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Amperometric Determination of Ascorbic Acid at a Thin Layer Flow Cell

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Abstract □ A thin layer flow cell with cell volume of 8 μl was constructed. Diffusion currents of ascorbic acid was directly proportional to the 1/3 power of volume flow rates. A linear dynamic range was obtained at the concentration range between 10^{-7} *M* and 10^{-4} *M* of ascorbic acid with a detection limit of 10^{-8} *M*. Ascorbic acid in the multivitamin product was amperometrically determined at TLFC after simply dissolving mg range ground product in 100m*l* of pH 7.0 phosphate buffer.

Keywords □ Thin layer Flow Cell, Amperometry, Voltammetry, Ascorbic acid.

Ascorbic acid is effective as antiscorbutic agent and plays an important role in the biological oxidation-reduction system[1]. The commonly used assays are direct titrations with oxidizing agents such as iodine and dichlorophenol-indophenol[2]. Potassium ferricyanide in acid media has been also used to titrate ascorbic acid in vitamin prepartations[3]. A colorimetric method has been reported to measure the Mo blue formed by reacting ascorbic acid with ammonium molybdate.[4] An electrochemical method has been used to determine ascorbic acid in pharmaceutical dosage forms using a tubular carbon electrode[5].

Direct titrations suffer from interfering substances as well as extensive manipulations. Although colorimetric method is more sensitive than titrations, it requires procedure for making colored product and is still less sensitive than the electrochemically oxidizing method.

This paper describes construction of a thin layer flow cell (TLFC) with cell volume of 8 μl and its application for the determination of ascorbic acid in the multivitamin products at a volume flow rate of 0.6m*l*/min. Compared with a TLFC, tubular carbon electrode had a cell volume of several m*l* and employed volume flow rates between 5 and 12 m*l*/min[5]. Consequently a TLFC requires quite less amount of sample and offers capability of being used as an electrochemical detector for high performance liquid chromatograph.

EXPERIMENTAL METHODS

Thin layer flow cell

Transparent Lucite bar was machined into a cylindrical form with a dimension of diameter 3.8cm × height 3.6cm. As shown in Figure 1, rectangular flow channel was first made using a 1/32 inch drill bit. The entrance and exit channel for solution flow were widened using a #3 drill bit to the depth of 1cm in order to make thread using a 1/4-28 tap. The plugs for these threaded entrance and exit were the standard plastic tube end fittings.

A working electrode compartment was made by drilling up to the flow channel with a #17 drill bit (i.d. 3mm) and then widening this hole with a #15 flat end drill bit (i.d. 4mm) up to the position 0.1mm above the flow channel. The entrance of a working electrode compartment was threaded with a #3 drill bit and a 1/4-28 tap as written above. Graphite rod (purity 99.8%, density 1.85, Dong Yang Carbon ISEM3, Japan) purchased from the local market was machined into a cylindrical form with a dimension of dia. 4mm × h. 8mm. The side facing solution flow was polished using aluminum oxide power with particle sizes ranging between 22.5 μ -3 μ . A polished graphite electrode, soldered copper disk and wire for electrical contact and a threaded plastic plug were inserted into the compartment in order. By screwing the plastic plug on the copper disk and graphite electrode a tight pressure fit was obtained in the working electrode compartment.

A reference electrode compartment was made by drilling up to the flow channel with a 1/32 inch drill bit, widening this hole with a #15 flat end drill bit up to the position 1 mm above the channel and making entry thread as written above. A dialysis membrane with a diameter of 4 mm was inserted at the bottom of the compartment. In order to fix the

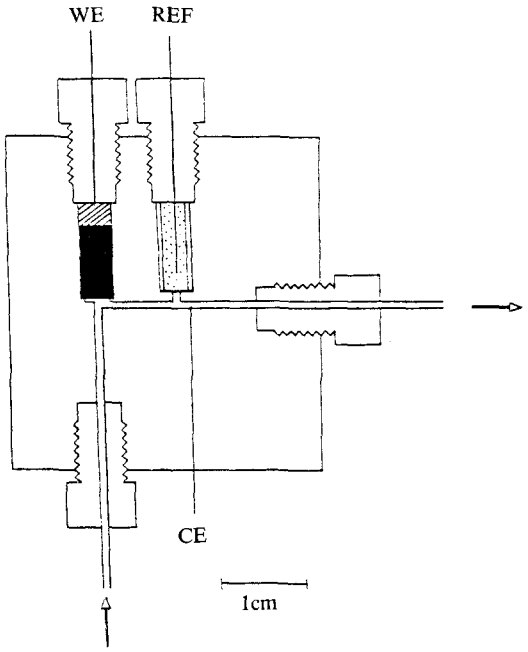

Fig. 1. Thin Layer Flow Cell
WE: graphite working electrode
REF: Ag/AgCl (Sat. KCl) reference electrode
CE: platinum wire counter electrode

