Al-Aqsa University Journal (Natural Sciences Series),Vol.20, Pages 61-82, Jan.2016
ISSN 2070-3155

Determination of ephedrine hydrochloride in pharmaceutical formulations and in urine samples using a new coated graphite electrode

Dr. Hazem M. Abu Shawish*
Dr. Ghada I. Khraish**

الملخص

تقدير هيدروكلوريد الايفيدرين في العينات الدوائية والبول باستخدام قطب الكربون المغطى

تم استحداث طريقة جديدة لتقدير المادة الدوائية الفعالة هيدروكلوريد الافيدرين في صورته الصيدلانية (الحقن) إضافة إلي قياسه في السوائل البيولوجية مثل البول. وعليه تم تحضير قطب من الكربون المغطى بالغشاء الحساس الذي يحتوي على المادة الفعالة وهي الافيدرين–فوسفوموليبديت وكذلك المادة الملدنة DOP. أظهر هذا القطب مواصفات ممتازة في تقدير ايون الافيدرين، إذ أعطى القطب مدى استجابة خطية ما بين (8.5×10^{-7} to 1.0×10^{-2} M) مول/لتر وبميل نيرنستي مقداره (57.8±0.03 mV/decade) وبحد تحسس مقداره (4.5×10^{-7}) مول/لتر وقد تراوحت قيمة درجة الحموضة المناسبة لعمل القطب ما بين (3.2 – 7.3). تم حساب معامل الانتقائية $K_{i,j}^{Pot}$ بوجود أيونات موجبة مختلفة، حيث أبدى القطب انتقائية عالية إتجاه أيون الافيدرين. وكان تقدير دواء الإفيدرين في المستحضرات الدوائية وعينات البول باستخدام هذا القطب وبطريقة الإضافات القياسية والمنحنى العياري والمعايرة الجهدية على درجة عالية من الدقة والحساسية.

Abstract

A novel approach for the determination of ephedrine hydrochloride (EDCl) in pharmaceutical formulations and in urine samples is presented. New coated graphite selective electrode for EDCl based on ephedrine-phosphotungstate (PD-PT) as an ion-exchanger dissolved in plasticizer DOP and their potentiometric characteristics were discussed. The electrode exhibited a good Nernstian slope of 57.8±0.03 mV/decade with a linear concentration range from 8.5×10^{-7} to 1.0×10^{-2} M for the ephedrine ion. The limit of detection was 4.5×10^{-7} M. It had response time of 8 sec, useable in pH range of 3.2 – 7.3 and temperature of 20–60 ^{o}C. The coated

* **Chemistry Department_ College of Sciences_ Al-Aqsa University_ Gaza_ Palestine.**
** **Faculty ofhealth professions_ Palestine polytechnic_ Gaza_ Palestine.**

electrode shows clear discrimination of ephedrine hydrochloride from several inorganic, organic ions, sugars and some common drug excipients. The sensor was applied for determination of ephedrine hydrochloride in urine and in pharmaceutical formulations using potentiometric determination, standard addition and the calibration curve methods.
The results are satisfactory with excellent percentage recovery comparable or better than those obtained by other routine methods.

Keywords: potentiometry; ephedrine hydrochloride; coated wire electrode; ion-selective electrode

1. Introduction

Ephedrine hydrochloride is a sympathomimetic amine named: (1R, 2S)-2-methylamino-1phenylpropan-1-ol) shown in Figure 1. It is used to excite the central nervous system, the systole of blood vessel and as appetite suppressant [1-4]. Recently, ephedrine assessment in food products, pharmaceutical formulations and detection of its toxicity and abuse has gained a growing interest.

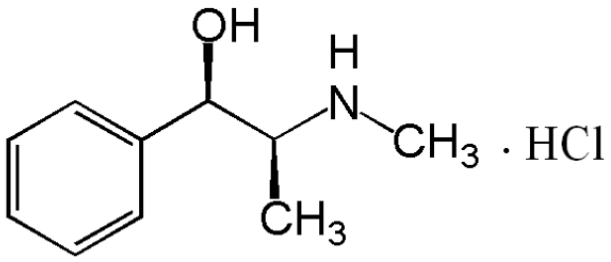

Figure 1. The chemical structure of ephedrine hydrochloride.

Methods for estimating ephedrine hydrochloride (EDCl) employ HPLC [5-7], liquid chromatography-mass spectrometry [8-10], capillary electrophoresis [11-14], gas chromatography-mass spectrometry [15-17], thermal analysis [18] , sectrophotometry [19–24], spectrofluorimetry [25], voltammetry [26, 27], potentiometry [28] as well as utilization of ion selective electrode [29,30]. These detection methods have excellent

detection limits and selectivity. However, their relatively high costs and complicated instrument setup make them difficult to employ for routine analysis. Consequently, there is need for devising a selective, fast, accurate, inexpensive and tool for determination of ephedrine.

Potentiometric methods with ion-selective electrodes (ISEs) are effective in the analysis of ephedrinein real samples for their advantages of simple design, construction and manipulation, reasonable selectivity, fast response time, applicability to coloured and turbid solutions and possible interfacing with automated and computerized systems.[31–35]. Therefore, ISEs are more desirable alternatives. Two of ISEs based on PVC membrane [29, 30] have been made for determination of ephedrine hydrochloride.

