18. Vitamin Methods

4. A Simple Potentiometric Method for Determining Ascorbic Acid, suitable for use with Coloured Extracts

By Leslie J. Harris, L. W. Mapson and Y. L. Wang, *From the Nutritional Laboratory, University of Cambridge and Medical Research Council*

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In the preceding paper further evidence was given of the reliability of the chemical test for estimating ascorbic acid [Harris & Ray, 1933; Birch *et al.* 1933], depending on its extraction in a strongly ionizing acid in such a manner as to prevent oxidation, followed by a rapidly performed titration at a highly acid reaction against 'Tillmans's indicator', 2:6-dichlorophenolindophenol. As there mentioned, the determination of the end-point is more difficult when, as occasionally happens, coloured extracts have to be titrated, e.g. from certain deeply pigmented fruits such as black currants. In their original description of the procedure, Birch *et al.* [1933] referred to the use of electrometric measurement as an alternative to the usual visual observation of the change in colour of the redox indicator at the end-point of the titration. Our aim in this paper is to discuss further the theory of the electrochemical reaction, and in particular we describe a potentiometric procedure, employing a novel type of electrode, which furnishes a simple and convenient substitute for use when the visual method is unsuitable. It circumvents any difficulties due to the presence of pigments or turbidity in the extract; the results correspond well with those observed colorimetrically, but are even more precise, duplicate determinations on solutions containing 0.2–0.02 mg./ml. being found to differ by no more than about 0.2–2 %, i.e. the order of accuracy is the same as that normally attaching to the volumetric apparatus employed, burettes, pipettes, measuring flasks etc. The method has been in use for routine determinations in this and other laboratories for the past 2–3 years.

Previous methods proposed for coloured extracts

Chloroform method. McHenry & Graham [1935] suggested titration in the presence of a layer of chloroform which selectively dissolves any excess of dye but not the interfering pigments; by a process of trial and error the end-point could thus be read in the chloroform layer. The technique, however, is tedious and requires considerable experience. Moreover, certain interfering pigments, such as those present in strawberries and red pepper, are unfortunately soluble in the chloroform.

Photoelectric method. Bessey [1938] suggested a photoelectric process for estimating vitamin C in turbid and coloured solutions, based on the original method of Mindlin & Butler [1937] for determining vitamin C in plasma. In this method the decrease in colour of 2:6-dichlorophenolindophenol produced by the addition of an amount of vitamin C extract insufficient to cause complete reduction of the dye is measured by means of a photoelectric colorimeter. The procedure is somewhat laborious as it involves three separate measurements for a single determination. A more serious objection is that the 'blank coloration' due to the natural pigment may be a very large fraction of the total colour to be measured, so that the percentage error may be considerable. The potentiometric method, on the other hand, is concerned with a specific property of the reaction which remains unmasked by the presence of fortuitous colours.

Electrometric determinations. Following the suggestion of Birch *et al.* [1933], papers describing potentiometric measurements have been published by Becker & di Gleria [1937] and by Kirk & Tressler [1939]. Becker & di Gleria used a platinum plate as the measuring electrode. An inspection of their titration curves reveals a continuous drift in potential throughout the whole course of the titration with the dye, which makes it impossible to locate the end-point of the titration with accuracy. Kirk & Tressler [1939] employed a bimetallic electrode of platinum and tungsten and measured the change of potential by means of a proprietary instrument termed a 'titrimeter'. Their results are open to the same criticism as are those of Becker & di Gleria [1937].

Fig. 1. Flat base and sharp end-point obtained by present method (curve 1), and for comparison the drifting potentials and indistinct end-points obtained by methods of Becker & di Gleria (curves 2 and 3) and Kirk & Tressler (4).

Fig. 1 shows the drifting potential and indistinct end-points by these methods as compared with the stable potential and sharp end-point given by the procedure recommended in this paper. The experiencing of these difficulties of unstable potentials and ill-defined end-points has probably deterred other workers from making general use of a potentiometric method for estimating ascorbic acid.