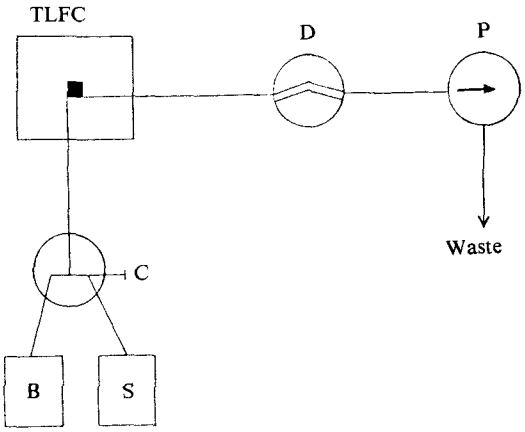

Fig. 2. Flow Stream Arrangement
B: buffer solution
S: sample solution
C: three way stop-cock
TLFC: thin layer flow cell
D: pulse damper
P: peristaltic pump

membrance to the bottom, a Teflon tube was inserted next to the membrance. Saturated potassium chloride solution was added into the Teflon tube. A threaded plastic plug having a AgCl-coated Ag wire at the center[6] was carefully screwed into the compartment by placing the AgCl-coated Ag wire in the saturated KCl solution.

A counter electrode was a platinum wire protruded a little bit into the flow channel. This was made by force-fitting the platinum wire through a thin channel drilled perpendicularly from the surface of the Lucite cell body up to the flow channel.

Both Ag/AgCl reference electrode and pt counter electrode were positioned downstream from the graphite working electrode.

Flow system

The flow stream arrangement is shown in Figure 2. A variable speed peristaltic pump (Eyela MP-3, Japan) was placed downstream from the TLFC. Thus solution was pulled into the TLFC rather than pushed. This "pull" system minimizes hold up volume before the electrochemical cell.

A glass capillary bore three way stop-cock was used to allow switching between sample solution and buffer solution. A short Teflon tubing was used to connect the exit of a three way stop-cock and the entry of the TLFC. In order to remove periodic peristaltic pump noise a large rubber tube serving as a pulse damper was placed in the flowing stream between TLFC and a pump. The volume flow rates were varied between 0.62ml/min and 2.68ml/ min.

Measurement system

All electrochemical measurements were made by employing a polarographic analyzer (EG&G Princeton Applied Research 174A, U.S.A.), a X-Y recorder (EG&G, Princeton Applied Research RE0074, U.S.A.) and a strip chart recorder (Philips PM8222, Netherlands).

Reagents

All chemicals were reagent grade. A 0.05 *M* pH 3.0 acetic acid solution was prepared by diluting acetic acid (Shinyo pure chem. Co., Japan) with doubly distilled water and adding 0.01*M* sodium nitrate (Junsei chem. Co., Japan) as a supporting electrolyte. A pH 7.0 phosphate buffer was prepared by dissolving appropriate amounts of KH_2PO_4 (Junsei chem. Co., Japan) and $Na_2HPO_4 \cdot 12H_2O$ (Yakuri pure chem. Co., Japan) in doubly distilled water to make total ionic strength of 0.2*M*. Ascorbic acid standard solution was prepared by dissolving L-ascorbic acid (Shinyo pure chem. Co., Japan) in a previously deaerated pH 3.0 acetic acid solution or pH 7.0 phosphate buffer. It was stored

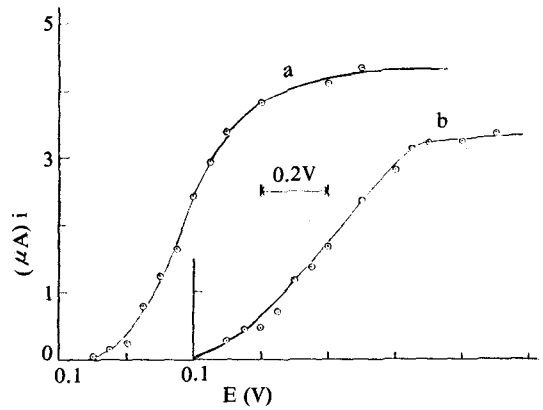

Fig. 3. Voltammograms of 1.00×10^{-3} *M* Ascorbic acid
a: ascorbic acid in pH 3.0 acetic acid solution
b: ascorbic acid in pH 7.0 phosphate buffer
Potential (E(V)) was measured *vs.* a Ag/AgCl (sat. KCl) reference electrode.

under purified nitrogen gas during the experiments. Nitrogen gas (Yugene gas, Seoul, Korea) was purified by passing it through two glass scrubbing towers; the first, containing acidified ammonium metavanadate with zinc amalgam to remove oxygen traces and the second, containing distilled water to remove acid.

