Talanta 103 (2013) 330–336

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

New potentiometric sensors based on selective recognition sites for determination of ephedrine in some pharmaceuticals and biological fluids

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ARTICLE INFO

Article history:
Received 7 September 2012
Received in revised form
19 October 2012
Accepted 20 October 2012
Available online 2 November 2012

Keywords:
Triacetyl-β-cyclodextrin
Carboxylated PVC
Potentiometry
Pharmaceutical analysis
Ephedrine PVC membrane sensors
Flow injection analysis

ABSTRACT

New cost-effective potentiometric membrane sensors with cylindrical configuration responsive to ephedrine are described. The sensors setup is, based on the use of triacetyl-β-cyclodextrin [(triacetyl-β-CD)] as a neutral ionophore embedded in a plasticized poly (vinyl chloride) (PVC) matrix (sensor I) and carboxylated poly(vinyl chloride) [(PVC–COOH)] as a simultaneous plastic matrix and ion exchanger (sensor II). Both sensors showed significant enhancement of response towards ephedrinium cation (EPD^+) over a concentration range of 3.0×10^{-5}–8.0×10^{-3} mol L^{-1} at pH 4–9 and 3–8 with low detection limits of 5.7×10^{-6} and 6.2×10^{-6} mol L^{-1} for sensors (I) and (II), respectively. The sensors displayed near-Nernstian cationic slope of 57.0 and 55.6 mV $decade^{-1}$ for EPD^+ and the effects of lipophilic salts and various foreign common ions were examined. The sensors were also satisfactorily used as tubular detectors in a double channel flow injection system. The intrinsic characteristics of the detectors in a low dispersion manifold under hydrodynamic mode of operation were determined and compared with data obtained under batch mode of operation. Validation of the method revealed good performance characteristics including long life span, good selectivity for EPD^+ over a wide variety of other organic compounds, long term stability, high reproducibility, fast response, low detection limit, wide measurement range, acceptable accuracy and precision. Applications of the sensors to the determination of EPD^+ in pharmaceutical formulations and spiked biological fluid samples were carried out and compared with standard techniques. Notably, the sensors introduced offer several advantages over many of those previously described that are amenable to quality control/quality assurance assessment of the homogeneity, stability and purity of ephedrine drug tablets.

1. Introduction

Ephedrine is a sympathomimetic amine that has central nervous stimulating properties [1]. It is used to excite the central nervous system, the systole of blood vessel and lyses of spasm of bronchial smooth muscle [2]. Ephedrine is also used as appetite suppressant, decongestant and to treat hypotension. The drug has an improving effect on the physical performance and is included in the doping list of pharmacological forbidden substances indicated by the medical commission of the international Olympic committee [3]. Recently, ephedrine assessment in food products, pharmaceutical formulations, human fluids of athletes and detection of drug toxicity and abuse, has gained a growing interest.

Several analytical techniques have been reported for the determination of ephedrine in dietary supplements by liquid chromatography [4,5], liquid chromatography coupled to mass spectrometry [6,7], capillary electrophoresis [8,9], gas chromatography-mass spectrometry [10,11], thermal analysis [12], spectrophotometry [13–15], spectrofluorimetry [16] and voltammetry [17,18]. Most of these methods require prior derivatization or extraction step, and involve the use of expensive equipment. On the other hand, potentiometric sensors offer simple, selective and sensitive technique for drug analysis, therefore, has had wide applications in this field. However, few potentiometric membrane sensors have been developed and used for ephedrine determination [19–28]. Most of these sensors involve the use of ephedrine ion-pair complexes as electro-active materials that exhibit poor selectivity, limited range of linear response and long response time [19,20,24–26,28]. Response characteristics of these sensors are presented in (Table 1).

Since cyclodextrins (CDs) have unique ability to form inclusion complexes (host/guest) with a great variety of molecular species [29], these compounds have been used as molecular probes for the determination of some organic compounds [30,31] and to form super molecular aggregates with drugs [32]. In this context, sensors for determining diclofenac, amantadine, midazolam and diazepam drugs have been suggested [33–35]. Because of their complexation ability with various pharmaceutical compounds,

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http://dx.doi.org/10.1016/j.talanta.2012.10.067

Table 1
Response characteristics of some potentiometric ephedrine sensors.

