Int. J. Electrochem. Sci., 7 **(2012)** 4443 - 4464

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Construction of Different Types of Ion-Selective Electrodes. Characteristic Performances and Validation for Direct Potentiometric Determination of Orphenadrine Citrate.

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Received: 13 March 2012 / *Accepted:* 11 April 2012 / *Published:* 1 May 2012

Three types of ion-selective electrodes (carbon paste, PVC membrane and screen printed electrodes) have been proposed for determining orphenadrine citrate (OphC) in pure solution and pharmaceutical preparation based on the ion-pair formation between OphC with sodium tetraphenylborate. The three types of electrodes were prepared using five types of plasticizers with each type of electrodes. The electrodes showed a linear response with a good Nernstian slope of 57.20 ± 0.70, 56.81 ± 1.6 and 57.09 ± 0.2 mV decade^{-1} over the concentration range 10^{-6} to 10^{-2} mol L^{-1} with CPE, PVC membrane and SPEs, respectively. The standard electrode potentials, $E°$, were determined at 10, 20, 30, 40 and 50 °C and used to calculate the isothermal coefficient ($dE°/dT$) of the electrodes. Temperatures higher than 60 °C affect the electrodes performance. The electrodes proved highly selective with selectivity coefficients ranging from 1.063-5.573, 1.323-6.798 and 1.073-6.504 for CPE, PVC membrane and SPE, respectively. The detection limits (signal/noise [S/N] = 3) were 1.016×10^{-6}, 0.984×10^{-6} and 0.992×10^{-6} mol L^{-1} for CPE, PVC membrane and SPE, respectively. The practical applications of these electrodes were demonstrated by measuring the concentrations of OphC in pure solutions and pharmaceutical preparations with satisfactory results. The reliability and stability of the electrodes gave a good possibility for applying the technique to routine analysis.

Keywords: orphenadrine citrate, pharmaceutical analysis, potentiometry, tetraphenyl borate, CPE, PVC, SPE.

1. INTRODUCTION

Orphenadrine citrate (OphC) has the IUPAC name N,N-dimethyl-2-[(o-methyl-α-phenylbenzyl) oxy]-ethylamine citrate (1:1). It occurs as a white, crystalline powder having a bitter

taste. It is practically odourless, soluble in hot water and slightly soluble in alcohols. Orphenadrine citrate (Figure. 1) is used to temporarily relieve pain caused by skeletal muscle relaxant [1]. It is an analogue of the antihistamine diphenhydramine [2-4], it is used in the treatment of Parkinson's disease [4] and to alleviate some of the troublesome symptoms of the disease, especially the involuntary resting tremor. Orphenadrine is used also as an analgesic both alone and in association with non-steroidal anti-inflammatory drugs [5]. Ion-selective membrane electrodes (ISEs) are now widely used for the direct potentiometric determination of ion activities or ion concentrations in different samples. Their advantages are simple design, low cost, adequate selectivity, low detection limit, high accuracy, wide concentration range and applicability to coloured and turbid solutions. Although potentiometric methods of analysis using ion-selective electrodes are cheap, simple and applicable to samples, only one potentiometric atenolol determination based on polymeric membrane with limited dynamic range is described [6].

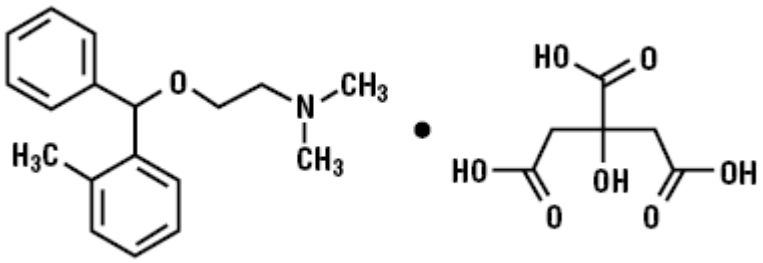


Figure 1. Structure formula of orphenadrine citrate

Several methods of analytical studies reported for the determination of OphC in pure form and pharmaceutical preparations by spectrophotometer [1, 3, 7-12], gas chromatography [13], gas chromatography-mass spectrophotometry [14-16], gas liquid chromatography [17], liquid chromatography-mass spectrophotometry [18,19], thin layer chromatography [20, 21], HPLC [22-25] and HPLC/MS-MS method [26].

