Studies About the Determination of Ascorbic Acid by Differential Pulse Voltammetry

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Summary: Ascorbic acid(Vitamin–C) is one of the most important ingredients of food, fruits, vegetables and many pharmaceutical tablets and preparations. A differential pulse voltammetric method has been developed for the analysis of ascorbic acid in acetate buffer. Effect of pH was studied on the determination of ascorbic acid. At lower pH one electron and at higher pH two electron transfer reaction takes place. At higher pH sensitivity of the method increases significantly. Air(oxygen) and higher pH accelerate the decomposition of ascorbic acid. Hanging mercury drop electrode (HMDE) was used for the determination of ascorbic acid as working electrode. Initial potential was –0.3V with scan rate of 10mV/s and the peak appeared at about +0.05V. Ascorbic acid can be measured in the nanogram range with very good accuracy. After developing and optimizing the method different pharmaceutical tablets were analysed for levels of Vitamin-C. After dissolution of tablets in water aliquots were taken into acetate buffer and determined applying the standard addition method. Different types of fruit juices were also analysed for Vitamin-C. Levels of ascorbic acid in juices were in the ranges of 24 to 225 ug/100 ml.

Introduction

Ascorbic acid (AA) is present naturally in different food materials, fruits etc. and may be lost during storage and cooking due to its oxidation. It is also added to certain juices, food preparations and pharmaceutical preparations. It participates in numerous biological events concerning electron transfer reactions, hydroxylation, and oxidative metabolism of aromatic amino acids and coexists in brain with several neurotransmitter amines. It is added into pharmaceutical preparations and human diet. Its analysis in blood, urine etc is also required. Thus analysis of Vitamin-C in food materials and body fluids is important [1- 4].

Different analytical techniques are used for the analysis of AA [5]. Mostly used analytical techniques are spectrophotometric [6-9] or electroanalytical. Conventional methods of analysis [2] are time consuming due to the lengthy procedures. Electroanalytical techniques mostly used are potentiometry [10], amperometry [11-12], coulometry [13], polarography [14] and voltammetry [15-22] etc. Polarography and voltammetry have numerous applications in medicine, biochemistry and pharmacy in addition to inorganic compounds. Voltammetry is a very good analytical technique and can be used for the analysis of organic compounds [3-4] proteins, enzymes and vitamins [4-5] heavy and toxic metals [4, 23] anions [24] and may other compounds. Polarography has been used for the study of vitamins A, B, C, E, K and folic acid etc. Differential pulse voltammetry is a very sensitive, reliable and rapid method of analysis. It does not require any chemicals for complexation, extraction and separation etc. This method can be used for the analysis of AA using carbon paste electrode [25], platinum electrode and mercury electrode.

In this work a differential pulse voltammetric method has been worked out using hanging mercury drop electrode for the analysis of ascorbic acid. Effect of pH has been investigated on the analysis of AA. Vitamin-C was measured in different types of juices and pharmaceutical tablets and results are reported and discussed.

Results and Discussion

Vitamin-C is often used as antioxidant. This is because AA (I) is oxidized very easily to give dehydroascorbic acid (II). This is unstable and undergoes a subsequent hydration reaction to give the gemdiol

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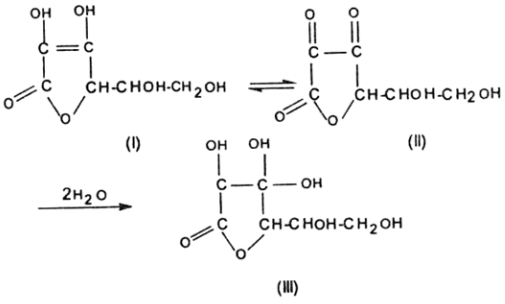
form (III). This oxidation reaction has been used for the voltammetric determination [3] of AA. The character of this anodic wave the change in half-wave potential, and the proposed reaction mechanisms are similar to the other endiols. Oxidation reaction of AA is given below.



The oxidation is reversible[26] whereas the subsequent hydration reaction is irreversible [27] with a rate constant 1 x 10^{-3} s^{-1} [28]. On a chemically modified glassy carbon electrode cyclic voltammetric studies [29] showed that the slope of the peak potential for AA between pH 2.0 and 4.0 is about 54±2 mV pH^{-1}, indicating a 1 e^-/1 H^+ reaction was involved in the oxidation process. At higher pH values (4.0-8.0) the slope decreased to about 35±2 mV pH^{-1}, suggesting a $2e^-/1H^+$ transfer reaction. The overall electrode reaction can be classified as an electrochemical reaction followed by a chemical reaction process as previously reported [30].

