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Flow method based on cloud point extraction for fluorometric determination of epinephrine in human urine

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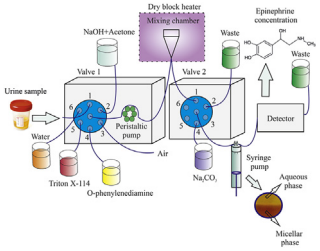
HIGHLIGHTS

- SWIA combined with cloud point extraction and fluorometric detection.
- On-line cloud point extraction for fluorometric epinephrine determination.
- First automated fluorometric determination of epinephrine in human urine.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel stepwise injection fluorometric method for the determination of epinephrine in human urine has been developed. In the current study, the stepwise injection analysis (SWIA) was successfully combined with on-line in-syringe cloud point extraction (CPE) and fluorometric detection. The procedure was based on the epinephrine derivatization in the presence of o-phenylenediamine followed by the preconcentration stage based on the CPE with the nonionic surfactant Triton X-114. After the phase separation into a syringe of the flow system, the micellar phase containing the epinephrine derivative was transported to a fluorometric detector. The excitation and emission wavelengths were set at 447 nm and 550 nm, respectively. The conditions of epinephrine derivatization and CPE have been studied. The calibration plot constructed using the developed procedure was linear in the range of $1 \cdot 10^{-11}$ $-5 \cdot 10^{-7}$ mol L^{-1}. The limit of detection, calculated as 3 σ of a blank test (n = 10), was found to be $3 \cdot 10^{-12}$ mol L^{-1}. The proposed method was successfully applied for the determination of epinephrine in human urine samples.

1. Introduction

Epinephrine or adrenaline ((R)-4-(1-hydroxy-2-(methylamino) ethyl)benzene-1,2-diol) is a physiologically active substance, hormone and mediator involved in cell–cell interactions of humans and animals [1]. Epinephrine is normally synthesized by the adrenal glands and certain neurons [2]. It exists as an organic cation in the nervous tissue and biological body fluids; and many diseases are related to the changes of its concentration in body fluids [3]. The diseases such as hypertensive crisis, angina, asthma attacks, hepatitis and cirrhosis of the liver and brain injury cause the changes in the concentration of catecholamines including epinephrine in biological fluids [4–6]. The determination of epinephrine and norepinephrine in urine allows to establish localization of adrenal and nervous tissue tumors [7,8]. Thus, the determination of epinephrine has attracted much attention for the diagnostic

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purposes. The determination of epinephrine in biological fluids such as blood plasma, urine and saliva is a frequently requested task in clinical laboratories.

Nowadays, a variety of analytical techniques, such as electrochemical [9,10], spectrofluorimetry [11,12], chemiluminescence [13], high-performance liquid chromatography (HPLC) [14,15], has been developed for the epinephrine determination in biological fluids. Although the techniques are widely recognized for the analysis of complex sample matrixes, these are laborious and time-consuming not only in relation the sample pre-treatment but also with respect to the analytical determination.

Also several automated techniques based on the flow systems have been reported for the determination of epinephrine using different detectors such as spectrophotometric [16–22], chemiluminescent [23,24], spectrofluorimetric [25] and potentiometric [26] (Table 1). The integrated systems based on flow injection manifold and HPLC for the determination of epinephrine in biological samples have been also developed [27,28]. The technique described in Ref. [27] assumes off-line solid-phase extraction of catecholamines from urine samples. Another technique involves on-line extraction of the catecholamines directly from urine by using an phenylboronic acid affinity column [28].

In general, the analytical techniques for the determination of epinephrine in biological fluids include different procedures of the sample preparation to eliminate interfering matrix effects of the samples. The liquid–liquid [29] and solid-phase [30,31] extraction methods are among them.

One environmentally friendly alternative to the traditional liquid–liquid extraction is the cloud point extraction (CPE) with nonionic surfactants [32]. The CPE method has significant advantages due to the possible mild extraction conditions (low temperature), low toxicity and biocompatibility of nonionic surfactants. The CPE is based on the clouding phenomenon of the aqueous solutions of many nonionic surfactants what appears at the certain temperature called the cloud point temperature (CP). At the temperature at or above the CP the aqueous solution of nonionic surfactant could split into two phases. The CPE involves introducing a surfactant solution into an aqueous sample containing analyte. Normally, the analyte partitioning favors its concentration in one of the phases, namely, in the surfactant-rich phase (or micellar phase) due to the effective solubilization capacity of the surfactant aggregates (micelles). It is well-known, that temperature and a salting-out agent influence on phase separation. The CPs of solutions of nonionic ethoxylated surfactants such as Triton X-114 could be decreased significantly. As a result of salting-out effect, an addition of the electrolyte into the solution can significantly decrease the CPs, i.e. promote the phase separation [33]. The magnitude of these salt-induced effects depends on salts nature, i.e. anion and cation compositions, concentrations of surfactant and salt [34].

To the best of our knowledge, the procedure for the determination of epinephrine in biological fluids with CPE has not been reported yet. Also the flow fluorometric determination of epinephrine in human urine has not been still developed.

