Determination of Ascorbic Acid in Pharmaceutical Dosage Forms Based on Oxidation at the Tubular Carbon Electrode

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Abstract □ A method for the determination of ascorbic acid in pharmaceutical dosage forms based on electrochemical oxidation at the tubular carbon electrode is presented. This method is highly specific and may be used to determine ascorbic acid in the presence of other vitamins commonly found in multivitamin products. A comparison with two commonly employed procedures of analysis shows this new method to have fewer manipulative steps and to give comparable precision and accuracy. Although automation was not used in this study, the method could readily be incorporated in automated systems because it employs the technique of continuous analysis in flowing streams.

Keyphrases □ Ascorbic acid in multivitamin dosage forms—analysis, tubular carbon electrode oxidation □ Tubular carbon electrode—oxidative analysis, ascorbic acid in multivitamin preparations □ Multivitamin dosage forms—analysis, ascorbic acid, tubular carbon electrode oxidation

A wide variety of chemical methods for the determination of ascorbic acid were comprehensively reviewed in the texts edited by Freed (1) and Higuchi and Brochmann-Hanssen (2). Direct titrations using oxidizing agents such as iodine and 2,6-dichloroindophenol are often satisfactory if reducing interferences can be removed. Other methods commonly used to assay ascorbic acid in pharmaceuticals are based on the reaction of the vitamin with suitable reagents to form colored products. All of these methods suffer from a lack of specificity and/or require extensive manipulations and separation techniques. Automation of the manipulative steps is one way in which the colorimetric methods can be simplified (3); however, the possibility of absorbing interferences still remains.

Another analytical method which may be used to determine ascorbic acid is polarography. The oxidation of ascorbic acid at the dropping mercury electrode and the advantages of this method over other commonly employed methods were summarized by Brezina and Zuman (4). These authors concluded that polarography is more specific and requires fewer steps in sample preparation than colorimetric and titration methods. The main limitations of polarography have been the relatively low sensitivity and the limited anodic voltage range of the mercury electrode, especially in the presence of chloride ion.

The recently introduced tubular carbon electrode (5) is more sensitive than the dropping mercury electrode and has a greater anodic voltage range. In addition, the tubular electrode is used to make continuous measurements on a flowing stream of sample solution and thus is readily incorporated in automated or semiautomated systems. In this paper, a method for the determination of ascorbic acid in pharmaceutical dosage forms containing other vitamins is presented and compared to two commonly used methods. This new method is based on continuous analysis in flowing streams by oxidation of ascorbic acid at the tubular carbon electrode.

EXPERIMENTAL

Instrumentation—The electrode assembly and a diagram of the flow system are shown in Fig. 1. The tubular carbon electrode described previously (5) was modified by adding a platinum counter electrode and replacing the salt bridge with a saturated calomel reference electrode[1]. Carbon rods of about 10 mm. were used throughout this study. Solutions were pumped by means of a peristaltic pump[2]. Tygon tubing (formula R3603), 0.15 cm. (0.0625 in.) i.d., was used in the pump and the remainder of the flow system. A

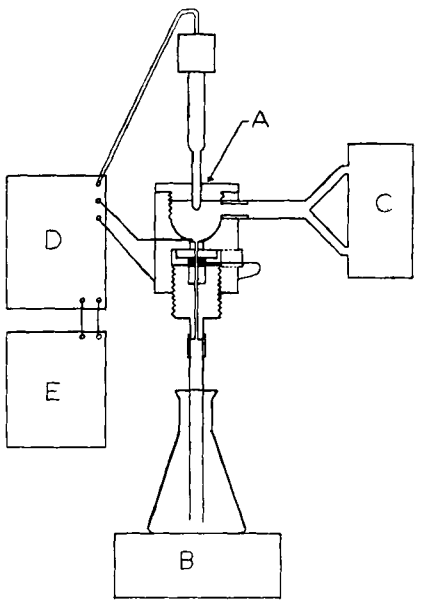

Figure 1—*Flow system and tubular carbon electrode. Key: A, electrode assembly; B, magnetic stirrer; C, pump; D, polarograph; and E, recorder.*

[1] Coleman No. 3-152.
[2] Harvard Apparatus model 1201.

Table I—Vitamin Content of Products Tested

Products	Components
Vitamin C tablets	
A	Ascorbic acid
B	Ascorbic acid
Multivitamin tablets	
C	Vitamin A, D, C, B_1, B_6, B_{12}, biotin, niacinamide, calcium pantothenate
D	Vitamin A, D_2, C, B_1, B_2, B_6, B_{12}, niacinamide
Multivitamin liquids	
E	Vitamin A, D_2, C, B_1, B_2, B_6, B_{12}, niacinamide
F	Vitamin A, D, E, C, B_1, B_2, B_6, B_{12}, niacinamide, pantothenic acid

polarography system[3] was used to record the current–voltage and current–time curves.

