measurements of diameter were replicated within 0.08 mm or within 2% of potency.

Using the equipment, procedures, and designs described here, proper computational procedures, and a very favorable assay system, a single determination (three plates) may be expected to be within 2% of the true value. Furthermore, no increase in effort or expense is required. As shown by the consideration of the influence of error of zone measurement upon error in sample potency, the tests cannot be greatly improved. The modified method requires fewer plates and less operator time than the standard FDA method. The accuracy of this plate method should be compared with the five- to 10-fold greater accuracy obtainable from an automated method for turbidimetric assays (14). Such differences in inherent accuracy is one reason to prefer turbidimetric methods when they are applicable.

The two-dose design of FDA was a less satisfactory method than the single-dose assay because it seemed to be excessively sensitive to the slope of the calibration line.

## REFERENCES


(1) L. F. Knudsen and W. A. Randall, *J. Bacteriol.*, **50,** 187(1945).

(2) *Fed. Regist.*, **10,** 11478(1945).

(3) F. Kavanagh, *J. Pharm. Sci.*, **63,** 1459(1974).

(4) "Code of Federal Regulations," Title 21, Part 141.1 and 141.101, 1949.

(5) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics," Medical Encyclopedia, New York, N.Y., 1955.

(6) J. Deutschberger and A. Kirshbaum, *Antibiot. Chemother.*, **9,** 752(1959).

(7) *Fed. Regist.*, **22,** 10443(1957).

(8) W. H. Schmidt and A. J. Moyer, *J. Bacteriol.*, **47,** 199(1944).

(9) K. E. Cooper and A. H. Linton, *J. Gen. Microbiol.*, **7,** 8(1952).

(10) N. R. Kuzel and H. F. Coffey, American Society for Microbiology, Miami meeting, 1966.

(11) F. Kavanagh, in "Analytical Microbiology," vol. 2, F. Kavanagh, Ed., Academic, New York, N.Y., 1972, chap. 2.2.

(12) K. E. Cooper, in "Analytical Microbiology," F. Kavanagh, Ed., Academic, New York, N.Y., 1963, chap. 1.

(13) F. Kavanagh, *Advan. Appl. Microbiol.*, **2,** 65(1960).

(14) F. Kavanagh, *J. Pharm. Sci.*, **60,** 1859(1971).


## ACKNOWLEDGMENTS AND ADDRESSES


Received May 7, 1973, from *Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication December 10, 1974.

The author thanks Helen Caster for preparing the assays and measuring the zone sizes, Richard Byers for constructing the special computer program, Sue Barnes for the analyses of variance, and Linda Roush for putting the manuscript into legible form.


# Automated Constant-Current Coulometric Assay System for Ascorbic Acid and Sodium Ascorbate


**S. A. MOROS ˣ, C. M. HAMILTON, J. E. HEVERAN, J. J. DONAHUE, and S. OLIVERI-VIGH**



**Abstract** □ The performance of an automated constant-current coulometric system for the assay of ascorbic acid and sodium ascorbate is described. After loading, it is capable of analyzing 25 samples and printing out the titer values with no operator attention for 2.5 hr. Under optimum conditions, ascertained by evaluating various electrochemical parameters, the accuracy and precision (95% ts) were found to be ±0.3%.



**Keyphrases** □ Ascorbic acid and sodium ascorbate—automated constant-current coulometric assay system □ Coulometry, constant current, automated—analysis, ascorbic acid and sodium ascorbate


Coulometric analysis of ascorbic acid, using both controlled-potential and constant-current methods, was reported previously. The constant-current method with iodine generated at the anode was used for the determination of small quantities (about 0.5 mg) of ascorbic acid with biamperometric ("dead-stop") end-point detection (1). The reproducibility was found to be ±0.1% *SD*. A platinum anode controlled at +1.1 v *versus* the saturated calomel reference electrode was used to oxidize 15–100 mg of ascorbic acid within 0.5–1 hr, with an average accuracy of ±0.7 mg (2).