Solid contact electrodes are effective [36-38] for elimination of the internal solution with advantages such as good mechanical stability, simplicity, and possibility of miniaturization.[39, 40] which attracted the attention of researchers in recent years.[41,42]

As no potentiometric coated wire electrode have been suggested for the determination of ephedrine hydrochloride, the present work describes construction, potentiometric characterization, and analytical application of a new coated graphite electrode (CGE) for ephedrine hydrochloride based on the ion-exchanger, namely, ephedrine-phosphotungstate dissolved in dioctyl phthalate (DOP)

as a plasticizer. The electrode exhibited prominent characteristics including: near Nernstian slope, wide concentration range, low detection limit and short response time. The electrode used successfully for the determination of ephedrine ion in ampoule as well as in urine sample

2. Experimental

2.1. Reagents

Doubly distilled water was used throughout all experiments. Ephedrine hydrochloride (EDCl) and pharmaceutical preparation (Ampoules 30

mg/mL) were provided by the General Administration of Pharmacy Ministry of Health (Gaza-Palestaine). Silicotungstic acid (STA) $H_4[SiW_{12}O_{40}]$, silicomolybdic acid (SMA) $H_4[SiMo_{12}O_{40}]$, phosphotungstic acid (PTA) $H_3[PW_{12}O_{40}]$, phosphomolybdic acid (PMA) $H_3[PMo_{12}O_{40}]$ and sodium tetraphenylborate (Na-TPB) $Na[C_{24}H_{20}B]$were obtained from Sigma. Dibutyl phthalate (DBP), dioctyl phthalate (DOP), 2-nitrophenyloctyl ether (2-NPOE), tris(2-ethylhexyl)phosphate (DOPh), poly vinyl chloride (PVC) of high relative molecular weight and tetrahydrofuran (THF) were obtained from Aldrich chemical company. All other reagents were pure.

2.2. Apparatus

A saturated calomel electrode (SCE), used as reference electrode was obtained from Sigma–Aldrich Co. (St Louis, MO, USA). Potentiometric and pH measurements were performed using a Pocket pH/mV meter (WTW) (pH315i) from Wissenschaftlich-Technische Werkstatten GmbH, Weilheim, Germany. Emf measurements with CGE were carried out with the following cell assemblies: Hg, Hg_2Cl_2 (s), KCl(sat.) || sample solution || membrane/silver wire. The performance of the electrodes were investigated by measuring the emfs of ED solutions in a concentrations ranging 1.0×10^{-7} to 1.0×1.0^{-2} [M] by serial dilution. Each solution was stirred and the potential reading was recorded when it became stable, and plotted as a logarithmic function of ED ion activities.

2.3. Preparation of ion-exchangers

Several ion-exchangers including: ephedrine silicotungstate (ED_4-ST), silicomolybdate (ED_4-SM), phosphotungstate(ED_3-PT), phosphomolybdate (ED_3-PM) and tetraphenylborate (ED-TPB) were prepared by adding a hot solution of 100 mL of 10^{-2} M ephedrine hydrochloride to an appropriate volume of individual solutions of STA, SMA, PTA, PMA or Na-TPB 10^{-2} M each. The precipitates were filtered off, washed thoroughly with distilled water, dried at room temperature and ground to fine powders prior to their use as the active substances for preparing the sensors of EDCl.

2.4. Preparation of coated wire electrodes (CWEs)

The coated-wire electrodes (CWEs) were prepared according to a previously reported method [43,44]. Varying amounts of individual ion-exchangers, PVC and plasticizer were dissolved in 5ml THF. The solvent was evaporated at room temperature to obtain oily concentrated mixture. Standardized dimension (1 mm diameter X 12 cm length) of silver, platinum, gold, copper and graphite were insulated by tight polyethylene tubes leaving a 2 cm length exposed for coating purposes and 1cm at the other end for connection (Figure 2). The surfaces of each type were individual coated with the active membrane by quickly dipping the exposed end into the concentrated membrane solution. The film was allowed to dry in air for about 1 min. The process was repeated ten times until a plastic film of approximately 1.0 mm thickness was formed. The prepared electrodes were preconditioned by soaking in 10^{-3} M solution of the ephedrine drug for about 30 min.

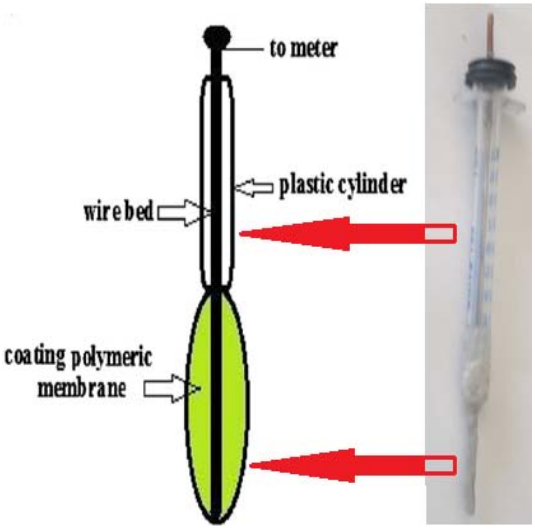

Figure 2. Silver coated wire electrode

2.5.Interferences

The influence of some inorganic and organic cations, sugars and some excipients or additives on the ISE were investigated. The matched potential method (MPM) [45] was employed since it is independent of the Nicolsky-Eisenman equation, or any of its modifications. This method was recommended in 2000 by IUPAC, as a method that gives analytically relevant practical selectivity coefficient values. According to this method, the activity of (EDCl) was increased from a_A= 1.0 x10^{-5} M (reference solution) to á$_A$ = 5.0x10^{-5} M, and the change in potential (ΔE) corresponding to this increase were measured. Furthermore, a solution of potentially interfering ions of concentration a_B in the range 1.0x10^{-1} – 1.0x10^{-2} M is added to a fresh1.0x10^{-5} M (reference solution) until the same potential change (ΔE) was recorded. The selectivity factors, were then evaluated using the following equation:

$$K_{A,B}^{MPM} = \frac{(a'_A - a_A)}{a_B}$$

Since MPM in this case, is time consuming due to the need to prepare many solutions, consequently the separate solution method (SSM) [45] was applied. The latter method requires two potential measurements, firstly, the potential is measured in a solution containing a known amount of the ion for which the electrode is selective, and secondly, the potential is measured in a solution containing the interferent. These potential values were used to calculate the selectivity coefficient values using the following equation:

$$\log K_{ED,J^{z+}}^{pot} = \frac{E_2 - E_1}{S} + \log [ED] - \log [J^{z+}]^{1/z}$$

Where E_1 and E_2 are the electrode potentials for a 10^{-2} M solution of each of the ephedrine drug and interferent cations, J^{z+}, respectively, S is the slope of the calibration curve and z is the charge of the interferent ion.