Multivitamin products were purchased from local drug stores. For interference study, riboflavin, vitamin B_6 and vitamin B_{12} were donated from Dong-A pharmaceutical company.

Procedures

Before starting each experiment, a TLFC was washed by pumping doubly distilled water and was scanned from zero volt to 1.0 volt using buffer solution. When adsorption peaks appeared with a scan indicating unclean graphite electrode, it was removed from the TLFC, and then the tip of the graphite electrode was scrubbed on the coarse and fine sand papers followed by polishing with aluminum oxide. A volume flow rate was set at 0.64ml/min except experiments measuring diffusion currents depending on volume flow rates.

For voltammetric studies, both pH 3.0 acetic acid solution and pH 7.0 phosphate buffer were used to prepare 1.00×10^{-3} *M* ascorbic acid solutions. Potentials were applied to the graphite working electrode from 0.20 volt to 1.00 volt with intervals of 0.050 V in current-rising region and 0.10 V in plateau region. At each applied potential, phosphate buffer (or acetic acid solution) and ascorbic acid solution were alternatively pumped through the TLFC while measuring currents.

For amperometric studies, a potential of 0.80 volt *vs.* a Ag/AgCl reference electrode was applied to a graphite working electrode and currents were measured with buffer and ascorbic acid solution (or sample solution). For determinations of ascorbic acid in multivitamin products, an amount of granules or ground tablets was weighed in the range between 0.0010 g and 0.0030 g. It was simply dissolved in 100ml of pH 7.0 phosphate buffer, subsequently followed by amperometric measurements.

RESULTS AND DISCUSSION

Voltammetric studies

Two voltammograms are shown in Figure 3; Figure 3-a for 1.00×10^{-3} *M* ascorbic acid in pH 3.0 acetic acid solution and Figure 3-b for 1.00×10^{-3} *M* ascorbic acid in pH 7.0 phosphate buffer. Only Faradaic currents were plotted in the voltammograms. Although both voltammograms are drawn out, they reached limiting current plateau at 0.80 volt *vs.* a Ag/AgCl reference electrode.

Diffusion currents depending on volume flow rates

Diffusion currents of 1.00×10^{-4} *M* ascorbic acid in pH 3.0 acetic acid solution were measured at six different volume flow rates such as 0.62ml/min, 0.99ml/min, 1.38ml/min, 1.83ml/min, 2.23ml/min and 2.68ml/min. Figure 4 shows plots of i_d *vs.* V_f in ml/sec on a logarithmic scale. For three replicate runs, slopes are 0.36, 0.30, 0.32 with a average value of 0.33. This experimental value is in agreement with a theory in which diffusion current is directly proportional to the 1/3 power of volume flow rate for a flat electrode in an enclosed rectangular channel with fully developed laminar flow parallel to the surface[7].

Linear dynamic range

A stock solution of 1.00×10^{-4} *M* ascorbic acid

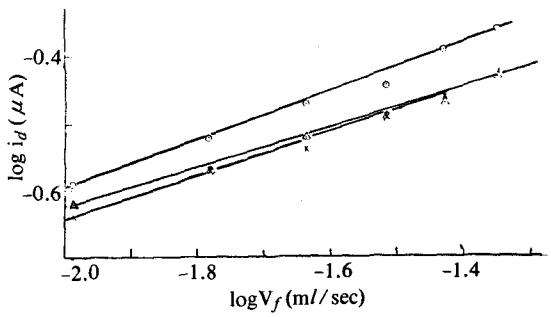

Fig. 4. Diffusion currents depending on volume flow rates

was prepared using pH 7.0 phosphate buffer. This stock solution was then appropriately diluted with pH 7.0 phosphate buffer to prepare 1.00×10^{-5} *M*, 1.00×10^{-6} *M* and 1.00×10^{-7} *M* ascorbic acid solutions. Four replicate amperometric measurements were made at each concentration. A linear plot of average i_d *vs.* concentration of ascorbic acid is shown in Figure 5 on a logarithmic scale. Relative standard deviations are less than 1.5% except 5% for 1.00×10^{-7} *M*, indicating reasonable precision of the present analytical method. A concentration of 1.00×10^{-8} *M* ascorbic acid could also be determined although it yielded diffusion currents six times larger than the expected ones from the plot of Figure 5. Relative standard deviation of 1.00×10^{-8} *M* was 13% for four measurements.

Interference study

Vitamin B complex, niacinamide, calcium pantothenate, biotin, sodium sulfite and eleven compounds commonly found in pharmaceutical dosage forms have been investigated to determine if they interfere assay of ascorbic acid at the tubular carbon electrode[5]. None of these compounds was reported to have an oxidation current in the voltage range between -0.10 V and 0.80 V *vs.* a saturated calomel reference electrode. Only sodium lauryl sulfate affected the current-voltage curve of ascorbic acid by lowering the limiting current at a concentration of 0.5 (w/v) %.

