrier [8, 14, 20].

One carbohydrate polymer candidate for encapsulation of protein and other active compounds is chitosan (Ch). It has excellent mechanical, biocompatible, biodegradable, nontoxic, mucoadhesive, and antimicrobial properties [20–22]. Ch entraps compounds by forming covalent or ionic cross-linked networks with glyoxal, glutaraldehyde, and tripolyphosphate (Tp) [21, 23]. In this study, ferrous gluconate (FeG) was used as an iron source because of its high bioavailability and stability under oxidation compared with other ionic ferrous salts [6, 15, 19]. The standard oxidation potential of Fe^{2+} at 25°C (298 K) is -0.77 V. The challenge in iron microencapsulation is dealing with its instability with respect to its environment. Several issues may occur during the preparation of iron microparticles, including changes of the iron state due to oxidation. Previous work succeeded in producing FeG microparticles using Ch, acetic acid (Ac), and Tp as the encapsulating material, solvent, and cross-linking agent, respectively [15]. That study showed that the amount of encapsulated ferrous iron was less than the total iron. This might be because some of the ferrous iron was transformed into ferric compounds during microparticles preparation. Oxidation of ferrous iron into ferric ones is not desirable, since the intestines can only absorb iron in its ferrous form [15, 17, 18]. Hence, further investigation of the redox behaviour of iron is urgently required.

Cyclic voltammetry (CV) is commonly applied method to observe redox potential behaviour by measuring the current generated from the electron transfer between an electrode and an electrolyte solution as a function of changes in potential. This experiment utilises a cell equipped with three electrodes: the reference, working, and auxiliary electrodes [24–29]. CV is frequently used in applied electrochemistry, analytical electrochemistry, and surface coating [24, 25, 28–30]. In contrast, there is a lack of information regarding its application in the food and health fields. Previous work examined the redox properties of ferrous and ferric upon addition of nicotine, and the correlation with neurodegenerative disease [26]. The ability of nicotine to form a complex reaction with free iron was proved by the change from reversible to quasi-reversible performances, as well as a reduced rate of electron transfer and the apparent rate constant (k_s); thus, it successfully reduced iron reactivity [26]. Another study investigated the structure antioxidant activity relationship (SAR) of flavonoids towards different free radicals by ferric reducing antioxidant power (FRAP) with slight modification using 96-well microplates [27]. Their oxidation potentials were analysed by the CV method and found to be in good agreement with the FRAP results [27].

Currently, there is insufficient information about the redox activity of FeG complex solution for preparation of Ch-Tp microparticle. Therefore, the aim of this study was to

investigate the likelihood of FeG oxidation using the CV electrochemical method. However, the electrochemical properties of FeG in the presence of Ac, Ch, and Tp should be confirmed by laboratory experiments. Hence, the ferrous oxidation rate of FeG was also discussed in this paper.

2. Materials and Methods

2.1. Materials. Ferrous gluconate was purchased from Dalian Chemical & Export Group Co. Ltd, China. Chitosan was supplied from CV. ChiMultiguna Cirebon, Indonesia. Acetic acid (99.99%), sodium tripolyphosphate, hydroxylamine hydrochloride, sodium acetate, and 1,10-phenanthroline were provided by Merck. All solutions were prepared with aquadest from PT. Brataco, Indonesia. The molecular structure of each material is described in Figure 1.

2.2. Preparation of Solutions. Four electrolyte solutions were prepared to investigate the effect of each compound normally used in the FeG microparticles preparation using Ch and Tp as encapsulating material and cross-linking agent, respectively. The electrochemical behaviour of ferrous compounds in all formulations was observed using the CV method.

2.2.1. Ferrous Gluconate Solution (FeG). FeG solutions of varying concentrations (5, 10, and 15 mM) were prepared by dissolving FeG in oxygen-free aquadest. These solutions are referred to as the blank samples.

2.2.2. Ferrous Gluconate-Acetic Acid (FeG-Ac) Solution. This formulation was similar to the FeG solution, except that the solvent was replaced with 2.5% (v/v) Ac. Various concentrations of FeG (5, 10, and 15 mM) were investigated.

2.2.3. Ferrous Gluconate-Acetic Acid-Chitosan (FeG-Ac-Ch) Solution. FeG-Ac-Ch solution was prepared by dissolving FeG in 2.5% (v/v) of Ac. Afterwards, different concentrations of Ch (0.25, 0.50, and 0.75% w/v) were prepared. The density and viscosity of this solution were also analysed due to its physical changes.