Sensing material	Linear range (mol L^{-1})	Slope (mV decade^{-1})	Detection limit (mol L^{-1})	Interferent, (Log K^{pot} EPD,B)	Reference
Ephedrine-5-nitrobarbiturate	1.0×10^{-5}–1.0×10^{-2}	55.0	4.5×10^{-6}	(Quinine + 0.14), (Caffeine + 0.23), (Strychnin + 0.44), (Nicotine − 0.09), (Ca^{2+} − 1.14), (Ba^{2+} − 1.27).	[19]
Ephedrine-flavinate	1.0×10^{-5}–1.0×10^{-2}	55.2	1.0×10^{-5}	(Methylurea − 1.39), (Piperidine − 1.45), (Glycine − 1.96), (Epinephrine 0.00), (Dimethylamine − 0.77), (NH_4^+ − 2.4).	[20]
– Ephedrine-reineckate	1.0×10^{-5} – 1.0×10^{-2}	50.0	4.0×10^{-6}	(Lignocaine + 0.74), (Promethiazine + 0.65), (Valine − 0.88), (Maltose − 1.12), (Glucose − 0.77), (Sucrose − 1.72), (Na + − 1.42).	[21]
– Ephedrine-tetraphenylborate + ephedrine-reineckate	4.5×10^{-5}-1.0×10^{-1}	49.0	1.0×10^{-5}	(Lignocaine + 0.95), (Promethiazine + 0.6), (Valine − 0.88), (tryptophane − 1.77), (Glucose − 0.32), (Thiamphenicol − 1.49), (Na^+ − 1.42), (K^+ − 1.65), (NH_4^+ − 1.55).	[21]
Ephedrine-reineckate	2.0×10^{-6} – 1.0×10^{-1}	56.0	1.6×10^{-6}	(Quinine + 0.82), (Phenylephrine − 0.3), (Pyridoxine − 0.82), (Tryptophan − 0.32), (Caffeine − 0.64).	[23]
Tetrabutylammonium bromide /ephedrine-5-nitrobarbiturate	5.0×10^{-5} – 1.0×10^{-2}	55.7	1.0×10^{-5}	(Dextrometorphane − 0.85), (Caffeine − 0.24).	[24]
Tetrabutylammonium bromide /ephedrine-tetraphenylborate	1.0×10^{-5}–1.0×10^{-2}	58.2	5.0×10^{-5}	(Dextrometorphane − 0.04), (Caffeine 0.00), (NH_4^+ − 1.96), (K^+ − 1.82)	[25]
Ephedrine-tetrakis (p-chlorophenyl) borate	2.0×10^{-5}–1.0×10^{-1}	57.5	1.0×10^{-5}	(Atropine + 0.64), (Quinidine + 2.16), (Pilocarpine + 0.38), (Strychnine + 1.67), (K^+ − 2.23).	[27]
Triacetyl-β-CD	3.1×10^{-5} – 1.0×10^{-2}	54.0	5.7×10^{-6}	(Caffeine − 3.2), (phenylalanine − 3.0), (Glutamine − 3.0), (Histidine − 3.2), (Glycine − 3.3), (Urea − 3.2), (Ca + − 3.9).	**This work**
PVC–COOH	7.2×10^{-5} – 1.0×10^{-2}	57.6	1.7×10^{-5}	(Caffeine − 2.6), (phenylalanine − 2.4), (Glutamine − 2.7), (Histidine − 2.6), (Glycine − 2.6), (Urea − 2.7), (Ca + − 2.7).	

cyclodextrins have been the subject of intensive electrochemical research that focuses mainly on both their interactions in solution and in thin films attached to the electrode surfaces. Preparation of CDs modified electrodes would, therefore, open new avenues for new electrochemical sensors and, thereby, widens their applications in pharmaceutical analysis

In the present work, two novel potentiometric membrane sensors for ephedrine drug are developed. Sensor (I) is based on the use of triacetyl-β-cyclodextrin as an ionophore embedded in plasticized PVC matrix membrane. Sensor (II) utilizes a carboxylated PVC membrane plasticized with DOP without using any other membrane ingredients. This allows the drug cations in the test solution to interact with the fixed carboxylic functional group in the membrane such as to form a self-generated electro-active layer on the membrane surface of the sensor. Both sensors are examined, characterized and used to determine ephedrine in some drug formulations and biological fluids.

2. Experimental

2.1. Equipments

Potentiometric measurements were performed at 25 ± 1 °C with an Orion digital pH/mV meter (model SA 720) using ephedrine PVC membrane sensors in conjunction with an Orion Ag/AgCl double junction reference electrode (model 90-02) filled with 10%(m/v) KNO_3 solution in the outer compartment and Ross glass pH combination electrode (Orion 81-02) was used for pH measurements. The potentials were measured for stirred solutions using the following electrochemical cell: Ag/AgCl/10^{-3} mol L^{-1}EPD/membrane/sample test solution/Ag/AgCl double junction reference electrode.

The flow injection analysis (FIA) system manifold consisted of a two-channel Ismatec- MS REGLO model peristaltic pump. The manifold was connected with polyethylene tubing (Tygon, 0.7 mm i.d.) and an Omnifit injection valve (Rheodyne, Model 7125) with sample loop of 100 μL volume. The potential signals were recorded using an Orion pH/mV meter (model SA 720) connected to a PC through the interface ADC 16 (Pico Technology, UK) and Pico Log for windows (version 5.07) software.

2.2. Reagents and materials

All reagents were of analytical grade and used as received without further purification. Doubly distilled water was used throughout. High molecular weight poly (vinyl chloride) PVC, tetrahydrofuran (THF), carboxylated PVC containing 1.8% COOH residue, triacetyl-β-cyclodextrin, Tris (hydroxyl methyl) amino methane and 2-nitrophenyl phenyl ether (2-NPPE) were obtained from Sigma-Aldrich (St.Louis, Mo). Dioctylphthalate (DOP), dioctylsebacate (DOS), potassium tetraphenylborate (K-TPB) and tetradecyl dimethylbenzyl ammonium bromide were obtained from Fluka (Ronkonoma, NY).

A fresh 1.0×10^{-2} mol L^{-1} Tris buffer solution of pH 7.4 was prepared daily. A 1.0×10^{-1} mol L^{-1} aqueous stock solution of EPD^+ was freshly prepared. Working solutions (1.0×10^{-2}–1.0×10^{-6} mol L^{-1}) were daily prepared by accurate dilutions and stored in brown bottles.

2.3. Sensor and detector preparation

Ephedrine sensor was prepared by mixing a 2 mg portion of triacetyl-β-cyclodextrin, 133 mg of the plasticizer (DOS, DOP or 2-NPPE), 66 mg PVC and 1.0 mg (K-TPB) and dissolved in ~2 mL THF in a glass ring (2.2 cm diameter) placed on a glass plate. Carboxylated PVC based membrane sensor was prepared by mixing 66 mg PVC–COOH with 133 mg of the plasticizer and dissolved in 2 mL THF. The resulting mixture was transferred to a glass ring (2.2 cm diameter) and left to stand over night at room temperature to evaporate the solvent slowly. The resulting membrane was peeled off from the glass ring and discs of 9 mm i.d were cut out and glued onto a 7-mm i.d PVC body using THF. The tube was filled with 1.0×10^{-3} mol L^{-1} ephedrine hydrochloride (EPD^+) as internal solution. A 3 mm diameter Ag/AgCl coated wire was used as an internal reference electrode. The sensors were conditioned by soaking in a 1.0×10^{-2} mol L^{-1} aqueous EPD^+ solution for 24 h before use and were stored in distilled water between measurements. The sensors were stored in the same solution when not in use.

