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Short communication

Simultaneous determination of rutin and ascorbic acid in a sequential injection lab-at-valve system

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ABSTRACT

A green, simple, accurate and highly sensitive sequential injection lab-at-valve procedure has been developed for the simultaneous determination of ascorbic acid (Asc) and rutin using 18-molybdo-2-phosphate Wells-Dawson heteropoly anion (18-MPA). The method is based on the dependence of the reaction rate between 18-MPA and reducing agents on the solution pH. Only Asc is capable of interacting with 18-MPA at pH 4.7, while at pH 7.4 the reaction with both Asc and rutin proceeds simultaneously. In order to improve the precision and sensitivity of the analysis, to minimize reagent consumption and to remove the Schlieren effect, the manifold for the sequential injection analysis was supplemented with external reaction chamber, and the reaction mixture was segmented. By the reduction of 18-MPA with reducing agents one- and two-electron heteropoly blues are formed. The fraction of one-electron heteropoly blue increases at low concentrations of the reducer. Measurement of the absorbance at a wavelength corresponding to the isobestic point allows strictly linear calibration graphs to be obtained. The calibration curves were linear in the concentration ranges of 0.3–24 mg L^{-1} and 0.2–14 mg L^{-1} with detection limits of 0.13 mg L^{-1} and 0.09 mg L^{-1} for rutin and Asc, respectively. The determination of rutin was possible in the presence of up to a 20-fold molar excess of Asc. The method was applied to the determination of Asc and rutin in ascorutin tablets with acceptable accuracy and precision (1–2%).

1. Introduction

By introducing a reaction chamber (RC) into the flow manifold, the obtained system exploits the characteristics of both flow and batch systems. As a result, such a system combines the advantages of the automated control of flows, including high sampling frequency, complete and precise control of reactant volumes and timing of operations, low cost, low consumption of the reagents and low effluent production – thus, the principles considered of green analytical chemistry – with the wide application range typical for batch systems [1].

Although systems in which RC is incorporated into sequential injection analysis (SIA) manifold were attributed to flow-batch analysis (FBA) systems [1], it is more logical to describe them as a separate technique. In accordance with this, the "Lab-at-valve" (LAV) concept was introduced by Grudpan [2]. In the SI-LAV system, sample processing, chemical reaction and/or detection are carried out in a designed LAV unit attached to the port of a multiposition selection valve. A LAV unit can be easily fabricated with relatively low-cost materials and available instrument/machine tools.

As follows from new trends in existing flow methods, increasing attention is being paid to multi-component analysis. Nevertheless, a review of the literature shows that contrary to the numerous developments in flow injection analysis (FIA) [3], only a limited number of papers have appeared in this field dealing with other techniques, including SIA [4] and FBA [5].

Rutin is a haemostatic drug used in the treatment of diseases characterised by capillary bleeding and increased capillary fragility [6]. It is often used together with ascorbic acid (Asc), and in this combination, it reduces capillary permeability and fragility more efficiently, also due to the inhibition of hyaluronidase activity. Rutin belongs to the group of bioflavonoids, and in line with Asc it participates in redox processes. Both compounds have antiox-

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idant properties and co-exist in plants. A few methods for the simultaneous determination of Asc and rutin in their combined dosages have been reported, including chemometric-assisted UV spectrophotometry [7], electrochemical methods [8], HPLC [9] and the SIA technique coupled with solid phase extraction [10].

Recently, the ammonium salt of 18-molybdo-2-phosphate heteropoly anion $P_2Mo_{18}O_{62}^{6-}$ (18-MPA) was proposed as a reagent for the determination of reducing agents, and several simple, fast, automated, sensitive and rather selective sequential injection methods have been developed for the determination of some reducing compounds such as Asc, *p*-aminophenol, epinephrine and cysteine [11–14].

In the present work, a novel, simple, highly sensitive, environmentally friendly, and cost-effective SI-LAV method has been developed for the simultaneous determination of Asc and rutin. The chemistry used in the determination is based on the dependence of the reaction rate between 18-MPA as reagent and reducing agents on solution pH. Only Asc is capable of interacting with 18-MPA at pH 4.7, while at pH 7.4 the reaction with both Asc and rutin proceeds simultaneously.

