Electrochemical properties of ascorbic acid and folic acid under acidic solution

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Abstract. Ascorbic acid and folic acid are very important vitamins which have some crucial roles in human body’s metabolism, including enhancing iron absorption. Regardless of its roles, these vitamins are also known to be unstable and easily degraded in the processing by oxygen, pH, temperature, and UV light. Nowadays, microencapsulation may be a proper technique to overcome some of these shortcomings. However, there is a possibility of the changes in vitamin stability during encapsulation processes, particularly while using chitosan and acetic acid as wall material and solvent, respectively. In this study, cyclic voltammetry method was carried out for investigating the electrochemical properties of ascorbic acid and folic acid under acidic medium. The effect of scan rate (0.1; 0.2; 0.3 V/s) on the observed current was also studied. Furthermore, vitamin-aquadest solutions were also studied as a referred sample. Cyclic voltammetry was conducted using a three electrodes configuration connected to an electrochemical analyser. Platinum wire, graphite, and Ag/AgCl were employed as the working, auxiliary and reference electrodes, respectively. All the solutions were maintained for free oxygen by the degassing process. In general, the recorded currents of all solutions were increased while using a higher scan rate. Cyclic voltammogram also showed that ascorbic acid had two anodic peaks and two cathodic peaks which indicated that ascorbic acid undergoes reversible reaction. However, folic acid only had one anodic peak in water which represented an oxidation reaction. Furthermore, the electrochemical properties of ascorbic acid have a similar profile with folic acid while were in acidic solutions. Both vitamins have more positives anodic peak; hence depicted that acid could suspend the oxidation reaction.

Keywords: ascorbic acid, folic acid, cyclic voltammetry, acidic solution

Introduction
Ascorbic acid (AA) and folic acid (FA) are water soluble vitamins which recognized to be involved in the metabolism of human. AA has important roles in immune response activation, dermatological properties, and osteogenesis [1, 2]. FA plays essential functions in DNA biosynthesis and reduces the risk of neural tube defects in the baby [3]. Furthermore, both AA and FA also act as an enhancer in iron absorption [4-6]. However, regardless of its advantages, AA and FA are unstable substances and easily degraded by UV lights, temperature, pH, and oxygen [2, 5]. Hence, microencapsulation might be an appropriate technique to overcome some of these shortcomings; thus, the vitamins could act their roles properly. However, the properties of AA and FA might change during encapsulation processes, particularly while using chitosan and acetic acid as wall material and solvent, respectively. In this study,
electrochemical analyses were carried out for investigating the stability of AA and FA under acidic solution. Commonly, cyclic voltammetry (CV) is applied to analyze the redox behaviors by measuring the current acquired from the electron transfer between the electrolyte solution and electrode. The counter, reference, and working-electrodes were used in this cell equipment [7-12]. The CV method is frequently utilized in the analytical and applied electrochemistry fields [7, 8, 11, 12].

Many studies were conducted to investigate the AA and FA properties by using the CV method. The previous research determined simultaneously dopamine, AA, and uric acid using carbon ionic liquid electrode [13]. Another study was carried out to detect the dopamine in the presence of AA and uric acid by a carbon nanotubes-ionic gel electrode [14]. Ganesh and Kumara (2016) were used poly modified carbon paste electrode for recording electrochemical properties of FA in phosphate buffer solution (PBS) [15]. However, there is a lack of information regarding the application of CV for observing the stability of AA and FA under acidic solution in the field of microencapsulation.

The main objective of the present works was to study the electrochemical properties of AA and FA in aquadest and in acidic solutions using a CV method. The effect of scan rate on the observed current was also investigated for all solutions.

Material and method

Materials.
AA and acetic acid (Ac) (99.99%) were provided from Merck. FA was supplied from Dalian Chemical &Export Group Co. Ltd China. All solutions were prepared with aquadest from PT. Brataco Indonesia.

Solutions preparation.
Four electrolyte solutions were well prepared to investigate the stability of AA and FA. The solutions were formulated by dispersing AA and FA into 100 ml of aquadest and 100 ml of 2.5% (v/v) acetic acid under magnetic stirrer until the homogenous phase was obtained. The electrochemical behavior of AA and FA was analyzed using the CV method.

