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An Amperometric Proton Selective Sensor with an Elliptic Micro-hole Liquid/Gel Interface for Vitamin-C Quantification

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ABSTRACT :

An amperometric ascorbic acid selective sensor utilizing the transfer reaction of proton liberated from the dissociation of ascorbic acid in aqueous solution across an elliptic micro-hole water/organic gel interface is demonstrated. This redox inactive sensing platform offers an alternative way for the detection of ascorbic acid to avoid a fouling effect which is one of the major concerns in redox based sensing systems. The detection principle is simply measuring the current change with respect to the assisted transfer of protons by a proton selective ionophore (*e.g.*, ETH 1778) across the micro-hole interface between the water and the polyvinylchloride-2-nitrophenyloctylether gel phase. The assisted transfer reaction of protons generated from ascorbic acid across the polarized micro-hole interface was first characterized using cyclic voltammetry. An improved sensitivity for the quantitative analysis of ascorbic acid was achieved using differential pulse stripping voltammetry with a linear response ranging from 1 to 100 μM concentrations of ascorbic acid. As a demonstration, the developed sensor was applied for analyzing the content of vitamin-C in different types of commercial pharmaceutical tablets and syrups, and a satisfactory recovery from these samples were also obtained.

Keywords : Amperometric proton selective sensor, Micro-ITIES, Liquid/liquid interface, Ascorbic acid, Assisted ion transfer, Ionophore, ETH 1778.

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1. Introduction

Ascorbic acid (AA), known as vitamin C is one of the most important components in the diet of human as well as animal species.[1] For example, it plays a vital role in various biological metabolic functions such as the synthesis of collagen, carnitine and several neurotransmitters as well as in immune response and wound healing.[2,3] An inadequate dietary intake of AA has a crucial impact on the drug metabolism thus its deficiency leads to symptoms of some diseases, e.g. scurvy and gingival bleeding.[4] In addition, due to the antioxidant properties, it acts as a free radical scavenger in the body which may play a significant role to decrease the risk of some diseases such as cancers, cardiovascular failures, and strokes.[5] AA has usually used for the treatment and prevention of common cold, infertility and mental illness, and could potentially be used for inhibiting the human immunodeficiency virus (HIV) expression.[6] Apart from its biological activity, it has broader applications as antioxidants and preservatives in food and pharmaceuticals industries.[7] However, AA could degrade in the presence of light and heat[8] thus the monitoring of its concentrations is highly essential for insuring the quality from production plants to consumer levels. Therefore, the development of simple and inexpensive methods for the routine and reliable analysis of ascorbic acid is extremely impor-

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tant in the food and pharmaceutical industries.

During the last decades, a wide spectrum of analytical techniques have been reported for the quantification of ascorbic acid; conventional spectroscopy,[9] titrimetry,[10] fluorometry,[11] calorimetry[12] and high performance liquid chromatography[13] have been widely used but they are mostly time consuming in addition to the demerits of requiring complex sample pretreatment processes and expensive equipments and trained operators. On the other hands, electrochemical sensing platform can be a superb alternative for ascorbic acid analysis with their diverse advantages including simplicity, high selectivity, rapid response and relatively low cost. Several electrochemical methods measuring the oxidation of AA have been demonstrated utilizing conventional metallic electrode materials.[14,15] The major drawback of this method is the high overpotential which creates the fouling occurred by the adsorption of the oxidation products on the electrode surface and thus decreases the reproducibility and sensitivity of the electrode.[16,17] Recently modified electrodes like self-assembled monolayer modified electrodes,[18] nanostructured electrodes,[19] sol-gel fabricated electrodes[20] and polymer modified electrodes[21] have been suggested to resolve this fouling effect by lowering the overpotential of AA oxidation.

In contrast to redox based processes, the redox inactive electrochemical detection method utilizing charge transfer reactions across the interface between two immiscible electrolyte solutions (ITIES) can be an excellent sensing platform to overcome the fouling effect occurred due to the overpotential. The sensors based on ion transfer reactions across ITIES can also be highly selective by incorporating ion selective ionophores in the organic phase to help assisting the transfer of ion of interests. The assisted transfer reaction has successfully been applied for the sensing of various ionic species including metal ions, biomolecules as well as food additives.[22,23] In order to utilize such a selective ion transfer reaction as a convenient sensing platform, there have been extensive efforts made by means of both gelifying one of the phases usually the organic phase with polyvinylchloride gel and manipulating the interface into a micro-hole or arrays of microhole supported on polymeric supporting films as well as micropipette tips.[24-26] We have recently developed an amperometric ion selective sensor based on single elliptic microhole liquid/gel interface created on a food wrapping film and applied for a reproducible glucose biosensing.[27] In this work, we demonstrate a simple amperometric proton selective sensors utilizing an elliptic microhole liquid/gel interface for the quantitative analysis of ascorbic acid. Dissociated protons from ascorbic acid in aqueous sample were detected via measuring the current change associated with the proton transfer assisted by the proton selective ionophore across the polarized microhole liquid/gel interface. The selectivity of the assisted proton transfer reaction is critically dominated by the choice of ionophores usually present in the organic phase. We choose octadecyl isonicotinate (also known as ETH 1778) which is one of the most widely used proton selective ionophores.[27] The proton transfer reaction of AA samples assisted by the ETH 1778 was first characterized using cyclic voltammetry. The use of differential pulse stripping voltammetry was then investigated for enhancing the sensitivity of AA quantification. The sensor was finally applied for the direct detection and recovery of ascorbic acid from six different pharmaceutical products including tablets and syrups.