2.2.4. Ferrous Gluconate-Acetic Acid-Chitosan-Tripolyphosphate (FeG-Ac-Ch-Tp) Solution. FeG-Ac-Ch-Tp solution was made by dissolving FeG in 2.5% (v/v) Ac. The Ch was then added and mixed until the solution was homogeneous. Next, Tp solution in various concentrations (0.25, 0.5, and 0.75% w/v) was added using a syringe to the FeG-Ac-Ch solution. The amount of Tp solution was 5% of the total volume.

2.3. Electrochemical Analysis: CV Method. A cyclic voltammogram was acquired using a configuration of three electrodes connected to an electrochemical analyser (Autolab Metrohm Potentiostat, Galvanostat PGSTAT 302 N). Platinum wire, graphite, and Ag/AgCl (with an electrolyte concentration of 3 M KCl) were used as the working, auxiliary, and reference

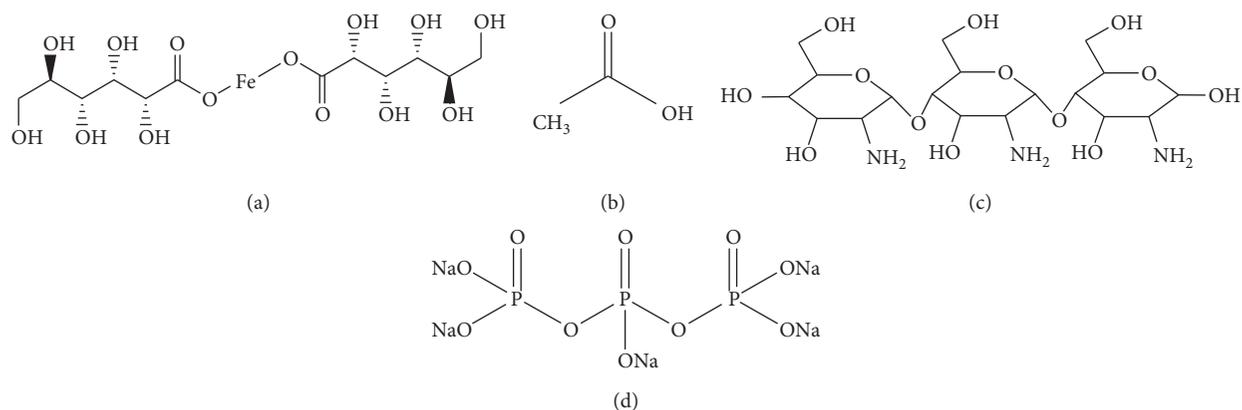


FIGURE 1: Molecular structure of (a) FeG, (b) Ac, (c) Ch, and (d) Tp.

electrodes, respectively. Furthermore, the solvent used was kept free of oxygen by degassing for 5 minutes prior to each experiment using an Elmasonic S40H sonicator. Each solution was mixed at room temperature in a closed system to avoid further oxygen interference.

2.4. pH Measurement. The acid-base behaviours of all investigated solutions were determined using a Hanna digital pH meter at 25°C. Both standard pH buffers (4.0 and 6.8) were utilised for calibration. The pH measurement was carried out prior to electrochemical analysis and again after the analysis was completed.

2.5. Density Measurement. The pycnometer method was used to determine the density of the FeG-Ac-Ch solution. The pycnometer was purchased from PT. Iwaki Glass Indonesia and calibrated using the ISO 4787:1984 and ISO 3507:1999 methods. The measurements were carried out three times at 25°C for each sample, and then the final density values were averaged.

2.6. Viscosity Measurement. The viscosity of the FeG-Ac-Ch solution was determined following the standard ASTM D445-17 method.

2.7. Ferrous and Total Iron Measurement. The amounts of ferrous and total iron in the aqueous solution were determined using the phenanthroline method, as described in a previous study [14]. In this method, 1,10-phenanthroline, sodium acetate, and hydroxylamine hydrochloride are utilised as the complexing agent, pH buffer, and reducing agent, respectively. The colour absorbance was analysed using a Thermo Scientific Spectrophotometer (GENESYS 10S) at a wavelength of 510 nm.