Ephedrine detector for flow injection analysis was prepared as previously described [36] by mixing 2 mg of triacetyl-β-cyclodextrin, 66 mg PVC, 133 mg DOS and 1 mg K-TPB with 2 mL THF. The

clear solution was deposited drop wise on Tygon tube window of ≈ 0.5 cm length and 2 mm id. After each addition, the mixture was allowed to evaporate slowly at room temperature to yield a thin film. This operation was repeated until a membrane with a thickness of approximately 0.1 mm was formed. The sensor was conditioned by soaking in 1.0×10^{-2} mol L^{-1} aqueous ephedrine hydrochloride solution for 24 h and was stored in the same solution when not in use. The ephedrine sensor was closely fitted in the tube at 10 cm distance from the valve. The end of the tube was placed in a beaker where a double-junction Ag/AgCl reference electrode was placed downstream from the detector just before the solution went to the waste. A carrier stream containing 1.0×10^{-2} mol L^{-1} Tris-buffer of pH 7.4 was pumped at a constant flow rate of 3 mL min^{-1}. To avoid slight pulsation originating from the peristaltic pump, grounding connection was made for flow system.

2.4. EMF measurement and sensor calibration

Triacetyl-β-cyclodextrin and carboxylated PVC membrane based sensors were calibrated by immersion with an Ag/AgCl double junction reference electrode into a 25 mL beaker containing 10 mL of 1.0×10^{-2} mol L^{-1} tris buffer solution of pH 7.4. Portions (0.25–1.0 mL) of 1.0×10^{-6}–1.0×10^{-3} mol L^{-1} of standard EPD^+ solutions were successively added and the potential response of the stirred solutions was measured after stabilization to ± 0.2 mV. A calibration plot was constructed by plotting the emf reading against the logarithm of EPD^+ concentrations. The plot was used for subsequent determination of unknown concentrations of ephedrine drug.

For FIA measurements, a series of 100 μL portions of EPD^+ test solutions spanning the concentration range from 1.0×10^{-2} to 1.0×10^{-6} mol L^{-1} were injected into a flow stream of 1.0×10^{-2} mol L^{-1} tris buffer of pH 7.4, flowing at a rate of 3 mL min^{-1}. The ephedrine sensor was used as a working sensor against Ag/AgCl double junction reference electrode. Each solution was measured in triplicate. The average potentials at maximum heights were plotted against log [EPD^+].

2.5. Determination of ephedrine in pharmaceutical drugs

Ephedrine hydrochloride injection (30 mg EPD^+ $ampoule^{-1}$), Sudophine syrup (2 mg EPD^+ mL^{-1}), Koffex syrup (0.6 mg EPD^+ mL^{-1}), and Asmacid tablet (15 mg EPD^+ $tablet^{-1}$) were analyzed. Stock solutions of these commercial samples were prepared by placing 0.1 mL of the injection, 0.5 mg of the syrup or 28 mg of the tablet (from 3 powdered tablets, previously weighed) into 25 mL Erlenmeyer flasks. Few drops of 0.1 mol L^{-1} HCl was added for complete dissolution of the sample, and the solution was completed to the mark with 1.0×10^{-2} mol L^{-1} tris buffer solution of pH 7.4. The EPD^+ test solutions were measured as described above and the potential reading was compared with a calibration plot prepared from 1.0×10^{-5} to 1.0×10^{-3} mol L^{-1} standard EPD^+ solutions under similar conditions.

2.6. Determination of ephedrine in biological fluids

Aliquots of human blood (1–3 mL) were transferred to 15 mL poly propylene sample tube containing 1.0 mL of 0.01% EDTA as anti-coagulant, thoroughly mixed and left for 10 min before being centrifuged. A 500 μL aliquot of the supernatant liquid was transferred, without removal of any particulate matter, to a 50 mL measuring flask and completed to the mark with 1.0×10^{-2} mol L^{-1} tris buffer solution of pH 7.4. A 10.0 mL aliquot was transferred to 25 mL beaker. The working ephedrine and reference electrodes were immersed in the solution. The potential reading was recorded after reaching the equilibrium response (10–20 s). Portions (0.25 mL) of standard 1.0×10^{-2} mol L^{-1} EPD^+ were added to the same solution, thoroughly mixed, and the potential measured.