The constant-current method employing electrogenerated iodine affords the electrochemical equivalent to the USP assay and offers a time advantage over the potentiostatic technique. Therefore, it was chosen for automation. A modular system (Fig. 1) consisting of an electrode station, electrolyte dispenser, current source, sample changer, control module, and

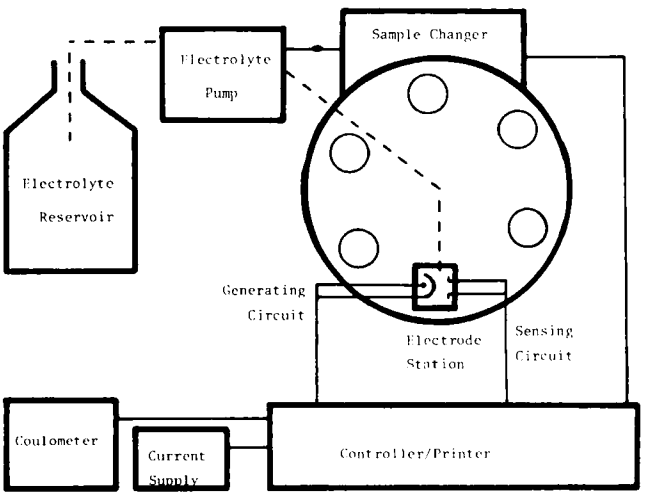


**Figure 1**—*System block diagram and interrelation of components.*

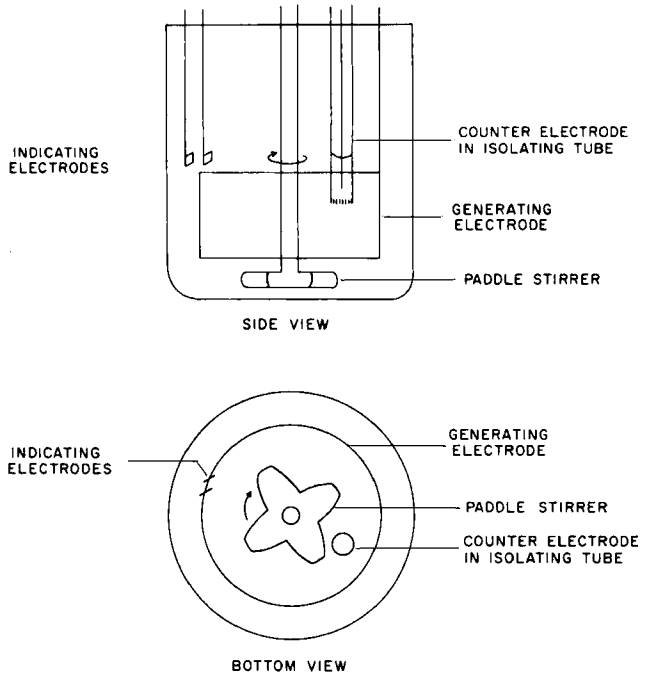


**Figure 2**—*Cell geometry.*

printer was assembled, and a study of various electrochemical parameters was undertaken to define the optimum conditions for the assay.

## EXPERIMENTAL

**Electrode Station**—The electrode station was a cluster of components including the electrolyte delivery tip, the stirrer, and the generating and sensing electrodes, all mounted on a stationary arm bearing the stirring motor and appropriate electrical wiring. The glass stirrer consisted of four 2 mm-thick blades or paddles (each ~10-mm diameter) fused to the end of a 5-mm diameter glass rod to form a flat cloverleaf. At 560 rpm, efficient stirring was obtained without a vortex being formed or air entrained as observed with propeller-shaped stirrers.

The relative positions of the stirrer and electrodes, *i.e.*, cell geometry, are indicated in Fig. 2. The generating electrode (anode) was a cylinder of platinum screen (40-mm diameter × 25-mm height) coaxial with the shaft of the stirrer whose blades were positioned just below this electrode. The counter electrode (cathode) was a 5-mm length of platinum wire immersed in a few milliliters of electrolyte contained in a sintered-glass isolation tube located within the anode cylinder. The indicating electrode pair (5-mm platinum foil squares with the faces set parallel and 2 mm apart) was positioned outside the field between the anode and cathode.

**Electrolyte Dispenser**—A metering pump[1] delivered the requisite volume (80 ml) of electrolyte from a reservoir prior to each titration. The electrolyte, 0.5 *M* KI in 0.4 m*M* $H_2SO_4$, was treated with a steady stream of nitrogen passing through a submerged sintered-glass gas-dispersion tube to prevent air oxidation.

**Current Source**—A constant-current source, based on conventional operational amplifier circuitry, provided 25–200 mamp with an accuracy of ±0.2% at a voltage compliance of 18 v. The 10-μamp polarizing current for the indicating electrodes was similarly obtained from conventional transistor circuitry.