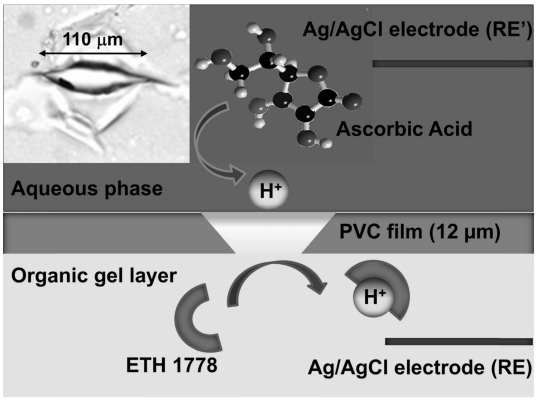
2. Experimental

2.1. Chemicals

L-ascorbic acid (Sigma-Aldrich), polyvinylchloride (PVC, high molecular weight, Sigma-Aldrich), 2-nitrophenyloctylether (NPOE, Fluka), octadecyl isonicotinate (ETH1778, Fluka), tetrabutylammonium chloride (TBACl, Aldrich) and lithium chloride (LiCl, Fluka) were all used as received. The supporting electrolyte for the organic phase was tetrabutylammonium tetrakis (4-chloro-phenyl) borate (TBATPBCl) prepared as described in the previous paper.[28] Millipore-filtered water was used for preparing all aqueous solutions.

2.2. Fabrication of single micro-hole ITIES

A single microhole was created by simply punching a thin PVC supporting film (12 μm thick)[27] with a sharp needle. An elliptic single microhole was formed due to the variation in the mechanical punching force when using the needle. The major diameter of the ellipse was about 110 ± 10 μm and the minor diameter was about 15 ± 5 μm (See Scheme 1 inset). The organic gel layer was prepared by dissolving PVC (3% m/m) in a solution of NPOE including the supporting electrolyte, 10 mM TBATPBCl as well as the ionophore, ETH 1778, at 120°C for 30 minutes. A



Scheme 1. A simplified schematic showing an ascorbic acid sensing methodology via measuring the current associated with the assisted transfer of protons across the microhole-ITIES by the proton selective ionophore, ETH 1778. Protons in the aqueous phase are readily released by the dissociation process of ascorbic acid. Inset shows an optical microscopy image for an elliptic microhole interface created on a PVC supporting film.

twelve microliter of the PVC-NPOE mixture was then hot casted at 80°C on the exit side of the microhole on PVC supporting film and allowed to cool down for a minimum of 6 h to form the gelified organic layer.

2.3. Electrochemical measurements

All electrochemical experiments were carried out using a computer-controlled potentiostat (Autolab PGSTAT30 Ecochemie) without any further IR drop compensation and all data were acquired using the General Purpose Electrochemical System (GPES) version 4.9 software. Cyclic voltammetry and differential pulse stripping voltammetry were employed to characterize proton transfer reactions and further sensing capabilities for ascorbic acid analysis.

3. Results and Discussions

We have chosen to develop proton selective sensors for ascorbic acid quantification since the ascorbic acid possesses a relatively high dissociation constant ($k_d = 5 \times 10^{-5}$) as well as diffusion coefficient in water ($D_w = 5.6 \times 10^{-6}$ cm^2/s) allowing the easy release of proton in aqueous solution.[29] Scheme 1 represents the principle for the selective sensing of protons released from ascorbic acid utilizing the ion transfer reaction across an elliptic microhole liquid/gel interface. Ascorbic acid in water phase releases proton which then transfers across a polarized elliptic micro liquid/gel interface by the aid of the proton selective ionophore, ETH 1778, present in the organic gel phase. The current associated with the assisted transfer of proton across the polarized micro-interface is then measured. The use of ionophore is to help a very hydrophilic proton usually transferring at the end of positive potential to transfer in the middle of potential window by means of lowering the Gibbs transfer energy required for the transfer of proton from the water to the organic phase.[24] The potential window is set by