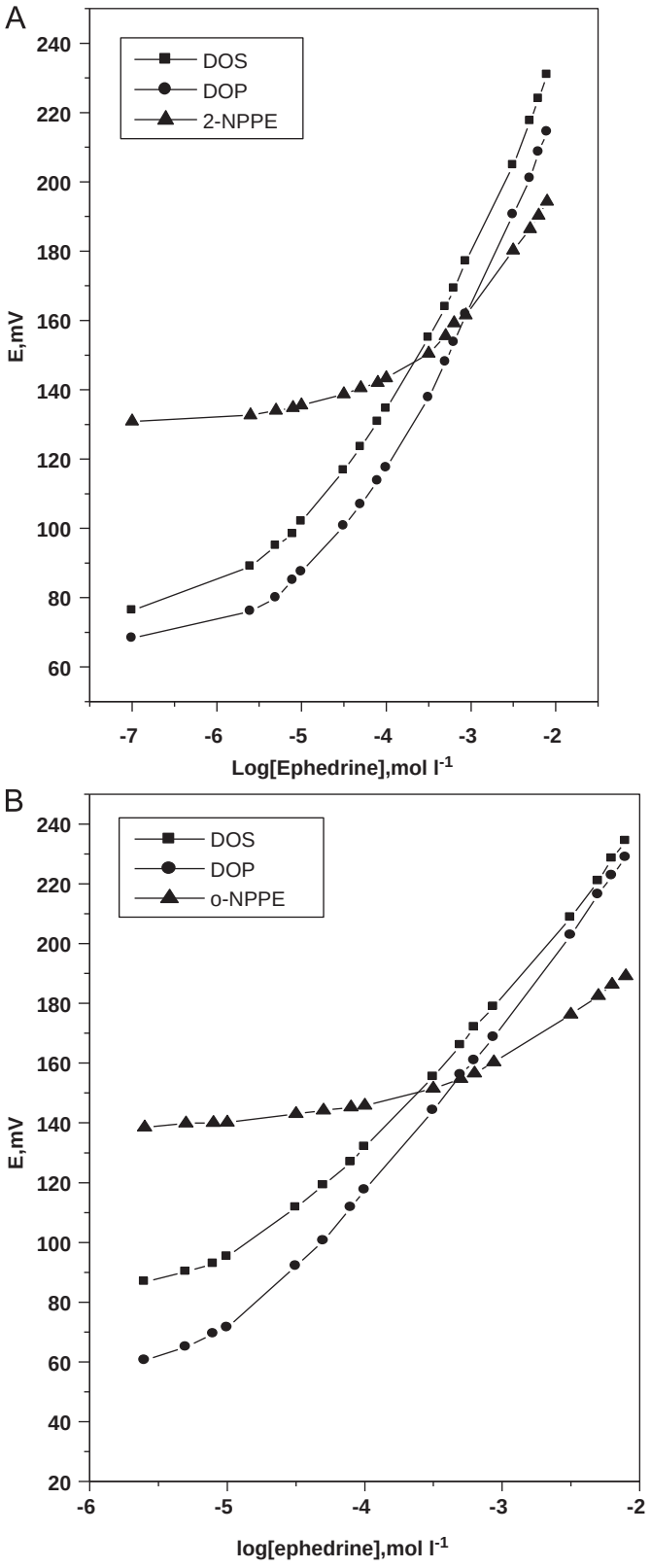

Fig. 1. Effect of membrane plasticizers on the potentiometric response of EPD^+ sensors: (A) Triacetyl-β-CD/PVC and (B) PVC–COOH based membranes in 10^{-2} mol L^{-1} tris buffer pH 7.4.

Known addition (spiking) technique was followed and the concentration of EPD^+ was measured.

For urine samples, a 10.0 mL aliquot of the human urine sample was transferred to a 100 mL calibrated flask, diluted to the mark with 1.0×10^{-2} mol L^{-1} tris buffer of pH 7.4 and mixed well. A 10.0 mL portion was transferred to 25 mL beaker; the working ephedrine and reference electrodes were immersed in the solution. The potential readings were recorded after reaching the equilibrium response (10–20 s). Portions (0.25 mL) of standard 1.0×10^{-2} mol L^{-1} EPD^+ were added to the same solution, thoroughly mixed, and the potential was then measured. Known addition (spiking) technique was followed and the concentration of EPD^+ was measured.

3. Results and discussions

3.1. Performance characteristics of ephedrine sensors

Membrane sensors responsive for ephedrine were prepared, characterized and used for the drug analysis. Since the membrane composition and nature of the plasticizer influence the sensitivity and selectivity of most potentiometric sensors, the effect of membrane composition on the response of ephedrine sensor was investigated (Fig. 1). For this purpose, the performance characteristics of some membranes with different compositions were electrochemically evaluated according to IUPAC recommendations [37] at 25 °C and the results were listed in Table 2. The optimum membrane composition was 32.7 wt% PVC, 1.0 wt% triacetyl-β-cyclodextrin, 65.8 wt% of the plasticizer (DOS, DOP or 2-NPPE), and 0.5 wt% TPB^- as ion excluder (sensor I) and 33 wt% PVC–COOH with 67 wt% DOS, DOP or 2-NPOE as a solvent mediator (sensor II). Triacetyl-β-cyclodextrin has the ability to form an inclusion complex with hydrophobic guest molecules such as ephedrine, because its cavity is exo-hydrophilic endo- hydrophobic [29]. The size of the cyclodextrin cavity fits to the size of the ephedrine guest molecule. The β-cyclodextrin derivative used in this work has a seven α-(1, 4) glycosyl units and a cavity size of 6.0–6.5 Å, which can fit and accommodate the ephedrine guest molecule. Triacetyl-β-cyclodextrin also acts as neutral carrier incorporating strong multiple, hydrogen bond donor groups (–OH) on its surface which assist conformational adjustments of ephedrine for maximum van der Waals forces [38]. On the other hand, carboxylated PVC acts as both a polymeric matrix and an ion exchanger.

3.2. Origin of sensor response

The potentiometric response properties of sensors based on neutral or charged ionophore strongly depend on the amount of ionic sites trapped in their membrane phase. It was found that addition of an ionic site (e.g., TPB^-) to the membrane bulk of ephedrine sensor incorporating (triacetyl-β-cyclodextrin) ionophore is necessary for obtaining a Nernstian response and to improve the membrane selectivity (sensor I). This ionic additive decreases the membrane resistance, and reduces interference by lipophilic counter-ions [39,40]. The amount of the added ionic site (potassium tetraphenylborate, K–TPB) to the membrane bulks was optimized to obtain the highest ephedrine selectivity. A 30 mmol% TPB^- addition, relative to the ionophore, was found to improve both sensor selectivity and detection limit for membranes plasticized with DOS and containing triacetyl-β-cyclodextrin ionophore.

Ephedrine sensor incorporating only plasticized carboxylated poly (vinyl chloride) (sensor II), was found to respond to EPD^+ *via* ion exchange mechanism. Upon soaking the sensor in ephedrine test solution, a self-generated electro-active membrane is formed due to the reaction of the –COOH of the polymer with the basic amine moiety of ephedrine. Addition of ionic additives (e.g., TPB^-) to this membrane sensor neither improves the selectivity nor decreases the detection limit of ephedrine.

It can be concluded that, the response mechanism of sensors containing triacetyl-β-cyclodextrin ionophore is based on a neutral carrier mechanism. The ionophore exhibits strong affinity towards EPD^+ ions to create positively charged complexes in the membrane phase while the lipophilic anionic site stabilizes this complex. On the other hand, sensors containing only PVC–COOH respond through an ion exchange mechanism. In this case, addition of an anionic site to the membrane shows no significant improvement of either the detection limit or selectivity of the sensor.