In the present study vitamin B_2, B_6, and B_{12} were checked for their interference, since Mason *et al* found none of compounds contained in multivitamin products interfered the assay of ascorbic acid based on the electrochemical oxidation at a carbon electrode. Differential pulse voltammetric and voltammetric studies of each vitamin B complex at a concentration of 1.00×10^{-4} *M* in pH 7.0 phosphate buffer did not show any oxidation current in the potential range between zero and 0.90 V *vs.* a Ag/AgCl reference electrode.

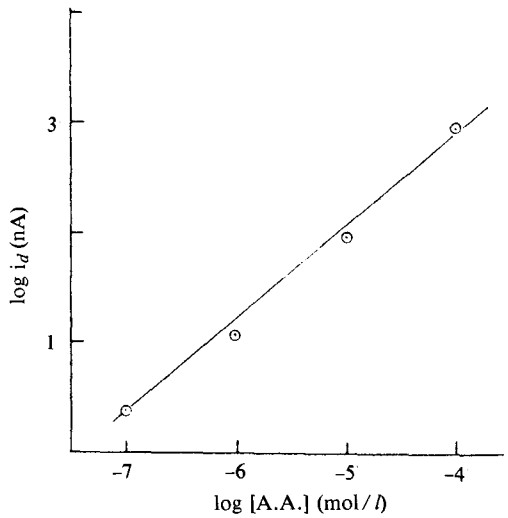

Fig. 5. Diffusion currents *vs.* concentrations of ascorbic acid

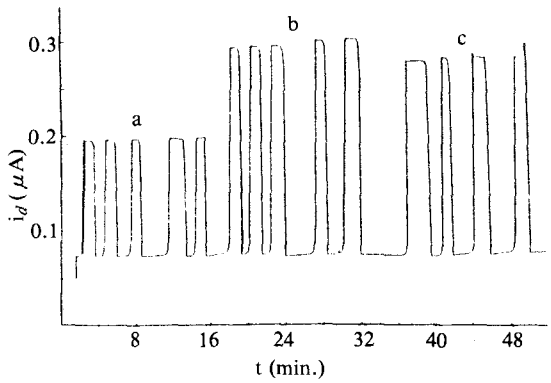

Fig. 6. Amperometric analysis of ascorbic acid in multivitamin products using TLFC.

a,b: sample solutions prepared from multivitamin products

c: 3.52×10^{-5} *M* ascorbic acid in pH 7.0 phosphate buffer

Determination of Ascorbic acid in multivitamin products

Figure 6 shows experimental data of two sample solutions and ascorbic acid standard solution at the concentration of 3.52×10^{-5} *M*. Five replicate runs for sample solutions and four replicate runs for standard solution show good precision of this method. Flat current plateau for each measurement indicates stability of a graphite working electrode in TLFC. It is not necessarily required to wait until getting the current plateau. If fast analysis is required, a three way stop-cock may be switched from sample to buffer as soon as current reaches its maximum value. From the calibration curve of standard ascorbic acid solutions, concentrations of ascorbic acid in the sample solutions were found. The amount of ascorbic acid per a tablet or a package was then calculated and compared with the amount declared by the pharmaceutical companies as shown in Table I.

CONCLUSION

Thin layer flow cell containing a graphite work-

Table I. Ascorbic acid in multivitamin products

No	Amount (mg) declared per a tablet	Experimental data ave. ± S.D. (mg) for 3 replicate runs	Components besides Ascorbic acid
1	200	205.2 ± 4.3	Vit. B_2, B_6
2	500	510.0 ± 10.4	Vit. B_1, B_2, B_6, B_{12} niacinamide, Ca-pantothenate
3	500	490.0 ± 7.5	Vit. B_2 Ca-pantothenate
4	500*	528.0 ± 3.7	Vit. B_6 Ca-pantothenate
5	500	523.0 ± 10.0	Ca-pantothenate

* mg per a package

ing electrode, a Ag/AgCl reference electrode and a platinum wire counter electrode with cell volume of 8 μl was constructed. Diffusion currents of ascorbic acid was directly proportional to the 1/3 power of volume flow rates. Since 1.00×10^{-7} M ascorbic acid was easily determined at a volume flow rate of 0.64 ml/min and two minutes were enough to reach the current plateau, twenty nanogram of ascorbic acid may be analyzed with the present amperometric method using a TLFC. More concentrated ascorbic acid solution up to 1.00×10^{-4} M could be determined with good precision (RSD less than 1.5%). Ascorbic acid in the pharmaceutical product was analyzed by simply dissolving ground product in pH 7.0 phosphate buffer to make concentration in the order of 10^{-5} M and measuring diffusion currents at TLFC.

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