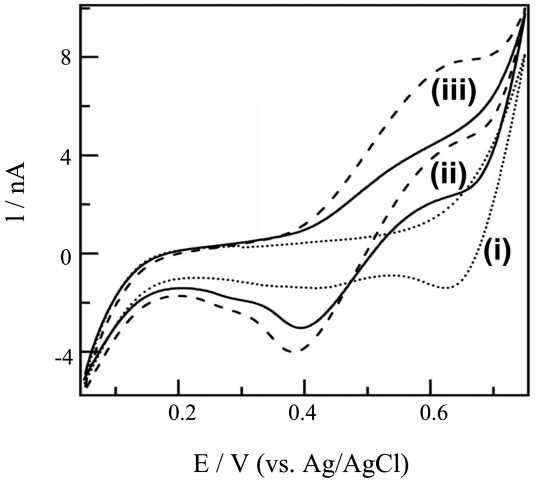
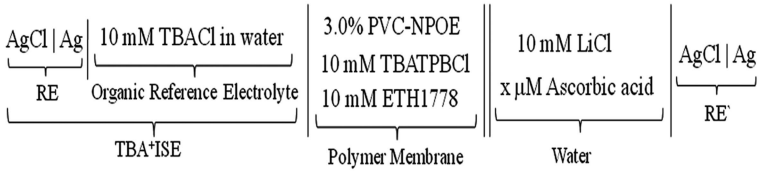


Fig. 1. Cyclic voltammograms for the assisted transfer of protons dissociated from ascorbic acid by ETH1778 present in the PVC-NPOE gel layer using Cell 1. (i) 10 mM LiCl in the absence of ascorbic acid and (ii) 40 μM and (iii) 100 μM ascorbic acid were present in the aqueous phase. 10 mM TBATPBCl was used as an organic supporting electrolyte. Scan rate was 20 mV/s.



Cell 1

both Li^+ and H^+ ion transfer from the water to the organic phase at the positive potential and TBA^+ ion transferring from the organic to the aqueous phase (see Fig. 1). The electrochemical cell setup is shown as Cell 1 where LiCl and TBATPBCl are are used as the aqueous and organic supporting electrolyte, respectively.

Fig. 1 shows cyclic voltammograms characterizing the proton ion transfer reaction assisted by ETH 1778 across the single elliptic microhole water/organic gel interface. In the absence of ascorbic acid, Li^+ ion transfer occurs from the water to the organic gel phase and TBA^+ ion from the organic gel to the aqueous phase. After addition of ascorbic acid, a steady state voltammogram in the forward scan and peak in the reverse scan was observed due to the assisted transfer of protons by ETH 1778 in the organic phase. The steady-state current on the forward scan was observed due to the fact that the proton transfer from the water phase to the organic layer across the micro-hole interface was dominated by the hemi-spherical diffusion flux of protons. This current was linearly increased as a function of the ascorbic acid concentration. The peak shaped voltammogram at the reverse scan was occurred. This is a typical phenomenon for the ion transfer reaction when using the gelified organic phase which slows the diffusion rate of ionic species in the organic phase. In order to utilize the steady state current at the forward scan as a sensitive proton sensing purpose, the excess of ionophore concentration (10 mM) was maintained for keeping the condition of $D_{H^+}[H^+] \ll D_L[L]$ where the linear dependence of the steady state current on the proton concentration can occur.[27)]

In order to enhance our proton sensing capabilities, a differential pulse stripping voltammetry was used with a preconcentration step by applying the proton transfer potential (about 0.3 V) for a certain period. This allows the released proton to transfer from the water to the organic phase and to be accumulated in the organic phase. The deposition time was optimized (data not shown) with a period of 30 s. After the preconcentration period, the proton accumulated in the organic gel layer followed by the complexation with the proton selective ionophore, ETH 1778 present in the organic phase is then stripped from the gel to the aqueous phase and the response is recorded as a function of difference pulsing potential steps. Fig. 2 demonstrates a series of differential pulse stripping voltammograms for various concentrations of ascorbic acid ranging from 1 to 100 μM and a linear response of negative peak current was achieved with respect to the ascorbic acid concentration (see Fig. 3). In Fig. 3 all data points at each concentration of ascorbic acid were averaged