3.3. Method validation

The validity of the proposed potentiometric method for determining ephedrine was assessed by measuring the range, lower limit of detection (LOD), accuracy (recovery), precision or repeatability (CV_w), between-day variability (CV_b), linearity (correlation coefficient) and sensitivity (slope) [41,42]. Data obtained with six batches (six determinations each) of EPD^+ solutions are shown in Table 2.

3.3.1. Linear range, sensitivity and lower detection limit

Ephedrine sensors based on triacetyl-β-cyclodextrin (sensor I), exhibit a near Nernstian response towards EPD^+ ions with cationic slopes of 54.0 mV ($r^2=0.998$), 54.7 mV ($r^2=0.996$) and 25.0 mV ($r^2=0.997$) per decade and detection limits of 1.0×10^{-5}, 2.0×10^{-5} and 3.0×10^{-5} mol L^{-1} for the membranes plasticized with DOS, DOP and 2-NPPE, respectively.

Table 2
Potentiometric response characteristics of ephedrine sensors based on triacetyl β-CD ionophore and carboxylated-PVC membranes.

Parameter	Triacetyl-β-CD/DOS	Triacetyl-β-CD/DOS + 30 mmol TPB^-	PVC–COOH/DOP	PVC–COOH/DOS	PVC–COOH DOS + 30 mmol TPB^-
Slope (mV decade^{-1})	54.0 ± 0.4	57.0 ± 03	55.7 ± 1.2	53.1 ± 1.3	57.6 ± 1.0
Correlation coefficient (r^2)	0.998	0.998	0.998	0.996	0.998
Linear range (mol L^{-1})	$7.2\times10^{-5}-7.9\times10^{-3}$	$3.1\times10^{-5}-7.9\times10^{-3}$	$3.1\times10^{-5}-7.9\times10^{-3}$	$3.1\times10^{-5}-7.9\times10^{-3}$	$7.2\times10^{-5}-7.9\times10^{-3}$
Detection limit (mol L^{-1})	1.0×10^{-5}	5.7×10^{-6}	6.2×10^{-6}	2.2×10^{-5}	1.7×10^{-5}
Working range (pH)	3–8	3–8	4–9	4–9	4–9
Response time (s)	10–20	10–20	10–20	10–20	10–20
Life span (week)	5	5	8	8	8
Standard deviation σ_v(mV)	0.8	0.8	1.2	1.4	1.7
Accuracy (%)	99.3	99.5	98.6	96	95
Precision CV_w (%)	0.4	0.4	0.6	0.7	0.8
Between-day variability CV_b (%)	0.9	0.8	0.9	1.0	1.6

Ephedrine sensor based on PVC–COOH (sensor II) exhibits a potentiometric response towards EPD^+ ions with near Nernstian slopes of 53.1 mV ($r^2=0.996$), 55.6 mV ($r^2=0.998$) and 25.0 mV ($r^2=0.997$) per decade with detection limits of 2.2×10^{-5}, 6.2×10^{-6} and 7.7×10^{-5} mol L^{-1} for membranes plasticized with DOS, DOP and 2-NPPE, respectively. These results reveal that the dielectric constant of the membrane plasticizer significantly influences the sensor response. Fig. 1 shows that a low dielectric constant plasticizer (e.g. DOS, $\varepsilon=4.8$) is more suitable compared with other plasticizers with higher dielectric constant (e.g., DOP, $\varepsilon=7$ and 2-NPPE, $\varepsilon=24$). It has been reported that plastic membranes plasticizer with low dielectric constant is more appropriate for monovalent ions [40].

3.3.2. Accuracy and precision

The accuracy (trueness) and precision (relative standard deviation, RSD) of the results obtained by sensors I and II were calculated according to the following equations [41]

$$\text{Accuracy}\% = (x/\mu)100 \quad (1)$$

$$\text{Precision}\% = (SD/x)100 \quad (2)$$

where: x, μ and SD are the average measured concentration, reference-value and standard deviation, respectively. The results obtained are given in Table 2.

3.3.3. Effect of pH and response time

The influence of the pH on the potential response of triacetyl-β-cyclodextrin and PVC–COOH membrane based sensors was tested using 1.0×10^{-4} and 1.0×10^{-3} mol L^{-1} ephedrine solutions over the pH range 2–10. Adjustment of pH was carried out using NaOH and/or HCl. The pH-potential profiles show that ephedrine membrane sensors display good stability and constant potential reading over the pH range 3–8 and 4–9 for triacetyl-β-cyclodextrin and PVC–COOH membrane based sensors, respectively. The sensors exhibit, however, a sharp decrease in potential response at pH values $>$ pH 9, probably originating from the precipitation of the free basic drug. At pH <3, there is a noticeable decrease in potential probably due to interference from the high hydrogen ions concentration.

The time required to achieve a steady-state potential of ephedrine sensors within ±0.2 mV of the final equilibrium value was examined after successive immersion of the sensors in a series of ephedrine solutions, each has a10 fold difference, from low to high concentrations. The response time was found to be <10–15 s for all ephedrine solutions of concentrations in the linear range of the calibration curves indicating fast response of the sensors. The potentials remains constant for ~10 min (drift <0.5 mV). The long term reproducibility of the calibration slope, based on measuring the standard deviation of the potential over a period of 8 weeks, is within ±1.2 and ±1.5 mV decade^{-1} ($n=6$) for sensors I and II, respectively. In general, detection limits, response times, linear range and calibration slopes were reproducible within $\pm3\%$ over a period of at least 8 weeks.

