Indirect Potentiometric Titration of Ascorbic Acid in Pharmaceutical Preparations Using Copper Based Mercury Film Electrode

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A simple and rapid potentiometric method for the estimation of ascorbic acid in pharmaceutical dosage forms has been developed. The method is based on treating ascorbic acid with iodine and titration of the iodide produced equivalent to ascorbic acid with silver nitrate using Copper Based Mercury Film Electrode (CBMFE) as an indicator electrode. Interference study was carried to check possible interference of usual excipients and other vitamins. The precision and accuracy of the method was assessed by the application of lack-of-fit test and other statistical methods. The results of the proposed method and British Pharmacopoeia method were compared using *F* and *t*-statistical tests of significance.

Key words ascorbic acid; pharmaceutical preparation; indirect potentiometric titration; copper based mercury film electrode

Ascorbic acid (vitamin-C) is essential to health of human beings. It is employed for the prevention and treatment of scurvy. Many methods have been reported in comprehensive reviews for the determination of ascorbic acid.[1—5] These methods include spectrophotometric methods using reagents such as dichlorophenolindophenol,[6] Eriochromecyamine,[7] Fast Blue Salt-B,[8] silicon molybdenum heteropoly blue,[9] copper sulphate in the presence of neocuprine,[10] *etc*. Chromatographic techniques such as HPLC with electrochemical detection,[11] liquid chromatography with electrochemical detection,[12] ion-suppression reverse phase chromatography,[13] capillary zone electrophoresis,[14] *etc.* have also been applied for ascorbic acid assay. A number of different types of voltammetric methods making use of a variety of electrodes have also been developed.[15—18] Most of the methods developed for ascorbic acid are visual and potentiometric titrants such as ceric ammonium sulphate,[19] *N*-chlorosuccinimide,[20] peroxymono sulphate,[21] hexacyanoferrate(III),[22] mercury(II) nitrate,[23] silver nitrate,[24] copper(II) sulphate,[25] codine[26] and *N*-bromosuccinimide.[27]

The widely used British Pharmacopoeia (BP) method recommends visual titration of ascorbic acid[28] with cerium(IV).[29] Visual titrimetric methods cannot be applied successfully for the ascorbic acid assay in coloured and opaque pharmaceutical solutions and also require large sample size.

In the present communication a simple, rapid, precise and accurate potentiometric method using copper based mercury film electrode (CBMFE) as an indicator electrode is reported. CBMFE has recently been applied by the authors as an indicator electrode for the assay for the direct titration of ascorbic acid with copper sulphate in the presence of ammonium thiocyanate[30] and also for the assay of isoniazid[31] and sulphamethoxazole.[32]

The method was successfully applied for the assay of ascorbic acid in pure and pharmaceutical dosage forms. The experimental data were analysed statistically to validate the proposed method. A 'lack-of-fit' test which involves the application of analysis of variance in regression analysis was adopted for assessing the data obtained in replicate analysis of pure ascorbic acid.[33—35] The results obtained in the analysis of ascorbic acid in pharmaceutical dosage forms were statistically compared with that of BP method by the application of *F*- and *t*-tests.[36]

Experimental

CBMFE was prepared as reported in our earlier communications.[30,31] A commercially available plastic sleeved copper wire of 1 mm diameter was used to prepare CBMFE. About 1 cm portion of one end of the wire was polished by abrasion with a fine emery paper and washed with water followed by treatment with Con HNO_3 for a few seconds and finally rinsed with water. A thin layer of mercury was coated over the polished wire by dipping 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.

Ascorbic Acid Solution (1.0 mg/ml) The stock solution of ascorbic acid was prepared and standardised potentiometrically by titration with potassium hexacyanoferrate(III).[37]

Iodine in Ethanol (0.1 M) This was prepared freshly and passed through anion exchange resins (DOWNEX 1-XB, chloride form, 100—200 mesh) packed in small tube to remove traces of iodide.

Silver Nitrate Solution (0.1 M) This was prepared by dissolving silver nitrate in water and stored in an amber coloured bottle.

Potassium Hydrogen Phthalate–Nitrate Buffer (pH 3.0) This was prepared by adjusting the pH of 0.1 M potassium hydrogen phthalate solution to 3.0 with 0.5 M nitric acid.

Recommended Procedure for the Indirect Titration of Ascorbic Acid To an aliquot containing 1.0—20.0 mg of ascorbic acid, 0.1 M iodine in ethanol was added dropwise until the solution turned light yellow, followed by addition of 10.0 ml of potassium hydrogen phthalate–nitrate buffer (pH 3) and diluted to 50 ml. The solution was titrated against 0.01—0.1 M $AgNO_3$ using CBMFE as an indicator electrode and saturated calomel electrode (double junction) as a reference electrode.

Recommended Procedure of Ascorbic Acid Assay in Pharmaceutical Dosage Forms The following pharmaceutical sample preparations were prepared with deaerated water and kept protected from light.