2. Experimental

2.1. Reagents and apparatus

Ultrapure water was produced by a Millipore™ water purification system (Millipore, Bedford, MA, USA) and was then used throughout the experiments. The ammonium salt of 18-MPA $(NH_4)_6P_2Mo_{18}O_{62} \times 14H_2O$ (18-MPC) was synthesized and recrystallized as previously reported [13]. A 0.01 M solution of 18-MPC was prepared by dissolving 0.7855 g of the salt in water and diluting to 25 mL. L-ascorbic acid (>99.7% purity), rutin trihydrate (>99% purity), methanol (for HPLC, > 99.9%), disodium hydrogen phosphate dodecahydrate, and sodium dihydrogen phosphate dihydrate were purchased from Fluka Analytical (Sigma-Aldrich, Buchs, Switzerland). A sample of the 0.01 M ascorbic acid stock solution was prepared by dissolving an accurately weighed amount in methanol. The stock solution of 1 mM rutin was prepared by dissolving and diluting 66.4 mg of $C_{27}H_{30}O_{16} \times 3H_2O$ to a final volume of 100 mL with methanol. Both of the last-mentioned solutions were preserved in a refrigerator to prevent untimely oxidation with oxygen dissolved in solvent. The Asc and rutin solutions were thus stable for at least two or four days, respectively. In order to prevent the untimely oxidation of rutin and ascorbic acid during the analysis, the dissolved oxygen was removed from the water used for the preparation of the standard and sample solutions by purging with nitrogen at a flow rate of 25 mL s^{-1} for 30 min. The following commercially available Ascorutin® tablets were analysed: 1) 100 mg of Asc and 20 mg of rutin trihydrate per 0.5 g tablet (Zentiva, Prague, Czech republic) and 2) 50 mg of Asc and 50 mg of rutin trihydrate per 0.33 g tablet (Kyiv vitamin factory, Kyiv, Ukraine). An acetate buffer solution with pH 4.7 ± 0.2 was prepared by mixing 10.1 g of sodium acetate and 4.0 mL of glacial acetic acid in a 250 mL flask and filling up to the mark with water. The phosphate buffer solution of pH 7.4 ± 0.1 was prepared by dissolving 1.17 g of $NaH_2PO_4 \times 2H_2O$ and 7.78 g of $Na_2HPO_4 \times 12H_2O$ in water and filling up to a volume of 500 mL (final concentrations 0.03 M and 0.087 M in NaH_2PO_4 and Na_2HPO_4, respectively). The absorbance measurements were performed on a Lightwave II UV–vis spectrophotometer (Biochrom Ltd., Cambridge, UK) with a 1.0 cm quartz cell. An Orion 720A pH meter (Orion Research Co., Boston, MA, USA) was used for measuring the pH.

2.2. SI-LAV system

A commercial FIAlab® 3500 system (FIAlab® Instruments Inc., Bellevue, WA, USA) equipped with a syringe pump (syringe reservoir 5 mL) and an 8-port selection Cheminert valve (Valco Instrument Co., Houston, TX, USA) was used. This SIA set-up was supplemented with an LS–1 tungsten halogen lamp as the visible light source, a USB4000-UV-VIS diode array spectrophotometer (both Ocean Optics Inc., Dunedin, FL, USA), and a microvolume SMA-Z flow cell with a 20 mm optical path length. The entire SIA system was controlled by the FIAlab software package (version 5.0). Flow lines were made from 0.75 mm i.d. PTFE tubing. A 2 mL microcentrifuge polypropylene tube with 1.2 cm i.d. width and a funnel-shaped inlet at the bottom was used as the reaction chamber. The SIA manifold used for the simultaneous determination of Asc and rutin is shown schematically in Fig. 1.

2.3. General SI-LAV procedure

The overall analytical procedure consisted of four stages: washing the RC, delivering the reaction components into the RC, carrying out the chemical reaction and measuring the analytical signal. At the first stage, the flow-rate is set at 100 μL s^{-1}; the syringe pump valve is switched to position IN; and the syringe pump is filled with 1500 μL of ultra pure water used as the carrier solution. Next, the syringe pump valve is switched to position OUT, and 450 μL of water is driven into the RC through port 2 of the multi-position valve. By the reverse movement of the syringe pump (500 μL) the washing is first directed back into holding coil (HC) and then into the waste reservoir through port 1 (600 μL).

At the second stage, the flow-rate is reduced to 50 μL s^{-1}, and 150 μL of air is aspirated into the HC through port 8, followed by 250 μL of sample and 40 μL of 0.15 mM 18-MPC introduced through ports 5 and port 6, respectively. After that, 20 μL of buffer solution with pH 4.7 ± 0.2 (port 3) or pH 7.4 ± 0.2 (port 4) are drawn into the HC. The obtained mixture is moved into the RC with 360 μL of water, thus leaving 100 μL of air in HC. Isolation of the reaction mixture from the carrier is necessary to retain sample homogenization.

At the third stage, 560 μL of air is introduced into the HC, and then 570 μL of air is passed through the solution in the RC. In this way, the fully homogenized solution is obtained at the earliest possible time. In order to complete the reduction of 18-MPA with analytes, the reaction mixture is maintained for 240 s.

At the measurement stage, the spectrometer reference scan is made. The coloured solution is first dispensed into the HC (400 μL), and then 320 μL of this solution is forced out through port 7 into the Z-flow cell at 30 μL s^{-1} (at higher flow rates the probability of the appearance of bubbles on the walls of flow cell increases), and the flow is stopped for 20 s. The measured absorbances are averaged during this time period. The response is measured at 920 nm. Finally, the remaining solution and the water contained in the system are directed through port 1 to the waste reservoir by emptying the syringe pump. The occasional washing of the system with methanol was found to be a very efficient method for avoiding the risk of air bubbles being trapped on the inner walls of the tubes and flow cell.

2.4. Sample preparation of ascorutin tablets

Five tablets were accurately weighed and crushed to a powder. The amount equivalent to one tablet was weighed, dissolved by gentle warming in methanol, transferred to a 25-mL volumetric flask, and the volume was filled up with water. The solution was then filtered through a Whatman no. 41 paper filter to separate the insoluble sample matrix. Then a 0.25 or 0.5 mL of this solution was