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Short communication

Flow injection spectrophotometric determination of L-ascorbic acid in biological matters

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Abstract

A method for spectrophotometric determination of L-ascorbic acid (vitamin C) in tissues by flow injection analysis is reported. The procedure is based on the oxidation of analyse with iron(III) and 2,2′-dipyridyl. Concentrations of vitamin C in the range of 0.5–20 ppm have been determined with a relative standard deviation 1.2% ($n = 15$). The injection rate was 40 samples per h. The method was applied to the determination of L-ascorbic acid in rat's tissues (blood serum, brain, and liver) and compared favourably with an independent reference method based on spectrophotometry. © 2002 Published by Elsevier Science B.V.

Keywords: Flow injection analysis; L-ascorbic acid; Tissues

1. Introduction

L-ascorbic acid is a vitamin soluble in water and its deficiency in human body causes scurvy. Its symptoms in adults are gingivitis, susceptibility of blood vessels to damage and bleeding, changes in bones and cartilage and retarded wound healing [1–3]. L-ascorbic acid is necessary in redox processes taking place in cell [2]. It is reversibly oxidised to L-dehydroascorbic acid and partially metabolised to inactive sulphide and oxalic acid, which is expelled in urine. It is well absorbed from the digestive system and easily reaches the tissues.

Healthy organism contains 1.5 g of L-ascorbic acid and daily requirement for L-ascorbic acid is estimated for 30–100 mg. L-ascorbic acid is not synthesised by humans, but it is an essential dietary vitamin for the species. Its main sources are: citrus, briar-rose and black currant fruits, tomatoes and horseradish root [3–5].

Clinical deficiency in vitamin C leads to reduce drug metabolism and immuno-competence and thus affects social and work functions. Appropriate intake levels of vitamin C for each of its physiological functions have not been fully defined.

L-ascorbic acid with L-dehydroascorbic acid composes oxidation–reduction system able to transport electrons and hydrogen in reversible processes, thus playing an important role in the

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tissue respiration and many other metabolic processes.

Several methods have been proposed for the determination of L-ascorbic acid. For a broad collection of older method, the reader is referred to the literature cited by Seib [6] and Serbell [7]. Numerous techniques have been used for the determination of L-ascorbic acid based on the reducing property and ability to produce coloured compounds. These methods have been reviewed previously [3,5,8]. Since the introduction of flow-injection analysis [9], many flow systems have been developed for the determination of L-ascorbic acid [10–14]. Flow-injection analysis (FIA) has found wide application in various fields of routine analysis, including pharmaceutical [15].

This paper describes a flow injection method for the determination of vitamin C in rat's tissues. The versatility and simplicity of the FIA technique with spectrophotometric detection allow its adaptation at relatively low cost to the different requirements of a variety of analytical problems.

2. Experimental

2.1. Reagents

A solution of L-ascorbic acid (vitamin C, $C_6H_8O_6$, H_2Asc), from Fluke was prepared by dissolving the requisite amount of sample in distilled water. The solution was prepared fresh every day and kept in the dark and cold to minimise oxidation.

Iron (III)-2,2′-dipyridyl $(C_5H_4N)_2$ reagent was prepared by dissolving 0.176 g of ammonium iron(III) sulphate dodecahydrate $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 800 ml of water containing 10 ml of 5 M sulphuric acid. Then 2.5 g of 2,2′-dipyridyl was added and after dissolving by heating at 80 °C and cooling, the solution was diluted to 1 l with distilled water. A working solution was prepared by adding few drops 2% solution of cerric sulphate $Ce(SO_4)_2$ in 50% sulphuric acid to ensure complete oxidation of traces of iron(II). The colour of the solution was changed from red to yellow.

All chemicals used were of analytical grade.

2.2. Materials

The study was conducted on 15 male Wistar rats of initial body weight of 180–200 g. The animals put to sleep under ether narcosis and the blood from the heart (for cloth and heparin) as well as the brain and liver were collected. The blood collected for cloth after coagulation was subject to centrifugation and serum was separated. The brain and liver after washing in physiological saline were weighed and homogenised in Teflon tube, then tissues were centrifugation. The blood serum are centrifuged at $3000 \times g$ for 15 min. Tissues: brain or liver were isolated and homogenised in Teflon tube, then centrifuged at $3000 \times g$ for 20 min.