2.6. Analytical application

2.6.1. Calibration graph method

In the calibration graph method, different amounts of EDCl were added to a 50 ml volume of water comprising a concentration range from 1.0×10^{-7} to 1.0×10^{-2} M and the measured potential was recorded using the present electrode. Data was plotted with the measured potential versus logarithm of the ED^{+} activity and the resulting graph was used for subsequent determinations of unknown ED concentrations.

2.6.2. Standard addition method

The standard addition method in which small increments(10–100 μL) of (0.1 M) EDCl solution were added to 50.0 mL aliquot to produce series of solutions, 5.0×10^{-6} to 1.0×10^{-5} M EDCl. The potentials were monitored, after each addition, at 25 ± 0.1 ◦C and was used to calculate the concentration of EDCl in the drug samples.

2.6.3. Potentiometric titration

Potentiometric titration of 5 mL of 1.0×10^{-2} M EDCl solution were transferred to a 25 mL beaker, and titrated with a standard solution of PTA using the prepared ED electrode as indicator electrode. The end points were determined from the S-shaped curve.

2.6.4. Analysis of ephedrine hydrochloride in spiked urine samples

Urine samples (5.0 mL) were spiked with EDCl and stirred for 5 min, prior to transferring to a 25-mL volumetric flask. Volume was completed to the mark to give 5.0×10^{-6} to 1.0×10^{-5} (M) EDCl. Solutions were analyzed using either the standard additions or the calibration graph method for EDCl determination.

3. Results and Discussion

3.1. Composition of the electrodes

The performance characteristics of an electrode depends on the nature of the ion-exchangers and its lipophilicitiy [43] in addition to the type of the

plasticizer employed [46]. The influences of membrane composition, the amount of ion-exchanger, nature and amount of plasticizersand the effect of operational conditions such as temperature, response time, presence of interferents and pH, etc on the potential response of the proposed sensor, were investigated. The results are summarized in Table 1.

3.1.2. Effect of ion-Exchanger

The influence of the amount of the ion-exchanger on the potential response of the electrode was studied and the results are summarized in Table 1. It can be seen, that the electrode without the ion-exchanger (electrode No. 1) showed insignificant selectivity towards ephedrine ions. The ion-exchangers of ED-ST, ED-SM, ED-PT, ED-PM and ED-TPB were prepared and tested as modifiers for the present electrode. The potentiometric response of the electrodes modified by ED-ST, ED-SM, ED-PM and ED-TPB was found to be non satisfactory comparatively an electrode modified by ED-PT gives better, stable and reproducible results. On the other hand, it is observed that an increase in the amount of the ion-exchanger to 0.5%, produce best results (electrode No. 3). Further addition of the ion-exchanger (electrodes No. 4&5) hamperedthe results, most probably due to some inhomogeneities and possible saturation of the membrane [47].

3.2. Selection of the plasticizer

The plasticizer should have a high capacity to dissolve the substrate and other additives present in the membrane. Since the nature of the plasticizer influences the dielectric constant of the membrane and the mobility of the ion-exchanger molecules[48, 49], The plasticizers viz. (2-NPOE), (DOP), (DBP), (DOPh) [35] were employed to study the effect on the electrochemical behavior of the electrodes. Table 1, Figure 3, demonstrate that the use of DOP results in a Nernstian linear plot over a wide concentration range. This is due to the ability of DOP to extract ephedrine ions from aqueous solutions to the organic membrane phase. Among the different compositions studied, the electrode containing 0.5% ED-PT,

51.0% DOP and 48.5% PVC exhibited favorable response characteristics. Therefore, this composition was used to study various operational parameters of the electrodes. The electrochemical performance characteristics of the electrode was systematically evaluated in accordance with the procedures of the IUPAC recommendations [50].

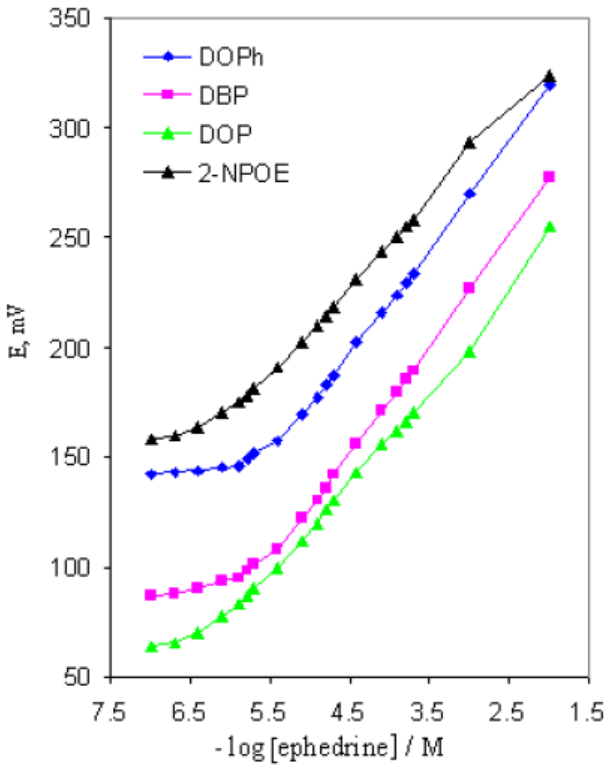

Figure 3. Effect of different level of various plasticizers on the measured potential