3.3.4. Sensors selectivity

The effect of some interferents on the potentiometric determination of EPD^+ was evaluated by measuring the selectivity coefficients using the fixed interference method [37] using 1.0×10^{-2} mol L^{-1} of the interferents. Table 3 summarizes the potentiometric selectivity characteristics of membranes containing, triacetyl-β-cyclodextrin and PVC–COOH with and without lipophilic anionic additive (TPB^-). The selectivity order for PVC–COOH membrane based sensor was: $EPD^+ >$ phenylalanine $>$ serine $>$ $Na^+ >$ histidine $=$ $K^+ >$ glutamine $>$ cysteine $=$ glycine $>$ $NH_4^+ >$ caffeine $>$ urea $>$ Ca^{2+}. For PVC–COOH/TPB^- membrane based sensor, the selectivity order was very close to the sensor with PVC–COOH only: $EPD^+ >$ phenylalanine $>$ $K^+ >$ cysteine $=$ serine $>$ $NH_4^+ >$ $Na^+ =$ caffeine $>$ histidine $>$ glycine $>$ glutamine $>$ urea $>$ Ca^{2+}.

Table 3
Potentiometric selectivity coefficients ($Log\ K^{pot}_{EPD,B}$) of ephedrine PVC membrane sensors.

Interferent, B	Triacetyl-β-CD/DOS	Triacetyl-β-CD/DOS + 30 mmol TPB^-	PVC-COOH/DOS	PVC-COOH DOS + 30 mmol TPB^-
Ephedrine	0	0	0	0
Caffeine	−3.2	−3.2	−2.7	−2.6
Phenylalanine	−2.9	−3.0	−2.5	−2.4
Glutamine	−2.9	−3.0	−2.6	−2.7
Histidine	−3.1	−3.2	−2.6	−2.6
Glycine	−3.0	−3.1	−2.7	−2.6
Cysteine	−3.0	−3.3	−2.7	−2.6
Urea	−3.0	−3.2	−2.7	−2.7
NH_4^+	−2.9	−3.0	−2.7	−2.6
Ca^{2+}	−3.6	−3.9	−3.7	−2.7
K^+	−2.9	−3.1	−2.6	−2.5
Na^+	−2.9	−3.1	−2.5	−2.6
Serine	−2.85	−3.19	−2.51	−2.59

The selectivity patterns of triacetyl-β-cyclodextrin membrane with and without a lipophilic anionic additive (TPB^-) in the membrane sensor was investigated. The selectivity order of triacetyl-β-cyclodextrin membrane based sensor, is $EPD^+ \gg Ca^{2+} >$ serine $>$ $Na^+ >$ glutamine $>$ phenylalanine $>$ $NH_4^+ >$ $K^+ >$ glycine $=$ urea $=$ cysteine $>$ histidine $>$ caffeine. Addition of TPB^- to the membrane (30 mmol% relative to the ionophore) was found to improve the selectivity behavior such that: $EPD^+ \gg$ glutamine $=$ $NH_4^+ >$ phenylalanine $>$ $Na^+ >$ $K^+ >$ glycine $>$ histidine $=$ urea $>$ serine $=$ caffeine $>$ cysteine $>$ Ca^{2+}. The selectivity differences between membranes containing the neutral carrier only and those containing neutral carrier plus TPB^- (30 mmol% relative to the ionophore) could be due to direct interaction between the complexed cations and the counter-anion sites in the membrane.

For membrane sensors containing PVC–COOH with and without an anionic additive (TPB^-), the response mechanism for ephedrinium cation is based on the ion-exchange properties between EPD^+ cations and the H^+ of –COOH in the polymer matrix. The electrostatic interaction plays the dominate role for the cation transfer across the organic/water interface. The hydration energy of the analyte cations is overcome by the electrostatic affinity. The selectivity sequence is, however, determined by the order of the hydration energy or by the hydrophilicity of the tested cations.

It is well established that the selectivity of neutral cation-selective carrier-based liquid-polymeric membrane sensors can be optimized by the addition of lipophilic anionic additives in the membrane. These additive sites reduce membrane resistance, minimize interference by anions at high sample activities, increase the availability of the free carrier for cations complexing, and improve the membrane selectivity. The optimum concentration of such lipophilic additives in the membrane phase depends in part on the charge of the primary ion and its complexation stoichiometry with the carrier relative to that of the interfering ion [39]. In general, the selectivity of the sensor based on triacety-β-cyclodextrin for ephedrine is much better than that of carboxylated-PVC based sensor. Furthermore, the validation study shows that ephedrine sensor based on triacetyl-β-cyclodextrin/TPB (sensor I) gives better response characteristics in terms of detection limit and selectivity. It was, therefore, used for all subsequent measurements.

3.4. Determination of ephedrine (EPD^+)

Replicate potentiometric measurements using sensor I ($n=10$) of 20.0 μg mL^{-1} internal quality control (IQC) EPD^+ sample and

Table 4
Determination of ephedrine in some pharmaceutical preparations using triacetyl-β-CD/DOS-TPB membrane based sensor.

Trade name and source	Nominal content	Found	Average recovery[a] (%)
Koffe x, (syrup) (Mina Pharm.Co., Egypt)	0.6 mg mL $^{-1}$	0.59 ± 0.08 mg mL^{-1}	98.3 ± 2.3
Sudophine, (syrup) (EVA Pharma, Egypt)	2 mg mL^{-1}	1.98 ± 0.29 mg mL^{-1}	99.0 ± 2.0
Asmacid, (tablet) (CID CO., Egypt)	15 mg tablet^{-1}	14.70 ± 0.58 mg tablet^{-1}	98.0 ± 1.9
Ephedrine hydrochloride, (injection)	30 mg ampoule^{-1}	29.32 ± 0.47 mg mpoule^{-1}	97.7 ± 1.6

[a] Average of six measurements.

calculation of the student's (t) value at 95% confidence level were made using the following equation:

$$t_{exp} = [\mu - x]\mathrm{x}\sqrt{n}/s \quad (3)$$

where: μ is the concentration of the initial internal quality control sample, x is the average concentration found, n is the number of replicates and s is the standard deviation of measurements. No statistical difference was detected between the practically obtained (t_{exp}=0.985) and the theoretically tabulated (t=1.833) values. Thus the null hypothesis was retained.