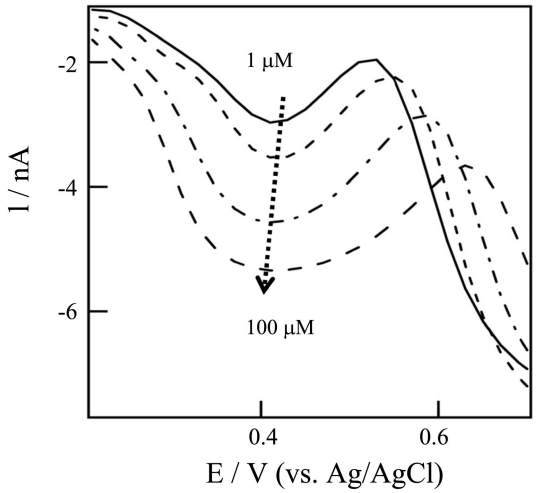


Fig. 2. A series of differential pulse stripping voltammograms for various concentrations of ascorbic acid. The scan was directed from high to low potentials to drive H^+ ion transfer from the PVC-NPOE gel layer to the aqueous phase. 1, 20, 40 and 100 μM of ascorbic acid were used and the deposition potential of 0.55 V for 30 s was applied prior to analysis. The pulse conditions were as follows: potential increment = 20 mV, pulse potential = 50 mV, pulse duration = 50 ms and scan rate = 20 mV/s.

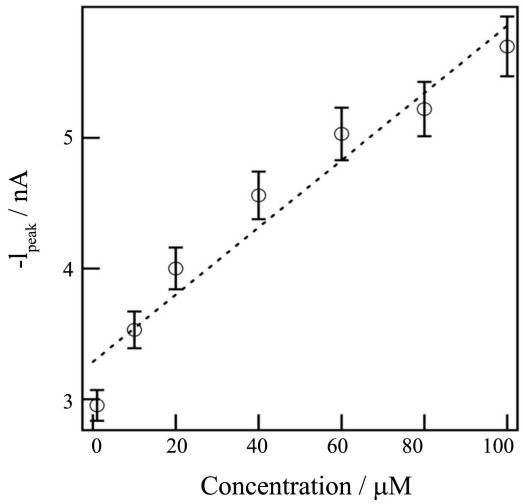


Fig. 3. A plot of averaged negative peak current values with respect to various concentrations of ascorbic acid using four different proton selective elliptic microhole-ITIES sensors.

Table 1. Summary showing the amount of ascorbic acid in various commercial vitamin-C tablets and syrups measured using the proton selective sensor and the percentage of recovery from each sample

Commercial vitamin-C products	Measured amount of vitamin-C (mg)	Recovery (%)
Tablet A	1000	104
Tablet B	1000	92
Tablet C	1000	97
Syrup A	300	105
Syrup B	500	103
Syrup C	205	94

from four different sets of the proton selective sensors. Excellent reproducibility with a linear fit over the ascorbic acid concentration was also obtained within the error of less than 5%.

As a final demonstration, we have applied the proton selective sensor for the analysis of real samples including six different pharmaceutical vitamin-C tablets and syrups. The major components of tablet and syrup are starch, glucose and other neutral compounds in addition to vitamin-C and our sensor has shown no interfering effects of these major compounds. In addition, the vitamin-C samples chosen in our assays did not contain any other distinctive proton-releasing compounds, however, it must be mentioned that the developed sensor could be affected to a certain degree if there are any other major proton releasing compounds except AA present in real samples. This could possibly be resolved by varying the sensor operation conditions such as pH of buffer solutions. Each sample was first dissolved in Millipore water and a fixed concentration (100 μM) of each sample was then taken and added in the aqueous phase and the amount of proton released from the sample was analyzed using differential pulse stripping voltammetry. The same deposition time and pulse step for the data shown in Fig. 3 were used. The obtained peak currents for each sample were then correlated to the amount of ascorbic acid using the calibration plot in Fig. 3 and these were finally compared to the reported vitamin-C quantity from the manufacturer of each commercial sample. Table 1 summarizes the comparison of our sensor analysis versus the manufacturer values. Satisfactory recovery results were observed within the error range of recovery varying between 92% and 105%, which can be regarded as a reliable error range in pharmaceutical and food industrial sensing platform.

4. Conclusions

We have demonstrated a simple amperometric proton selective sensor utilizing the ion transfer reaction across the elliptic micro-hole water/organic gel interface for the quantitative analysis of ascorbic acid in real samples such as commercial pharmaceutical tablets and syrups. As an example, the amount of vitamin-C in various pharmaceutical products was successfully analyzed; the percentages of recovery are in good agreement with the given values from the manufacturer. We envision that the highly selective and sensitive as well as cost-effective amperometric proton selective sensors can be powerfully applied for assuring quality from production plant to consumer level in food and pharmaceutical industries.

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