3. Results and Discussion

3.1. Determination of the Proper Conditions for Cyclic Voltammetry Analysis. In this study, various scan rates were applied, and different concentrations of FeG, Ch, and Tp

were used to determine the appropriate conditions for CV analysis, so that a clear cyclic voltammogram could be obtained. Figure 2 depicts the reduction oxidation activity of each proposed solution at various scan rates (ν) in the potential (V) range of -0.80 to 0.0 V. In general, the cathodic peaks were not distinct in the four formulations, while anodic peaks were slightly observable. The recorded current peak (i_p) decreased as slower ν was applied, which is in good agreement with previous reports [24, 26, 28, 31]. This result could be explained by considering the size of diffusion layer around the electrode and the time required to observe it. Since a slower scan rate was applied, a cyclic voltammogram would take longer to record and there was greater growth of the diffusion layer compared with a faster rate. As a result, the electron flux to the electrode surface was smaller; hence, a lower peak current was obtained, as the current is proportional to the flux towards the electrode. This is shown in equation (1), where C is the concentration of FeG complex solution, D is the diffusion coefficient, A is the surface area of the working electrode, and α is the transfer coefficient [32]. However, the anodic potentials also became more positive. Furthermore, a higher and clear current was obtained using the higher ν ; thus a value for ν of 0.2 V/s was applied in later investigations.

$$i_p = 2.99 \times 10^5 \nu^{1/2} CD^{1/2} A \alpha^{1/2}. \quad (1)$$

Figure 3 shows the cyclic voltammograms for FeG and FeG-Ac solutions at various FeG concentrations (5, 10, and 15 mM) at $\nu = 0.2$ V/s, in the V range of -1.0 to 0 V. In general, a higher current was recorded as the FeG concentration increased, due to the increase transfer of electron towards the electrode. These results are similar to those of the previous reports [24, 26, 28]. Because FeG concentrations of 10 mM and 15 mM did not represent the significant recorded current (Figure 2(b)), a concentration of 10 mM was used for the next set of observations.

The electrochemical behaviour of FeG was also investigated using different Ch and Tp concentration, as shown in Figures 4(a) and 4(b), respectively. All experiments were conducted at $\nu = 0.2$ V/s in a V range of -0.8 to 0 V. Figure 4 shows that an increase in the Ch and Tp concentrations did not significantly alter the observed current (i), which might