EXPERIMENTAL

We began our investigation with a bright platinum electrode, using the apparatus shown in Fig. 2.

Preliminary experiments with a bright platinum electrode

The procedure was briefly as follows. A measured quantity of the extract to be examined was placed in tube *F*. With the electrode and agar bridge in position, and N_2 or CO_2 bubbling through the solution at a rapid constant rate, a standardized solution of 2:6-dichlorophenolindophenol was added from the burette, 0.1 ml. or more at a time, and the reading of the potentiometer taken after each addition. Towards the end-point of the titration the dye was added at a slower rate. The results were plotted on a graph, and the end-point of the titration was taken as the point of intersection of two tangents, one drawn along the line connecting the basal potentials before the end-point and the other along the part of steepest rise of potential after the end-point.

During the course of the investigation, the following difficulties were encountered: (1) the initial potential of the bright platinum electrode in a solution of 5% metaphosphoric acid containing the vitamin was found to depend upon its pre-treatment and might vary from 100 to 600 mV. when measured against a saturated calomel half-cell; (2) the electrode took a long time to reach a true equilibrium with the solution; (3) there was usually a continuous drift of potential during the course of the titration. This last finding agrees with the observation of the other workers mentioned above. In view of these objections, we considered it better to abandon the use of bright platinum and to search for a more suitable electrode.

Fig. 2. Apparatus used for potentiometric determination of ascorbic acid. *A*, saturated-KCl calomel half-cell; *B*, connecting vessel with saturated KCl; *C*, agar bridge; *D*, CO_2 or N_2 bubbling tube for agitating extract during titration; *E*, microburette; *F*, vessel containing extract; *G*, needle electrode.

Requirements of a perfect electrode

An ideal electrode for the purpose would be one that fulfils the following requirements:

(1) it must give in a metaphosphoric acid extract of the vitamin a reproducible e.m.f. which does not change appreciably with each addition of the dye until the end-point of titration is reached; (2) it must be capable of attaining equilibrium with the surrounding solution virtually instantaneously; (3) the initial e.m.f. should be low compared with the e.m.f. after the addition of an excess of the dye, so that the end-point can readily be determined, and should preferably be controllable; (4) the response of the electrode to an excess of the dye should be prompt and reproducible.

The Hg-Pt double electrode

With the above considerations in mind we tried out a number of different electrodes including gold, silver and mercury. Gold resembled platinum in showing the continuous drift of potential, while mercury gave only a slight response to an excess of the dye (10–20 mV.). It was found that an electrode consisting of a bright platinum wire very thinly coated electrolytically with mercury answered most of the requirements listed above. The e.m.f. of this electrode in a metaphosphoric acid extract of vitamin C can be regulated to any desired value within certain limits by the addition of halogen ions, and is not changed appreciably with each addition of the dye until the end-point of titration is reached, when it responds promptly with a large rise of e.m.f.

Preparation of the electrode

An ordinary bright platinum electrode is cleaned with warm 6N HNO₃, followed by alcoholic KOH, and rinsed with distilled water. It is plated by being immersed in about 100 ml. of a 1% solution of HgCl₂ for 30–60 sec. with the application of a potential of 1.5 V. from a dry battery, the electrode being made the cathode while another platinum electrode 1–2 cm. distant serves as the anode. After plating it is washed with distilled water and is then ready for use. Less satisfactory results were found when other mercury salts were used for electroplating (Table 1).