Tablets Twenty tablets containing ascorbic acid were weighed and powdered. An appropriate amount of powdered sample equivalent to 250 mg of ascorbic acid was dissolved in about 50 ml of water and the residue if any, was filtered using Whatmann No. 41 filter paper and washed 5—6 times with water. The combined filterate and washings was diluted to 250 ml. Exactly 5.0 ml of this solution was taken for titration.

Injection A certain volume of the injection fluid, equivalent to about 100 mg of 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.

Procedure for Ascorbic Acid Assay in Pharmaceutical Preparations For analysis of tablets, capsules, oral drops and injections samples were prepared as described and 5.0 ml of the sample solution was taken for analysis.

Results and Discussion

Iodine oxidises ascorbic acid to dehydroascorbic acid.

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$$C_6H_8O_6+I_2 \rightarrow C_6H_6O_6+2HI$$

The reaction is spontaneous over a wide range of pH. However a pH of 2—3 is essential for quantitative oxidation at low concentrations of ascorbic acid.[38)]

Indirect titration of 1.0 to 20.0 mg of ascorbic acid was investigated. Since CBMFE showed characteristic potentiometric response towards iodide ion, the titration was monitored with CBMFE as an indicator electrode. During titration, the equilibrium potential was established within a few seconds and CBMFE showed stable potentiometric response towards iodide formed, and the precipitate of silver iodide coagulated as soon as the end point was over. For titration of 1.0—20.0 mg of ascorbic acid, 0.1 to 1.2 ml of 0.1 M iodine in ethanol was required. The pH of 20.0 mg of ascorbic acid in the presence of excess iodine was 2.6. Titration was carried out with various amounts of iodine in ethanol to study the effect of amount of iodine on titration. The presence of twice the equivalent amount of iodine required did not affect the results. The titration was carried at various pH values to study the effect of pH. Ascorbic acid could be determined accurately in the pH range 2—4. The pH can be maintained with potassium hydrogen phthalate–nitrate buffer. The titration curve for the titration of 14.44 mg of ascorbic acid is given in Fig. 1. A potential break of 54 mV was observed at the end point for the addition of 0.02 ml of 0.1 M $AgNO_3$. The end point was accurately located from the first derivative curve.

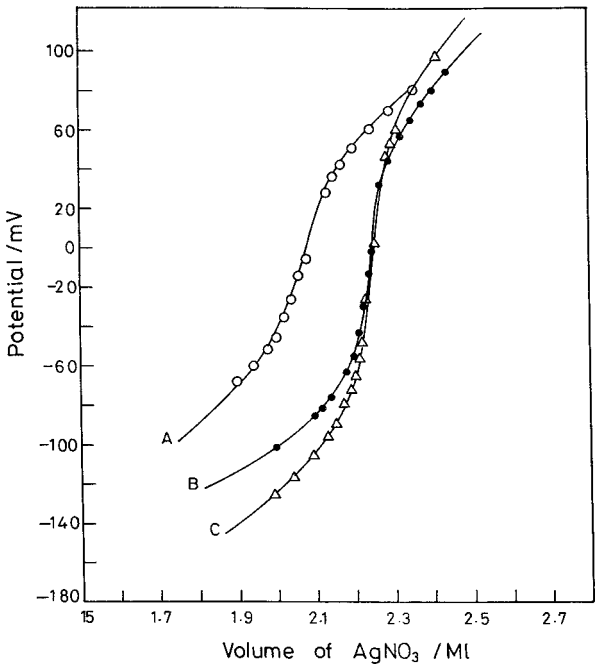

Fig. 1. Indirect Potentiometric Titration of Ascorbic Acid

Curve A - 4.66 mg, B - 9.76 mg, C - 14.44 mg of ascorbic acid with 0.025 M, 0.049 M and 0.10 M $AgNO_3$ respectively.

Precision and Accuracy To assess the precision and accuracy of the proposed method, seven replicate titrations were performed with each of nine standard solutions of different concentrations. The results are given in Table 1. The overall relative standard deviation and the mean percentage recovery for 63 determinations were 0.94% and 99.92% respectively and mean standard analytical error was 0.03. Student's *t*-value was applied to compare the amount taken for analysis and the amount found by seven replications at each concentration and *t*-value was calculated at each concentration level. The mean value of Student's *t* obtained for overall nine concentrations was 1.51 which was less than two-tailed critical *t*-value of 2.57 for six degrees of freedom at 5% level of significance. It indicated the absence of any systematic error in the analysis.

Further, a statistical significance test namely lack-of-fit was applied to analyse the experimental data given in Table 1 to check any systematic error in the analysis. This test was preformed to check whether the data fitted a linear model indicating agreement between the amount taken and found for the analysis. In the test, a hypothesis that the data fit a linear model is considered and *F*-value is calculated. The application of the lack-of-fit test to the data given in Table 1 yeilded a *F*-value of 2.09 which was less than the critical value of 2.18 for (7, 54) degrees of freedom at 5% level of significance. It indicated that the data fit a linear model and the absence of any systematic error in the analysis.

