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First investigation of electrochemical behavior and Detection of 2-O-(β-D-glucopyranosyl) ascorbic acid

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In this work, the electrochemical behavior of 2-O-(β-D-glucopyranosyl) ascorbic acid, a vitamin C analogue, was reported, with glassy carbon electrode as working electrode. It is the first report regarding the study of electrochemical property of 2-O-(β-D-glucopyranosyl) ascorbic acid. In addition, an analytical method for 2-O-(β-D-glucopyranosyl) ascorbic acid detection based on its electrochemical oxidation signal was established. In the range of 100 nM to 300 μM, the proposed electrochemical method was allowed for linear detection. The detection limit was calculated as 30 nM based on S/N=3. This result was more sensitive than the two HPLC-based analytical protocols that had been reported.

Keywords: *Lycium barbarum*; Electrochemical behavior; Chemical component; Chinese traditional medicine; Electrochemical oxidation

1. INTRODUCTION

Lycium barbarum L. is a precious plant with high edible and medicinal value. It is a deciduous shrub of the genus *Lycium* in the family Solanaceae, mainly distributed in northwest China [1–6]. The analysis of specific chemical components of *Lycium barbarum* and the study of its medicinal effects are important for the comprehensive development and deep processing of *Lycium barbarum* [7–11].

The polysaccharides and flavonoid polyphenolic compounds contained in *Lycium barbarum* are good natural antioxidants. Their antioxidant property is mainly reflected in scavenging free radicals, reducing malondialdehyde and enhancing superoxide dismutase [12–16]. *Lycium barbarum*

polysaccharides can inhibit the decrease of cell viability caused by 6-hydroxydopamine (6-OHDA), reduce the proportion of apoptotic cells, and inhibit the nuclear concentration caused by 6-OHDA In addition, polysaccharides of *Lycium barbarum* also reduce the accumulation of reactive oxygen species (ROS) and nitric oxide, decrease the level of protein-bound 3-nitrotyrosine (3NT), thereby inhibiting the apoptosis of PC12 cells [17].

Lycium barbarum polysaccharides are mainly composed of rhamnose, galactose, glucose, mannose, xylose, galacturonic acid, and a variety of amino acids or lipids [18,19]. It has high activity and complex composition. Their content is one of the important criteria to identify the quality of *Lycium barbarum*, and also a key concern in the process of deep processing and comprehensive development and utilization of *Lycium barbarum* [20,21]. However, polysaccharides in plants are not considered as an objective evaluation criterion [22,23]. Therefore, betaine has been another indicator to evaluate the quality of *Lycium barbarum*. However, betaine is not a unique component of *Lycium barbarum*, so it is difficult to be adopted as the key indicator to measure the quality of *Lycium barbarum*.

In 2004, Toyoda-Ono et al. isolated and purified 2-O-β-D-glucosyl-L-ascorbic acid from the dried fruit of *Lycium barbarum* [24]. 2-O-β-D-glucosyl-L-ascorbic acid is the only new, stable, and natural L-AA precursor substance found in natural resources. Its content is 0.5% of the net weight of the dried fruit of *Lycium barbarum*. 2-O-β-D-glucosyl-L-ascorbic acid is a unique component of *Lycium barbarum*, so its content can be measured to identify the quality of *Lycium barbarum* extract [25,26]. It will hopefully serve as a basis for establishing new quality standards for *Lycium barbarum* extracts. Few studies have been reported on how to detect the content of 2-O-β-D-glucosyl-L-ascorbic acid in *Lycium barbarum* extracts by now [27–30]. Electrochemical analysis is a sophisticated modern instrumental technology for determining the composition and content of substances based on their electrochemical properties in solution. Electrochemical analysis measures changes in electrical signals, including resistance, conductivity, potential, current, power, or current-voltage curves. Therefore, it can be identified and analyzed by testing instruments without signal conversion. Compared with traditional analytical methods, electrochemical analysis has the advantages of small size, low power consumption, simple operation, and short analysis time. At the same time, the electrochemical method has high detection sensitivity, which has been regarded as a sensitive and efficient method for the analysis of trace and tracer substances for a long time.

In this work, the behavior of 2-O-β-D-glucosyl-L-ascorbic acid using a glassy carbon electrode was investigated for the first time. It was found that 2-O-β-D-glucosyl-L-ascorbic acid and ascorbic acid have very different electrochemical behaviors. In addition, the oxidation of 2-O-β-D-glucosyl-L-ascorbic acid on the electrode surface can be used as a detection tool.