Table 1. *Comparison of different Hg salts tested for use in the electro-plating bath*

Salt	Concentration	Time of plating	Attainment of equilibrium by electrode in titration	Whether electrode needs prior 'sensitization' by soaking in dye
HgCl ₂	1%	30–60 sec.	Very rapid before end-point, slightly less rapid after end-point	No
Cyanide (HgO + excess KCN)	1%	10–30 sec.	Very slow (10–30 min.)	Yes
HgNO ₃	1%	20–120 sec.	Slow	Yes
Hg ₂ Cl ₂	Saturated	<1 min.	Drifts (like bright Pt)	—
		1–10 min.	Rapid	No
		>15 min.	Slow	Yes
Mercuric acetate	1%	<1 min.	Drifts (like bright Pt)	—
		>1 min.	Rapid	No

Properties and mode of function of the electrode

The following are the characteristic properties of the Hg-Pt electrode prepared as described above:

(1) *Relation between electrode-potential and halide concentration.* In an acid solution of vitamin C the potential of the electrode with respect to the solution can be varied within certain limits by the addition of halides: the higher the concentration of halogen ions the lower the potential. Other ions, including SO₄²⁻, NO₃⁻, Ac⁻, PO₄³⁻, Ca⁺⁺, K⁺, Na⁺, NH₄⁺ were found to have no significant effect on the potential. The potentials observed in different concentrations of halogen ions agree well with the calculated values based on the assumption that they depend on the electrode reaction: 2Hg = Hg₂⁺⁺ + 2e, which in turn depends on the concentration of halogen ions. Table 2 gives the calculated and the observed values referring to saturated calomel half-cell as zero.

Table 2. *Observed and calculated potentials with the Hg-Pt electrode*

ml. of saturated KCl solution per 10 ml. of 5% metaphosphoric acid	ml. of saturated KBr solution per 10 ml. of 5% metaphosphoric acid	Normality	E (calculated) mV.	E (observed) mV.
0.10	—	0.046	105	101–106
0.20	—	0.093	86	80–82
—	0.01	0.0055	59	50–60
—	0.10	0.055	-1	-16

The calculated values of E are based on the electrode reactions: $2\text{Hg} = \text{Hg}_2^{++} + 2e$; $E = E_0 + \frac{RT}{nF} \ln [\text{Hg}_2^{++}]$; $E_0 = 0.550$ V. referred to saturated calomel electrode; $[\text{Hg}_2^{++}][\text{Cl}^-]^2 = 3.1 \times 10^{-18}$; $[\text{Hg}_2^{++}][\text{Br}^-]^2 = 1.3 \times 10^{-21}$.

The calculated values are not strictly accurate owing to the use of concentrations instead of activities in the calculation. However, the errors introduced should not be serious in view of the low dilutions of the salt used.

(2) *Comparison of effects of halides.* The effectiveness of the different halogen ions in lowering the potential of the electrode is of the order: $\text{I}^- > \text{Br}^- > \text{Cl}^-$. This is to be expected from the varying solubility-products of the different mercurous halides:

Iodide ions depress the concentration of Hg_2^{++} ions most effectively, so that the potential of the electrode in a definite concentration of iodide ions should be more negative than in an equal concentration of other halogen ions. However, iodides cannot be used in the titration owing to their reaction with the indophenol dye. KCl is easily available in pure form and was therefore used throughout the experiments.

(3) *Optimal concentration of halide.* The potential of the electrode is independent of the hydrogen ion concentration of the surrounding solution between pH 6.5 and 1.4 (other pH values have not been tried), and of the amount of ascorbic acid present (Tables 3, 4). It depends solely on the concentration of halogen ions. In practice we

Table 3. *Lack of correlation between pH and e.m.f. with the Hg-Pt electrode.*
Relation between e.m.f. and concentration of chloride

Solution	pH	Concentration of KCl, M	Observed e.m.f. in mV. (referred to saturated calomel electrode)
Metaphosphoric acid: 5 %	1.39	0.0463	+ 102
1 %	1.83	0.0463	+ 102
0.1 %	3.63	0.0463	+ 103
5 %	1.39	0.093	+ 80
1 %	1.83	0.093	+ 79
0.1 %	3.63	0.093	+ 80
Phosphate buffer, M/10	6.50	0.093	+ 78
Acetic acid, M/1	2.37	0.0463	+ 103
Acetate buffer: M/1	4.50	0.0463	+ 104
M/1	5.50	0.0463	+ 104

