

Short communication

Automatic potentiometric flow titration procedure for ascorbic acid determination in pharmaceutical formulations

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Abstract

A flow procedure for the determination of ascorbic acid in pharmaceutical formulations exploiting potentiometric titration is described. The method is based on the reduction of IO_3^- by ascorbic acid and the detection was carried out employing a flow-through ion selective electrode for iodide. The flow network controlled by a microcomputer was designed to implement multicommutation for ease of operation and robustness. The titration system allowed the determination of ascorbic acid in pharmaceutical formulations with concentrations ranging from 7.5 to 15.0 mmol l^{-1} . No significant differences at the 95% confidence level were observed in comparison with results obtained by a manual procedure. Merit figures of results such as a relative standard deviation of 1.0% ($n=6$) and a reagent consumption of 21.4 mg IO_3^- per determination were obtained. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Volumetric titration was proposed 200 years ago and today is still widely used in research and routine analysis [1,2]. Features such as ease operation, specificity and accuracy are advantages afforded by this analytical process. As detection techniques, potentiometry and spectrophotometry have been widely used [3,4]. Aiming of the improvement of throughput and also a reduction in

reagent consumption, analytical procedures based on flow analysis have been proposed [5–9]. To attain these goals, strategies such as use of a mixing chamber [5], variation of the flow rate ratio (linearly or exponentially) [7–9], binary sampling [10] and air monosegmented flow [11,12] have been proposed.

Flow networks using a mixing chamber in the analytical path were designed for titration procedures for the spectrophotometric determination of ascorbic acid in pharmaceutical formulation [13], tartaric acid and total acidity in wines [14,15]. A difficulty that occurs in the determination of

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ascorbic acid by spectrophotometry is interference caused by the colour of the sample. To overcome this difficulty, some authors have employed a sample dilution step prior to the analysis [16,18].

In this work, we develop an automated titration flow procedure for the determination of ascorbic acid in pharmaceutical formulations, employing a flow network based on the multicommutation process [19]. The method selected was based on the reaction of ascorbic acid with IO_3^- followed by potentiometric detection using as a sensor of iodine an ion selective electrode [20]. A sweeping sample volume strategy was exploited to obtain an automatic flow titration procedure, using a single pumping channel to displace both titrand and titrant solutions. Furthermore, operational simplicity, robustness and the ability to process the untreated sample are also anticipated advantages of associating solution handling by multicommutation and detection using Na ion selective electrode.

2. Experimental

2.1. Apparatus

The system consisted of a IPC-4 Ismatec peristaltic pump with tygon pumping tubing, a microH 2002 Crison potentiometer furnished with a flow-through ion selective electrode sensitive to iodine [17] and a Ag/AgCl 900029 ORION reference electrode, a 486 microcomputer furnished with an Advantech PCL 711S electronic interface card (American Advantech Corp., San Jose, CA, USA), running a software written in Quick Basic 4.5, and a home-made electronic interface to drive the solenoid valves [19]. The flow network comprised a 161T031 NResearch three-way solenoid valve (Neptune Research, MA, USA), and a TPFE helical reaction coil, 100 cm long and 0.8 mm i.d.

2.2. Reagents and samples

All chemicals were of analytical grade. Freshly distilled and deionized water was used throughout.

A $0.1 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$ solution was prepared by diluting 3 ml of concentrated acid (ca. 97%) to 500 ml with water.

A $15 \times 10^{-3} \text{ mol l}^{-1}$ L-ascorbic acid standard solution was prepared by dissolving 0.5284 g in 200 ml of $0.1 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$ solution. Afterwards, the working solutions 7.5×10^{-3} , 9.0×10^{-3} , 10.5×10^{-3} , 12.0×10^{-3} , $13.5 \times 10^{-3} \text{ mol l}^{-1}$ L-ascorbic acid were prepared by appropriate dilution of the above solution with the $0.1 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$.

A $8.35 \times 10^{-3} \text{ mol l}^{-1} \text{ IO}_3^-$ solution was prepared by dissolving 0.357 g of KIO_3 in 200 ml of the $0.1 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$.

A 1% (m/v) starch solution was prepared by dissolving 1.0 g in hot water and making the volume up to 100 ml.

Samples of pharmaceutical formulations were prepared by powdering the tablets employing a porcelain capsule. The mass (0.3–0.6 g, depending on the formulation) was weighed and dissolved in 100 ml of the $0.1 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$. The sample and reference solutions were prepared daily and stored in amber bottles to avoid oxidation by light.