Chemicals—All chemicals employed were of the highest quality commercially available. Standard solutions of ascorbic acid were prepared using the powder[4]. All vitamin products were purchased from local pharmacies. All solutions used in the electrochemical studies were prepared using 0.05 *M* acetic acid (pH 3.0), with 0.01 *M* sodium nitrate as supporting electrolyte. The acid medium was used to suppress autoxidation of the ascorbic acid.

Procedures—The tubular carbon electrode was cleaned each day with ethyl acetate, and a calibration plot of limiting current *versus* ascorbic acid concentration was prepared at the volume flow rate selected for that day. Volume flow rates between 5 and 12 ml./min. were commonly employed and were controlled to ±1%. These calibration curves were used directly to calculate the ascorbic acid concentration in unknown samples. Standard and sample solutions were prepared to contain approximately 1×10^{-4} *M* ascorbic acid in the following manner. Solid dosage forms were weighed and powdered, and a weighed aliquot was diluted directly in the acetic acid medium. Liquid dosage forms were diluted directly; for viscous liquids the samples were quantitatively transferred from 2.0-ml. volumetric flasks. These dilutions (usually 1:10,000) were pumped directly through the tubular carbon electrode. If the sample solution contained undissolved solids, a pledget of glass wool was placed over the intake tube to act as a filter. A current–voltage curve was obtained for each product by scanning anodically from zero volts *versus* the saturated calomel reference electrode (SCE) at 0.5 v./min. Once the current–voltage curve for a product indicated no interferences, the electrode potential was set at 0.75 v. *versus* the reference electrode, and only the limiting current was measured for subsequent samples. Intermittent ascorbic acid standards were run to check the electrode calibration.

To determine if other vitamins or compounds commonly found in pharmaceutical dosage forms alter the current–voltage curve of ascorbic acid or are oxidized at the tubular carbon electrode, the following studies were performed. A 10^{-4} *M* solution of each water-soluble vitamin[5] listed in Table I was prepared with and without 10^{-4} *M* ascorbic acid. Each solution was pumped through the tubular carbon electrode, and the voltage was scanned from −0.10 to 0.80 v. *versus* the SCE. This experiment was repeated for 10^{-4} *M* sodium sulfite solutions and also for 0.50% solutions or suspensions of the compounds listed in Table II.

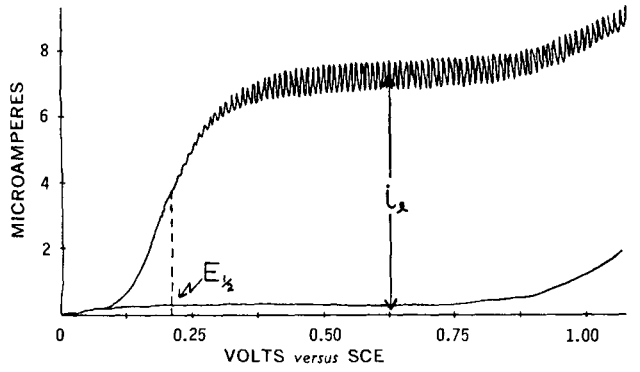

Figure 2—*Ascorbic acid current–voltage curve: a 2.6 × 10⁻⁴ M solution in 0.05 M acetic acid; flow rate, 11 ml./min.; scan rate, 0.5 v./min.*

Table II—Compounds Tested for Interference with Ascorbic Acid Assay Using Tubular Carbon Electrode

Talc	Magnesium stearate
Dextrose	Sodium lauryl sulfate
Sucrose	Cornstarch
Gum tragacanth	Sorbitan monooleate[a]
Acacia	Polysorbate 40[b]
Gelatin	

[a] Span 80. [b] Tween 40.

RESULTS AND DISCUSSION

The current–voltage curve shown in Fig. 2 was typical of the data obtained for oxidation of ascorbic acid at the tubular carbon electrode. For all but one product, the half-wave potential of 0.23 ± 0.01 v. *versus* the SCE and the shape of the current–voltage curve did not change during a day's work. For the one product, the wave became less sharp with successive runs and periodic cleaning of the electrode with ethyl acetate was necessary, probably to remove absorbed dye from the electrode surface.

The limiting current was linear with respect to ascorbic acid concentration over the 10^{-6}–10^{-3} *M* range. A typical calibration plot had a slope of 7.0 μamp./10^{-4} *M* with a zero intercept. A plot of logarithm limiting current *versus* logarithm flow rate had a slope of 0.33, which agrees with theoretical considerations of the tubular electrode geometry (6).

Although the concentrations tested greatly exceeded those expected when the pharmaceutical dosage forms are diluted for analysis, none of the vitamins listed in Table I, sodium sulfite, or the compounds listed in Table II had an oxidation current at the tubular carbon electrode under the conditions studied; only sodium lauryl sulfate affected the current voltage curve of ascorbic acid. Sodium lauryl sulfate (0.50% solution) lowered the limiting current of the 10^{-4} *M* ascorbic acid solution by 20%. This effect diminished as the concentration of the sodium lauryl sulfate was lowered and was less than 1% at a concentration of 0.01% w/v.