Table 1. Composition and slope of calibration curves for ED-CGE at

No.	Composition (%)				*C.R	*LOD	*R.S.D	*$R_{(s)}$
	*I.E.	*PVC	*P	*S				
Effect of different percentages of the ion-exchanger								
1	-	48.2	51.8 (DOP)	42±0.2	1.4×10^{-5}-1.0×10^{-2}	3.4×10^{-5}	0.95	16
2	0.2 (ED-PT)	48.6	51.2 (DOP)	52±0.4	5.1×10^{-6}–1.0×10^{-2}	3.7×10^{-6}	0.21	10
3	0.5 (ED-PT)*	48.5	51.0 (DOP)	57±0.8	9.3×10^{-7}–1.0×10^{-2}	6.2×10^{-7}	0.24	6
4	1.0 (ED-PT)	48.2	50.8 (DOP)	53±0.4	$2.5\text{x}10^{-6}$ –$1.0\text{x}10^{-2}$	1.3×10^{-6}	0.24	8
5	2.0 (ED-PT)	47.8	50.2 (DOP)	50±0.2	4.7×10^{-6}-1.0×10^{-2}	2.4×10^{-6}	0.17	12
Effect of different plasticizers								
6	0.5 (ED-PT)	48.5	51.0 (DOPh)	46±0.7	6.3×10^{-6}-1.0×10^{-2}	3.2×10^{-6}	1.25	18
7	0.5 (ED-PT)	48.5	51.0 (DBP)	53±0.3	4.1×10^{-6}–1.0×10^{-2}	2.2×10^{-6}	1.01	12
8	0.5 (ED-PT)	48.5	51.0(2-NPOE)	47±0.3	1.7×10^{-6}- 1.0×10^{-2}	7.6×10^{-7}	0.65	16
9	0.5 (ED-PT)*	48.5	51.0 (DOP)	57±0.8	9.3×10^{-7}–1.0×10^{-2}	6.2×10^{-7}	0.24	6
Effect of the type of ion-exchangers								
10	0.5 (ED-SM)	48.5	51.0 (DOP)	44±0.9	7.1×10^{-5}–1.0×10^{-2}	4.3×10^{-5}	0.88	15
11	0.5 (ED-ST)	48.5	51.0 (DOP)	42±0.3	2.3×10^{-5}–1.0×10^{-2}	1.2×10^{-6}	0.74	23
12	0.5 (ED-PT)	48.5	51.0 (DOP)	57±0.8	9.3×10^{-7}–1.0×10^{-2}	6.2×10^{-7}	0.24	6
13	0.5 (ED-PM)	48.5	51.0 (DOP)	51±1.0	6.8×10^{-6}–1.0×10^{-2}	3.5×10^{-6}	0.83	15
14	0.5 (ED-TPB)	48.5	51.0 (DOP)	54±0.71	2.6×10^{-6}- 1.0×10^{-2}	1.2×10^{-6}	0.35	12

25.0±0.1 °C.

*I.E: Ion-exchanger, *PVC: poly vinyl chloride, *P: plasticizer, *S: slope (mV/decade), *C.R.: concentration range (M), *LOD: limit of detection (M), *R(s): response time(s),*RSD: relative standard deviation, the number of replicate measurements = 5.*: Selected composition.

3.3. Effect of temperature

To study the thermal stability of the electrode, calibration graphs were constructed at different test solution temperatures including: 20- 60 ^{o}C. The slopes, response times, concentration ranges and the detection limits were obtained from each the calibration plot. It was thus concluded that there was no significant difference in the performance characteristics measured at

different temperature. This indicates fairly high thermal stability of the electrode.

3.4. Effect of potentially interfering ions

Selectivity is an important characteristic of a sensor [51].The effect of the interfering ions on the response factors measured by electrodes was evaluated by (SSM) and (MPM) [45]. The results, presented in Table 2, show that the electrode display significantly high selectivity for ephedrine over many common organic and inorganic compounds, sugars, amino acids and drugs that may taken during treatment with ephedrine.

3.5. Effect of pH on response functions

The influence of pH on the response of the CGE was investigated for EDCl solutions covering the range (1×10^{-4} and 1×10^{-3} M). Figure 4 demonstrates that the variation in potential due to pH change was minimal in the pH range 2.9–7.7. However, there was significant reduction of the potential at pH values lower than 2.9.This may be due to H^+ ion interference. On the other hand, the potential decreased gradually at pH values higherthan 7.7. The decrease may be attributed to the effect of the hydroxyl ion on the electrode.