The concentration of L-ascorbic acid was assayed in supernatant by FIA method with spectrophotometric detection.

2.3. Methods

The flow injection assembly is shown in Fig. 1 and is included a multi-channel peristaltic pump type 304 (Electromedical Coop, Poland), a laboratory-made rotary injection valve with exchangeable sample loops. Spectrophotometric measurements were made using a spectrophotometer Specol 10 (Carl Zeiss Jena, Germany) equipped with a laboratory-made flow through cuvette and connected to a model TZ 4620 recorder (Laboratorni pristroje, Czechoslovakia). The PVC tubing used (0.7 mm i.d.) was connected with perspex connectors.

3. Results and discussion

3.1. General procedure for flow injection determination of L-ascorbic acid

3.1.1. Calibration procedure

Sample solution (100 μl) containing 1–20 mg dm^{-3} of L-ascorbic acid was injected into deionised water carrier and subsequently merged with stream of iron(III)-2,2′-dipyridyl reagent. Product of reaction was carried to the spectrophotometer flow-cell and the absorbance read at 510 nm.

L-ascorbic acid levels were analysed with the use of flow injection analysis method (FIA) with spectrophotometric detection [15]. Double channel system was applied, with water as a carrier and Fe(III) 2,2′-dipyridyl solution as a reagent (Fig. 1). The supernatant was injected to the carrier stream. In the reaction spiral L-ascorbic acid reduces Fe(III) to Fe(II) and the red coloured complex is created. The concentration of L-ascorbic acid was calculated on the basis of the complex absorbance. A spectrophotometer Specol 10 was used at 510 nm wavelength.

The values obtained by the presented FIA method were compared with the values obtained by the spectrophotometric Kyaw's method [16].

3.2. Optimisation of variables

In order to optimise the proposed FIA method, the influence of various experimental parameters on the peak height and reproducibility of the results was studied. These included flow rate, reaction coil length, injection volume and concentration of reagent.

3.2.1. Flow rate and reaction coil length

Both parameters are closely related and a change of one or the another causes a great influence on the peak height. The peristaltic pump controls the flow rate. Increasing the flow rate in the range 0.5–1.8 $cm^3\ min^{-1}$ was accompanied by a slight decrease in the peak height. A final flow rate of 1.2 $cm^3\ min^{-1}$ was chosen.

The reaction coil length, which was defined as the total length of tubing from the point of samples introduction to the detector, is interchangeable with longer or shorter tubing of the same type and diameter. It was therefore decided to use a 3 m long reaction coil.

3.2.2. Sample volume

The sample volume was varied between 50 and 700 μl by changing the sample loop length in the injection valve. The peak height and peak width was increased with sample volume. The volume of 100 μl was a compromise between sensitive and sample volume.

3.2.3. Concentration of 2,2′-dipyridyl $(C_5H_4N)_2$ and of ammonium salt $NH_4Fe(SO_4)_2 \cdot 12H_2O$

The effect of concentration of the reagents was studied in the range 1×10^{-3}–5×10^{-3} M of ammonium salt and 0.2–0.5% of 2,2′-dipyridyl. A 3.6×10^{-3} M ammonium salt and 0.25% 2,2′-dipyridyl concentration was chosen as the optimum for high sensitivity.

3.3. Analytical evaluation

Typical calibration peaks for L-ascorbic acid obtained under the optimised conditions is shown in Fig. 2.

The calibration graph (Fig. 3.) obtained from these results was linear over the range 0.5–20 ppm with a regression coefficient of 0.9993. Analytical features of the proposed method were summarised in Table 1 [17].

