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Flow Injection Determination of Vitamin C in Pharmaceutical Preparations by Differential Electrolytic Potentiometry

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Abstract

Differential electrolytic potentiometry (DEP) was coupled with Flow injection analysis (FIA) technique for the determination of ascorbic acid in pharmaceutical preparations. Platinum electrodes were used as an indicating system to follow the oxidation of vitamin C with either potassium iodate, or potassium permanganate in an acidic medium. Univariate method was employed to optimize the variables such as the current density, the flow rate, the concentrations of the oxidants and the sulfuric acid. A current density of 40 μAcm^{-2} and a flow rate of 25 $\mu lsec^{-1}$ were found to be optimum. The optimum concentrations of iodate and permanganate were 8.35 mM and 0.11 mM respectively. On using iodate as an oxidant the method showed a linear range of 12-130 μgml^{-1}, a detection limit of 9 μgml^{-1} and R2 of 0.999. In case of permanganate a linear range of 18-36 μgml^{-1}, a detection limit of 11 μgml^{-1} and an R2 of 0.996 were obtained. The procedure was applied successfully to the determination of vitamin C in commercial tablets. The results of this study were found to be statistically comparable with those obtained by official methods.

Keywords Flow injection, DEP, ascorbic acid, pharmaceutical, potassium iodate

1. Introduction

Ascorbic acid (Fig. 1), a water soluble vitamin, is essential to health of human beings. It is used in the treatment and prevention of scurvy.

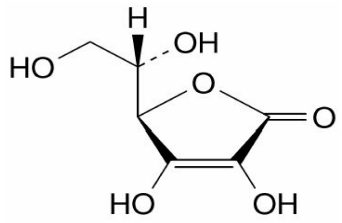

Figure 1. Structure of ascorbic acid (vitamin-C)

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] and copper sulphate in the presence of neocuprine [10]. Chromatographic techniques such as HPLC with electrochemical detection [11], liquid chromatography with electrochemical detection [12], ion-suppression reverse phase chromatography [13] and capillary zone electrophoresis, [14] have also been applied for ascorbic acid assay. A number of different types of voltammetric methods making use of a variety of electrodes have been developed [15- 18]. Most of the methods developed for the determination of ascorbic acid are either visual or potentiometric titrations. In the latter, 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 with cerium(IV) [28-29]. However, visual titrimetric methods require large samples and suffer from serious limitations if the sample is colored or opaque.

In such samples, the exact location of the end-point by using indicators becomes difficult. Researchers have tried to offer other alternative methods that can successfully locate the end-points even in colored or opaque samples. Among those methods was the technique of differential electrolytic potentiometry (DEP). This technique consists of polarizing two identical electrodes with a stabilized small current and measuring the potential differences between them during the course of the titration. The direct current dc.DEP technique has been applied to various types of titrimetric reactions in both aqueous [30-32] and non-aqueous media [33-37] using different types of electrodes. Using this technique the polarized electrodes respond faster, the apparatus is simple and the salt bridge problems of the reference cell are eliminated.

At present, flow injection and sequential injection FIA/SIA techniques allow automated handling of micro liters amount of sample and reagent solutions with a strict control of reaction conditions. These techniques produce less hazardous waste. Moreover they are suitable for the analysis of a large number of samples. FIA was coupled with DEP for the determination of chloride [30]. The same setup was applied for the determination of ascorbic acid by using Ce(IV)as an oxidant. However, due to the effects of mixed potentials resulting from the existing ratios of Ce(IV)/ Ce(III) and ascorbic acid / dehydroascorbic acid on the resulting signals a second line was introduced.. In this line a standard solution of ascorbic acid was propelled.[31]. To avoid this effect it was decided to apply other oxidants like iodate and permanganate. By coupling DEP with SIA, in this study, a new opportunity is opened for the assay of vitamin C. In this method, two identical platinum electrodes were polarized with a heavily stabilized small direct current. This proposed