Ascorbic acid can be measured in the whole pH range by voltammetry; its peak height is directly proportional to concentration. For actual determination pH 2-7 is the best. At higher pH the oxidation of ascorbic acid is caused too easily by traces of oxygen. The most important step in the determination of ascorbic acid is the preparation of sample before the actual analysis. The determination is influenced by the facile oxidation of ascorbic acid. This oxidation is brought about very often by means of air especially in the presence of oxidases and becomes more rapid the higher the pH of the solution containing the dissolved AA.

Ascorbic acid has been measured on carbon paste electrode[25] versus SCE because of its better working range on the anodic side and for AA anodic wave is measured because of its oxidation. Solid electrodes like carbon paste electrode have some inherited problems of surface renewal and require very fine polishing and other treatments to get reproducible results. In this work a HMDE was used. AA was measured in acetate buffer without any interference. Very good voltammograms were obtained. Voltammograms of AA of sample and standard additions are given in Fig.1. Precision of the method was very good. Peak height versus AA concentration is quite linear Fig. 2. To see the accuracy of the method studies were done about the recovery of the added standards. Known amounts of AA were added to actual samples. Samples were measured for the levels of AA before the addition of standards and then after the addition of standards. It can be seen that recoveries of the added standards to the samples were quite good (Table.1).

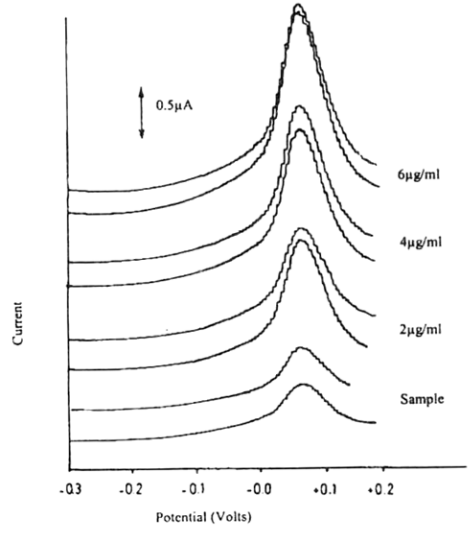


Fig.1: Voltammetric peaks of Vit-C, Initial potential –0.3 V, scan rate 10 mV/sec., sensitivity 5 µA, background electrolyte is acetate buffer (pH=4.70)

Table-1: Recoveries of the Added AA to Samples

	Added µg/100 ml	Measured µg/100 ml	Recovery %
Sample-1	-	206±8	-
Sample-1	100	302±7	96
Sample-2	-	115±12	-
Sample-2	100	212±9	97

Different analytical methods for the determination of ascorbic acid have been reviewed [32]. Voltammetric method is very good method and

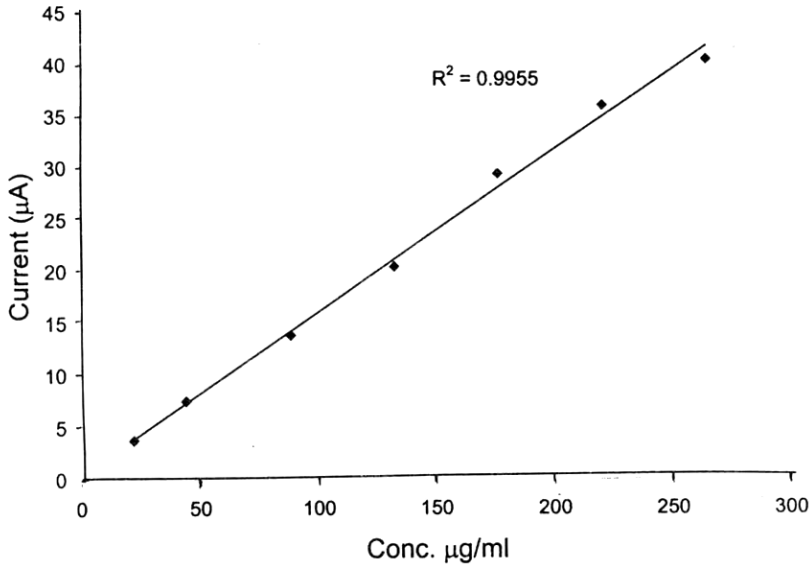


Fig. 2: Calibration curve of ascorbic acid

can be used for the analysis of AA in a variety of materials. AA can be determined in all types of materials including plants, fruits, vegetables and nuts etc. The material to be analysed is ground into a powder form and AA is extracted with acetate buffer. 3% metaphosphoric acid is also used for extraction of AA mixed with 8% acetic acid. For materials in which ascorbic acid cannot be measured directly such as potatoes, sugar beet determination can be carried out after extraction of the dry substance with absolute methanol. The extracts are decanted (40 ml) mixed with 1M acetic acid (10 ml). pH is adjusted and measured directly.