**Sample Changer**—By means of an elevator mechanism, a turntable[2] with 50 positions for 100-ml beakers presented 25 preweighed samples and rinse water alternately to a fixed electrode station where the electrolysis was carried out in the sequence given under *System Operation.* As each revolution was completed, the entire turntable was automatically displaced relative to the electrode station to present beakers in the next circle. After the last circle of samples was presented, the turntable returned to the starting position and the "end of cycle" signal was displayed.

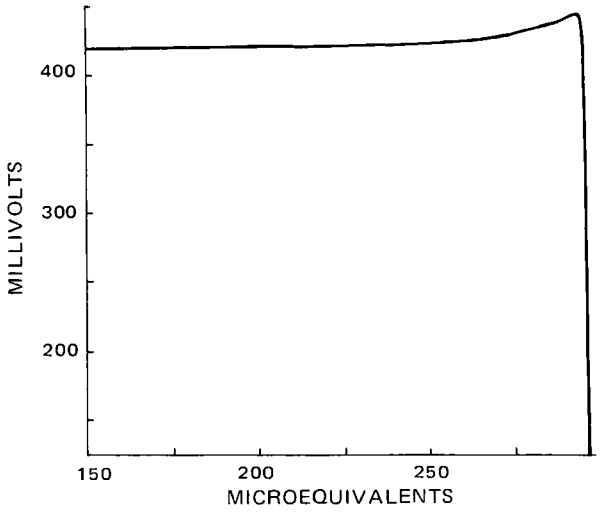


**Figure 3**—*End-point region of titration curve.*

**Control Module and Printer**—The operation of the entire system was controlled by a printing coulometer[3] incorporating the aforementioned current sources, a sensing and control circuit, a coulometer, and a printer. The sensing circuit displayed the potential across the indicating electrodes and compared it with a preset end-point potential, ending the titration when the potentials became equal. An adjustable time delay of up to 180 sec in the sensing circuit precluded false end-point signals, which could result from switching transients occurring at the start of the titration. An electronic integrator, accurate to 0.1%, provided a continuous digital readout in microequivalents and drove a printer for the final readout.

**System Operation**—The listed components formed a constant-current coulometric system operating in the following sequence. As each sample was presented to the electrode station, the electrolyte dispenser delivered electrolyte and the stirrer was activated. After 30 sec of stirring to dissolve the sample, the generating current was started to begin the titration. At the end-point, the control module stopped the generating current, printed both the sample number and titer in microequivalents, reset the integrator and printer, and sent a signal to the sample changer. This procedure resulted in the titrated sample being replaced by a beaker of rinse water for washing the electrodes and the next preweighed sample being presented to the electrode station.

**Electrochemical Parameters**—The generating current was selected to provide the highest titration rate possible consistent with 100% current efficiency for iodine generation. This current corresponded to a current density of 5 mamp/cm$^2$ of anode area in the 0.5 *M* KI electrolyte employed and did not increase significantly at higher concentrations. Above this current, the precision of the titration decreased and gas formation became evident at the anode.

The remaining electrochemical parameters were dictated by the titration curve; the end-point region is depicted in Fig. 3. The potential between the indicating electrodes rises gradually (20–30 mv) during the titration and more sharply just before excess iodine appears, whereupon the potential falls rapidly almost to zero. For the end-point, the comparator voltage was set 40 mv below the peak potential after several trials. This choice allowed for some variation in electrode response during a run but minimized the value and variability of the blank correction required for a larger difference.

The response of the indicating electrodes became less stable and the potential difference between them decreased during a series of titrations of ascorbic acid unless the electrolyte was acidified. The response was degraded during titration of sodium ascorbate to the

[1] Vision Laboratories Beaker Butler.
[2] Vision Laboratories Li'l Squirt.
[3] Ryaby Associates.