2.3. Procedure

The sweeping sample volume approach as proposed in this work consists in the insertion of titrand solution aliquots into the stream of the titrant solution. After the first run, the volume of the sample aliquot is increased by a constant value to carry out the next one. This strategy is maintained until the titration end point is attained. At constant flow rate, the titrand solution volume can be changed by controlling the sample loading time according to the set of equations described below:

$$\Delta t_n = t_0 + n \Delta t_i, \quad (1)$$

$$\Delta t_d = \Delta t_p - n \Delta t_n, \quad (2)$$

$$v_s = \varphi \Delta t_n, \quad (3)$$

$$n = 0, 1, 2, \dots, (\Delta t_p - 2t_0)/\Delta t_i, \quad (4)$$

where t_0 is the initial time; Δt_p , run period; Δt_n , sample loading time, valve switched on; Δt_d , delay time with valve switched off; Δt_i , increment in

sample loading time; v_s , sample aliquot volume; and φ , flow rate in $\mu\text{l s}^{-1}$.

The system designed to implement the sweeping approach is shown in Fig. 1. In this configuration, the solenoid valve (V) is switched off and the titrant solution (KIO_3) is flowing through the analytical path. When the software was run, the values of the parameters defined in these equations were supplied. Afterwards, an aliquot of the sample solution was inserted into the reaction coil (B) by switching the solenoid valve on. This was done by sending an electric pulse from the microcomputer to the valve V through the digital interface with a duration $\Delta t_n = t_0$ (see Eq. (1), first run, $n = 0$). Afterwards, the valve was switched off for a delay time Δt_d to complete the run period (Δt_p). This cycle was repeated several times, increasing the sample aliquot volume, as indicated by Eq. (1) until the end point of the titration was reached.

The microcomputer read the generated signal coming from the analogue output of the potentiometer through the analogue input A_0 of the PCL711S interface card. Data acquisition was undertaken at the same time that titration procedure was running and the measurements were processed as function of time until the end point of the titration. The IO_3^- solution was used as the carrier stream, so that the analytical signal (mV) was positive. When ascorbic acid solution was inserted into the carrier stream, the magnitude of

the signal decreased as a result of the reaction of ascorbic acid with IO_3^- ions. The signal acquires a negative polarity in an excess of the titrand solution. This feature was elected as a criterion to halt the analytical procedure. A plot of the measurements was displayed on the microcomputer screen to allow observation of the titration curve in real time.

To minimise the pulsing effect caused by the peristaltic pump and so improving the precision of the sampled solution volume, the sampling step was synchronised with the pumping pulsation. This was achieved via the microcomputer by monitoring the tachometer signal generated by the peristaltic pump prior to each analytical run. To allow the implementation of this step, the analogue input A_1 of the PCL711S interface card was coupled to the output of the peristaltic pump tachometer.

3. Results and discussion

The ion selective electrode for iodide was based on the homogeneous crystalline membranes without an inner reference solution [20], and its response concerning to iodide ions reflects the reaction of IO_3^- with ascorbic acid as indicated by the following chemical equation:

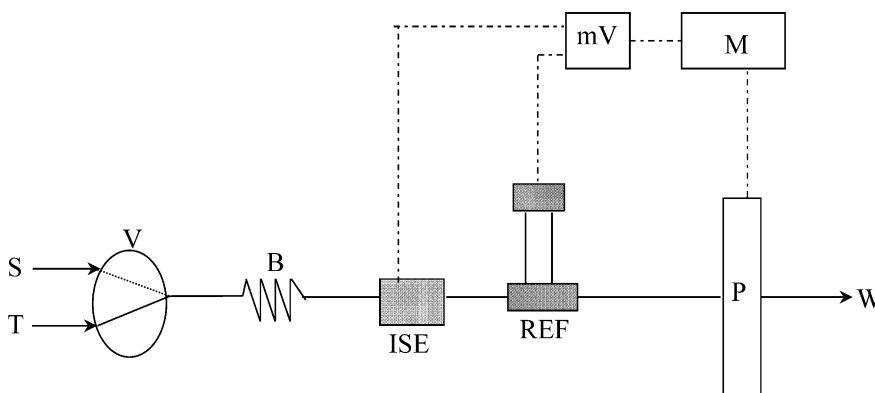


Fig. 1. Flow diagram of the system. V, three-way solenoid valve; B, helical reaction coil, 100 cm long and 0.8 mm i.d.; ISE, flow-through ion selective electrode; REF, reference electrode; P, peristaltic pump; mV, potentiometer; M, microcomputer; S, sample solution; T, titrant solution, flow rate 1.8 ml min^{-1} ; W, waste. Arrows indicate pumping direction. Solid and dashed lines within the valve symbol indicate the flow pathways when the valve is on or off, respectively.

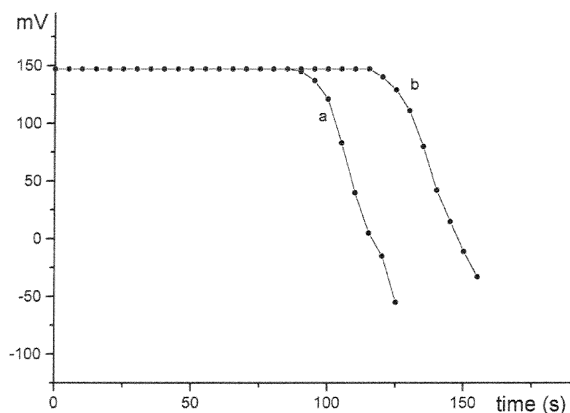


Fig. 2. Titration profiles. Curves *a* and *b* correspond to 9.0 and 12.0 mmol l⁻¹ ascorbic acid solutions, respectively.