2. EXPERIMENTAL AND INSTRUMENTS

2-O-β-D-glucosyl-L-ascorbic acid was purchased from Chengdu Biopurify Phytochemicals Ltd. All other chemicals were analytical grade and used without purification. PBS under different pH conditions were used as electrolyte. All electrochemical fingerprint recordings were conducted using a CHI760 electrochemical workstation. A commercial glassy carbon electrode (GCE), an Ag/AgCl

electrode and a Pt electrode were used as working electrode, reference electrode and counter electrode, respectively.

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) were used to characterize the electrodes and electrochemical behavior of 2-O-β-D-glucosyl-L-ascorbic acid.

3. RESULTS AND DISCUSSION

The performance of the electrodes was first investigated in the work. Figure 1A shows the CV curves of GCE in 2 mM $K_3[Fe(CN)_6]$. A pair of very obvious electrochemical redox peaks can be seen on the surface of the electrodes. The distance between the oxidation peak and the reduction peak was 99 mV, indicating the excellent conductivity of GCE. Figure 1B shows the EIS plots of GCE in 2 mM $K_3[Fe(CN)_6]$. The electrodes presented a very small impedance. The studies of both CV and EIS proved that GCE is a suitable electrode material for sensing research. In addition, glassy carbon itself does not generate electrochemical reaction in the commonly used sensing potentials, which does not interfere with our study of the electrochemical behavior of the target substance on the electrode surface.

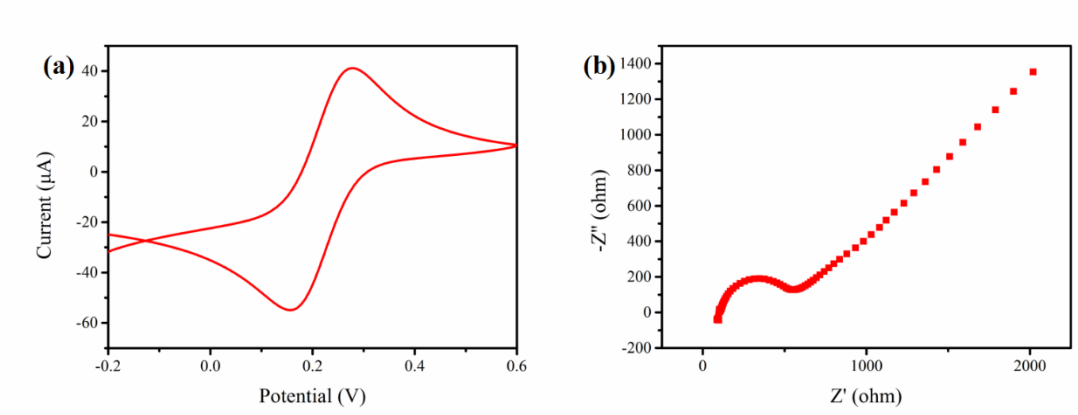

Figure 1. (A) CV and (B) EIS of GCE recorded in 2 mM $K_3[Fe(CN)_6]$. Scan rate: 50 mV/s.

Figure 2 shows the CV profiles of GCE in the absence and presence of 2-O-β-D-glucosyl-L-ascorbic acid under pH7 PBS. The GCE showed no electrochemical redox behavior in the absence of 2-O-β-D-glucosyl-L-ascorbic acid. However, a distinct oxidation peak located at 0.84 V was noticed when 2-O-β-D-glucosyl-L-ascorbic acid was added in PBS. L-AA was unstable and easily oxidized to dehydroascorbic acid, which degraded and lost physiological activity in aqueous solution and air. It was found that the hydrogen atom at the C2 position in the molecular structure of L-AA was replaced by glycosides. The oxidation rate of the substituted molecules (named AA2G) was greatly reduced, which improved the stability of the aqueous solution, and made the molecules heat resistant and less susceptible to degradation [31,32]. In the early 1990s, an AA2G was successively discovered from guinea pig urine of guinea pigs and rats as well as from *Lycium barbarum*, and an AA2G was discovered with the structure glucose group attached at the C2 position with α/β-1, 4 glycosidic bonds, which were called