Table I—Precision Studies

| Sample | Weight, mg | Current, mamp | Electrolyte Sulfuric Acid Concentration, m*M* | Degrees of Freedom | Precision, % (95% Confidence Level) |
|---|---|---|---|---|---|
| Ascorbic acid | | | | | |
| A | 30 | 200 | 0 | 46 | 0.4 |
| B | 30 | 200 | 0 | 11 | 0.4 |
| A | 30 | 100 | 0 | 17 | 0.3 |
| B | 30 | 100 | 0 | 67 | 0.3 |
| C | 30 | 120 | 0 | 30 | 0.3 |
| B | 100 | 120 | 0 | 8 | $0.1_5$ |
| D | 30 | 125 | 0.4 | 22 | 0.2 |
| D | 400 | (USP manual titration) | | 14 | 0.6 |
| Sodium ascorbate | | | | | |
| E | 30 | 120 | 0.1 | 26 | 0.5 |
| F | 30 | 120 | 0.1 | 17 | 0.9 |
| E | 30 | 120 | 0.4 | 50 | 0.3 |

extent that only a few samples could be titrated. In both cases, the formation of a coating on the indicating electrodes appeared to be involved; rinsing with a few drops of concentrated sulfuric acid restored the original electrode condition. Incorporating a trace of acid in the electrolyte minimized electrode contamination, so a full load of 25 samples could be titrated with the same end-point setting.

**Other Analytical Parameters**—The blank could not be directly determined because the potential obtained in acidified potassium iodide electrolyte was substantially lower than that obtained in the presence of sample. Instead, the blank was determined by extrapolating to zero sample weight, having titrated various weights of sample. Typical blank values were equivalent to 0.5% assay, but variations in these values were obtained with lots and suppliers of potassium iodide. These values were confirmed by manual titration with 0.1 *N* $I_2$ solution; the values ranged from 2 to 6 microequivalents of iodine/80 ml of electrolyte, values that would, if neglected, adversely affect the assay values.

Stability studies of acidified 0.5 *M* KI sparged with nitrogen showed no significant deterioration for up to 3 weeks.

A 30-mg sample size represents a compromise among such factors as titration time, weighing error, blank variability, and sample homogeneity. When using the maximum current for the electrochemical conditions (electrode area, stirring, temperature, *etc.*) employed, 5 min was required for titrating samples of the size recommended.

## RESULTS AND DISCUSSION

Numerous determinations of the precision of the method were made on replicate samples of various lots of ascorbic acid or sodium ascorbate (Table I). Generating current levels of 200 mamp afford somewhat poorer precision than 120-mamp levels in the titration of ascorbic acid. Increasing the sample size to 100 mg (from the recommended 30 mg) improves the precision at the cost of prolonged titration time. The addition of acid permits the titration to be carried out in a reasonable time with excellent precision, ±0.2% (95% probability). Similarly, sodium ascorbate titrations are best carried out at the 0.4 m*M* acid concentration recommended. In this case, the use of a slightly lower acid concentration significantly reduces the precision of the measurement. In summary, the precision of the recommended method exceeds that of the USP XVIII (3) manual titration (different operators weighed and titrated the samples to avoid bias).

The automated system, standardized electronically, was compared to chemical standardization by ascorbic acid and arsenic trioxide to determine the accuracy of the system. Ascorbic acid, assayed as 100.0% by manual iodometric titrations, gave a coulometric value of 99.7%. Arsenic trioxide (National Bureau of Standards), when dissolved in base, acidified, and titrated, gave an average of 100.2% for 12 determinations.

The automated system described is capable of analyzing 25 samples in less than 4 hr, apportioned as follows: electrolyte and rinse preparations, 30 min; sample weighing, 35 min; titrator operation (100 mamp), 2.5 hr; cleanup, 15 min; and calculations, 10 min. The total time can be reduced by approximately 30 min if the remaining samples are weighed as the first few are being titrated. In any event, the operator's attention is required for only 90 min to carry out the auxiliary functions.

This automated system is similar in principle to the USP method and: *(a)* is equivalent to, or exceeds, the manual method in precision and accuracy; *(b)* is significantly less time consuming than the manual method; and *(c)* eliminates the need for preparation, standardization, and storage of the titrant.

While the prototype system is being evaluated for other applications, an equivalent system is in daily operation for monitoring the quality control of ascorbic acid production. Incorporating automatic weighing equipment and digital output capability would permit calculations to be performed by a computer, virtually eliminating all sources of human error.

## REFERENCES

(1) W. Jedrzejewski, *Chem. Anal. (Warsaw)*, **2**, 453(1957).

(2) K. S. V. Santhanam and V. R. Krishnan, *Anal. Chem.*, **33**, 1493(1961).

(3) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 51.

## ACKNOWLEDGMENTS AND ADDRESSES

Received July 29, 1974, from the *Analytical Research Laboratory, Quality Control Department, Hoffmann-La Roche Inc., Nutley, NJ 07110*

Accepted for publication December 10, 1974.

[x] To whom inquiries should be directed.