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Short communication

Potentiometric determination of ascorbic acid in pharmaceutical preparations using a copper based mercury film electrode

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1. Introduction

Many methods have been developed for the determination of ascorbic acid and quality control of vitamin C containing products. These methods include spectrophotometry [1–5], chromatography [6–9], visual and potentiometric redox titrations using titrants such as chloramine-T [10], cerium(IV) [11], *N*-bromophthalimide, *N*-bromosaccharin [12], polyvinylpyrrolidone-iodine [13], *N*-chlorosuccinamide [14], dibromamine-T [15], vanadium(V) [16] and ferricyanide [17]. The other potentiometric methods reported make use of various types of ion-selective electrodes [20–25]. Biamperometric titration using peroxymonosulphate [18] and ferricyanide [19] as titrants and a number of different types of voltammetric methods have also been developed [26–29]. Ascorbic acid has also been titrated with copper sulphate directly in the presence of thiocyanate using visual indicators [30,31] and potentiometrically using a Cu(II)-selective electrode [32]. British Pharmacopoeia (BP) methods which are widely used in Europe and India are visual titration of ascorbic acid with iodine [33] and cerium(IV) [34]. Visual titrimetric methods are not preferable for ascorbic acid assay in coloured and opaque pharmaceutical solutions.

In the present communication, a simple, rapid, precise and accurate method is reported. The proposed method is based on the direct potentiometric titration of ascorbic acid with $CuSO_4$ using a copper based mercury film electrode (CBMFE) as the indicator electrode. The method was successfully applied for ascorbic acid assay in pharmaceutical dosage forms. For statistical validation of the proposed method, a 'lack of fit test' which involves the application of analysis of variance in regression analysis was adopted to assess the data obtained in replicate analysis of pure ascorbic acid [35–37]. *F* and *t*-tests were applied to compare the results of ascorbic acid assay in pharmaceutical preparations with that of the BP method.

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2. Experimental

2.1. Apparatus

2.1.1. Preparation of CBMFE

A plastic sleeved copper wire (99% purity) of 15 cm length and 1 mm thickness was taken and the plastic sleeve was removed at one end to expose about 1 cm copper wire. An epoxy seal was applied at the junction of the copper wire and the plastic sleeve to impede entry of solution into the sleeve. The copper wire was cleaned well by abrasion with a fine emery paper and washed with water followed by treatment with concentrated HNO_3 for a few seconds and finally rinsed with water. The polished copper wire was coated with mercury as a thin film by dipping the wire in acidified mercuric nitrate (0.02 M) solution for 10 min. The electrode surface was gently wiped with a filter paper and rinsed with water.

The titration cell consisted of the CBMFE as the indicator electrode, a saturated calomel electrode (SCE) as the reference electrode and a 50 ml beaker. The solution was continuously stirred using a mini magnetic stirrer and potentials were measured with a digital pH/mV meter (precision ± 1 mV) at ambient temperature (29 ± 1°C).

2.2. Reagents

All chemicals were of analytical grade and solutions were prepared with deionised distilled water.

Copper sulphate (0.1 M) solution was prepared by dissolving appropriate amounts of copper sulphate in water containing a few drops of concentrated sulphuric acid and diluted to the required volume. The solution was diluted appropriately before use.

Ammonium thiocyanate (0.1 M) was prepared by dissolving the appropriate amount of the reagent in water.

Acetate buffer (pH 4.7). Acetic acid (1 M) and sodium acetate (1 M) solutions were mixed appropriately to prepare a pH 4.7 acetate buffer solution.

Ascorbic acid. The stock solution of ascorbic acid containing 1–2 mg/ml in deaerated water was prepared and standardised potentiometrically [38] against ferricyanide, using Pt as the indicator electrode and SCE as the reference electrode, just before use, and kept protected from light.

Mercuric nitrate solution (0.02 M) was prepared by dissolving 1.7 g mercuric nitrate monohydrate in 400 ml water containing 10 ml nitric acid (2 M) and diluting to 500 ml.