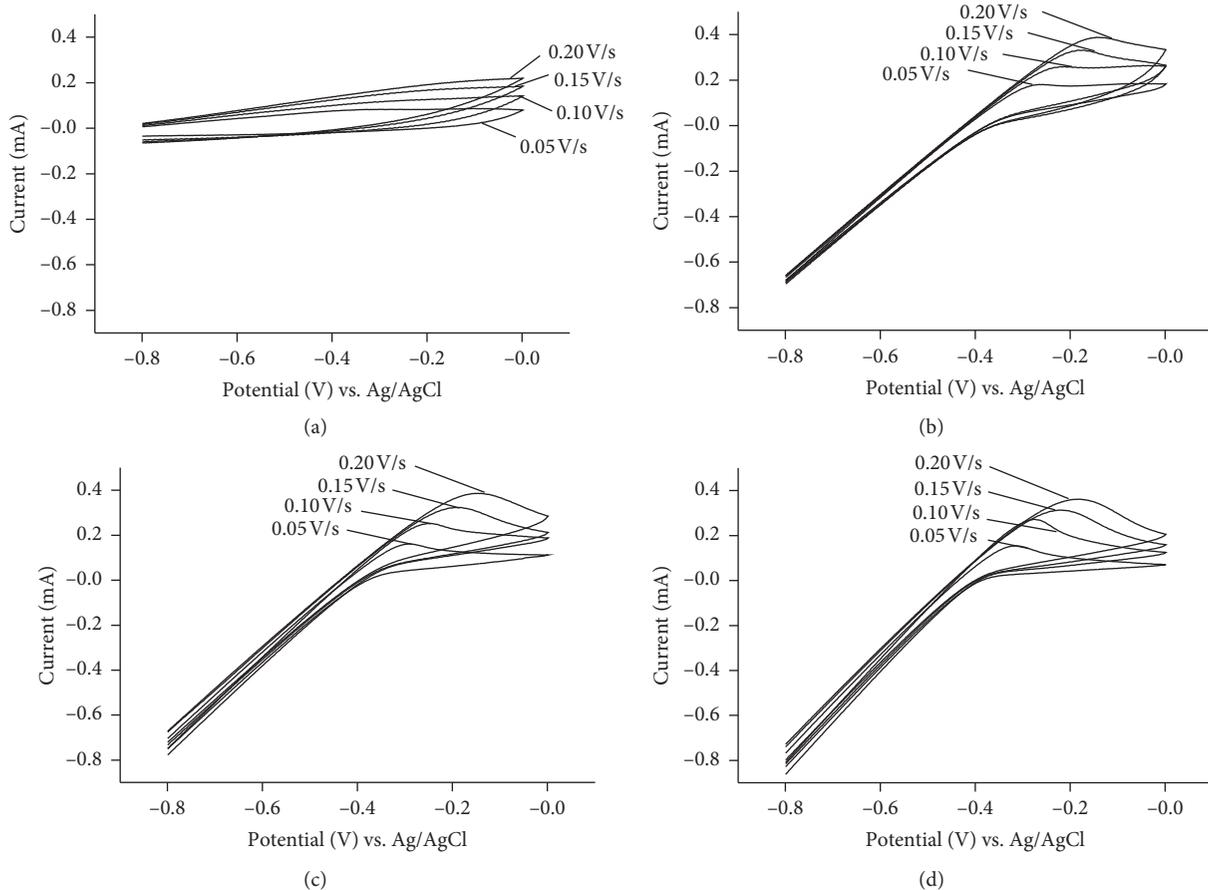


FIGURE 2: Cyclic voltammograms of (a) FeG 10 mM solution, (b) FeG 10 mM-Ac 2.5% solution, (c) FeG 10 mM-Ac 2.5%-Ch 0.5% solution, and (d) FeG 10 mM-Ac 2.5%-Ch 0.5%-Tp 0.25% solution, at ν values of 0.05, 0.10, 0.15, and 0.20 V/s.

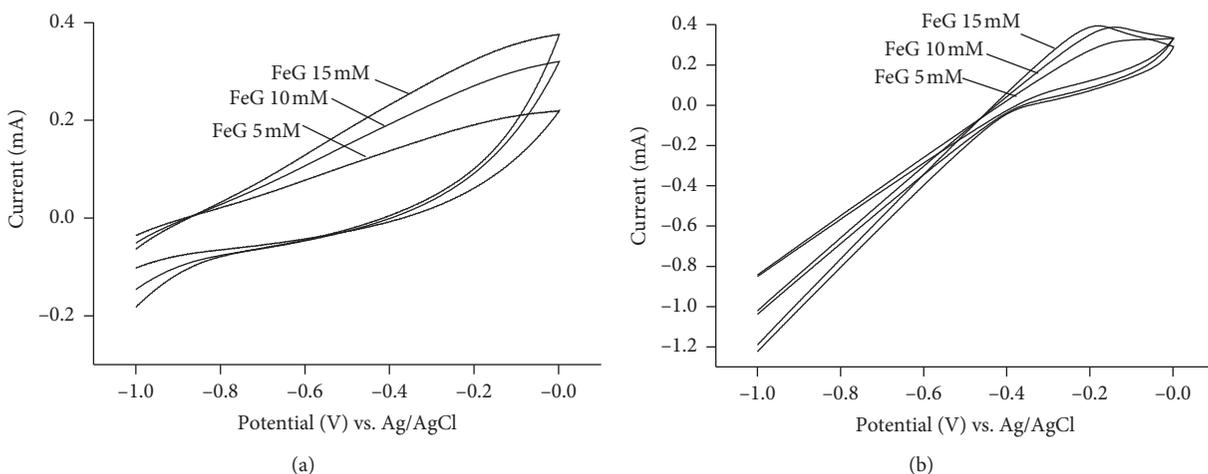


FIGURE 3: Cyclic voltammograms of (a) FeG solution and (b) FeG-Ac 2.5% solution with varying concentrations of FeG (5, 10, and 15 mM) at $\nu = 0.20$ V/s.

be caused by a fixed amount of oxidised component. However, the value of V for each i_p became more negative with the increasing of Ch concentration. Given these observations, a higher concentration of chitosan induced ferrous compounds being more susceptible to entering ferric

compounds. The density and viscosity were also analysed, due to the physical changes following Ch addition. A higher Ch concentration would increase density and viscosity values of FeG-Ac solution (Table 1). The high viscosity may have disrupted the electron transfer towards the electrode,