Assessment of the analytical usefulness of the EPD$^+$ sensor was performed using sensor I for ephedrine determination in some pharmaceutical preparations. The drug tablets were powdered, dissolved in Tris buffer of pH 7.4 and directly measured without further treatment. The results were compared with a calibration plot prepared with standard ephedrine solutions. The mean average recovery obtained was 97.7% (n=6) with a mean standard deviation of ±0.5% (Table 4). It is noted that, no interference is usually caused by active or inactive ingredients and diluents commonly used in the drug formulations.

3.5. Flow injection measurement (FIA) of ephedrine

The flow cell used for EPD$^+$ assessment was designed with a constant geometry and a minimum "dead space" and to accommodate small sensor size. Such setup avoids large dispersion of the sample in the cell and gave high response with short recovery time. A tubular-type detector that incorporates a triacetyl-β-cyclodextrin/TPB$^-$ based membrane sensor was prepared and used under hydrodynamic mode of operation for continuous monitoring of EPD$^+$. A linear relationship was obtained between the FIA signals and EPD$^+$ concentrations over the range 1.0×10^{-5}–1.0×10^{-1} mol L^{-1} and with a lower detection limit of 3.5×10^{-5} mol L^{-1} (Fig. 2). The slope of the calibration plot was near-Nernstian (62.1 ± 0.3 mV decade^{-1}). The recommended optimal flow rate was chosen to be 3 mL min^{-1}. Under these conditions, the relative standard deviation of the FIA signals is ±2% for 1.0×10^{-5}–1.0×0^{-1} mol L^{-1} EPD$^+$.

3.6. Determination of ephedrine (EPD$^+$) in biological fluids

Application of the method for determining ephedrine in biological fluids was tested by spiking aliquots of human urine and plasma samples with a known concentration of standard EPD$^+$ in 1.0×10^{-2} mol L^{-1} Tris buffer of pH 7.4. An internal quality control sample from certified reference material (1.0×10^{-2} mol L^{-1}) was spiked into 10 mL of urine and plasma test solutions to evaluate the method procedure and recovery using the following equation:

$$\text{Recovery, \%} = [(x_s - x)/x_{add}]\,100 \quad (4)$$

where x_s, x and x_{add} are the results of spiked sample, mean results of un-spiked sample and of added (spiked) reference, respectively.

The results show recoveries range from 94.5 to 98.2 ± 5.6% with urine samples and from 95.2 to 102.4 ± 3.3% for plasma samples (Table 5). This confirms the suitability and applicability

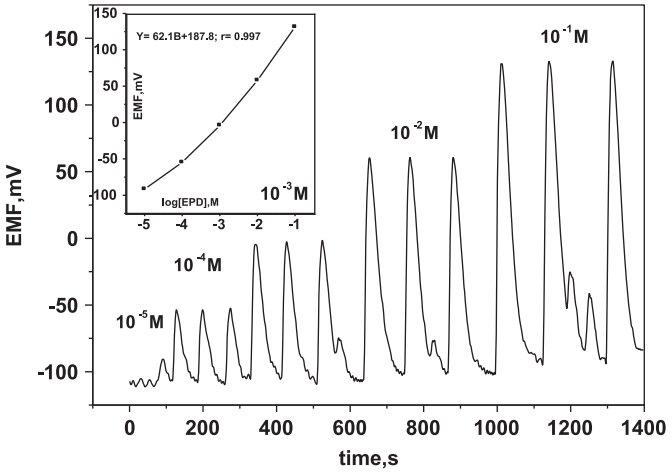

Fig. 2. Transient potentiometric signals of ephedrine using Triacetyl-β-CD/–PVC+DOS based membrane detector. Conditions: carrier solution, 10^{-2} mol L^{-1} tris buffer (pH 7.4); flow rate, 3 mL min^{-1}; injection valve 100 μL. Inset: calibration graph obtained under hydrodynamic mode.

Table 5
Determination of ephedrine in some biological fluids using triacetyl-β-CD/DOS-TPB membrane based sensor.

Sample	(mmol L^{-1})		
	Added	**Found**	**Recovery[a] (%)**
Plasma	0.91	0.89 ± 0.03	97.8 ± 3.2
	1.66	1.70 ± 0.01	102.4 ± 3.8
	2.10	2.00 ± 0.03	95.2 ± 3.0
Urine	0.91	0.86 ± 0.05	94.5 ± 2.5
	1.66	1.63 ± 0.03	98.2 ± 2.4
	2.10	2.06 ± 0.06	98.1 ± 2.2

[a] Average of six measurements.

of the method for accurate routine analysis of ephedrine in pharmaceutical formulations and biological fluids. In general, the present sensors offer some clear advantages over many of those previously described. Apart from the enhanced selectivity of the present sensors compared to other studies [19,20,24,25], the response time is shorter (< 10 s) compared to 60–600 s [24,26] and the limit of detection is lower (5.7×10^{-6} mol L^{-1}) compared to 1.0–3.2×10^{-5} mol L^{-1} [20,24,26,28].

4. Conclusions

Potentiometric ephedrine-selective membrane sensors are developed based on the use of triacetyl-β-cyclodextrin as neutral ionophore, dioctylsebacate (DOS) as a plasticizer, Na-TPB as an anionic excluder and PVC as a polymeric matrix. Fast, stable, reproducible and selective response of the sensors toward EPD$^+$ ions is obtained.