In the present work, screen printed (SPE), carbon paste (CPE) and plastic membrane electrodes of the conventional type have been constructed and their performance characteristics were studied. The electrodes are based on the interaction of the sodium tetraphenylborate (Na^+TPB^-) with the orphenadrine drug [Oph^+] to form the ion-pair which utilized to study the life time of the above-mentioned electrodes by the changes that appear on these voltage until they lose their sensitivity and all effects such as pH, temperature, selectivity, etc. The electrodes were used successfully as sensors to determine OphC in pure form and pharmaceutical preparation. Method validation was studied.

2. EXPERIMENTAL

2.1. Materials

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. Distilled water was used throughout all experiments. They

included OphC provided by Misr Company for Pharmaceutical Industry, Egypt. Glucose, sucrose, starch, maltose, lactose, fructose, glycine, sodium fluoride and chloride salts of calcium, nickel, potassium, ammonium, cadmium and cobalt were used as interfering materials.

For making ISE membrane the following reagents were used: *o*-nitrophenyloctylether (*o*-NPOE) was supplied from Fluka, while di-n-octyl phthalate (DOP), dibutylphthalate (DBP) and dioctyl sebacate (DOS) were supplied from BDH. In addition, tricresylphosphate (TCP), polyvinylchloride (PVC relative high molecular weight) and graphite powder (synthetic 1 – 2μm) were supplied from Aldrich.

Sodium tetraphenylborate (NaTPB, Sigma-Aldrich, Germany), phosphotungstic acid (PTA, Fluka, Switzerland), phosphomolybdic acid (PMA, Fluka, Switzerland) and ammonium reineckate salts (RN, Fluka, Switzerland) were used for precipitation of different ion pairs. Cyclohexanone and acetone were supplied from Fluka (Switzerland).

OphC pharmaceutical preparations "Norflex" were purchased from Sedico (OphC 100 mg per tablet), Egypt.

2.2. Apparatus

Elemental analysis (C, H, N) were determined at the Microanalytical Center at Cairo University using CHNS-932 (LECO) Vario Elemental analyzers. Laboratory potential measurements were performed using Jenway 3505 pH-meter. Silver-silver chloride double-junction reference electrode (Metrohm 6.0222.100) in conjugation with different drug ion selective electrode was used. pH measurements were done using HANNA, model 211, Romania.

2.3. Standard solutions

2.3.1. Orphenadrine citrate solution

Stock OphC solution (1.0×10^{-2} mol L^{-1}) was prepared by dissolving the proper weight of the drug (461 mg) into smaller amount of distilled water, heated with stirring till the drug completely dissolved. The resulting solution was then made up to 100 mL with distilled water in a measuring flask.

To compare the sensitivity of electrodes with the drug once and with the pharmaceutical preparation, Norflex (white tablets 100% OphC) used, take 5 tablets and ground them well then calculate the right weight to prepare 10^{-2} mol L^{-1}, dissolved in smaller amount of distilled water, heated with stirring then filtered using filter paper to get rid of insoluble materials, transferred quantitatively to 100 mL in volumetric flask. Then content was estimated via potentiometric titration with NaTPB using CPE, PVC and SPE (plasticized with *o*-NPOE) as sensing electrodes. The method was repeated several times to check the accuracy and reproducibility of the proposed method.

2.3.2. Tetraphenylborate solution (TPB^-)

1×10^{-2} mol L^{-1} NaTPB solution was prepared by dissolving 1811 mg into 500 mL distilled water, adjusted to pH = 9 by adding sodium hydroxide and completed to the desired volume with water. The resulting solution was standardized potentiometrically against standard (1×10^{-2} mol L^{-1}) thallium (I) acetate solution [27].

2.3.3. Interfering ions solutions

A 10^{-3} mol L^{-1} standard solution each of glycine, glucose, sucrose, starch, fructose, maltose, lactose, sodium fluoride, chloride salts of calcium, ammonium, nickel, potassium, cadmium and cobalt were prepared by dissolving the proper weights into 100 mL bidistilled water.