The aim of this work was to develop an automated method for the fluorometric determination of epinephrine in human urine. The stepwise injection analysis, originally suggested by our lab, assumes the mixing of the solutions in a mixing chamber (MC) by bubbling to achieve physical and chemical equilibrium state [35,36]. CPE was previously coupled with flow-injection [37], multi-pumping flow [38] and lab-in-syringe [39] systems to improve precision and sample throughput, and to minimize waste generation. In our research SWIA was successfully combined with on-line in-syringe CPE and fluorometric detection. It was found that the epinephrine derivatization product 1-methyl-1H-pyrrolo[2,3-b]pheazin-3-ol (MPP) could be preconcentrated by CPE method using nonionic surfactant Triton X-114 and fluorescence of MPP in micellar phase is observed.

2. Experimental

2.1. Chemicals

The 0.01 mol L^{-1} stock solution of epinephrine (Sigma–Aldrich, USA) was prepared by dissolving reagent in 0.1 mol L^{-1} acetic acid and stored in a refrigerator. The working solutions of epinephrine were daily prepared by appropriate dilution of the stock solution with deionized water. The nonionic surfactant Triton X-114 ((1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol, n = 8) (AppliChem, Germany) was used without further purification. The 0.05 mol L^{-1} solution of o-phenylenediamine (Sigma–Aldrich, USA) was prepared by dissolving reagent in 0.1 mol L^{-1} HCl and stored in a refrigerator. The 0.1 mol L^{-1} NaOH (Sigma–Aldrich, USA) was prepared by dissolving reagent in 2% (v/v) solution of acetone.

All other reagents used were of analytical reagent grade. Deionized water was purified by means of a Direct-Q™ water purification system and was used throughout the experiments.

Table 1
Comparison of the flow methods for determination of epinephrine.

Method	Sample	Sample preparation	Linear range, mol L^{-1}	LOD, mol L^{-1}	Throughput, h^{-1}	Reference
FIA-UV/Vis	PP	Ultra-sound extraction	$2.7\cdot10^{-5}$–$1.1\cdot10^{-3}$	$2.2\cdot10^{-5}$	120	[16]
SWIA-UV/Vis	PP	Dissolution	$1.5\cdot10^{-6}$–$3\cdot10^{-4}$	$0.5\cdot10^{-6}$	20	[17]
FIA-UV/Vis	PP	Dissolution	$2\cdot10^{-5}$–$2\cdot10^{-4}$	$9.7\cdot10^{-7}$	130	[18]
FIA-UV/Vis	PP	Ultra-sound extraction	$5\cdot10^{-6}$–$1\cdot10^{-3}$	$4\cdot10^{-7}$	120	[19]
FIA-UV/Vis	PP	Dissolution	$1.1\cdot10^{-6}$–$1.1\cdot10^{-5}$	$0.5\cdot10^{-6}$	130	[20]
FIA-UV/Vis	PP	Dissolution	$5.5\cdot10^{-9}$–$6.5\cdot10^{-8}$	$0.93\cdot10^{-9}$	12	[21]
FIA-UV/Vis	PP	Dissolution	$6.4\cdot10^{-6}$–$3\cdot10^{-4}$	$0.48\cdot10^{-6}$	80	[22]
FBA-CL	PP	Dissolution	$5.1\cdot10^{-8}$–$1.2\cdot10^{-5}$	$11.5\cdot10^{-9}$	28	[23]
FIA-CL	PP	Dissolution	$1.1\cdot10^{-8}$–$1.1\cdot10^{-7}$	$4.4\cdot10^{-9}$	60	[24]
FIA-FL	PP	Dissolution	0.027–0.14	$5\cdot10^{-3}$	32	[25]
SIA-LOV	PP	Dissolution	$2\cdot10^{-4}$–$2.5\cdot10^{-3}$	$0.18\cdot10^{-3}$	32	[26]
FIA-HPLC	Urine	Solid-phase extraction	$2.7\cdot10^{-8}$–$2.6\cdot10^{-7}$	$1.4\cdot10^{-9}$	3	[27]
FIA-HPLC	Urine	Dilution,on-line extraction	$1.8\cdot10^{-8}$–$2.5\cdot10^{-6}$	$5.5\cdot10^{-9}$	16	[28]
SWIA-FL	Urine	Dilution, on-line derivatization and CPE	$1\cdot10^{-11}$–$5\cdot10^{-7}$	$3\cdot10^{-12}$	3	This work

FIA-UV/Vis, FIA-CL, FIA-FL – Flow injection analysis with spectrophotometric, chemiluminescence and fluorometric detection, respectively; FBA-CL – Flow batch analysis with chemiluminescence detection; FIA-HPLC – Flow injection analysis coupled with high performance liquid chromatography; SIA-LOV – Sequential injection analysis lab-on-valve; SWIA-UV/Vis, SWIA-FL – Stepwise injection analysis with spectrophotometric and fluorometric detection, respectively; PP – Pharmaceutical formulations; CPE – cloud point extraction.