Table 4. *Lack of correlation between concentration of ascorbic acid and e.m.f. with the Hg-Pt electrode*

Solution	Concentration of ascorbic acid mg./ml.	Concentration of KCl, M	Observed e.m.f. in mV. (referred to saturated calomel electrode)
Metaphosphoric acid: 5 %	Nil	0.0463	+ 102
	0.004	0.0463	+ 101
	0.04	0.0463	+ 100
	0.40	0.0463	+ 101

found a concentration of KCl corresponding to 0.1 ml. of saturated KCl in 10 ml. of the vitamin extract (or 0.046 N KCl) to be the most satisfactory for the estimation, for reasons to be explained later. The electrode in this concentration of KCl gives an e.m.f. of about 100 mV. when measured against a saturated calomel half-cell.

(4) *End-point.* With each addition of the dye during the titration, the e.m.f. remains virtually constant until the end-point is reached, and then there follows an abrupt rise of potential from 100 mV. to about 310–350 mV. This sharp change of potential makes it possible to determine the end-point of the titration with great accuracy.

(5) *Probable mode of action of electrode.* Before the end-point is reached the electrode behaves in every way like an ordinary mercury electrode, but as soon as an excess of the dye is present it becomes entirely different from a mercury electrode which under the same condition responds with an increase of only 10–30 mV. This property of the new electrode is very similar to that of a platinum electrode, which also registers an e.m.f. of 310–350 mV. in presence of an excess of the dye. Apparently the electrode functions as a double electrode; as a mercury electrode before the end-point, and as a platinum electrode after the end-point, when, under the oxidizing action of the dye, the thin film of mercury on the electrode is converted into Hg_2^{++} . This explanation is supported by the following experimental facts: (a) if the coating of mercury is made heavy by electrolysis in a solution of a mercury salt which is more highly ionized than mercuric chloride, e.g. mercurous nitrate, or by more prolonged electrolysis in a solution of HgCl_2 , then the

response of the electrode to an excess of the dye becomes very sluggish, at first rising only 10–30 mV. like a mercury electrode, and only after being immersed in the dye for several minutes ('sensitization') does its potential rise to 310–350 mV.; (b) if the concentration of halogen ions in the extract to be titrated is increased, the electrode becomes less and less sensitive to an excess of the dye just like the heavily plated electrode. The obvious explanation is that the high concentration of halogen ions reverses the forward reaction of $2\text{Hg} = \text{Hg}_2^{++}$, preventing the conversion of mercury into mercurous chloride. It is for this reason that we choose to use a low concentration of KCl (i.e. about 0.046 *N*, equivalent to 0.1 ml. of saturated KCl in 10 ml. of extract).

(6) *Life of electrode.* The electrode can be used for several consecutive titrations without the need of replating. It seems that the reaction $2\text{Hg} \rightarrow \text{Hg}_2^{++}$ which takes place on the surface of the electrode is readily reversible, being reversed in a reducing medium and displaced forward in the presence of excess of an oxidizing agent.

Detailed working procedure

Electrode. Prepare an electrode according to the directions given above. The Pt wire should protrude about 1 cm. below the seal, which should be made of cobalt glass. The electrode may generally be used a number of times during a day's work without replating. The electrode should be replated at the start of each day's work.

In place of the HgCl_2 bath, a 1% solution of mercuric acetate or a saturated solution of calomel may also be used, but these are not recommended as the time of electrolysis has then to be prolonged to about 5 min. (a shorter time causes these electrodes to behave like a bright Pt electrode, while with a longer time they do not respond adequately at the end-point unless previously 'sensitized' by contact with excess of dye: cf. Table 1).