2.4. Electrodes preparation

2.4.1. Carbon paste electrode preparation

The sensing electrodes were prepared by intimate mixing accurately weight 500 mg of highly pure graphite powder and plasticizer (0.2 mL of DOP, TCP, DBP, DOS or *o*-NPOE). This matrix was thoroughly mixed in the mortar and the resulted past was used to fill the electrode body [28, 29]. A fresh surface was obtained by gently pushing the stainless-steel screw forward and polishing the new carbon-paste surface with filter paper to obtain a shiny new surface.

2.4.2. PVC membrane preparation

For PVC electrode, five membranes were prepared using the cocktail consisting of 240 mg plasticizer "DOP, DBP, TCP, DOS and *o*-NPOE", 240 mg PVC and 6 mL THF. The cocktail was stirred for 5 min and poured into Petri dish "5 cm" diameter. After 24 h of slow evaporation of solvent, a master membrane with 0.11 mm thickness was obtained which was mounted on the softened end of the PVC tubing with the help of adhesive solution prepared by dissolving PVC in THF. The PVC closed tube with the membrane was filled with 0.25 mL of 1 mol L^{-1} KCl and completed to 25 mL with 0.01 mol L^{-1} OphC drug solution under investigation using Ag/AgCl as internal reference electrode.

2.4.3. Preparation of the screen printed electrodes

Disposal SPE was performed by using a manual screen printer, an array of 12 electrodes was printed on a flexible X-ray film by forcing the prepared conductive ink to penetrate through the mesh of a screen stencil. A screen consisting of a heavy duty polyester fabric (I 003 M Sefar Pet 1000 with mesh count of 36) was pre-tensioned to ca 30x40 cm wooden frame. For the stainless steel template, steel sheet were pre-tensioned to a steel frame and contain grooves with the same electrode dimensions

[28, 29]. The homemade printing ink was prepared by thoroughly mixing the cyclohexanone-acetone mixture 1:1, as a solvent for the binding material with 450 mg of *o*-NPOE, 1.25 mg polyvinyl chloride then 0.75 mg of the carbon powder was added and after stirring for 15 min, the ink was sonicated and applied for printing of the electrodes [28, 29]. The influence of the plasticizer choice on the electrode performances has been studied as the electrode plasticized with *o*-NPOE is compared with those plasticized with DBP, DOP, DOS and TCP. The SPEs were stored in a dry state at room temperature [28, 29].

2.5. Procedures

2.5.1. Study of the experimental conditions

2.5.1.1. Identification of slope of the studied electrodes:

The electrochemical performance characteristics of the two studied OphC-selective electrodes were evaluated according to IUPAC standards [30].

Sensors calibration was carried out by measuring the potential of 10^{-6}–10^{-2} mol L^{-1} drug solutions starting from low to high concentrations. The potentials were plotted as a function of drug concentrations. Sensors life spans were examined by repeated monitoring of the change in the potential break and total potential jump of the drug titration periodically. The detection limit was taken at the point of intersection of the extrapolated linear segment of the drug calibration graph.

The dynamic response times of the electrodes (SPE, PVC membrane and CPE) were tested for the concentrations of 10^{-6}–10^{-2} mol L^{-1} OphC solutions. The sequence of measurements was from low to high concentrations. The time required for the electrodes to reach value within ±2 mV from the final equilibrium potential after increasing OphC concentration level by ten folds was measured.

2.5.1.2. Effect of pH on the electrodes response

The effect of pH on the potential values of the three electrodes systems was studied over the pH range of 2–11 at 1-pH interval by immersing electrodes in 10^{-2} and 10^{-4} mol L^{-1} OphC solutions. The pH was gradually increased or decreased by adding aliquots of diluted sodium hydroxide or hydrochloric acid solutions, respectively. The potential obtained at each pH was recorded.

2.5.1.3. Effect of temperature

The effect of temperature on the performance of the potentiometric electrodes was evaluated in a thermostat at different temperatures ranged from 10-60 °C.

2.5.1.4. Effect of titrants

3 mL of 10^{-2} mol L^{-1} OphC drug solution was potentiometrically titrated against different titrants including NaTPB, RN, PTA and PMA using CPE, PVC and SPE (plasticized with o-NPOE) as

sensing electrodes where the total potential change and the potential break for each titrant were calculated at the end point.