Electrochemical analysis – CV method.
The cyclic voltammogram was observed using three electrode configurations connected with potentiostat-galvanostat (Autolab Metrohm PGSTAT 302 N). Ag/AgCl, Pt wire, and graphite were applied as the reference, working, and counter electrodes, respectively. In addition, solvent was always prepared for free of oxygen by degassing process using an Elmasonic S40H sonicator for 5 minutes prior to each analysis. The homogenizing process was conducted at room temperature in a closed system to keep off further oxygen interference.

pH measurement.
The pH value of each solution was determined using a HANNA digital pH meter at 25°C. The calibration was carried out using pH buffers 4.0 and 6.8.

Results and Discussions
The electrochemical properties of AA and FA in aquadest and acidic solutions were well investigated using cyclic voltammetry method in varying scan rate and potential range. The pH properties of AA and FA are shown in Table 1.

Electrochemical properties of AA and FA in varying scan rate
Cyclic voltammograms of AA 25 µM in aquadest, FA 25 µM in aquadest, AA 25 µM in acidic solution, and FA 25 µM in acidic solution were observed at varying scan rate (v) of 0.1, 0.2, 0.3 V/s in the potential (V) range of -1 to 0 V, as depicted in Figure 1a, 1b, 1c, and 1d, respectively. In general, the currents were higher recorded while using v 0.2 and 0.3 V/s in comparison with 0.1 V/s in all solutions. The previous research also represented a similar profile of cyclic voltammogram while using varying scan rate [7, 9, 11, 16].
This could be explained by considering the size of diffusion later around the electrode and the time required to observe. Since a faster scan rate was used, a cyclic voltammogram had a shorter time to observe and a diffusion layer did not have a chance to grow around the electrodes. Furthermore, the electron flux was faster towards the electrode surface; thus the higher current was well recorded. However, the anodic peak of each solution was not visible when applying 0.3 V/s (Figure 1c, 1d). This can probably be attributed to the fastest electron flux towards the electrode, hence the anodic peaks were not well observed. Hereafter, \( v \) at 0.1 V/s would be applied for later investigation, since the more obvious current was obtained by using this rate.

![Graphs](a)(b)(c)(d)

Figure 1. Cyclic voltammograms of (a) AA 25 \( \mu \)M in aquadest, (b) FA 25 \( \mu \)M in aquadest, (c) AA 25 \( \mu \)M in acidic solution, and (d) FA 25 \( \mu \)M in acidic solution in varying \( v \) value of 0.1; 0.2; and 0.3 V/s.

**Electrochemical properties of AA**

Cyclic voltammograms of AA 25 \( \mu \)M in aquadest and AA 25 \( \mu \)M in acidic solution were investigated at scan rates (\( v \)) 0.1 V/s, as depicted in Figure 2a and 2b, respectively. Commonly, electrochemical properties of AA in aquadest represented two anodic peaks (at -0.5 V; 0.75 V) and two cathodic peaks (at -1.0 V; 1.0 V) (Figure 2a). It indicated that there were two oxidation and two reduction reactions of AA. The results were in good agreement with the previous research [17]. The mechanism of AA oxidation in aquadest was shown in detail in Figure 3. The pH solution of AA in aquadest was 4.1 (Table 1). Thus, AA could be ionized into ascorbate anion, which initiated the 1st oxidation reaction to form ascorbyl radical. Furthermore, the 2nd oxidized form of AA, dehydroascorbic acid (DHA), could be synthesized by two-electron oxidation of AA or by one-electron oxidation of ascorbyl radical. Otherwise, one-electron reduction of DHA could generate ascorbyl radical, while one-electron reduction of ascorbyl radical could initiate ascorbate anion.

However, only one anodic peak (at -0.10 V) and one cathodic peak (at 0.35 V) was shown while AA was in acidic solution (Figure 2b). It indicated that one-electron oxidation of AA could initiate ascorbyl radical formation; otherwise one-electron reduction of ascorbyl radical could form ascorbate.
anion. The cyclic voltammogram also represented that acid might help AA to stay in its ascorbyl radical form and to avoid the 2nd oxidation.

Table 1. pH properties of AA and FA in aquadest and acidic solution

|          | AA   | FA   |
|----------|------|------|
| in aquadest | 4.1  | 3.3  |
| in acidic solution | 3.0  | 2.9  |

Figure 2. Cyclic voltammograms of (a) AA 25 µM in aquadest and (b) AA 25 µM in acidic solution with \( v \) value of 0.1 V/s.