Interference Study The effect of the presence of usual excipients and other vitamins in the proposed method was assessed by performing five replicate determinations of 5.0 mg of ascorbic acid in the presence of excipients and other vitamins. The presence of equivalent amount of thiamine, three fold amounts of lactose, four fold amounts of each of nicotinamide, calcium panthothenate, riboflavin, folic acid, sucrose and ten fold amounts of minerals such as potassium, zinc, magnesium and manganese as sulphates or nitrates which are often present is dosage forms also did not affect the results of

Table 1. Results of Seven Replicate Determination of Ascorbic Acid by Indirect Titration with $AgNO_3$ and Statistical Analysis of the Data

Sl. No.	Amount taken (mg) (μ)	Amount found (mg)	Mean ($\bar{x}$) ±Std. dev.	% Mean recovery	Std. analytical error [$s/\sqrt{n}$]	Student's-t $\frac{\mu-\bar{x}}{S/\sqrt{n}}$
1	1.19	1.18, 1.18, 1.18, 1.16, 1.16, 1.20, 1.20	1.18±0.0163	99.16	0.0062	1.620
2	2.32	2.34, 2.29, 2.34, 2.31, 2.25, 2.29, 2.29	2.30±0.0318	99.20	0.0120	1.543
3	4.66	4.66, 4.62, 4.69, 4.62, 4.56, 4.65, 4.67	4.64±0.0430	99.54	0.0162	1.319
4	6.16	6.19, 6.19, 6.15, 6.15, 6.11, 6.11, 6.06	6.14±0.0471	99.63	0.0178	1.282
5	9.76	9.72, 9.74, 9.67, 9.67, 9.85, 9.76, 9.85	9.75±0.0751	99.91	0.0284	0.302
6	12.50	12.48, 12.40, 12.40, 12.57, 12.62, 12.40, 12.44	12.47±0.0896	99.78	0.0338	0.802
7	14.44	14.35, 14.44, 14.53, 14.71, 14.44, 14.44, 14.44	14.48±0.1145	100.27	0.0433	0.924
8	16.80	16.99, 16.86, 16.87, 16.99, 16.74, 17.08, 17.15	16.95±0.1406	100.92	0.0531	2.904
9	19.68	20.12, 19.90, 19.68, 19.66, 19.81, 19.96, 19.85	19.85±0.1602	100.86	0.0605	2.878
			Mean	99.92	0.0300	1.510

The calculated value of *F* in lack of fit test was 2.09.

Table 2. Results of Determinations of Ascorbic Acid by Indirect Titration with $AgNO_3$ and by BP Method in Pharmaceutical Preparations and Statistical Analysis of the Data

S. No.	Brand name & stated amount	Amount found by proposed method (mg) Mean±Std. dev.	Amount found by BP method (mg) Mean±Std. dev.	$F^{a)}$	$t^{a)}$
1.	Tablets Celin (500 mg)	466.43±4.76	465.86±4.08	1.11	0.22
2.	Succee (500 mg)	451.00±3.00	454.00±3.27	1.19	1.79
3.	Nycee (500 mg)	456.00±2.31	455.71±2.87	1.54	0.21
4.	Vitamin C (500 mg)	477.14±5.01	473.14±2.48	4.09	1.89
5.	Oral drops Cecon (100 mg per ml)	83.29±0.49	82.57±0.79	2.60	2.04
6.	Cebion (100 mg per ml)	94.86±2.04	94.00±1.29	2.49	0.94
7.	Injection Tildoxon (100 mg per ampoule)	96.00±0.82	97.00±1.00	1.50	2.05

a) Calculated *F* and *t*-values for (6,6) and 12 degrees of freedom respectively at 5% level of significance.

the analysis. However, the presence of copper(II) and iodine as potassium iodide are likely to interfer. It indicates that the proposed method can be applied for ascorbic acid assay in pharmaceutical preparations.

Determination of Ascorbic Acid in Pharmaceutical Preparation The proposed method was successfully applied for ascorbic acid assay in pharmaaceutical preparations. Seven replicate determinations were performed on four tablets, two oral drops and an injection containing ascorbic acid, by the proposed method as well as by the BP method. The BP method involved in the direct titration of pharmaceutical sample preparation with iodine using starch as indicator. The results obtained are given in Table 2. The application of *F*-test for comparing the variances of the two methods yielded *F*-values which were less than the two-tailed critical value of 5.82 for (6, 6) degrees of freedom at 5% level of significance, for all pharmaceutical preparations analysed. It indicated no significant difference between the precision of two methods. The calculated value of Student's *t* for comparing the means of seven replicate determinations of ascorbic acid by two methods was also less than the critical value of 2.18 for 12 degrees of freedom at 5% level of significance for each of the pharmaceutical analysed. It indicated no significant difference in the results of the two methods.

The proposed method is rapid, simple and selective for the determination of ascorbic acid in pharmaceutical preparations.

Conclusion

The proposed method of indirect potentiometric titration is very simple precise and accurate. The method can successfully be applied for ascorbic acid assay even in coloured pharmaceutical dosage forms for which visual titrimetric and spectrophotometric methods suffer severe limitations. The sharp detection of end point with a large potential break renders the method sensitive and rapid, and may be used in flow injection analysis.

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