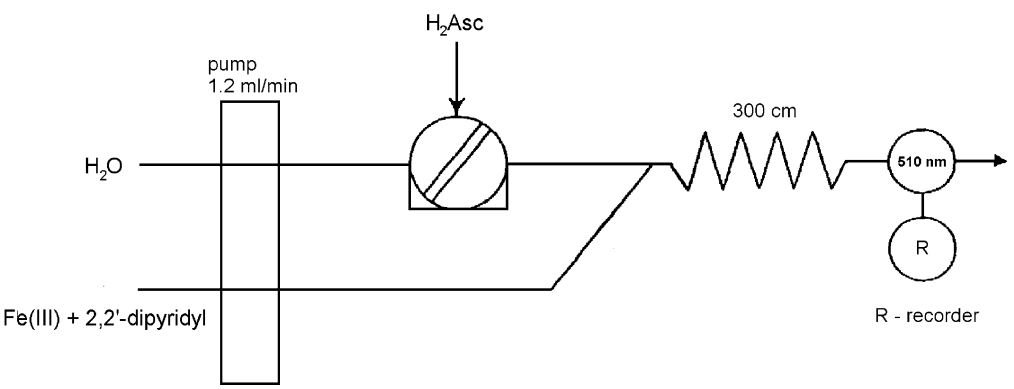

Fig. 1. Schematic diagram of the FI manifold used for the determination of L-ascorbic acid.

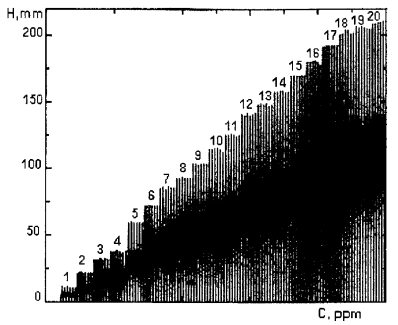

Fig. 2. Typical calibration peaks for L-ascorbic acid.

3.3.1. Effect of interference

In order to evaluate the selectivity of the developed method for the analysis of biological mater preparations, the effect of the presence of several species, which can occur in real samples with L-ascorbic acid, was investigated.

The tolerance level (interfering species/analyte); sacharose, glucose, lactose > 500 μg ml^{-1}, glutamic acid 30 μg ml^{-1}, citric acid 5 μg ml^{-1}, acetysalicylic acid 15 μg ml^{-1}.

3.4. Flow injection determination of L-ascorbic acid in biological mater

Concentrations of L-ascorbic acid in blood serum, brain and liver of rats are presented in Table 2.

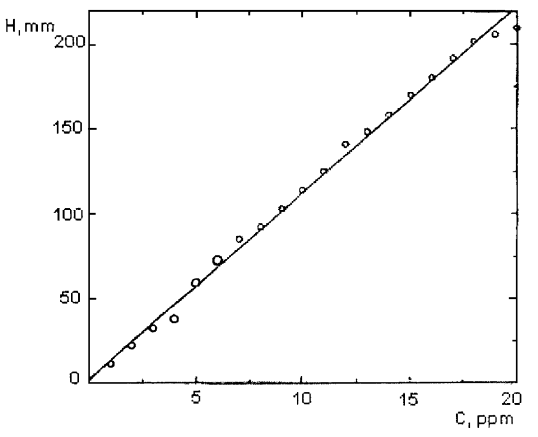

Fig. 3. The calibration graph used for the determination of L-ascorbic acid.

Table 1
Analytical features of the proposed FIA method

Parameter	
Linear range (mg· dm^{-3})	0.5–20
Regression equation ($y = ax + b$)	$a = 12.47 \pm 0.25$; $b = -2.8 \pm 1.6$
r^2	0.9993
Detection limit (mg· dm^{-3})	0.2
R.S.D. (%)	1.2
Sample throughput (samples per h)	40

Table 2
The concentration of L-ascorbic acid in tissues; methods, FIA and Kyaws, $n = 15$

Tissues	L-ascorbic acid levels FIA	L-ascorbic acid levels Kyaws
Blood serum (mg per 100 ml)	1.04 ± 0.081	1.01 ± 0.098
Liver (mg per 100 g)	13.5 ± 0.021	13.43 ± 0.034
Brain (mg per 100 g)	10.0 ± 0.057	9.89 ± 0.076

The proposed method is superior to other conventional method in that is fast and simple. The described method the determination of L-ascorbic acid in tissues may be a complement to the applied methods. Reagent consumption is minimum, precision and reproducibility of the adopted spectrophotometric systems are good and the values of RSD are low.

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