The electrochemical properties of FA 25 µM in aquadest and FA 25 µM in acidic solution were well recorded at \( v \) 0.1 V/s, as shown in Figure 4a and 4b, respectively. It depicted that FA had an anodic peak at -0.3 V and at -0.2 V; while FA was in aquadest and acidic solution, respectively. However, the cathodic peak of FA was not well and slightly observed (at 0.35 V) in aquadest and acidic solutions, respectively. The cyclic voltammograms exhibited that FA underwent oxidation reaction according to the mechanism represented in Figure 5. Two-electron oxidation of FA could form dehydrofolic acid. This result was in a good agreement with previous studies [15, 18]. In addition, the shifts of anodic peaks becoming more positive indicated that FA was more susceptible to oxidize in aquadest.

Figure 3. Oxidation mechanism of AA in aquadest

Electrochemical properties of FA

The electrochemical properties of FA 25 µM in aquadest and FA 25 µM in acidic solution were well recorded at \( v \) 0.1 V/s, as shown in Figure 4a and 4b, respectively. It depicted that FA had an anodic peak at -0.3 V and at -0.2 V; while FA was in aquadest and acidic solution, respectively. However, the cathodic peak of FA was not well and slightly observed (at 0.35 V) in aquadest and acidic solutions, respectively. The cyclic voltammograms exhibited that FA underwent oxidation reaction according to the mechanism represented in Figure 5. Two-electron oxidation of FA could form dehydrofolic acid. This result was in a good agreement with previous studies [15, 18]. In addition, the shifts of anodic peaks becoming more positive indicated that FA was more susceptible to oxidize in aquadest.
Figure 4. Cyclic voltammograms of (a) FA 25 µM in aquadest and (b) FA 25 µM in acidic solution with \( v \) value of 0.1 V/s.

\[
\begin{align*}
\text{Potential [V] vs. Ag/AgCl} & \quad \text{Current [mA]} \\
-1.0 & \rightarrow 0.0 \\
\end{align*}
\]

\[
\begin{align*}
\text{Potential [V] vs. Ag/AgCl} & \quad \text{Current [mA]} \\
-1.0 & \rightarrow 0.0 \\
\end{align*}
\]

(a) \hspace{1cm} (b)

Figure 5. Oxidation mechanism of FA in aquadest

\[
\begin{align*}
\text{Folic Acid} & \rightarrow \text{Dehydrofolic Acid} \\
\end{align*}
\]

Electrochemical properties of AA and FA in aquadest solution

The electrochemical properties of AA 25 µM and FA 25 µM in aquadest were well studied at \( v \) 0.1 V/s in the \( V \) range of -1 to 0.4 V, as shown in Figure 6. Cyclic voltammogram depicts that AA had one anodic peak (-0.5 V) and one cathodic peak (-0.4 V). However, FA had one anodic peak at -0.3 V while the cathodic peak was not distinct. It indicates that AA was oxidized first followed by FA since AA had a more negative potential. The previous research also represented similar results [18]. Voltammogram of 10-60 µM FA in the presence of 1000 µM AA depicted that AA had more negative potentials in comparison with FA.

Figure 6. Cyclic voltammograms of AA 25 µM in aquadest and FA 25 µM in aquadest with \( v \) value of 0.1 V/s in the \( V \) range of -1 to 0.4 V
Electrochemical properties of AA and FA in acidic solution

Cyclic voltammogram of AA 25 µM and FA 25 µM in acidic solution was well studied at v 0.1 V/s in the V range of 0.8 to 0.4 V, as depicted in Figure 7. In general, AA and FA have similar properties while were in acidic solution. Both vitamins had only one anodic peak at -1.8 V and the cathodic peak was not well recorded. It indicates that AA and FA more susceptible to oxidize in aquadest since they had a more negatives potential. It also represents that acid could suspend the oxidation process for both AA and FA.

![Cyclic voltammogram of AA 25 µM and FA 25 µM in acidic solution](image)

**Figure 7.** Cyclic voltammograms of AA 25 µM in acidic solution and FA 25 µM in acidic solution with v value of 0.1 V/s in the V range of -0.8 to 0.4 V

Conclusion

Electrochemical properties of AA and FA were investigated by cyclic voltammetry method. Commonly, the recorded currents of all solutions were increased while using a higher scan rate. Cyclic voltammogram also showed that AA had reversible reactions, including two oxidation and two reduction reactions while was in the water. However, FA only had one anodic peak in water which represented an oxidation reaction. In addition, the electrochemical properties of AA have a similar profile with FA while were in acidic solutions. Both vitamins have more positives anodic peak; hence represented that acid could suspend the oxidation reaction.

Acknowledgments

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