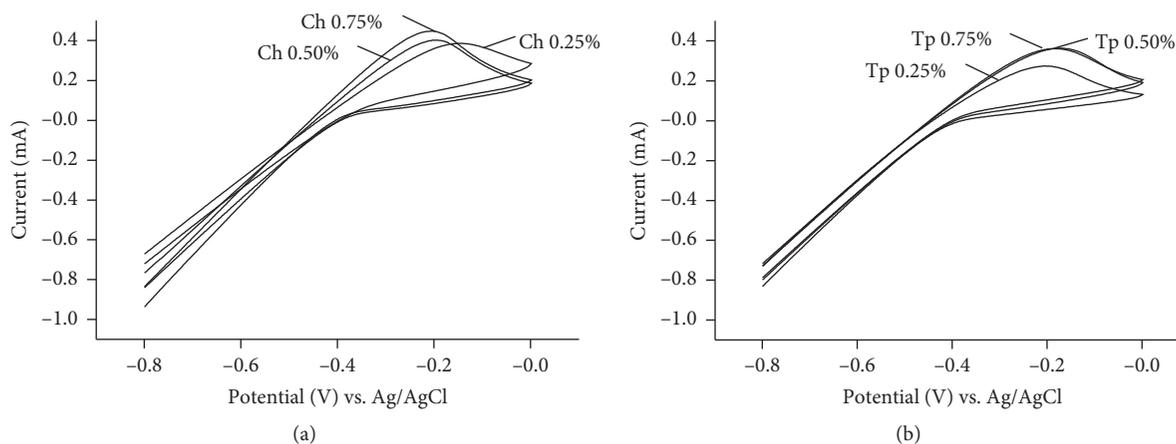


FIGURE 4: Cyclic voltammograms of solution containing (a) FeG 10 mM-Ac 2.5% at varying Ch concentrations and (b) FeG 10 mM-Ac 2.5%-Ch0.50% at various Tp concentrations, $\nu = 0.20$ V/s.

TABLE 1: Density and viscosity of FeG-Ac solution and FeG-Ac-Ch solution.

FeG-Ac solution	Density at 25°C (g/ml)			FeG-Ac solution	Viscosity at 25°C (Cst)		
	FeG-Ac-Ch solution at various Ch concentrations				FeG-Ac-Ch solution at various Ch concentrations		
	0.25%	0.50%	0.75%		0.25%	0.50%	0.75%
1.026	1.03	1.03	1.04	1.00	4.50	10.15	20.22

thus interrupting the current recording. However, the cyclic voltammogram also shows that the addition of 0.5% Ch led to obvious oxidation property as demonstrated by the negative shift of the anodic potential compared with the addition of 0.25% Ch. Furthermore, the addition of 0.75% Ch did not shift the anodic potential significantly; thus a Ch concentration of 0.5% was used for further experiments, so a clear potential alteration could be recorded.

Figure 4(b) shows that the increase in the Tp concentration changed the oxidation potentials from -0.20 V to -0.17 V and -0.19 V indicating that FeG was more prone to oxidise. Also, the increase of Tp concentration from 0.25% to 0.50% and 0.75% did not significantly alter the anodic potential; thus a Tp concentration of 0.25% was applied for further investigation, so an obvious potential shift would be observed.

3.2. Electrochemical Properties of FeG Complex Solutions for Preparation of Chitosan-Tripolyphosphate Microparticles.

The experiments were carried out using FeG 10 mM, Ac 2.5%, Ch 0.5%, and Tp 0.25% at $\nu = 0.2$ V/s, in the V range of -0.8 to 0 V. All the solutions were formulated to simulate the actual solution used in iron microparticle production. The electrochemical behaviours of each solution are represented in Figure 5. The values for the anodic current, anodic potential, and pH of each solution are also given in Table 2.

In general, the observed currents (i) became more positive and more negative with the presence of Ac, Ch, and Tp. The cyclic voltammogram for the FeG solution showed that there were no distinct oxidation and reduction peak. However, currents around zero were recorded during the

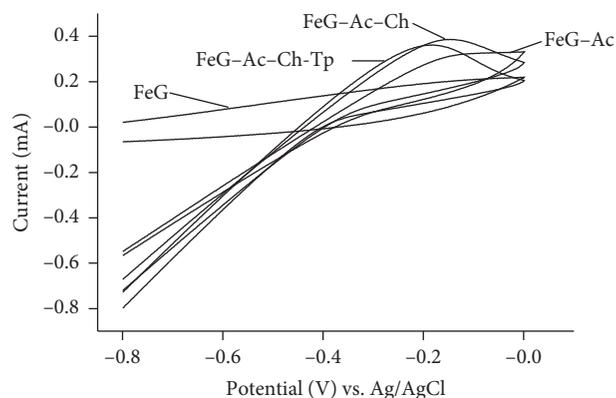


FIGURE 5: Cyclic voltammogram of complex solutions containing FeG 10 mM, Ac 2.5%, Ch 0.5%, and Tp 0.25% during iron microparticle preparation.

scanning process in the area of both oxidation and reduction; thus, a slight change of potential could deliver both anodic and cathodic currents.