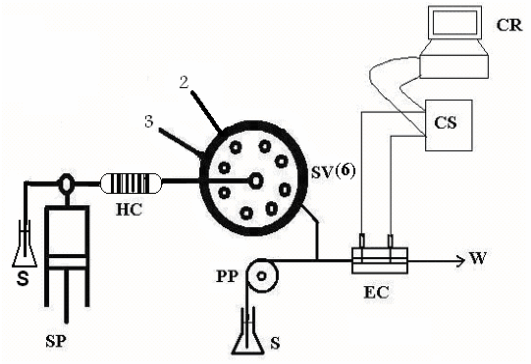

Figure 2. Schematic diagram for SIA/FIA manifold used for the determination of ascorbic acid. S; oxidant solution, SP; syringe pump, HC; holding coil, 2,3 drug and sulphuric acid solutions respectively, PP; peristaltic pump, EC; electrochemical cell containing two similar electrodes, CS; constant current source ,CR; computer readout, W; waste.

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method, SIA-DEP, is based on the oxidation of vitamin C with iodate or permanganate.

2. Experimental

2.1. Reagents

A solution of 0.1 mol l^{-1} H_2SO_4 was prepared by diluting 3.0 ml of concentrated acid (ca. 97%) to 500 ml with water. A standard solution of 1.5×10^{-3} mol l^{-1} L-ascorbic acid (Aldrich) was prepared by dissolving 0.5284 g in 200 ml of 0.1 mol l^{-1} H_2SO_4 solution. Afterwards, the working standard solutions of ascorbic acid were prepared by appropriate dilution with 0.1 mol l^{-1} H_2SO_4.

A solution of 8.35×10^{-3} mol l^{-1} IO_3^- was prepared by dissolving 0.357 g of KIO_3 in 200 ml of 0.1 mol l^{-1} H_2SO_4. A solution of $KMnO_4$ was prepared by dissolving 0.216g of $KMnO_4$ (Merck) in 250 ml deionized water, then working solutions were prepared by appropriate dilutions.

2.2 Apparatus

A Keithley Instruments model 224 programmable current source was used to polarize the electrodes.
LabJack U12 USB DAQ device with eight 12-bit analog inputs, 2 analog outputs, 20 digital I/O, and a 32-bit counter used to convert the signal from analog to digital.

A Perkin Elmer UV/Visible Lambda EZ210 spectrophotometer equipped with a high resolution concave diffraction grating and Seya-Mamioka mount. The instrument consists of a 2 nm spectral band pass slit and a wavelength range from 190 nm -1100 nm. A 10 mm rectangular shaped cell was used for taking spectrophotometric readings.

A flow cell of 4 cm length was fabricated from plexiglass. A canal was made in the middle of this cell and two ports were designed to accommodate the electrodes. The distance between the electrodes was 0.8 cm. This distance was found to be optimized to produce a signal of a considerable height.

2.3 FIA/SIA Instrument

The SIA system is the *FIALab 3500* (Medina, WA USA). It is composed of a syringe pump, a multi-position valve, a Z-flow cell with SMA fiber optic connectors as well as pump tubing and PC. The Syringe Pump is 24,000 steps with an optical encoder feedback and 1.5 seconds to 20 minutes per stroke of 2.5 ml size. It is > 99% accuracy at full stroke. The volume capacity of the syringe is 2500 µl. The Multi-Position Valve has eight ports with a standard pressure of 250 psi (gas)/600 psi (liquid); zero dead volume; chemically inert; port selection is usually done using the software program. The Flow Cell is a 40 mm path length plexiglass with two screw holes where the metal electrodes can be accommodated. Pump Tubing of 0.30" ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used for connecting the different units; and making the holding coil (190 cm long). An Alitea peristaltic pump which is built with the instrument was also used.

2.4 Electrodes

Platinum wire of a 1.0 mm diameter and 99.99 % purity was purchased from ALDRICH. Two electrodes, each of 2.0 cm length, were prepared from platinum and were cleaned using a solution of aqua regia.

2.5 SIA/FIA Procedure

Fresh working solutions of ascorbic acid and a solution of 0.1 M sulphuric acid were prepared and linked to the selector valve through ports 2 and 3, respectively. The oxidant was linked to the syringe at the in-position valve. This solution was also linked to the peristaltic pump to obtain a continuous stream that passes through the flow cell.