2.3. Dosage forms of ascorbic acid

Tablets: (1) Succee (IDPL, India). (2) Nycee (Jwalin Pharmaceuticals, India). (3) Vitamin C (Emerk, India). (4) Celin (Glaxo, India). Each tablet contains 500 mg ascorbic acid.

Oral drops: (1) Cebion (Emerk, India). (2) Cecon (Abbotte, India). Each oral drops contains 100 mg ascorbic acid/ml.

Capsules: (1) Becosule (Pfizer, India), a multivitamin capsule containing 150 mg ascorbic acid/capsule along with vitamins B_1, B_2, B_6, nicotinamide, calcium pantothenate and folic acid. (2) Cobadex (Glaxo, India), a multivitamin capsule containing 150 mg ascorbic acid/capsule along with vitamin B_{12} and other vitamins as in the Becosule capsule.

Syrup: Becosule (Pfizer, India), a multivitamin syrup containing 75 mg ascorbic acid/5 ml of syrup along with other vitamins as in the Becosule capsule.

Injections: (1) Tildoxon (Tablet India, India), ascorbic acid 100 mg/ml in a 5 ml vial, along with methylparaben and propylparaben. (2) Ascorbic acid Injection (Civil Drug, India), ascorbic acid 100 mg/ml in a 5 ml vial. (3) Visyneral Injection (US Vitamin, India), a multivitamin injection containing 50 mg ascorbic acid/ml along with vitamins A, B_1, B_2, B_6 and other vitamins and excipients.

2.4. Procedure for the titration of ascorbic acid

An aliquot of ascorbic acid (1–15 mg) was transferred into the titration cell, followed by 1.5 ml NH_4SCN and 1–2 ml acetate buffer. The solution was diluted to about 30 ml with water.

The pH of the solution was 4.7. A freshly prepared CBMFE and SCE were introduced into the titration cell. $CuSO_4$ solution (5×10^{-3}–3×10^{-2} M) was slowly added from a 5 ml semimicro burette (0.02 ml sub divisions). A white precipitate of cuprous thiocyanate was observed after the addition of about 0.3 ml of the titrant. The potential after each addition of the titrant was noted. The end point was located graphically. After each titration, the surface of the electrode was gently wiped with filter paper and rinsed with water. 1 ml 0.1 M $CuSO_4$ is equivalent to 8.806 mg ascorbic acid.

2.5. Procedure of ascorbic acid assay in pharmaceutical formulations

The following pharmaceutical sample preparations were prepared with deaerated water and kept protected from light.

2.5.1. Tablets

Twenty tablets containing ascorbic acid were weighed and pulverized. An appropriate amount of the pulverized sample, equivalent to about 250 mg ascorbic acid, was dissolved in about 30 ml of water and any residue was filtered using a Whatmann No. 41 filter paper and washed 5–6 times with water. The filtrate and washings were collected in a 250 ml volumetric flask and made up to the mark and 5.0 ml of this solution was taken for titration.

2.5.2. Ampoules, syrups and oral drops

A certain volume of the drug, equivalent to about 100 mg ascorbic acid, was transferred into a 100 ml volumetric flask and made up to the mark with water and 5.0 ml of the solution was taken for titration.

2.5.3. Multivitamin capsules

The contents of ten capsules were mixed well and an appropriate amount, equivalent to about 250 mg ascorbic acid, was taken and stirred well with 50 ml water for 5 min and filtered into a 250 ml volumetric flask quantitatively followed by dilution with water to the mark.

3. Results and discussion

The freshly prepared CBMFE had a smooth, uniform, stable and adhesive deposit of thin mercury film. The electrode showed potentiometric response towards cupric ions in aqueous solutions. Electrode performance was tested by measuring the potential of the electrode versus SCE in a series of Cu(II) solutions from 10^{-1} M to 10^{-7} M with constant ionic strength (0.1 M KNO_3) and pH 4.7 (acetate buffer). The electrode response was rapid, stable and reproducible (± 1 mV) when potential readings were measured eight consecutive times in 10^{-5}–10^{-1} M Cu(II) solution and the electrode displayed a linear Nernstian response. The response characteristics of CBMFE are listed in Table 1.