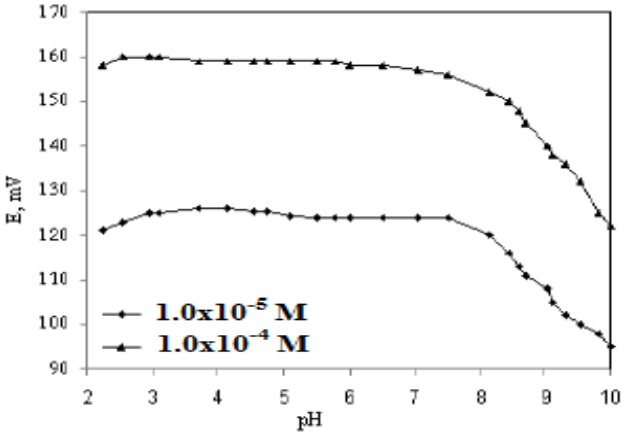

Figure 4. Effect of pH of the test solution on the potential response of proposed electrode

Table 2. Selectivity coefficient of various interfering ions for CGE

Interfering ions	SSM	MPM
NH^{+}	1.42×10^{-4}	8.48×10^{-5}
Na^{+}	5.62×10^{-4}	4.86×10^{-3}
K^{+}	4.95×10^{-4}	3.37×10^{-4}
Li^{+}	1.52×10^{-4}	5.11×10^{-5}
Mg^{2+}	5.05×10^{-4}	7.58×10^{-4}
Ca^{2+}	8.82×10^{-5}	5.53×10^{-5}
Ba^{2+}	1.28×10^{-4}	4.26×10^{-5}
Zn^{2+}	1.14×10^{-5}	5.18×10^{-5}
Ni^{2+}	3.41×10^{-4}	5.95×10^{-4}
Cd^{2+}	4.38×10^{-4}	7.43×10^{-4}
Co^{2+}	8.15×10^{-5}	2.77×10^{-4}
Al^{3+}	3.28×10^{-5}	2.88×10^{-5}
Ce^{3+}	9.55×10^{-5}	3.82×10^{-4}
captopril	2.42×10^{-4}	8.22×10^{-4}
spectinomycine	5.21×10^{-4}	9.76×10^{-4}
Dicolfine	3.86×10^{-4}	8.21×10^{-4}
Maltose	-	5.21×10^{-7}
D-Fractose	-	5.49×10^{-7}
glucose	-	4.92×10^{-7}
D-Galactose	-	2.92×10^{-6}

3.6. Effect of electrode bed

To investigate the effect of the bed nature on the efficiency of coated wire electrodes, the optimized coating mixture was used for preparation of electrodes with different conductive beds,namely silver, copper, graphite, gold and platinum. After conditioning, each electrode was examined in the concentration range from$1.0x10^{-7}$to $1.0x10^{-2}$ (M) EDCl. The dynamic range of concentration and the limit of detection of the electrodes were evaluated according to the IUPAC recommendations [31].Table 3 demonstrates, one can notice that allwires give inferior response towards EDCl as compared to that of graphite wire. graphite wire-coated electrode has a slope 57.8±0.03 (mV/decade), a linear concentration range from 8.5 x 10^{-7} to 1.0 $x10^{-2}$ M and limit of detection of 4.5 x 10^{-7}(M) for ephedrine ion. Therefore, graphite wire was used as the inner solid contact for the electrodes in this study.

Table 3: Analytical characteristics of various ED CWEs prepared by using the optimized membrane mixtures.

Type of wires	Linear Range (M)	Slope (mV/decade)	Detection Limit (M)	Resistivity ($\mu\Omega$ cm^{-1a})
silver	2.6×10^{-6}- 1.0×10^{-2}	50±0.4	1.4×10^{-6}	1.62
copper	7.9×10^{-6}–1.0×10^{-2}	53±0.3	6.3×10^{-6}	1.72
gold	3.3×10^{-6} -1.0×10^{-2}	52±1.0	1.8×10^{-6}	2. 65
platinum	2.2×10^{-4}-1.0×10^{-2}	33±0.4	1.6×10^{-4}	10.7
graphite	9.3×10^{-7}–1.0×10^{-2}	57±0.8	6.3×10^{-7}	1375

[a] Resistivity values of conductive beds, Reproduced from C. R. C. Handbook of Chemistry and Physics, 58th ed, 1978, CRC Press, West Palm Beach, Florida, F-170,171.

*** selected composition.**

3.7. Dynamic response time, repeatabilityand life time of the electrodes

The dynamic response time of the presented electrode was measured in accordance with the IUPAC recommendations [50]. The response time may be defined as the time between addition of the analyte to the sample solution and the time when a limiting potential was attained[50]. In this work, the

response time of each electrode was measured by varying the ED concentration over the range $1.0x10^{-5}$ to$1.0x10^{-2}$(M). As shown in Figure 5, the electrode reached equilibrium in about 5 s and no change was detected up to 5 min. The repeatability of the potential reading for the electrode was examined by subsequent measurement in $1.0x10^{-5}$ M ED solution immediately after measuring the first set of solutions in $1.0X10^{-4}$ M EDCl. The electrode potential for five replicate measurements using a $1.0x10^{-5}$ M solution averaged 117 (mV) and a standard deviation of 1.53. The corresponding values for a$1.0X10^{-4}$ (M) solution averaged 168 with a standard deviation of 0.67. Repeatability of the potential response of the electrode was established. The performance characteristics of the investigated electrode was further evaluated as a function of soaking time. By soaking the electrode in 10^{-3} M solution of EDCl for different intervals starting from 30 min up to 30 days. The slopes of the electrodes were measured and showed a gradual decrease in potential after 22 days. The life spans of the CWEs, in general, are less than those of the corresponding liquid contact electrodes. This may be attributed to poor mechanical adhesion of the PVC-based sensitive layer to the conductive bed [52].