The syringe was filled with 2500 µl of the permanganate solution by directing the two-way valve to the (in-position) mode, with a flow rate of 100 µl s^{-1}. The syringe pump was programmed to dispense about 800 µl of the carrier to clear out the flow cell and to flush the tubing.

A volume of 100 µl of a drug solution was aspirated into the holding coil and dispensed to the flow cell to flush the sample tubing. A volume of 100 µl of 0.1 M of sulphuric acid solution was aspirated into the holding coil and dispensed to the flow cell to flush the sample tubing. A volume of 100 µl of the drug solution was aspirated into the holding coil and while the peristaltic pump propelling the permanganate solution through the flow cell, a volume of 800 µl was dispensed to a T-shape injection port located 4 cm a head from the first electrode in the flow cell. The

solution is passed through the flow cell then the signal is measured.
The flow cell was then washed with 0.1 M sulphuric acid in order to allow the electrodes to equilibrate before injecting a new sample. A schematic diagram of the manifold is shown in Fig. 2.

2.6. Analysis of tablets
Five tablets of either vitamin C were finely powdered and a portion of this powder equivalent to 20 mg of the drug was accurately weighted. The sample was dissolved in enough amount of de-ionized water, and then different aliquots were delivered to each of the 10 ml volumetric flasks and diluted to the mark. These solutions were linked to the selector valve ports and analyzed as mentioned above.

3. Results and Discussion

In this method, vitamin-C is oxidized to the dehydroascorbic acid with either permanganate or iodate in sulfuric acid media.

$$MnO_4^- + C_6H_8O_6 + 6H^+ \rightarrow C_6H_6O_6 + Mn^{+2} + 4\,H_2O$$
$$IO_3^- + C_6H_8O_6 + 4H^+ \rightarrow C_6H_6O_6 + Mn^{+2} + 3\,H_2O$$

The kinetics of these reactions have been investigated (1) and were found to depend on the presence of the acid. Although, the oxidation of ascorbic with either permanganate or iodate cannot come to completion, however, it is followed by FIA technique. This is because the volumes and the time can be accurately controlled using the programmable computerized FIA/SIA system.

In DEP, the signal results from the difference in the potential of the anodically and cathoically polarized electrodes [27, 28]. Before injecting the drug solutions into the flowing stream, the reduction of MnO_4^- to Mn^{2+} or IO_3^- to I^-occurs at the cathode, hence a stable base potential for the system is established. When the sample is injected, vitamin C begins to react with MnO_4^- or IO_3^- while diffusing, so Mn^{2+} or I^- and the oxidation products are formed. Therefore, changes in the potentials of the two electrodes will take place, hence a signal will be generated and measured.

3.1 Optimization of the variables

The smoothness, the sharpness, and the symmetry of the differential curves obtained, depend on applying optimum conditions like the volume of the sample, the flow rate, the current density and the oxidant concentration. To investigate such conditions several experiments were performed using platinum electrodes as an indicating system. The effects of varying the concentrations of either KIO_3 or $KMnO_4$ were studied using a current density of 30µAcm^{-2} and a volume of 100 µl. In case of iodate, the concentration of $1.1x10^{-4}$ M was found to give the best signal while for the permanganate, the optimum concentration was $8.35x10^{-3}$ M. The optimum concentration of iodate was found to be $8.35x10^{-3}$ M and $1.66x10^{-4}$ M for permanganate. Those concentrations were used to study the effect of the aspirated volume of drug solution. A solution of 100 µgml^{-1} of ascorbic acid and a current density of 30 µAcm^{-2}. The results showed that the response increases with increasing the volume of the ascorbic acid up till 100 µl. beyond this volume the resulting peak becomes much broader. In addition, the effect of the current density was studied and it was noted that the response increases with an increase in the current density until the value 30 µAcm^{-2} is reached. On applying current densities above this value, the peaks became broader and demonstrate higher overpotentials.

3.2 Analytical Appraisal

The DEP FIA method was applied by running triplicates of standard solutions of ascorbic acid, using the above mentioned conditions. The results are shown in Fig.3. The calibration results are summarized in table 1.