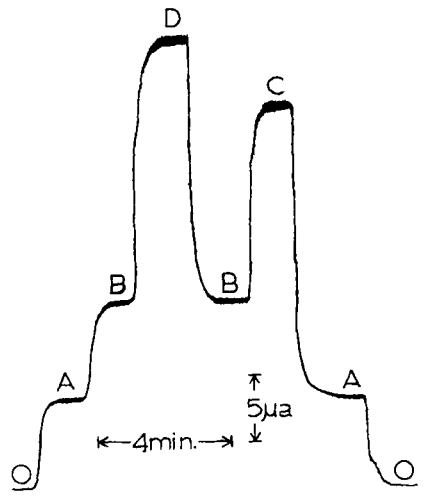

Figure 3—*Ascorbic acid current–time recorder trace. Key: O, 0.05 M acetic acid; A, 1 × 10⁻⁴ M; B, 2 × 10⁻⁴ M; C, 4 × 10⁻⁴ M; and D, 5 × 10⁻⁴ M. See text for details.*

[3] Health EU-401.
[4] Obtained from Baker Chemical Co., Phillipsburg, N. J.
[5] Water-solubilized α-tocopheryl acetate of U.S.V. Pharmaceutical, New York, N. Y., was used to prepare the vitamin E solutions.

Table III—Ascorbic Acid Analysis

Product	Amount Declared per Dose, mg.	Mean Amount (mg.) ± SD^a — TCE[b] Method	Mean Amount (mg.) ± SD^a — Independent Method
A	100	115.13 ± 2.78	111.81 ± 11.37[c]
B	100	97.77 ± 5.36	102.98 ± 6.38[c]
C	75	77.98 ± 2.78	79.32 ± 2.99[c]
D	75	76.32 ± 2.09	80.74 ± 2.65[c]
E	50/5 ml.	54.10 ± 0.44	53.81 ± 0.20[d]
F	60/0.6 ml.	61.41 ± 0.52	62.00 ± 0.21[d]

[a] Calculated on basis of five doses; each assayed by TCE and independent method. [b] Tubular carbon electrode method. [c] *Reference 8.* [d] *References 9* and *10.*

Several compounds commonly used as antioxidants undergo oxidation at carbon electrodes (7), but no interference by these compounds with the ascorbic acid assay is expected for two reasons:

1. The concentrations of antioxidants is usually low and will not be detectable in the samples diluted (usually 1:10,000) for analysis.

2. The half-wave potentials for most (7) antioxidants differ sufficiently from that of ascorbic acid to permit separation of the waves.

The absence of interferences in the multivitamin products used in this study is indicated by the fact that the shapes of the current–voltage curves for the products were identical to those for standard ascorbic acid solutions.

The vitamin content of the products studied is given in Table I. No alterations of the ascorbic acid current–voltage curves resulted from the other vitamins contained in these products. Table III shows the mean ascorbic acid content of these products determined by assaying five doses of each. Aliquots of each dose were assayed using both the tubular carbon electrode and the methods indicated in the table. The data for the solid dosage forms show variations in the content of the tablets as well as the relative precision of the methods used, while the data for the liquid dosage forms indicate only the relative precision of the methods. From this study it may be concluded that the tubular carbon electrode method gives comparable or superior precision and accuracy when compared to other methods commonly used in the analysis of ascorbic acid. The reproducibility of the response of the tubular carbon electrode was determined by assaying a single sample dilution five times. A standard deviation of the mean of 0.91% was found.

A recorder trace of limiting current *versus* time for a series of standard ascorbic acid solutions is shown in Fig. 3. This recorder trace indicated the time required to assay a single sample. Once the sample solutions had been prepared, between 25 and 30 ascorbic acid determinations could be performed each hour. Although automation was not employed in the present study, the flowing stream methodology could readily be incorporated in automated or semi-automated systems.

In comparison to the tubular carbon electrode method, the iodine titration was less specific while the colorimetric methods involved several manipulative steps and thus required a much longer time. In summary, it is concluded that the method introduced in this paper has comparable precision, accuracy, and freedom from interferences when compared with methods presently available for the analysis of ascorbic acid in multivitamin products. In addition, the method is faster and less complicated to perform.

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TLC of Coumarin Anticoagulants

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Abstract □ A simple, rapid, and reproducible TLC procedure for the separation and identification of five 4-hydroxycoumarin derivatives used as anticoagulants and rodenticides is presented.

Keyphrases □ Coumarin anticoagulants—TLC separation and identification □ Anticoagulants, coumarin—TLC separation and identification □ TLC—separation, identification of coumarin anticoagulants

The separation and identification of coumarin derivatives used as anticoagulants and rodenticides are of interest to the forensic toxicologist and the analytical chemist. Internal hemorrhages found in corpses upon autopsy is often considered presumptive evidence of death arising from intoxication by coumarin anticoagu-