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# Stepwise injection spectrophotometric determination of epinephrine

Andrey V. Bulatov[a,*], Anastasiia V. Petrova[a], Andriy B. Vishnikin[b], Aleksey L. Moskvin[a], Leonid N. Moskvin[a]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Saint-Petersburg State University, Saint-Petersburg, pr. Universitetskij 26, St. Petersburg 19850, Russia
[b] Department of Analytical Chemistry, Faculty of Chemistry, Oles Gonchar Dnipropetrovsk National University, Gagarina 72, Dnipropetrovsk 49010, Ukraine

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ABSTRACT

Simple, rapid and fully automated methods for the manual and automated spectrophotometric determination of epinephrine have been developed by using schemes of stepwise injection (SWIA) and sequential injection analysis (SIA) implemented in the same manifold. The determination is based on the formation of reduced form of 18-molybdodiphosphate heteropoly anion by its reaction with epinephrine. Using of the reaction vessel in the general SWIA configuration instead of a holding and reaction coil in the SIA manifold provides several essential advantages, including higher sensitivity and lower reagent consumption. The linear dependence of the analytical signal on the epinephrine concentration was preserved over the range of 1.5–30, 3.0–30, and 1.5–25 μmol $L^{-1}$ by using of SWIA, SIA and spectrophotometric analysis, respectively. The relative standard deviation for the SWIA determination of 10 μmol $L^{-1}$ epinephrine was 1.8% ($n$=10).



## 1. Introduction

Flow-injection analysis (FIA) [1] and sequential injection analysis (SIA) [2–5] are the well-established automated methods used in the analysis of pharmaceuticals. However, both methods have certain drawbacks. At least a part of them are caused by used non-equilibrium approach involving registration of the analytical signal by flow detector in the non-equilibrium conditions. Dispersion of reactants and reaction products zones along the hydraulic routes results in decreased sensitivity of FIA and SIA methods in comparison with similar non-automated methods. Besides, FIA and SIA are less effective in the case of the automation of multistage or slow reactions or when the redesign of the manifold is necessary by changing from one application to another.

To eliminate the above mentioned disadvantages, methods limiting the dispersion of the analytical form were proposed, including FIA/SIA with a mixing chamber [6,7], using of an automated micro batch analyzer [8], segmented flow analysis [9], or stepwise injection analysis (SWIA) [10,11]. SWIA provides the widest possibilities of solving the problems of the analysis automation without loss in the sensitivity due to dispersion as well as unification of the design of hydraulic schemes.

The SWIA and SIA manifolds are close to each other in many aspects; both of them allow easy manipulation with the direction of the flow. The SWIA and SIA manifolds include reversible pump and multiposition switching valve in which several inlets are commutated to a single outlet. As opposite to the SIA manifold, holding and reaction coil are replaced in the SWIA configuration by the cylindrical reaction vessel (RV) with a funnel-shaped inlet at the bottom. Finally, one of the inlets of switching valve is connected to the atmosphere or to a gas container with an inert gas ensuring a possibility for the intense and effective mixing of a reagent and sample solutions in the reaction vessel. One of the main distinctions between SWIA and previously known FIA/SIA techniques consists in the change of diffusion mass transfer between sample and reagent zones to more effective convective stirring. Thus, an analytical signal is measured under conditions, when it reaches a maximum value in the given analytical procedure.

By using of a SWIA method, the optimization of the flow variables of the manifold can be greatly simplified because the parameters of the analytical method found in batch conditions can be used almost without any changes. The combination of the SWIA manifold with such modules as an auxiliary vessel or a sorption column allows effectively to preconcentrate the analyte. By the carrying out of the extraction process or the absorption of the gaseous species in the reaction vessel, the configuration of the system is significantly simplified [11–14]. The configuration of the flow system is in many situations more flexible than that for the FIA/SIA methods and shows greater analytical efficiency. New possibilities are given by the integration of the reaction vessel with a measurement cell. At the same time, SWIA essentially concedes to the known flow methods in throughput but this feature is not always asked for in real analysis.

* Corresponding author. Tel.: +91 126113385; fax: +7812 372 44 21.
*E-mail address:* bulatov_andrey@mail.ru (A.V. Bulatov).

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Epinephrine is employed in cardiac resuscitation and in veterinary medicine as a treatment for anaphylaxis. Because of its vasoconstrictive properties, epinephrine is also added to local anesthetics to retard systemic absorption and prolong effect. It works by narrowing the blood vessels, increasing blood pressure and blood glucose levels.

Several flow injection procedures have been reported for epinephrine determination using different detectors such as spectrophotometric [15–20], chemiluminescent [7,21–23], spectrofluorimetric [24] and potentiometric [25]. The numerous color reactions for adrenaline determination described in the literature can be divided in three groups: (a) those depending mainly on the formation of a red oxidation product; (b) those depending on the presence of the catechol grouping, (c) miscellaneous. The reaction of oxidation is slow and 15–30 min is necessary for the complete development of the coloration. Several oxidants such as permanganate [26]; iodine [20], metavanadate [27], periodate [28], bromine [29] and phenanthroline-iron(III) complex [30] have been proposed.

The heteropoly anions were among first colorimetric reagents proposed for the determination of epinephrine. Folin uric acid reagent 18-tungstophosphate heteropoly acid rapidly reacts with adrenaline in strongly basic solution [31]. In these conditions color produced fades quite rapidly because heteropoly complexes are unstable in basic medium due to progressive destruction of the reagent by the alkaline hydrolysis [32]. In such conditions reaction is not specific. Besides uric acid, most easily oxidizable substances, particularly aromatic polyhydroxy compounds, give some blue color [33]. The reaction is much more sensitive than that based on the formation of orange adrenochrome.