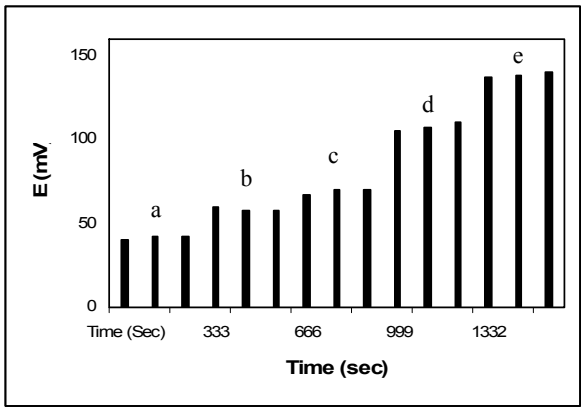

Figure 3. Triplicates of standard solutions of ascorbic acid (a, 0; b, 18; c, 24; d, 30; e, 36 µgml^{-1}) using permanganate as an oxidant, and applying the optimum conditions

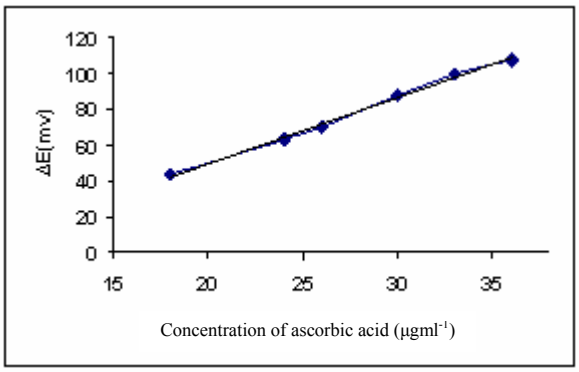

Figure 4. Calibration graph of ascorbic acid determination using iodate as an oxidant.

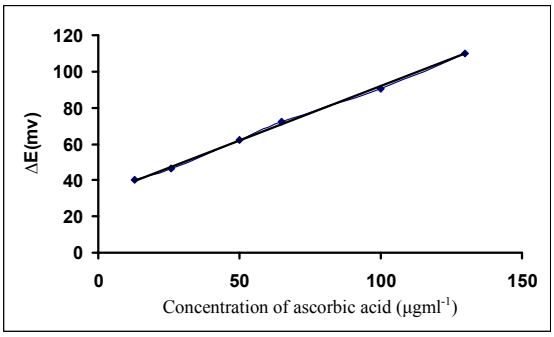

Figure.5. Calibration graph of ascorbic acid determination using permanganate as an oxidant.

Table1. Results obtained by DEP method using Platinum electrodes**.**

	Ascorbic acid	
	Using iodate	**using permanganate**
Concentration range, µgml^{-1}	13-130	18-36
Correlation coefficient	0.999	0.996
Slope	0.5979	3.693
Intercept	31.915	-24.19
Detection limit, µgml^{-1}	9.0	11
R.S.D, %	11	12

3.3 Application

The method was applied to the determination of ascorbic acid in drug tablets. Five 40 mg tablets of either ascorbic acid were finely powdered, an accurate weight is taken and stirred at room temperature with enough amount of water, the resulting solution is then filtered and a volume of 5 ml of this solution was quantitatively transferred to 100 ml volumetric flask and diluted to the mark with 0.01 M KNO_3 solution. Then the procedure was undertaken by applying the above mentioned conditions. The statistical values were calculated for both the adopted values and the standard method [28] and compared. The results are shown in Table2.

Table 2 Application of the proposed method

Drug	**Proposed method**		**Standard method**	
	Amount taken	**Recovery**	**Amount taken**	**Recovery**
	µgml^{-1}	**RSD%**	**µgml^{-1}**	**RSD%**
Ascorbic acid	50	98% ± 7	50	99% ± 4
	50	97% ± 8	50	98% ± 2

4. Conclusions

The present method reported for the first time the application of FIA/SIA combination coupled with DEP detection system for the determination of ascorbic acid in drug formulations. It has advantages of using small amounts of chemicals, fast, and economical. The method can be used for online analysis of the mentioned drugs.

Acknowledgments

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