It has been shown by Yoshida [39] that a mercury layer deposited on Au, Cu, Ni or Pt consists of stable mercury compounds with the base metal at their interface and a uniform metallic mercury layer on them. Consideration of the mercury–copper phase diagram [40] would suggest that the mercury film deposited on copper would contain about 0.003% w/w dissolved copper at 20°C [41]. The presence of copper in the mercury film may be responsible for CBMFE's response towards Cu(II) ions in solution. Further, Jangg and Kirchmayr [42] have reported that the potential of copper amalgam electrodes obey the Nernst equation over the range of concentrations from 10^{-5} M to 10^{-2} M Cu(II) ions.

Table 1
Response characteristics of CBMFE towards Cu(II)

Linearity range	10^{-5}–10^{-1} M
Slope (mV/decade of concentration)	31–35
Detection limit	2×10^{-6} M
Response time	15–30 s in 10^{-1}–10^{-2} M
	45–90 s in 10^{-3}–10^{-4} M
pH range where potential remains constant	pH 2–6 for 10^{-4}–10^{-5} M
	pH 1.5–4.5 for 10^{-3}–10^{-2} M
	pH 1.0–4.0 for 10^{-1} M

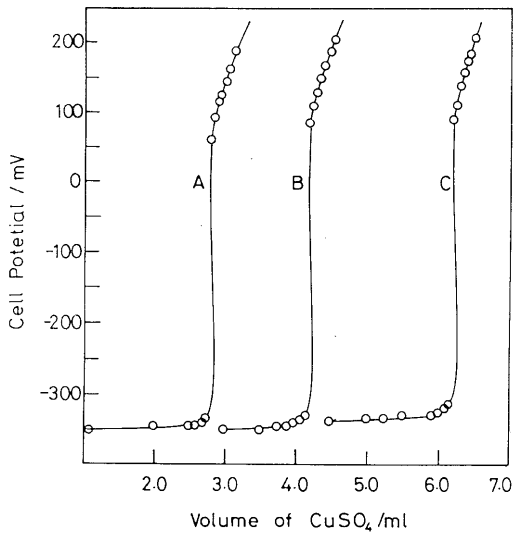

Fig. 1. Potentiometric titration of ascorbic acid with 0.0206 M $CuSO_4$ Curve A, 5.0; B, 7.71; and C, 11.30 mg of ascorbic acid.

3.1. Determination of ascorbic acid

Ascorbic acid reduces Cu(II) ions quantitatively in aqueous solution to Cu(I) ions in neutral or acidic medium (pH > 2.8)

$$2Cu^{2+} + C_6H_8O_6 \rightleftharpoons C_6H_6O_6 + 2Cu^{+} + 2H^{+}$$

The forward reaction is favoured in the presence of ammonium thiocyanate which forms sparingly soluble cuprous thiocyanate ($K_{sp} = 4.8 \times 10^{-15}$). Since CBMFE responds to the Cu(II) ion, this electrode could be applied with advantage to monitor the titration of 1–15 mg ascorbic acid with $CuSO_4$ solution. A potential break of 400 mV was observed for an addition of 0.02 ml $CuSO_4$ (0.0206 M) near the end point while titrating 5.0 mg ascorbic acid. Representative titration curves are shown in Fig. 1. The response of the CBMFE was fast and the equilibrium potential established within a few seconds before and after the end point. However, a wait of 1–2 min was necessary near the end point.