The electrode presented a linear response to the principal ion and a high selectivity concerning to the proton (pH range 2.2–12.5), which is an advantage since the reaction of ascorbic acid with IO₃⁻ occurred in acidic medium, thus allowing sample preparation in sulfuric acid as recommended elsewhere [21] to improve analyte preservation by avoiding oxidation by ambient oxygen.

The titrant solution was used as the carrier stream and the signal generated by the detector was positive and became negative in excess ascorbic acid as shown in Fig. 2. This feature was employed to indicate the end point and the software was designed to end the procedure when this effect occurred. The sweeping procedure could also be stopped when the maximum sample volume solution was attained, that is the condition expressed by Eq. (4), $n = (\Delta t_p - 2t_0) / \Delta t_i$. This occurs when the sample is very dilute, in which case a more dilute titrant solution must be employed.

An initial sample aliquot volume of 15 μ l ($\Delta t_0 = 0.5$ s) and the final volume of 360 μ l were chosen ($\Delta t_n = 12$ s), so that a fraction of analytical path (30–720 mm), from the inlet was filled with sample solution during the loading step. Prior to the next analysis, the interval (Δt_n) used to load the sample solution was increased by 0.1 s; therefore, the true sample aliquot was 3 μ l greater than the one before.

Variation of sample zone length can affect the mixing conditions, therefore, to locate the end

point with assurance it is necessary that the solutions attain merge. To obtain the suitable conditions, experiments were carried out with reaction coil lengths of 50, 75, 100, 125 and 150 cm. The results showed that the coil should be at least 100 cm long. With higher lengths, the maximum volume (360 μ l) settled for sample solution aliquot was attained and the procedure was stopped without detection of the end point. On the other hand, with a shorter coil length (< 100 cm), the temporal signal presented shoulders, thus indicating that good mixing between the titrand and titrant solutions was not attained.

Curves *a* and *b* of Fig. 2 present a plateau region followed by an abrupt decrease towards negative polarity, indicating an excess of ascorbic acid. The time interval elapsed to attain this condition increased when the sample solution was diluted. This effect is expected, considering that a diluted solution requires a higher volume to surpass the stoichiometric point. The time elapsed from the first run up to the detection of a signal with negative polarity was employed to obtain the analytical curve, which presented a linear response as indicated by the equation: $y = 32.2 - 0.103x$; $y = \text{mmol l}^{-1}$ ascorbic acid; and $x = \text{elapsed time in s}$ ($r = 0.9998$). The volume of the titrant solution should be obtained by multiplying the elapsed time by the flow rate of the carrier solution.

Once the best operational conditions were established samples of pharmaceutical preparations were analysed, yielding the results shown in Table 1. These samples were also analysed using the manual procedure, employing the method based on reaction with IO₃⁻ and using starch as a visual indicator. Accuracy was accessed by applying the paired *t*-test to the results and no significant difference at the 95% confidence level was observed. Other important analytical features, such as a reagent consumption of 21.4 mg KIO₃ per determination, a relative standard deviation of 1.0% ($n = 6$) for a typical sample containing 12.3 mmol l⁻¹ ascorbic acid, a linear response ranging from 7.5 up to 15 mmol l⁻¹ ascorbic acid ($r = 0.9997$), and an analytical throughput of 15 determinations per hour were also achieved.

Table 1
Results comparison

Drug	Supplier	Contents (mg)	Mass (g)	
			Proposed method	Reference method
Redoxon liquid (drops)	Roche	Vitamin C (200)	0.235 ± 0.001	0.234 ± 0.00
Redoxon (tablet effervescent)	Roche	Vitamin C (1000)	0.262 ± 0.001	0.253 ± 0.002
Vitamin C-Schering	Schering	Vitamin C (1000)	0.265 ± 0.001	0.267 ± 0.002
Cewin	Sanofi-synthelabo	Vitamin C (1000)	0.238 ± 0.001	0.236 ± 0.003
Ceklin (tablet effervescent)	Klinger	Vitamin C (1000)	0.208 ± 0.001	0.165 ± 0.003
Calcium-Sandoz	Sandoz	Vitamin C (500), calcium lacticogliconic (500), CaCO ₃ (327)	0.177 ± 0.137	0.218 ± 0.004
Cebion-Calcium	Merck	Vitamin C (500), CaCO ₃ (280)	0.184 ± 0.002	0.190 ± 0.001
Cebion (tablet effervescent)	Merck	Vitamin C (1000)	0.226 ± 0.009	0.218 ± 0.003

Average of three replicates; mean ± standard deviation.

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