AA2αG [33] and AA2βG [34] respectively. Studies showed that the antioxidant property of AA2βG is more durable than that of ascorbic acid, and it can stay in the body for a long time to exert antioxidant activity [35]. Thus, a completely different electrochemical behavior was observed from that of ascorbic acid. Normally, ascorbic acid will be electrochemically oxidized at around 0.2 V with a reversible electrochemical reduction when GCE is used as a working electrode. However, the oxidation potential of 2-O-β-D-glucosyl-L-ascorbic acid was as high as 0.82 V. Our previous study showed the ascorbic acid can be oxidized by an electrode at around 0 V after proper modification [36]. A well-defined oxidation peak was observed, while only small reduction peaks were observed due to the irreversible electrode processes. For 2-O-β-D-glucosyl-L-ascorbic acid, no reduction can be observed during scanning, which indicated compared with ascorbic acid, 2-O-β-D-glucosyl-L-ascorbic acid has a very good stability and is not easily oxidized.

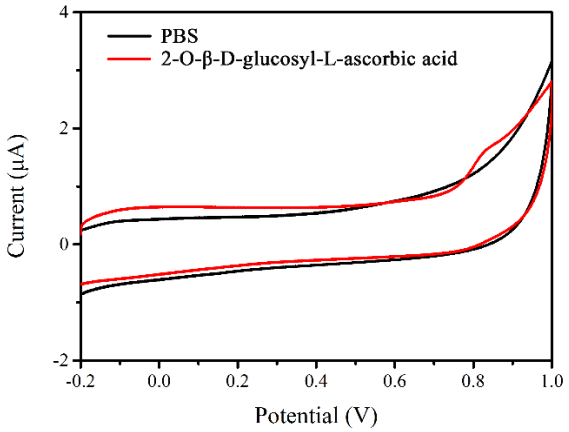

Figure 2. CV profiles of GCE recorded in PBS (pH=7.0) in the absence and presence of 2-O-β-D-glucosyl-L-ascorbic acid. Scan rate: 50 mV/s.

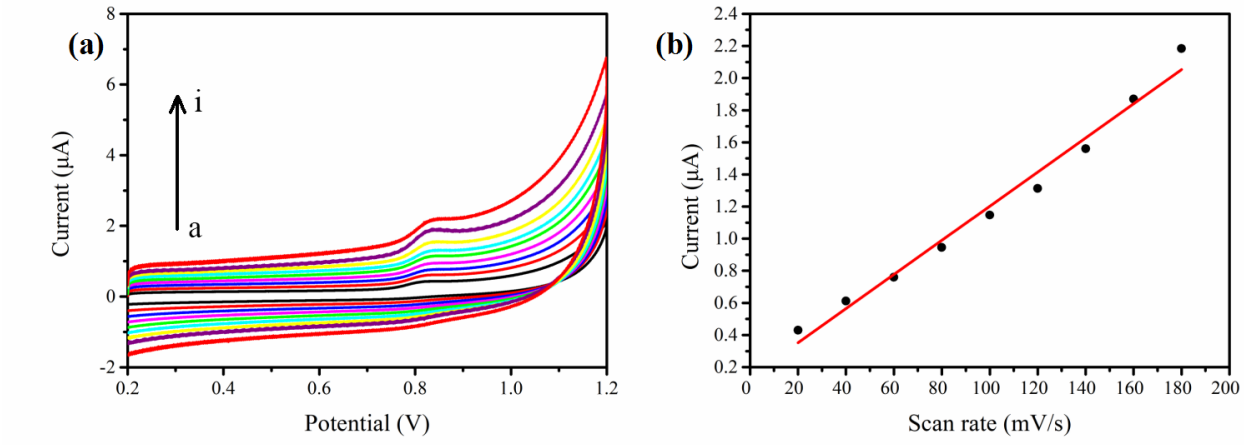

Figure 3. (a) CV profiles of GCE recorded in PBS (pH=7.0) in the presence of 2-O-β-D-glucosyl-L-ascorbic acid with different scan rates (a: 20 mV/s, b: 40 mV/s, c: 60 mV/s, d: 80 mV/s, e: 100 mV/s, f: 120 mV/s, g: 140 mV/s, h: 160 mV/s, i: 180 mV/s). (b) Plots of scan rate vs. peak current.