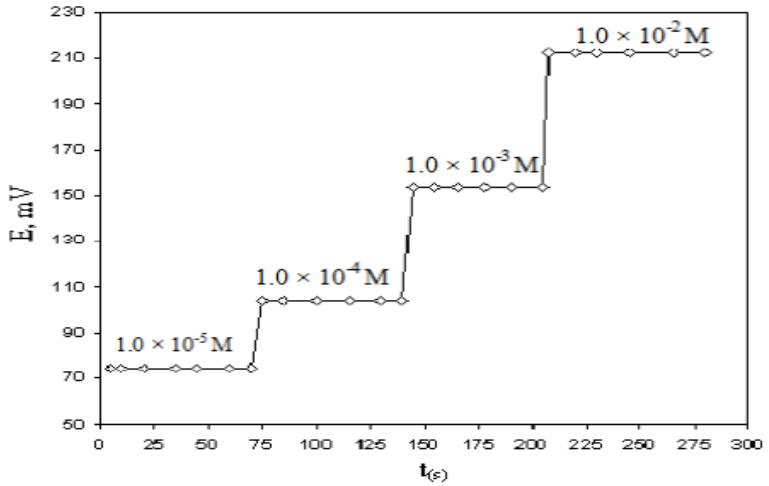

Figure 5. Typical potential–time plot for the electrode response

3.7. Analytical application

In order to test the analytical applicability of the proposed sensor, The proposed sensor was successfully applied fordetermination of ephedrine hydrochloride in ampoules and in urine samples using standard addition and calibration curve methods.Table 3 summarized the results after applying the above methods are compared with the values obtained from the official method [53]. F-test was used for comparing the precision resulting from the two methods, t-tests was used for comparing the methods accuracy. The calculated F-and t-test in Table 3 were less than critical values. There was no significant difference between the precision or the accuracy of the two methods at 95% confidence levels.

3.7.1.Titration of ephedrine solution with PTA

The ED-CGE was successfully used as an indicator electrode for potentiometric titrations of 5 ml of 0.01 M of EDCl with 0.0033 M PTA. The method for ephedrine ion (ED) titration is based on the decrease of (ED) concentration by precipitation with a PTA standard solution. Figure 6 illustrates that the amount of ephedrine can be accurately determined from the end point of the titration curve using the test electrode.

3.7.2. Recovery and determination of ephedrine ions in urine samples

Ephedrine is rapidly and completely absorbed after oral administration. It is rapidly and extensively distributed throughout the body and accumulates in the liver, lungs, kidneys, spleen and brain. Up to 95 % of an oral dose may be excreted in the urine in 24 h. 55 to 75 % was excreted as unchanged drug and the rest as metabolites. The urinary excretion of ephedrine is pH dependent increases in acidic urine. In alkaline urine, excretion is much lower, 20 – 35 % of the dose [54]. Recovery experiments were conducted by spiking urine samples with appropriate amounts of ephedrine ions, and determined by the electrode using the standard addition and the calibration curve methods. The results, shown in Tables 3 indicate recoveries and relative standard deviation values range between 97.8– 100.7 % and 0.71-1.186 respectively. It is noted

that the results are accurate and reproducible. Thus the sensor can be employed for quantification of ephedrine in urine samples.

Table 3: Results of real samples analysis

sample	Taken (M)	measured (M)	X%	R.S.D%	F-value	t-value
Ampoule						
	S 7.00×10^{-7}	6.93×10^{-7}	99.00	1.03	2.45	1.55
	C 5.50×10^{-6}	5.38×10^{-6}	97.80	0.95	2.21	1.64
Urine						
	S 7.00×10^{-7}	7.09×10^{-7}	101.3	1.86	3.13	2.19
	C 5.50×10^{-6}	5.54×10^{-6}	100.7	0.71	2.39	1.64

C: calibration curve, S: standard addition method R.S.D.: relative standard deviation X: recovery The critical value of F=9.28 and the critical value of t=3.707.

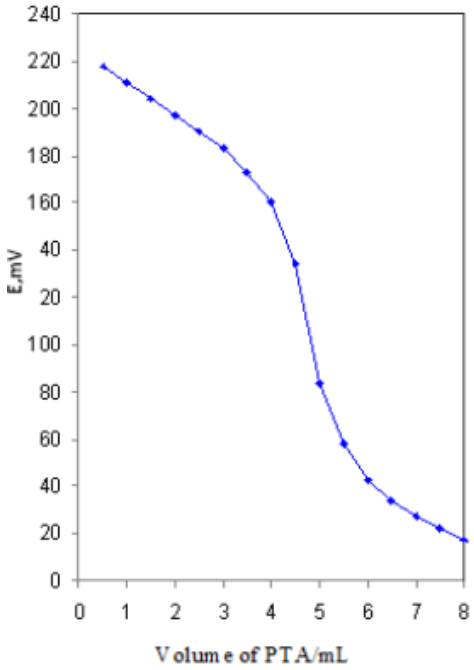

Figure 6. Potentiometric titration curve of 5.0 mL of 0.01 M solution of EDCl with 0.0033 M PTA.

4. Conclusions

The proposed CGE was based on the ion-exchangers namely ephedrine-phosphotungstate as the electro-active compound, might be a useful analytical tool and an interesting alternative for the determination of ephedrine hydrochloride in pharmaceutical preparations. The electrode shows high sensitivity, good selectivity, fast static response, long term stability and a wide concentration range.Notably, minimum sample pretreatment is required prior to detection body fluids such as urine.

5. References:

1- White S. Wong, Y. H. S.1999: "Guidelines for Therapeutic Drug Monitoring Services". The National Academy of Clinical Biochemistry, USA, 63

2- Konig, A. W. Ernst, K. 1983: Application of enantioselective capillary gas chromatography to the analysis of chiral pharmaceuticals, J. Chromatogr. 280, 135-141

3- Dictionary of Traditional Chinese Medicine, New Medical College of Jiangsu, People's Publisher of Shanghai, 1977, p. 2222.