2.5.1.5. Effect of foreign compounds on the electrodes selectivity

The selectivity coefficients for of many nitrogenous compounds such as starch, sugars and glycine was obtained by the matched method which is totally independent of the Nicolsky equation. To determine the selectivity coefficients by the matched method, a known activity ($a_{D'}$) of the primary ion solution is added into a reference solution that contains a fixed activity (a_D) of primary ions, and the corresponding potential change (ΔE) is recorded. Next, a solution of interfering specie is added to the reference solution until the same potential change (ΔE) is reached and the activity of interfering (a_B) is recorded. The change in potential produced at the constant background of the primary ion must be the same in both cases. Also, The potentiometric selectivity coefficients ($K^{Pot}_{OphC,I}$) were evaluated according to IUPAC guidelines using the separate solutions method [31, 32] in which the potential of cell compromising the membrane electrode and a reference electrode is measured with two separate solutions, A and B where A (OphC ions) and B (interfering ion) at the same activity $a_A = a_B$. Selectivity coefficients were calculated by the separate solutions method, where potentials were measured for 10^{-3} mol L^{-1} OphC solution and 10^{-3} mol L^{-1} interfering solution, separately, and then potentiometric selectivity coefficients were calculated [31, 32].

2.5.1.6. Studying the effect of soaking using time the proposed sensors

Freshly prepared electrodes must be soaked to activate the surface to form an infinitesimally thin gel layer at which ion exchange occurs. Storage was in the distilled water when not in use (in case of CPE and PVC membrane).

2.6. Potentiometric determination of OphC in pharmaceutical preparations

OphC was determined in pure solution and pharmaceutical preparations using the developed electrodes under both batch conditions (by standard addition and potentiometric titration). In standard addition method, known increments of 10^{-2} mol L^{-1} standard OphC solution were added to 25 mL aliquot of sample solution where the change in the potential readings was recorded for each increment and used to calculate the concentration of OphC in sample solution. For potentiometric titration, aliquots of the sample solutions containing 5.40–8.43 mg OphC were titrated against standard NaTPB solution. The titration process was monitored using OphC sensors in conjugation with the conventional Ag/AgCl reference electrode and the potential values were plotted against the titrant volume to estimate the end point.

3. RESULTS AND DISCUSSION

The development and application of ion-selective electrodes (ISEs) is of interest for pharmaceutical analysis because these sensors offer the advantages of simple design and operation, fast response, reasonable selectivity, low detection limit, high accuracy, wide concentration range applicability to colored and turbid solutions, and possible interfacing with automated and computerized systems. OphC reacted with sodium tetraphenylborate to form stable 1:1 water insoluble ion association complex, with low solubility product and suitable grain size precipitate, having the following suggested composition: $C_{42}H_{43}BNO$ with elemental analysis data: Found%: C = 82.52, H = 8.46 and N = 2.53, Calculated%: C = 82.3, H = 8.54 and N = 2.78.

3.1. Electrochemical behaviour of orphenadrine citrate with utilized electrodes

To obtain the electrochemical behavior, calibration was carried out by immersing the electrode in conjunction with the double junction Ag/AgCl reference electrode in solutions of OphC in the concentration range of 10^{-6}–10^{-2} mol L^{-1}. They were allowed to equilibrate whilst stirring and recording the e.m.f. readings. The electrodes showed a linear response over the concentration range with Nernstian slope of 57.20 ± 0.7, 56.81 ± 1.6 and 57.09 ± 0.2 mV $decade^{-1}$ for CPE, PVC membrane and SPE, respectively (Table 1). The E (mV)–p[OphC] profile was plotted as shown in Figure (2).

3.2. Effect of soaking time on the electrode performance

Table 1. Response characteristics of the investigated OphC electrodes.

Parameters	CPE	PVC	SPE
Slope (mV $decade^{-1}$)	57.20 ± 0.7	56.81 ± 1.6	57.09 ± 0.2
Intercept (mV)	421.21	376.12	493.26
Correlation coefficient	0.9694	0.9713	0.9856
Detection limit (mol L^{-1})	1.016×10^{-6}	0.984×10^{-6}	0.991×10^{-6}
Limit of quantitation (mol L^{-1})	3.389×10^{-6}	3.279×10^{-6}	3.305×10^{-6}
Response time for 10^{-3} mol L^{-1}, (s)	9	13	7
Working pH range	3.5-8	3.5-7.5	3-8
Concentration range, mol L^{-1}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}
Life span (weeks)	4-5	2-3	18-25
Average recovery (%)	97.33-98.66	97.63-98.03	98.63-99.10
RSD%[a]	0.33	0.28	0.32
Between day variability (CV[b] %)	0.69	0.95	0.81
Robustness[b]	99.02±1.02	98.45±1.11	99.32±0.97
Ruggedness[c]	98.88±0.62	98.22±1.05	99.15±0.76

[a] Average of four determinations.
[b] Variation in method parameters such as pH of buffer.
[c] Comparing the results by those obtained by using HANNA 211.