The purpose of this work was to develop a simple, rapid and fully automated methods for the routine manual and automated spectrophotometric determination of epinephrine by using schemes of stepwise injection and sequential injection analysis. The determination is based on the formation of reduced form of recently proposed reagent – 18-molybdodiphosphate heteropoly anion (18-MPA) – by its reaction with epinephrine [34,35].

## 2. Materials and methods

### 2.1. Reagents and solutions

The procedure for the synthesis of ammonium salt of 18-MPA described in [36] was modified as follows. Dissolve 100 g of $Na_2MoO_4 \times 2H_2O$ in 400 mL of $H_2O$, add 15 mL of 85% $H_3PO_4$ and 80 mL of concentrated HCl. Boil for 8 h with return condenser. When the solution turns from yellow to green due to reduction, add several mL of 3% $H_2O_2$. After cooling add sufficient solid $NH_4Cl$ assuring the precipitation of as much as possible of light-yellow ammonium salt of $PMo_{12}O_{40}^{3-}$. Usually, depending on the quantity of the 12-molybdophoshate HPA formed, about 20–30 g of $NH_4Cl$ is enough to ensure complete separation of 18-MPA from the main impurity. The yellow crystalline precipitate, after settling, is filtered on a Buchner funnel and discarded. To the clear solution add enough solid $NH_4Cl$ (~100 g) to saturate the solution. Every portion of the $NH_4Cl$ added must have been dissolved before adding a new one. To do this, the solution can be slightly heated (40–50 °C). The precipitated orange ammonium salt of 18-MPA is filtered on a Buchner funnel and sucked as dry as possible. The obtained preparation can be usually used without further purification. Yield: ~40 g.

0.01 mol $L^{-1}$ solution of 18-MPC is prepared by dissolving 0.7855 g of the synthesized salt and diluting to 25 mL with distilled water. If there is a small insoluble residue, filter the solution. The stock solutions of $2.5 \times 10^{-3}$ mol $L^{-1}$ epinephrine nitrate (Sigma-Aldrich) was daily prepared by dissolving accurately weighed amounts in 0.01 mol $L^{-1}$ hydrochloric acid, and stored in a refrigerator. 0.1 mol $L^{-1}$ sodium phosphate buffer (pH=7.0) was used for adjusting the pH of the samples to an optimum value.

### 2.2. Apparatus

Absorption spectra and absorbances were measured using a SF-26 (LOMO, Russia) and SHIMADZU UVmini-1240 (Shimadzu Scientific Instruments, Japan) spectrophotometers equipped with 10 or 50 mm light-path cell. The pH of solutions was measured using an EV-74 ion meter (ZIP, Homel, Belarus) equipped with glass and Ag/AgCl reference electrodes.

The flow systems for the SIA (Fig. 1(a)) and SWIA (Fig. 1(b)) determination of epinephrine were based on flow injection analyzer PIAKON-30-1 (Rosanalit, Saint-Petersburg, Russia). They included a bidirectional peristaltic pump ensuring a reverse flow, a six-port titanium valve, a 50 mm optical Z-flow through cell, and communication tubes (PTFE, 0.5 mm in inner diameter). A tungsten light source and a USB650 UV–VIS fibre optic CCD detector (OceanOptics, USA) were connected to the flow system via 600 μm i.d. optical fibres having SMA connectors.

In the case of exploitation of the analyzer in SIA mode (Fig. 1(a)), it included a holding coil (HC) (diameter 10 mm, length 40 mm) and reaction coil (diameter 10 mm, length 45 mm). In the other case, by exploitation of the analyzer in SWIA mode (Fig. 1(b)), the system included a reaction vessel (RV) which had cylindrical shape and was funnel-shaped at the bottom (glass tube 350 mm in height and 10 mm in inner diameter). The analyzer was controlled by the homemade programme written in QuickBasic language.

### 2.3. Spectrophotometric procedure for the determination of epinephrine

In a 25 mL volumetric flask, 1 mL of 2.5 mmol $L^{-1}$ 18-MPA, 1 mL of 0.1 mol $L^{-1}$ phosphate buffer, and the required amount of epinephrine were mixed. The flask was then filled with distilled water to the mark. Absorbance was measured after 5 min at 820 nm against water in a 50 mm glass cell.

### 2.4. Procedure for the SWIA determination of epinephrine

At the first stage of the measurements, the components of the reaction mixture are sequentially delivered through the ports of multiselection valve into reaction vessel in the following order: 170 μL of 0.2 mmol $L^{-1}$ 18-MPC (port **a**), 130 μL of sample solution (port **b**), 210 μL of phosphate buffer with pH 7.0 (port **c**). To stir the reaction mixture, a flow of argon gas was passed through the port **d** at a rate of 6 mL $min^{-1}$ into the reaction vessel for a 60 s. Then the reaction mixture was moved at 30 μL $s^{-1}$ from the reaction vessel into the photometric detector flow cell through port **e** by reverse movement of the peristaltic pump. The absorbance is measured under stopped-flow conditions for a 15 s and solution is discharged to waste. The system lines, reaction vessel and flow cell are washed with distilled water and argon gas using port **f, d** and **e**. At the second stage, the described sequence of the operations is repeated with the exception that distilled water is pumped instead of sample solution. The absorbance of the blank is measured. The difference between the absorbances measured at the second and first stages is used as analytical signal. The sequence and time of performing all steps of analysis were set by the program as a matrix and presented in Table 1.

### 2.5. Procedure for the SIA determination of epinephrine

Before the carrying out the measurement, the system is washed and filled with phosphate buffer used as carrier solution. The