Standard solution of 2:6-dichlorophenolindophenol. Dissolve 40 mg. of the dye in 40 ml. of hot boiled distilled water and filter. Cool to room temperature, add KCl to make a final concentration of 0.046 *N*, and make up to 50 ml. Standardize by direct titration against a solution of pure ascorbic acid of known strength. Check the latter by iodine titration. One ml. of the dye solution should be equivalent to 0.4 mg. of ascorbic acid.

Preparation of extract. Weigh out 5–10 g. of the specimen to be analysed (10 g. for a material of low, and 5 g. for one of high, potency), and cover it with 5% metaphosphoric acid in a mortar. Add about 2–4 g. of acid-washed sand and grind the material to a fine pulp. Centrifuge and wash the residue twice with 10–15 ml. of the acid. Combine the clear centrifugates and make up to 50 ml. or more depending on the concentration of the vitamin. A suitable dilution is about 4 mg. of ascorbic acid per 100 ml., so that 10 ml. of extract will require 1–2 ml. of the standard dye solution. The use of less than 0.5 ml. or more than 4 ml. of the latter is not recommended. For further recommendations regarding sampling and extracting see Harris & Olliver [1942].

Titration. Measure by pipette 10 ml. of the extract into tube *F* (see Fig. 2) and add 0.1 ml. of a saturated solution of KCl, so that the final concentration of the latter is about 0.046 *N*. With the electrode and agar bridge in position, pass a stream of N_2 or CO_2 into the solution at a rapid, constant rate. After about 30 sec. take the reading of the e.m.f. Add from a burette 0.01 ml. or more of the dye and take the reading again after 15 sec. Proceed in this way until the end-point is reached as indicated by a sharp rise of e.m.f. At this part of the curve, wait for 45–60 sec. before taking each reading. Carry on the titration a little beyond the end-point until the e.m.f. shows little further change with each addition of the dye, i.e. the curve begins to flatten out once again. It is advisable to run a preliminary rapid titration first in order to ascertain the approximate amount of the dye needed and then do a more careful titration. In this second titration, run in the dye rapidly to within 0.1 ml. of the volume required according to the preliminary finding, and then add slowly 0.02–0.03 ml. at a time, till the exact end-point is reached, again waiting 45–60 sec. before taking each reading on the rising part of the curve.

Calculation of result. Plot the results on a graph with the e.m.f. as ordinate and the volume of dye added as abscissa, and draw a smooth curve through all the points. Draw two tangents, one along the base line (which should be around 100–120 mV. for solutions of pure ascorbic acid and a little lower for some natural extracts), and the other along the steepest part of the rising e.m.f., as illustrated in Fig. 3. The end-point is taken as the point of intersection of the two tangents.

Fig. 3.

Fig. 4.

Fig. 3. Potentiometric titration curve for pure ascorbic acid, showing method of determining end-point.

Fig. 4. Potentiometric estimation of ascorbic acid in black-currant preparations.

Typical results

Fig. 3 shows curves obtained with pure ascorbic acid, and Figs. 4 and 5 with various fruits and vegetables etc.

Reproducibility and accuracy of the results

Table 5 illustrates the reproducibility of the results in a number of repeated determinations on specimens of pure ascorbic acid. The coefficient of variation is not more than about 1% for a solution containing 0.05 mg. of ascorbic acid per ml., or 0.1% for one containing 0.16 mg./ml.

As with all volumetric operations, the percentage accuracy of the method increases with increasing concentrations of the unknown and the reagent, the 'water blank' being then relatively less. This is illustrated in Fig. 6, the more steeply ascending curves given by the stronger solutions fixing the end-point more precisely. If the amount of unknown available is small it is better to make the extract as concentrated as possible and titrate with a small amount of dye from a microburette rather than to dilute the dye and use a macroburette.

Comparison with direct visual titration

As will be seen from Table 6, the values obtained by the potentiometric method on a number of fruit and vegetable extracts agree very well with those obtained by the direct visual technique [Harris & Ray, 1933; Birch *et al.* 1933].