In this study, Ac was used to dissolve Ch, and FeG was then added to the solution as encapsulated material. The stability of FeG in Ac should be investigated; hence, the first step was to analyse the FeG-Ac solution. The presence of Ac led to a decrease in the pH of the FeG-Ac solution. This may have been due to the partial dissociation of Ac to produce protons (H^+) in the aqueous phase, thereby increasing the acidity (Figure 1). Ac could also inhibit the polymerisation of iron in solution, thereby keeping it in its reactive state in the acidic medium [33, 34].

TABLE 2: Values of anodic peak, and pH of complex solutions containing FeG 10 mM, Ac 2.5%, Ch 0.50%, and Tp 0.25% at $\nu=0.20$ V/s.

	Anodic peak		pH prior to analysis	pH after analysis
	Current (mA)	Potential (V)		
FeG	---was not observable---		4.1	4.0
FeG-Ac	0.388	-0.145	3.3	3.3
FeG-Ac-Ch	0.386	-0.148	3.4	3.4
FeG-Ac-Ch-Tp	0.362	-0.204	3.6	3.6

The Ch in the FeG-Ac-Ch solution served as the encapsulating agent. Because of its rigid crystalline structure, it is only soluble in an acidic medium, such as Ac [20, 35]. The oxidation peak of FeG-Ac-Ch increased from 0.33 to 0.39 mA, while V became more negative, falling from -0.14 to -0.15 V (Table 1). This indicates that the ferrous iron was more susceptible to oxidation compared with the FeG-Ac solution. The presence of Ch also led to a slightly more alkaline pH (Table 1). Ch is a weak base polymer, since it has a large number of amino groups (Figure 1), and therefore increased the alkalinity of the FeG-Ac-Ch solution [21].

The chitosan application for oral delivery is closely related to controlled release systems; thus, the presence of Tp as a cross-linking agent is important [35, 36]. The oxidation peak of the FeG-Ac-Ch-Tp solution became more negative, dropping from 0.39 to 0.36 mA in the presence of 0.25% (w/v) Tp. However, the V also became more negative, which indicates that it was easier to transform ferrous iron in the FeG-Ac-Ch-Tp solution into ferric iron in comparison with the FeG-Ac and FeG-Ac-Ch solution. The addition of Tp also produced a more alkaline pH in the FeG-Ac-Ch-Tp solution in comparison with the FeG-Ac-Ch solution. This occurred because of the presence of Tp anion which attracts H^+ ions.

The pH of each solution was affected only by the presence of Ac, Ch, and Tp (Table 1). The pH value of each sample measured following the experiment did not significantly differ from the pH recorded prior to the experiment. In accordance with the cyclic voltammogram results, all four solutions showed a tendency of ferrous state transformation during iron microparticle preparation.

3.3. FeG Oxidation Rate in Aqueous Solutions. Four solutions were formulated to study the likelihood of FeG oxidation using CV method. However, the electrochemical properties of FeG in the presence of Ac, Ch, and Tp needed to be confirmed experimentally. Hence, the concentration of ferrous iron as a function of time was investigated for 75 min (the average time for iron microparticle preparation), as depicted in Figure 6. The observed solution was prepared using 10 mM of FeG, 0.5% Ch, and 0.25% Tp. A sample was collected every 15 min, for ferrous determination using the phenanthroline method.

Figure 6 shows that 30% of the ferrous iron in the FeG solution was lost in the first 15 min of the experiment. This result supports previous work reporting that about 16.6% of the ferrous iron was lost from FeG solution after storage in a closed container for a day [37]. In contrast, only 5% of the ferrous iron in the FeG-Ac solutions was lost at the first 15 min and it stabilised in the last 60 min of the experiment.