Combined ascorbic acid is a term used for compounds which themselves are polarographically inactive at the potential where free ascorbic acid undergoes oxidation. The combined form is converted into free ascorbic acid by hydrolysis in acid medium, if the plant material is treated with a hot solution of 2% sulfuric acid. All of the ascorbic acid can be determined whereas if 2% metaphosphoric acid is used, only the free acid is determined. The difference between the two determinations then gives the content of the combined AA. Average values of AA in different types of fruits and vegetables are given in Table-2.

Table-2: Average Values of AA in Different Types of Fruits and Vegetables (3)

Fruit/Vegetable Type	AA concentration mg%
Lemons	72
Oranges	59
Strawberries	43
Grape fruit	39
Apples	3
Red peppers	230
Green peppers	59
Cauliflower	80
Cabbage	75
Spinach	35
Potatoes	36
Tomatoes	30

Pharmaceutical Tablets

AA was determined in tablets by dissolving them in water and measuring in aqueous buffer, such as an acetate buffer of pH 4.7. The presence of compounds such as aspirin, calcium gluconate, histadine, vitamin K_3, vitamin A and D do not affect the determination of AA and the analysis of complex preparations can be carried out. If there is any interference these can be prevented by extraction with absolute ethanol. In this work two types of chewable Vitamin-C tablets and two types of Calcee (effervescent) tablets were analyzed. Tablets were

dissolved in 50 ml of dearated water. About 100µl of the sample were taken and measured in acetate buffer background electrolytes. Calculations were done using standard addition method. The chewable tablets usually contain: saccharine, silicic acid, sodium cyclamate, magnesium stearate, talc etc. and calcee tablets usually contain, tartaric acid, sod. bicarbonate, sod. Cyclamate, sod. Chloride and saccharine which do not cause any interference during the measurement of AA by voltammetry. For chewable tablets the prescribed levels of AA were 500mg per tablet whereas the measured valued were in the range of 430 mg to 510 mg. In the first type of tablets the levels of AA were about more than 98% where as in the second type of tablets the levels of AA were about 87%. Calcee tablets were measured in two sets of tablets collected from different sources. In the first set of tablets the levels of AA were about 90 % and in the second set the levels of AA were about 98%. Lower levels of AA in tablets than prescribed show either the oxidation of AA during preparation or storage or actually contain lower levels. For chewable tablets the first type of tablets contain nearly the prescribed levels of ascorbic acid while in the other type the levels are a little bit lower which may be due to some degradation with time during storage. Results about the measured values of AA in the tablets are given in (Table-3).

Table-3: Determination of AA in Pharmaceutical Tablets

Chewable tablets	Measured Value (mg)	Prescribed value (mg)	Measured %
S1-1	510±22	500	102
S1-2	490±15	500	98
S1-3	492±15	500	98
S2-1	445±12	500	89
S2-2	430±15	500	86
S2-3	435±16	500	87
Calcee tablets			
C-1	890±45	1000	89
C-2	920±30	1000	92
C-3	910±32	1000	91
C1-1	990±35	1000	99
C1-2	975±38	1000	97
C1-3	980±40	1000	98

Fruit Juices

Different types of fruit juices are available in the market. Some selected juices of certain brands were purchased and stored at -20^0C and thawed before analysis or these were analysed directly after purchase. Most of the juices were clear solutions and were analysed directly. In case of pulpy juices these were strained through gauze. 25 ml of IM acetate buffer of pH 4.7 was taken in the cell and deoxygenated with nitrogen and mixed with 25 ml of juice and again deoxygenated and then measured directly for AA by differential pulse voltammetry. But in most of the cases where concentration of AA was enough in the juices only 5ml of juice was taken and mixed with 45ml of 0.1M acetate buffer of pH 4.27. Peaks of AA obtained for juices were as good as for pure ascorbic acid. No distortion or broadening of peaks took place. No other peak was observed with the AA peak. The reason may be the clear oxidation reaction without any side reactions and relatively less positive potential, which not only produced well-defined and sharp peaks, but also got rid of interfering peaks. Lower pH values will shift the peak to more positive potential. Levels of AA in the different types of juices are given in (Table-4). It can be seen that levels of AA in Mango + orange mixed juices are higher than in mango and apple juices. Apple juices from two different companies varied appreciably regarding the levels of AA. This shows that addition of AA to juices by different companies is different.