4- International Olympic Committee Medical Code. World Anti-Doping Agency, website: / www.wada-ama.org

5- Hood J. D. and Cheung Y. H. 2003: A chromatographic method for rapid and simultaneous analysis of codeine phosphate, ephedrine HCl and chlorpheniramine maleate in cough–cold syrup formulation, J. Pharm. Biomed. Anal., 30 (5), 1595–1601

6- Ganzera, M. Lanser, C. Stuppner, H. 2005 : Simultaneous determination of Ephedra sinica and Citrus aurantium var. amara alkaloids by ion-pair chromatographyTalanta 66(4), 889- 894

7- Pellati F. and Benvenati S. 2008 : Determination of ephedrine alkaloids in Ephedra natural products using HPLC on a pentafluorophenylpropyl stationary phase J. Pharm. Biomed. Anal. 48(2), 254-263

8- Marchei E. Pellegrini M. Pacifici, R. Zuccaro P. Pichini, S. 2006 : A rapid and simple procedure for the determination of ephedrine alkaloids in

dietary supplements by gas chromatography–mass spectrometry, J. Pharm. Biomed. Anal. 41(5), 1633–1641

9- Sullivan, D. Wehrmann, Schmitz, J. Crowley, R. Eberhard, J. 2003: Determination of ephedra alkaloids by liquid chromatography/tandem mass spectrometry. J. AOAC Int. 86 (3), 471-475

10- Jacob P. Haller A. C. Duan M. Yu L. Peng M. . Benowitz N. L. 2004: Determination of ephedra alkaloid and caffeine concentrations in dietary supplements and biological fluids. J. Anal. Toxicol., 28(3), 152-159

11- Borst C. and Holzgrabe U. 2010 : Comparison of chiral electrophoretic separation methods for phenethylamines and application on impurity analysis, J. Pharm. Biomed. Anal. 53(5), 1201–1209

12- Wei F. Zhang M. Feng Y.Q. 2007: Combining poly (methacrylic acid-co-ethylene glycol dimethacrylate) monolith microextraction and on-line pre-concentration-capillary electrophoresis for analysis of ephedrine and pseudoephedrine in human plasma and urine, J. Chromatogr. B 850 (2007) 38-44

13- Fang H. Liu M. Zeng Z. 2006 : Solid-phase microextraction coupled with capillary electrophoresis to determine ephedrine derivatives in water and urine using a sol–gel derived butyl methacrylate/silicone fiber, Talanta 68(3), 979–986

14- Phinney W. K. Ihara T. Sander, L.C. 2005: Determination of ephedrine alkaloid stereoisomers in dietary supplements by capillary electrophoresis, J. Chromatogr. A, 1077, 90-97.

15- Li H.X. Ding M.Y. Lv K. Yu J.Y. 2001: Separation and determination of ephedrine alkaloids and tetramethylpyrazine in Ephedra sinicaStapf by gas chromatography-mass spectrometry, J. Chromatogr. Sci. 39(9), 370-374

16- Marchei, E. Pellegrini, M.Pacifici, R.Zuccaro P.Pichini, S. 2006:A rapid and simple procedure for the determination of ephedrine alkaloids in dietary supplements by gas chromatography–mass spectrometry J. Pharm. Biomed. Anal. 41, 1633–1641.

17- Betz M. J. Gay L. M. ossoba M.M. M. Adams, S. Portz, S. B. 1997: Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing MáHuáng.J. AOAC Int.,80 (2), 303-315

18- Prankerd, L. R. Elsabee, Z. M. 1995: Thermal analysis of chiral drug mixtures: the DSC behavior of mixtures of ephedrine HCl and pseudoephedrine HClenantiomersThermochim. Acta 248, 147-160.

19- Aman, T.Shahid, S. M. Rashid A.Khokhar I. 1997: J. Anal. Chem. 30, 1517.

20- Wallace, E. J. 1969 : Determination of phenethanolamine drugs in biologic specimens by ultraviolet spectrophotometry, J. Pharm. Sci., 58 (12), 1489-1492

21- Gala, B. Gomez Hens, A. Perez Bendito, D. 1994: Individual and simultaneous determination of ephedrine and phenylephrine by use of kinetic methodology, Fresenius J. Anal. Chem. 349 (12), 824-828

22- Ying W. Hong-wei X. 2007: J. Anal. Sci. 2, 246–248.

23- Senturk Z. Erk N. Ozkan A. S. Akay C. Cevheroglu S. 2002 : Determination of theophylline and ephedrine HCL in tablets by ratio-spectra derivative spectrophotometry and LC, J. Pharm. Biomed. Anal. 29, 291–298

24- Abdel-Ghani, N. T. Rizk, S. M. Mostafa, M. 2013: Extractive determination of ephedrine hydrochloride and bromhexine hydrochloride in pure solutions, pharmaceutical dosage form and urine samples SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy, 111, 131–141.

25- Tator, U.L.U.S. 2006: Highly sensitive spectrofluorimetric determination of ephedrine hydrochloride in pharmaceutical preparations, J.A.O.A.C. Int. 89 (5) 1263-1967

26- Mazzotta, E. Picca, A. R. Malitesta, C. Piletsky, A. S. Piletska, V. E. 2008: Development of a sensor prepared by entrapment of MIP particles in electrosynthesised polymer films for electrochemical detection of ephedrineBiosens. Bioelect., 23 (7) 1152-1156

27- De Marco A. Mecarelli E. 1967: Polarographic determination of ephedrine in liquid and solid pharmaceutical preparations, Farmaco 22 (12), 795-799.