The effect of scanning speed on the electrochemical oxidation of 2-O-β-D-glucosyl-L-ascorbic acid was further explored. As shown in Figure 3A, the current value of electrochemical oxidation of 2-O-β-D-glucosyl-L-ascorbic acid increased in line with the increase of the scanning speed. There was a very consistent positive correlation between scanning speed and current intensity. Figure 3B shows the plots of oxidation current vs. scan rate. The oxidation current was proportional with the scan rate. This electrochemical behavior indicated the oxidation of 2-O-β-D-glucosyl-L-ascorbic acid on the GCE surface was controlled by diffusion [37].

Figure 4A shows the CV profiles of GCE towards 2-O-β-D-glucosyl-L-ascorbic acid under different pH conditions. The oxidation current reached the maximum at pH 7.0. Therefore, pH 7.0 was selected for analytical sensing. It was also noticed that the oxidation potential was shifted when the pH changed. As shown in Figure 4B, the oxidation potential decreased when the pH value increased. However,2-O-β-D-glucosyl-L-ascorbic acid did not have the same potential as ascorbic acid with pH changed, so the mechanism of its electrochemical oxidation cannot be particularly clarified. Figure 5 shows the possible electrochemical oxidation reaction we proposed. We believe that two electrons were involved in the electrochemical oxidation [38–43].

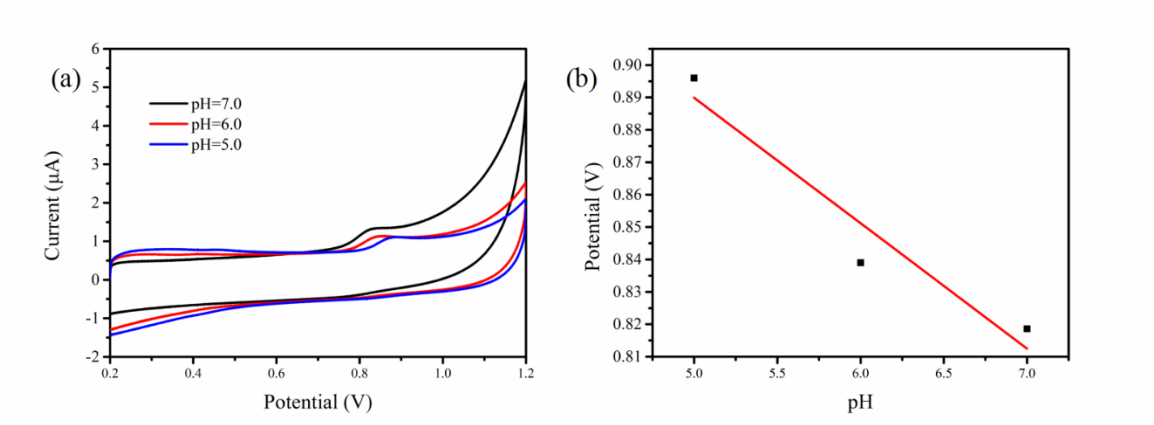

Figure 4. (a) CV profiles of GCE recorded in the presence of 2-O-β-D-glucosyl-L-ascorbic acid under different pH conditions in PBS. (b) Plots of scan rate vs. pH value.

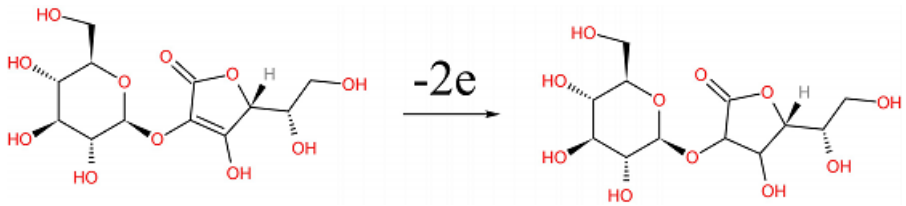

Figure 5. Proposed possible electrochemical oxidation reaction of 2-O-β-D-glucosyl-L-ascorbic acid.