The sensors display near-Nernstian response with calibration slope of 57.0 ± 0.4 mV decade^{-1}, offer a wide linear response range (3.1×10^{-5}–7.9–10^{-3} mol L^{-1}), provide low detection limit (5.7×10^{-6} mol L^{-1}), wide working pH range (3–8), fast response (10 s) and exhibit better selectivity than those previously reported EPD+ sensors. A carboxylated-PVC membrane without any ionophore is also examined. It gives good response toward EPD^+ drug with a near-Nernstian response of 55.6 ± 1.2 mV decade^{-1}, offer a wide linear response range (3.1×10^{-5}–7.9×10^{-3} mol L^{-1}), low detection limit (6.2×10^{-6} mol L^{-1}), high sensitivity, long-term stability and good selectivity. The sensors are used for determination of EPD^+ in human biological fluids and some drugs. No interferences are caused by most ions that normally present in biological fluids. Active and inactive ingredients and diluents commonly used in drug formulations do not interfere. Tubular triacetyl-β-cyclodextrin membrane sensor is prepared, incorporated in a flow through cell and used as detector for flow injection analysis (FIA) of ephedrine. This system facilitates the determination of EPD^+ with high sampling rate, and a low consumption of sample volume.

References

[1] W.A. Konig, K. Ernst, J. Chromatogr. 280 (1983) 135.
[2] Dictionary of Traditional Chinese Medicine, New Medical College of Jiangsu, People's Publisher of Shanghai, 1977, p. 2222.
[3] International Olympic Committee Medical Code. World Anti-Doping Agency, website: 〈www.wada-ama.org〉.
[4] M. Ganzera, C. Lanser, H. Stuppner, Talanta 66 (2005) 889.
[5] F. Pellati, S. Benvenati, J. Pharm. Biomed. Anal. 4 (2008) 254.
[6] D. Sullivan, J. Wehrmann, J. Schmitz, R. Crowley, J. Eberhard, J. AOAC Int. 86 (2003) 471.
[7] P. Jacob, C.A. Haller, M. Duan, L. Yu, M. Peng, N.L. Benowitz, J. Anal. Toxicol. 28 (2004) 152.
[8] F. Wei, M. Zhang, Y.Q. Feng, J. Chromatogr. B 850 (2007) 38.
[9] K.W. Phinney, T. Ihara, L.C. Sander, J. Chromatogr. A 1077 (2005) 90.
[10] H.X. Li, M.Y. Ding, K. Lv, J.Y. Yu, J. Chromatogr. Sci. 39 (2001) 370.
[11] J.M. Betz, M.L. Gay, M.M. Mossoba, S. Adams, B.S. Portz, J. AOAC Int. 80 (1997) 303.
[12] R.J. Prankerd, M.Z. Elsabee, Thermochim. Acta 248 (1995) 147.
[13] T. Aman, M.S. Shahid, A. Rashid, I. Khokhar, J. Anal. Chem. 30 (1997) 1517.
[14] J.E. Wallace, J. Pharm. Sci. 58 (1969) 1489.
[15] B. Gala, A. Gomez Hens, D. Perez Bendito, Fresenius J. Anal. Chem. 349 (1994) 824.
[16] U.L.U.S. Tator, J.A.O.A.C. Int. 89 (2006) 1263.
[17] E. Mazzotta, R.A. Picca, C. Malitesta, S.A. Piletsky, E.V. Piletska, Biosens. Bioelect. 23 (2008) 1152.
[18] A. De Marco, E. Mecarelli, IL Farmaco 22 (1967) 795.
[19] S.S.M. Hassan, M.M. Saoudi, Analyst 11 (1986) 1367.
[20] S.S.M. Hassan, G.A. Rechnitz, Anal. Chem. 58 (1986) 1052.
[21] M.M. Zareh, J. Mikrochem. Acta 126 (1997) 271.
[22] J. Zeng, Yaoxue Xuebao 17 (1982) 841.
[23] M.M. Zareh, R. El-Shiekh, R. El-Bahnasawy, D.O. Abo-El-Naga, Microchem. J. 60 (1998) 110.
[24] P.R. Chamorro, R.C. Diaz, Analyst 117 (1992) 1905.
[25] P.R. Chamorro, R.C. Diaz, Talanta 40 (1993) 1461.
[26] A. Qu, L. Sun, Z. Qu, Yaowu Fenxi Zazhi 13 (1993) 248.
[27] M.N.M.P. Alcada, J.L.F. Cd., M.C.B.M. Lima, J. Montengero, Pharm. Biomed. Anal. 10 (1992) 757.
[28] M.N.M.P. Alcada, J.L.F.C. Lima, M.C.B.M. Montengero, J. Pharm. Biomed. Anal. 13 (1995) 459.
[29] S. Muñoz-Botella, B. del Castillo, M.A. Martyn, Ars Pharm. 36 (1995) 187.
[30] M. Maafi, M.C. Mathedero, J. Aaron, Talanta 44 (1997) 2193.
[31] D.A. Lemer, Anal. Chim. Acta 227 (1989) 297.
[32] T. loftsson, A. Magnusdotir, M. Masson, S. Dottir, J. Pharm. Sci. 91 (2002) 2307.
[33] A.M. Pimenta, A.N. Araujo, M.C.B.S.M. Montenegro, Anal. Chim. Acta 470 (2002) 185.
[34] C.G. Amorim, A.N. Araujo, M.C.B.S.M. Montenegro, V.L. Silva, Electroanalysis 19 (2007) 2227.
[35] C.G. Amorim, A.N. Araujo, M.C.B.S.M. Montenegro, V.L. Silva, J. Pharm. Biomed. Anal. 48 (2008) 1064.
[36] Ayman H. Kamel, J. Pharm. Biomed. Anal. 45 (2007) 341.
[37] Y. Umezawa, P. Batlmann, K. Umezawa, K. Tohda, S. Amemiya, Pure Appl. Chem. 72 (2000) 1851.
[38] E.M.M. Del Valle, Proc. Biochem. 39 (2004) 1033.
[39] R.D. Armstrong, Electrochim. Acta 35 (1990) 1.
[40] R. Eugster, P.M. Gehrig, W.E. Morf, U.E. Spichiger, W. Simon, Anal. Chem. 63 (1991) 2285.
[41] U.S. FDA—Guidance for Industry (draft) Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls and Documentation, 2000.
[42] J.K. Taylor, Quality Assurance of Chemical Measurements, CRC Press, Florida, 1987.