Table-4: Determination of AA in Fresh Juices

Type of Juice	Measured Value (µg/100 ml)
Mango + Orange	
MOH-1	225±11
MOH-2	200±9
MOH-3	208±9
Mango	
MF-1	105±5
MF-2	120±8
MF-3	116±9
Apple Brand-1	
AH-1	124±10
AH-2	124±9
AH-3	116±12
Apple Brand-2	
AF-1	24±3
AF-2	28±2
AF-3	30±2

Effect of pH

Effect of pH was studied on the determination of AA by differential pulse voltammetry. Voltammetric peaks were taken at different pH values. In Table-5 are given the peak potentials of AA at different pH values. With the increase in pH value the peak potential decreases. It was observed that with the decrease of pH the peak potential shifts towards more positive potentials. Change in peak potential is appreciable. Slope of the peak potential

Table-5 Effect of pH on The Peak Potential of Vitamin-C

S. No	pH	Peak Potential (Volts)	Peak Height/ Current (μA)
1.	3.00	+ 0.18	2.68
2.	3.42	+ 0.15	2.92
3.	4.05	+ 0.12	2.91
4.	4.65	+ 0.08	5.75
5.	4.99	+ 0.06	5.74
6.	5.89	+ 0.025	5.58
7.	6.49	+ 0.01	5.56

from pH 3.0 to pH 4.05 is about 56 mV and slope of peak potential from pH 4.65 to 6.99 is about 37 mV, which shows that up to pH 4.05 $1e^-/H^+$ reaction takes place and from pH 4.65 to 6.49 $2e^-/H^+$ reaction takes place. It is also apparent from the increase in peak height at higher pH values. Peak heights for the same concentration of AA nearly become double at higher pH as compared to lower pH due to two electron transfer reaction instead of one electron transfer reaction. At lower pH the shift in potential is more than at higher pH. At lower pH the peak height is also reduced which reduces the sensitivity of the method. Although air oxidation and decomposition chances of AA are lower at lower pH but due to more positive potentials it may create some problems due to interference peaks and will also become difficult to measure at very low pH value due to the low working range of mercury in the positive side. Thus the pH value of acetate buffer is better for the measurement of AA. It is still in the acidic side and air oxidation of AA will not take place easily and at the same time it can be measured easily on the mercury electrode. Secondly the sensitivity of the method was better than at lower pH values.

Experimental

Apparatus

Polarographic analyzer model 174A and X-Y recorder model Re0089 from Princeton Applied Research, USA alongwith the hanging mercury drop electrode model 6.0335.000 of Metrohm, Switzerland was used for the analysis of AA in the differential pulse mode. Pulse height was 50 mV with scan rate of 10-mV/sec and clock time of 0.5 s. The three electrode system used consisted of a hanging mercury drop electrode as working electrode saturated Ag/AgCl electrode as reference electrode and platinum wire as counter electrode. pH meter used was Model 605 of Metrohm Switzerland. All the glassware used was of pyrex glass.

Chemicals

Ascorbic acid, Acetic Acid and Sod. Acetate p. a grade from E-Merck Germany were used and solutions were prepared as required. Standard solutions of Ascorbic acid were prepared fresh in deaerated deionized water and stored in the dark.

Procedure

The stock solution of ascorbic acid (1000 μg/ml) was prepared in acetate buffer after prior deaeration by pure nitrogen. The stock solution was kept in darkness. For each measurement the supporting electrolyte of acetate buffer was deaerated with pure nitrogen for about ten minutes, then sample was added and again deaerated and measured for AA. After each standard addition solution was deaerated prior to measurement. Initial potential selected was -0.3 V and scanned in the positive direction at a scan rate of 10 mV/sec and potential range selected was 0.75 volts. AA peak appeared on the positive side.

Conclusions

Hanging mercury drop electrode has been used advantageously for the analysis of AA after careful selection of pH and reference electrodes by differential pulse voltammetry (DPV). Effect of pH has been studied on the measurement of AA by DPV and slopes of the peak potentials between pH 3.0 and 4.05 and between pH 4.65 and 6.49 show one electron and two electron transfer reactions. The DPV method is very useful for the determination of Vitamin-C in pharmaceutical tablets and preparations for quality control. Some tablets contained lower values of Vitamin-C than the prescribed levels. This method was successfully applied for the determination of AA in fruit juices directly without any prior separation procedure. Levels of AA in different fruits and vegetables are also reviewed. Orange juices contain higher levels of Vitamin-C followed by mango and apple juices when compared of the same brand. Different brands of Juices of the same fruit have different levels of Vitamin-C. DPV method is sensitive, simple, selective, quick and reliable for the analysis of AA in different type of materials.

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