In order to fix optimum pH for the titration where the titration is quantitatively reproducible, 5.0 ml ascorbic acid at various pH between 2 and 6.5 were titrated against 0.01 M $CuSO_4$. The end point corresponding to quantitative recovery was reproducible in the pH range 2.8–6.1 and the end point volume varied below pH 2.8 and above 6.1, without change in ΔE at the inflection. Above pH 6.1 the variation in the end point could be due to hydrolysis of Cu(II) and acceleration of aerial oxidation and extensive degradation of the ascorbic acid in a basic medium [43]. Below pH 2.8, the oxidation of ascorbic acid could be slow, thus causing variation in the end point [44].

3.2. Precision and accuracy

In order to study the precision and accuracy of the proposed method, standard solutions containing eight different concentrations of ascorbic acid were prepared and six titrations were performed on each standard solution and the results obtained are given in Table 2. The overall mean recovery for 48 determinations was 99.68% while overall relative standard deviation (RSD%) and mean standard analytical error were 0.7348 and 0.0236 respectively. These statistical parameters reflect the reliability of the proposed method. Two tailed Student's t-test was applied for comparing the amount taken (μ) for analysis and the amount found ($\bar{x}$) at each concentration level. Student's t-value $|t|$ was calculated at each concentration level by stating a hypothesis that the mean of amount found by six replications ($\bar{x}$) was not significantly different from the amount taken for analysis (μ) [41]. The mean value of Student's t-value calculated for eight concentration levels was 1.498 which was less than the critical value of 2.57 at the 5% level of significance and 5 degrees of freedom. It indicated the absence of any systematic error in the proposed method.

Further, a regression analysis of the amount taken for analysis versus the amount found by seven replications at each concentration level was performed for the data given in Table 2 and a significance test namely a 'lack of fit test' was performed to check whether the data fitted a linear model indicating agreement between the amount taken and found for the analysis. The lack of fit test involves the application of analysis of variance in regression analysis. In the test, a hypothesis that the data fits a linear model is

Table 2
The results of six replicate determinations of ascorbic acid and statistical analysis of the data

Amount taken (mg)	Amount found (mg)	Mean ($\bar{x}$) $\pm$ S.D.	% Mean recovery	Standard analytical error ($s/\sqrt{n}$)	Student's t-value $\left(\frac{\bar{x}-\mu}{s/\sqrt{n}}\right)$
1.15	1.13, 1.13, 1.14, 1.14, 1.15, 1.15	1.14 ± 0.0089	99.13	0.0037	2.739
2.45	2.44, 2.45, 2.44, 2.46, 2.43, 2.42	2.44 ± 0.0141	99.59	0.0058	1.732
3.93	3.95, 3.93, 3.93, 3.95, 3.94, 3.91	3.93 ± 0.0152	100.13	0.0062	0.808
4.40	4.44, 4.44, 4.40, 4.38, 4.38, 4.37	4.40 ± 0.0313	100.04	0.0128	0.131
7.71	7.72, 7.64, 7.59, 7.59, 7.64, 7.70	7.65 ± 0.0543	99.18	0.0222	2.858
9.01	9.00, 9.00, 8.95, 8.98, 8.87, 8.87	8.94 ± 0.0609	99.28	0.0249	2.614
11.30	11.38, 11.19, 11.27, 11.19, 11.27, 11.38	11.28 ± 0.0853	99.82	0.0348	0.574
15.01	15.07, 15.07, 14.83, 14.83, 15.22, 15.29	15.05 ± 0.1919	100.28	0.0783	0.532
		Mean	99.68	0.0236	1.498

The calculated value of F in the 'lack of fit test' was 1.327.

considered and an F-value is calculated [35]. If the calculated value of F is less than the critical value, the hypothesis is retained. The calculated value of F for the data given in Table 2 was 1.32 which was less than the critical value of 2.34 at the 5% level of significance and (6,40) degrees of freedom. It indicated the data to fit a linear model and the absence of any systematic error in the analysis.