Advantages of potentiometric method

Theoretically, the potentiometric method should give more accurate results than any visual titration method because the former method records the true end-point of the reaction, while the latter requires a small excess of the dye to indicate the completion

Fig. 5. Typical determinations on plant and vegetable extracts, Hg-Pt electrode.

of the titration. That such is the case is supported by the fact that perceptibly higher values were obtained in many instances with the visual method (especially with the less accurate 'chloroform' technique), although the difference was usually very small. Another reason why the potentiometric value is, if anything, more accurate is that the end-point is derived as the mean of a series of readings taken during the whole course of the titration, both before and after the end-point; whereas in the visual method there is a single reading

only at the end-point. Again, the potentiometric method is not influenced by the subjective factor involved in detecting a colour change.

To sum up, the potentiometric method has the following advantages: (1) applicability to highly coloured extracts, (2) increased sensitiveness, (3) greater objectivity.

Fig. 6. Titration of strong, medium and weak solutions of ascorbic acid, showing effect on accuracy of locating the end-point. Curve 1 = 1 mg. of ascorbic acid dissolved in 10 ml.; Curve 2 = 1 mg. of ascorbic acid dissolved in 100 ml.; Curve 3 = 1 mg. of ascorbic acid dissolved in 250 ml.

Table 5. *Reproducibility and accuracy of results*

(A) 0.25 mg. ascorbic acid to 10 ml. solution. 1 ml. of dye = 0.49 mg. ascorbic acid			(B) 1.60 mg. ascorbic acid in 10 ml. solution. 1 ml. of dye = 0.52 mg. ascorbic acid		
Exp.	Vol. of dye required in duplicate titrations ml.	Corresponding wt. of ascorbic acid found mg.	Exp.	Vol. of dye required in duplicate titrations ml.	Corresponding wt. of ascorbic acid found mg.
1	0.52	0.255	1	3.095	1.609
2	0.51	0.250	2	3.090	1.607
3	0.515	0.252	3	3.090	1.607
4	0.51	0.250	4	3.090	1.607
5	0.51	0.250	5	3.092	1.608
6	0.51	0.250	6	3.096	1.610
Mean		0.251			1.608
Standard deviation		0.002			0.0013
Coefficient of variation		0.8 %			0.08 %

Table 6. *Ascorbic acid in various fruits and vegetables as determined by visual and potentiometric methods (Hg-Pt electrode)*

	Ascorbic acid, mg./100 g.			Ascorbic acid, mg./100 g.	
	Visual	Electro-metric		Visual	Electro-metric
Cabbage: savoy (1)	66.1	66.5	Raspberries, cooked. Pulp preserved 6 months with sulphite	15.9*	16.2
savoy (2)	73.6	73.9	Black currant purée: (1)	80.1*	78.7
Winningsstadt (1)	73.0	74.0	(2)	79.9*	78.9
Winningsstadt (2)	60.6	60.0	Strawberry jam	5.7*	5.6
Grass	87.0	85.0	Rose hips	423	430
Orange juice	51.3	51.0	Brussels sprouts	86.5	85
Mixed vegetables	12.1	12.0	Kale: marrow stem	180	176
Black currants, raw. Pulp preserved 6 months with sulphite	98*	97	thousand headed	140	138
Black currants, cooked. Pulp preserved 6 months with sulphite	113.5*	112	Turnip tops	109	108
Raspberries, raw. Pulp preserved 6 months with sulphite	7.7*	7.7	Cabbage, winter	57	56

* By the 'chloroform' method.

Fig. 7. Curve illustrating the slightly delayed equilibrium after end-point, with Hg-Pt electrode. *A* = reading after 5 sec.; *B* = reading after 1 min.; • = values established almost instantaneously.

Fig. 8. Variable and drifting potential as given by ordinary bright Pt electrode.