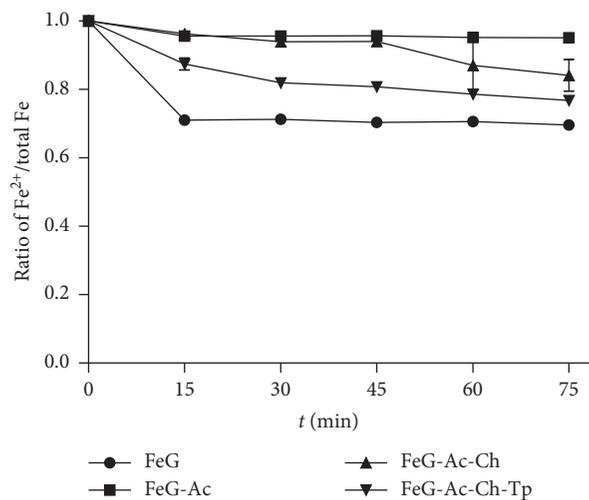


FIGURE 6: The oxidation rate of soluble ferrous iron in a batch test.

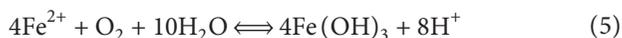
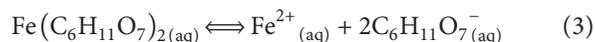
In a previous study, a kinetic equation for the oxidation of ferrous iron to the ferric iron in an aqueous solution, with O_2 as the electron acceptor, was given in equation (2) [34]. This equation demonstrates that the oxidation rate of ferrous iron will decrease by twofold for every unit decrease in pH. The presence of Ac decreased the pH from 4.1 to 3.3; the oxidation rate of FeG-Ac was very low in the more acidic solution (Table 2) [34].

$$-\frac{d[Fe^{2+}]}{dt} = k[Fe^{2+}][OH^-]^2 P_{O_2}. \quad (2)$$

However, the presence of Ch and Tp increased both the pH value and the oxidation rate of ferrous iron, although the rate was not as high as that of the FeG solution (Table 2 and Figure 6). Figure 6 also shows that ferrous iron concentration in FeG-Ac-Ch and FeG-Ac-Ch-Tp tended to decrease in the last 60 min of the experiment.

A possible mechanism for the reaction that occurred during iron microparticle preparation is described in the reaction below (equations (3)–(5)). Initially, both FeG and Ac were ionised in the aqueous phase, as described in equations (3) and (4), respectively. The normal oxidation mechanism for ferrous iron is shown in equation (5). However, the higher amount of H^+ produced by Ac ionisation (equation (4)) would increase the amount of H^+ in equation (5); thus, the equilibrium would be driven towards the left side, and iron would appear in its reactive state. Furthermore, the presence of Tp would increase the pH, thereby decreasing the amount of H^+ , and the equilibrium would shift towards the right side; thus, ferrous oxidation

would occur (equation (5)). The feasibility of FeG and Ac ionisation were shown by the dissociation constant for gluconic acid ($K_a = 1.99 \times 10^{-4}$) and Ac ($K_a = 1.76 \times 10^{-5}$).



In practice, Ch-Tp microparticles can be prepared by ionic gelation, freeze drying, spray drying, etc. However, this study of CV and oxidation rates indicates that the presence of Tp increases the likelihood that ferrous iron will be transformed into ferric iron. Therefore, spray drying might be a preferable method of microparticle preparation due to the low amount of Tp (3–12% of the total volume) in the feed solution [36, 38].

4. Conclusions

In this study, the likelihood of ferrous oxidation was investigated using CV and then confirmed experimentally. The results show that there was oxidation of the FeG complex solution used for preparation of Ch-Tp microparticles. This was evidenced by a more negative anodic potential and a decrease of ferrous concentration in the presence of Ch and Tp. The results demonstrate a likelihood of oxidation in the order $\text{FeG} > \text{FeG-Ac-Ch-Tp} > \text{FeG-Ac-Ch} > \text{FeG-Ac}$. These findings suggest that the spray drying method is preferable to minimise the transformation of ferrous iron into ferric iron.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This study was funded by Universitas Indonesia through the TADOK research grant contract number NKB-0164/UN2.R3.1/HKP.05.00/2019. The authors would also like to thank Dr. Yunita Sadeli, the Head of Corrosion Laboratory, Department of Metallurgical and Material Engineering, Universitas Indonesia, for permission to use the Autolab Metrohm Potentiostat device.

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