3.3. Study of possible source of interference

In order to apply the proposed method for ascorbic acid assay in pharmaceutical preparations, five replicate determinations of 2.5 mg ascorbic acid in the presence of 4- or 5-fold amounts of each of sucrose, lactose, magnesium stearate, thiamine, nicotinamide, calcium pantothenate, pyridoxine, riboflavine, cyanocobalamin, folic acid, glucose and ferrous sulphate were carried out. The results of the interference analysis showed that the error did not exceed 2–3%.

3.4. Validity of the proposed method

The proposed potentiometric method was applied successfully for ascorbic acid assay in various pharmaceutical dosage forms such as tablets, syrups, oral drops and injections. Six replicate titrations were performed using CBMFE as the indicator electrode with $CuSO_4$ as titrant and simultaneously with the BP method using iodine as titrant. The results are presented in Table 3. All sets of results were compared statistically by calculating the F-ratio and Student's t-values [45]. The calculated value of the F-ratio for comparing variance of the two methods for each sample was less than the two tailed critical F-value of 7.15 at the 5% level of significance and (5,5) degrees of freedom indicating that the precision of two methods did not differ significantly. The calculated value of Student's t-value for all the samples was also less than the two tailed critical t-value of 2.23 at the 5% level of significance and 10 degrees of freedom indicating that the results of the two methods did not differ significantly.

Table 3
Results of six replicate analyses of drug formulations by the proposed method and the BP method and statistical analysis of the data

Brand name	Labelled amount (mg)	Found by the proposed method (mean ± S.D.)	Found by the BP method (mean ± S.D.)	F*	t*
Tablets					
Succee	500/tab	495.33 ± 3.93	500.5 ± 4.18	1.13	2.20
Nycee	500/tab	495.50 ± 2.81	494.33 ± 3.67	1.71	0.62
Vitamin C	500/tab	488.17 ± 7.55	487.67 ± 9.11	1.46	0.10
Celin	500/tab	493.00 ± 2.97	489.00 ± 3.35	1.27	2.19
Oral drops					
Cebion	100/ml	95.83 ± 0.75	95.33 ± 1.03	1.89	0.96
Cecon	100/ml	93.83 ± 1.60	94.50 ± 1.38	1.34	0.78
Capsules					
Becosule	150/cap	145.33 ± 2.25	145.00 ± 2.68	1.42	0.23
Cobadex	150/cap	154.67 ± 0.82	153.33 ± 1.82	4.93	1.64
Syrup					
Becosule	75/ml	77.50 ± 1.64	78.33 ± 1.86	1.29	0.82
Injections					
Vitamin C	500/amp	405.33 ± 4.68	400.66 ± 3.08	2.31	2.04
Tildoxon	100/ml	95.17 ± 1.72	97.00 ± 2.00	1.35	1.70
Visyneral	500/amp	497.83 ± 6.60	491.00 ± 4.20	2.47	2.14

* Calculated two tailed F and t values at the 5% level of significance and (5,5) and 10 degrees of freedom respectively. Critical values of F and t are 7.15 and 2.23 respectively.

4. Conclusion

The CBMFE is shown to be a suitable indicator electrode for cuprimetric determination of ascorbic acid since the electrode displayed a rapid, reproducible and stable response towards Cu(II) in aqueous solutions. The ease of fabrication using commercially available low-cost copper wires and no need of further pretreatment of CBMFE before use are some of the advantages of the electrode. The CBMFE provides an inexpensive alternative to more expensive cupric ion-selective electrodes for potentiometric determinations of ascorbic acid in pharmaceutical preparations. The proposed method for ascorbic acid assay with CBMFE provides many advantages over visual titrimetric BP methods which includes sharp end point detection with a very large potential jump, sensitivity, selectivity, small consumption of reagents and sample and ease of operation using simple equipment. Moreover, the proposed method is precise, accurate, rapid and direct, involving no prior treatment of the sample and relatively free from interference from other vitamins and excipients commonly present along with ascorbic acid in drug formulations. The method can be successfully applied in quality control laboratories for ascorbic acid assay in vitamin C products.

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