Limitation of the electrode. Perhaps the only defect of the Pt-Hg electrode is the slight sluggishness at the end-point where the e.m.f. change is most rapid. Although its response to an excess of the dye is very prompt, the e.m.f. does not immediately come to a steady value, but usually takes 1–2 min. to reach its maximum value (Fig. 7). However, as the

Table 7. *Ascorbic acid in various materials as determined by the visual and the alternative potentiometric methods (polarized bright Pt electrode)*

Material	Ascorbic acid mg./100 g.		Ascorbic + dehydroascorbic acids mg./100 g.	
	Visual	Electrometric	Visual	Electrometric
Cabbage: (1)	54.0	53.0	54.2	53.5
(2)	72.2	72.3	74.3	73.7
cooked	12.9	13.0	12.9	13.1
dried	42.5	41.9	42.0	42.1
Peas: raw (1)	24.6	23.9	24.9	24.0
raw (2)	35.5	35.2	35.7	35.6
blanched	13.5	13.7	13.4	13.8
frozen after blanching	11.6	11.8	11.8	11.7
Spinach: raw	41.5	41.4	41.2	41.5
dried	13.9	13.4	14.1	13.7
Lettuce: fresh (1)	27.0	26.2	—	—
(2)	13.4	13.1	—	—
Cauliflower: flower	116	114	—	—
leaves	192	190	—	—
Beans, dwarf	20.4	22.0	—	—
Orange juice (1)	41	42	—	—
(2)	63	61	—	—
Lemon juice	34	33	—	—
Grapefruit juice	50	49.2	—	—
Strawberry juice	60*	57	—	—
Strawberry juice, canned	15.0*	14.4	—	—
Raspberry: fresh (1)	40.5*	40	—	—
fresh (2)	44.0*	44.1	—	—
cooked	34.5*	34.0	—	—
Tomatoes	15.6	15.0	—	—
Black currant: fresh	183*	174	184	173
cooked	153*	147	—	—
purée	84.0*	82.8	—	—
Plums	2.64*	2.67	—	—
Red currant, stewed	26.0*	25.3	—	—
Black currant juice concentrated	416*	408	—	—
Black currant juice concentrated	135*	130	—	—
Liver, rat: (1)	15.9	16.3	—	—
(2)	22.0	21.7	—	—
Kidney, rat	12.5	12.1	—	—
Suprarenals, rat	30.7	29.6	—	—

* By 'Chloroform' method.

Table 8. *Estimation of ascorbic acid in presence of cysteine*

Method of titration	Ascorbic acid found, mg.		% error
	(a) Solution of ascorbic acid 0.5 mg./ml.	(b) Solution of ascorbic acid plus cysteine, 0.5 mg. of each in 1 ml.	
Potentiometric, normal procedure:			
Titration completed in 2 min.	0.502	0.510	+1.6
	0.500	0.506	+1.2
	0.520	0.522	+0.4
	0.500	0.506	+1.2
Potentiometric, titration completed 3–5 min.	0.500	0.512	+2.4
Potentiometric, titration completed in 10 min.	0.500	0.541	+8.2
Visual, titration completed in 30–60 sec.	0.520	0.525	+1.0
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potential change after 1 min. following the addition of the dye is small in comparison with the change during the first few seconds, readings can be taken at 45–60 sec. without causing any appreciable error in the final result.

Alternative electrode for use with high concentrations of interfering reducing substances

When the concentration of interfering reducing substances is unusually high in comparison with the amount of ascorbic acid present, as in liver and kidney, the slight sluggishness at the end-point does constitute a serious disadvantage owing to the slow oxidation of the interfering substances during the minute of waiting.