For the quantitative analysis of 2-O-β-D-glucosyl-L-ascorbic acid, DPV investigations with different concentrations of 2-O-β-D-glucosyl-L-ascorbic acid were carried out. Figure 6A shows the DPV of 100 nM to 300 μM 2-O-β-D-glucosyl-L-ascorbic acid. As described in Introduction, the content of 2-O-β-D-glucosyl-L-ascorbic acid in the dried *Lycium barbarum* was 0.5% and there was no need for

ultra-sensitive detection of too high or too low concentrations. As shown in Figure 6B, the proposed electrochemical method was allowed for linear detection in the range of 100 nM to 300 μM. The detection limit was calculated as 30 nM based on S/N=3. Table 1 compares the performance of our proposed electrochemical detection with the existing HPLC. As can be seen from the table, our proposed method was competitive in the detection interval and detection limit. Since this technology does not require pre-treatment of the sample, it has strong application value.

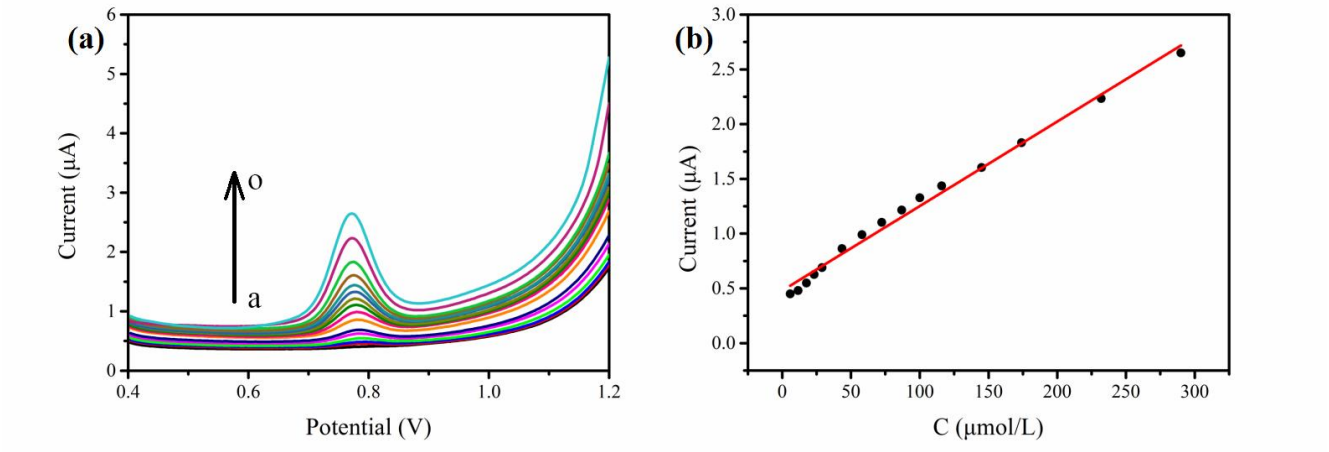

Figure 6. (a) DPV profiles of GCE recorded under pH7.0 PBS with different concentrations of 2-O-β-D-glucosyl-L-ascorbic acid (a: 100 nM, b: 0.5 μM, c: 5 μM, d: 25 μM, e: 30 μM, f: 50 μM, g: 60 μM, h: 75 μM, i: 90 μM, j: 100 μM, k: 120 μM, l: 150 μM, m: 175 μM, n: 230 μM, o: 300 μM). (b) Plots of concentration of 2-O-β-D-glucosyl-L-ascorbic acid vs. peak current.

Table 1. The performance of our proposed electrochemical detection with some other detection methods.

Methods	Linear detection range	Limit of detection	Reference
HPLC	30 μM to 295 μM	-	[44]
HPLC	5.61 μM to 230 μM	73.9 nM	[45]
Electrochemistry	100 nM to 300 μM	30 nM	This work

4. CONCLUSION

In this communication, the electrochemical behavior of 2-O-(β-D-glucopyranosyl) ascorbic acid was studied for the first time. Although 2-O-β-D-glucopyranosyl-L-ascorbic acid is an analogue of ascorbic acid, its electrochemical behavior is completely different from ascorbic acid. The results showed that the 2-O-β-D-glucopyranosyl-L-ascorbic acid was oxidized at a 0.83 V on the surface of GCE without reverse reduction process. The surface process during the oxidation of 2-O-β-D-glucopyranosyl-L-ascorbic acid was controlled by diffusion. In addition, in the range of 100 nM to 300 μM, the proposed electrochemical method was allowed linear detection. The detection limit was

calculated as 30 nM based on S/N=3. This result was much sensitive than previously reported HPLC methods.

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