28- Alcada, M.N.M.P. Lima, J.L.F.C. Montenegro, M.C.B.S.M. 1995: FIA titrations of ephedrine in pharmaceutical formulations with a PVC tetraphenylborate tubular electrode, J. Pharm. Biomed. Anal. 13, 459–464

29- Chamorro P.R. and Díaz R.C. 1993 : A double-membrane ephedrine selective electrode based on ephedrine-tetraphenylborate in poly (vinyl chloride) resin, Talanta 40, (9), 1461–1464

30- Hassan, S. M. S. Kamel A. H. Abd El-Naby H. 2013: New potentiometric sensors based on selective recognition sites for determination of ephedrine in some pharmaceuticals and biological fluids, Talanta, 103, 330–336

31- Khaled K. Hassan A. N. H. G. Mohamed, G. Seleim. A. A. 2010: Carbon paste and PVC electrodes for the flow injection potentiometric determination of dextromethorphan. Talanta, 81(1-2), 510-515

32- Vytras. K. 1989: The use of ion-selective electrodes in the determination of drug substances. J. Pharm. Biomed. Anal., 7(7), 789-812

33- Cosofret A. V. and Buck. P. R. 1993: Recent Advances in Pharmaceutical Analysis with Potentiometric Membrane Sensors. Crit. Rev. Anal. Chem., 24, 1-58

34- Abu-Shawish M. H. 2008 : Potentiometric Response of Modified Carbon

Paste Electrode Based on Mixed Ion-Exchangers. Electroanalysis, 20, 491.

35- Abu-Shawish M. H. Dalou A. A. Abu Ghalwa N. Khraish I. G. Hammad J. Basheer H. 2013: Determination of pethidine hydrochloride using potentiometric coated graphite and carbon paste electrodes, Drug Test. Analysis, 5, 213–221

36- Rubinova N. Torres, C. K. Bakker E 2007 : Solid-contact potentiometric polymer membrane microelectrodes for the detection of silver ions at the femtomole level. Sens.Actuators B, 121(1), 135-141

37- Ceresa A. Bakker E. Hattendorf B. Gunther D. Pretsch E. 2001: Potentiometric Polymeric Membrane Electrodes for Measurement of Environmental Samples at Trace Levels: New Requirements for Selectivities and Measuring Protocols, and Comparison with ICPMS.Anal. Chem. 72(2), 343-351

38- Pretsch. E. 2007: The new wave of ion-selective electrodes. TrAC, 26(1), 46-51.

39- Cattrall W. R. and Freiser. H. Coated wire ion-selective electrodes. Anal. Chem. 1971, 43, 1905-1906.

40- Bakker E. and Chumbimuni-Torres K. 2008 : Modern directions for potentiometric sensors. J. Braz. Chem. Soc. 2008, 19(4), 621-629.

41- Ibrahim H. Issa M. Y. Abu-Shawish M. H. 2007: Improving the detection limits of antispasmodic drugs electrodes by using modified membrane sensors with inner solid contact. J. Pharm. Biomed. Anal. 44, 8-15

42- Chumbimuni-Torres Y. K. Rubinova N. Radu A. Kubota, T. L. Bakker.E. 2006: Solid Contact Potentiometric Sensors for Trace Level Measurements. Anal. Chem. 78(4), 1318-1322.

43- Ibrahim H. Issa M. Y. Abu-Shawish, M. H. 2007: Improving the detection limits of antispasmodic drugs electrodes by using modified membrane sensors with inner solidcontact. J. Pharm. Biomed. Anal 44, 8-15.

44- Ardakani M. A. Pourhakak P. Salavati-Niasari M. 2006: Bis(2-hydroxyacetophenone) ethylenediimine as a neutral carrier in a coated-wire membrane electrode for lead(II) Anal. Sci 22 (2006) 865-870.

45- Umezawa Y. Buhlmann P. Umezawa K. Tohda K. Amemiya S. 2000: Potentiometric selectivity coefficients of ion-selective electrodes, Pure and Applied Chemistry. 72, 1851–2082.

46- Masadome T. Yang J. Imato T. 2004: Effect of Plasticizer on the Performance of the Surfactant-Selective Electrode Based on a Poly(Vinyl Chloride) Membrane with no Added Ion-Exchanger″. Micrchim. Acta. 144, 217-220

47- Arvand, M. Asadollahzadeh. A. S 2008: Ion-selective electrode for aluminum determination in pharmaceutical substances, tea leaves and water samples. Talanta, 75, 1046-1054.

48- Egorov V. V. Bolotin A. A. 2006: Ion-selective electrodes for determination
of organic ammonium ions: Ways for selectivity control. Talanta, 70, 1107-1116

49- Zhang, B. X. Han, X. Z. Fang, H. Z. Shen, L. G. Yu. Q. R. 2006: 5,10,15-Tris(pentafluorophenyl)corrole as highly selective neutral carrier for a silver ion-sensitive electrode. Anal. Chim. Acta, 562(2), 210-215

50- Buck, R. P., Lindner, E. 1994: IUPAC recommendation for nomenclature of ionselective electrodes. Pure and Applied Chemistry, 66, 2527‑2536.

51- Švancara I, Vytras K. Kalcher K. Walcarius A. Wang J. 2009: Carbon Paste Electrodes in Facts, Numbers, and Notes: A Review on the Occasion of the 50-Years Jubilee of Carbon Paste in Electrochemistry and Electroanalysis, Electroanalysis, 21, 7-28

52- Vlekkert V. H. Francis C. Grisel A. Rooij N. 1988: Solvent polymeric membranes combined with chemical solid-state sensors, Analyst, 113, 1029-1033.

53- British Pharmacopia, Vol 1 & II, (M.G. Lee), MHRA, Market Towers, 1 Nine Elms Lane, London SW8 5NQ, (2009).

54- Sever, S. P. Dring G. L. Williams T. R. 1975: The metabolism of (−)-ephedrine in man″. Europ. J. Clin. Pharm., 9, 193-198.