When working with such materials, we have found it preferable to rely on a modified use of the bright platinum electrode, which, in spite of its many disadvantages, does give a very quick response at the end-point. To avoid the drifting of potential in the preliminary phase of titration (Fig. 8), the contact key is tapped two or three times after each addition of the dye so as to polarize the electrode. In this way, the initial e.m.f. can be kept more or less constant until the end-point is reached, when in the presence of excess of the redox dye, the electrode is no longer polarizable and establishes an equilibrium immediately with the solution. The initial potential of the electrode is kept low by treating the electrode, before use, with alcoholic KOH or alkaline pyrogallate after the cleaning with HNO_3 . This step is necessary as otherwise the e.m.f. might be too high and the change at the end-point scarcely noticeable. It is understood, of course, that in using the platinum electrode in this way, the initial potentials are not true equilibrium values.

However, this does not influence the results appreciably so long as the initial e.m.f. is kept low. Table 7 gives some of the results obtained by this alternative electrometric method. A typical curve with the 'polarized' bright-platinum electrode is shown in Fig. 9.

Determinations in presence of cysteine, glutathione etc. In order to submit the method to as stringent a test as possible we have made some titrations on artificially prepared extracts containing as much as one part of cysteine to one of ascorbic acid. Even at such high concentrations the interference from cysteine was negligible provided that the titration was completed within 1–1½ min. (Table 8), following the usual procedure of one or two preliminary trial runs. Actually, however, free cysteine is not present in significant amounts in any but autolysed or hydrolysed protein-rich material, and is unlikely to cause trouble in practice.

Since the titration is carried out at a strongly acid reaction interference from glutathione is virtually eliminated, even if present in large excess.

Thiosulphate in urine is one of the few remaining substances known to occur in nature which has to be considered. However, as pointed out in the preceding paper [Harris & Olliver, 1942] it does not affect the validity of the 'saturation' test used for establishing the level of nutrition, the amount of thiosulphate being negligible compared with the greatly increased excretion of ascorbic acid after the large test doses.

Fig. 9. Comparison of curves with Hg-Pt electrode (below) and the alternative, 'polarized' bright Pt electrode (above). Cabbage extract.

To sum up, there is no evidence of interference with any ordinary fruit or vegetable product, and the Hg-Pt electrode can be used with confidence. If with an animal product a slowly falling potential on the rising part of the curve gives indication of interference from some non-specific more slowly reducing substance, the 'polarized' bright Pt electrode can be substituted.

Estimations in presence of SO₂

The potentiometric method finds a useful application in the estimation of ascorbic acid in deeply coloured 'sulphited' fruit pulps. The simplest procedure for removing the SO₂ is by addition of acetone. To 4 ml. of extract add 1 ml. of acetone, and perform the titration in the usual way, preferably with the Hg-Pt electrode. An alternative and equally effective method is to eliminate the SO₂ by a stream of O₂-free N₂, while a vacuum is applied. Details of control tests with the 'acetone' and 'de-gassing' methods are given in the following paper [Mapson, 1942].

Measurement of dehydroascorbic acid

The amount of dehydroascorbic acid ordinarily present in natural products is too small to be of practical significance. If, however, it should be desired to estimate it, titrations can be done, first, on the extract as usual, and, second, on the extract after treatment with H₂S (for details of procedure see Harris & Olliver [1942]). The difference gives the dehydroascorbic acid.

SUMMARY

1. A potentiometric method suitable for the estimation of vitamin C in coloured extracts is described.
2. The method involves the use of a special Hg-Pt double electrode, consisting of a mercury-coated Pt wire.
3. An explanation is given of the probable mode of function of the electrode.
4. The method has given good results with vegetable and fruit extracts, coloured or uncoloured.
5. Comparisons with the direct visual titration method of Harris and Ray have shown agreements to within 1-2%. In control tests ascorbic acid could be recovered with an error of less than 0.5-2% at concentrations of 0.2-0.02 mg./ml. respectively.
6. An alternative electrometric procedure is also described for estimating vitamin C in the presence of high concentrations of interfering reducing substances; it involves the use of a polarized bright Pt electrode.

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