The effect of soaking time on the performance of the electrodes was studied and the data obtained are listed in Table (2). The electrodes were soaked in Oph-TPB ion-pair suspended solution and the titration curves were plotted from which the total potential changes are recorded after different time intervals. The optimum time was found to be 1 hr soaking, as indicated by the values of total potential charge = 238, 156 and 241 mV/mL for CPE, PVC membrane and SPE plasticizer with *o*-NPOE, respectively) and potential break at the end point (220, 133 and 226 mV for CPE, PVC membrane and SPE, respectively).

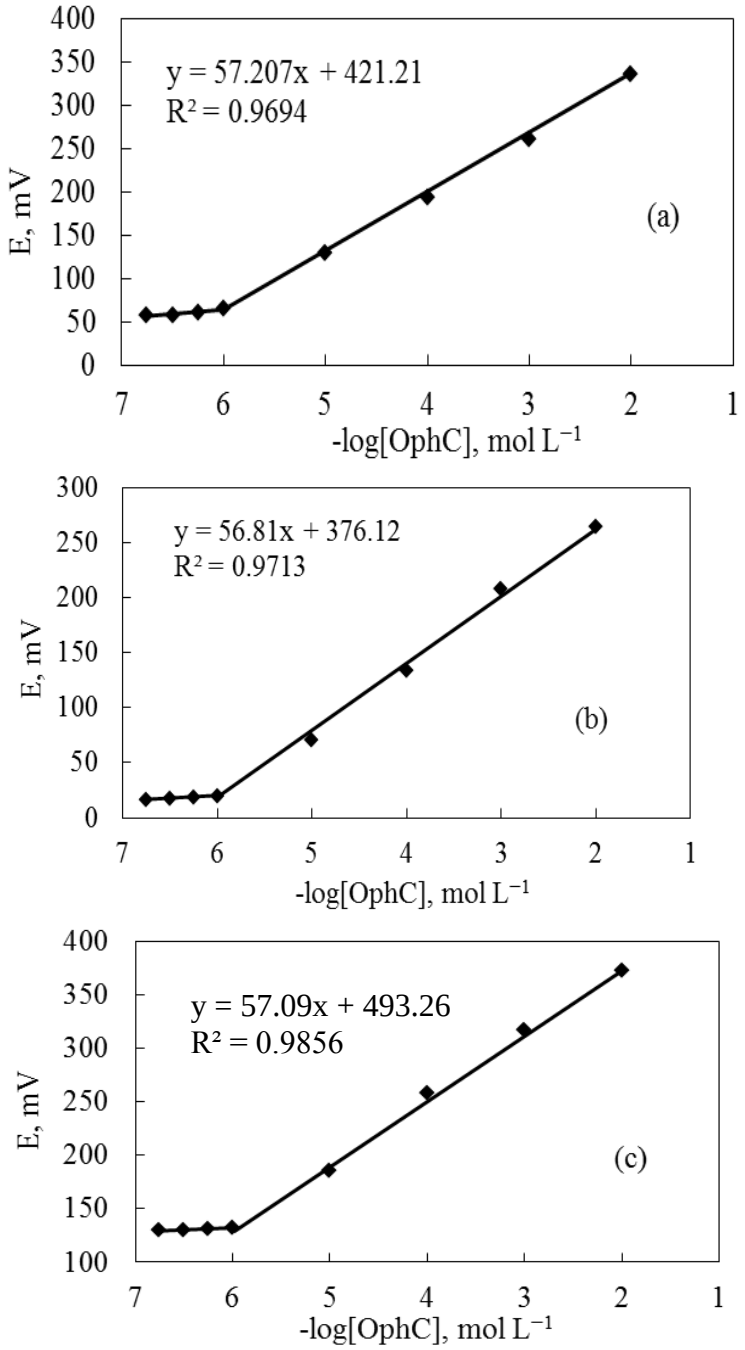


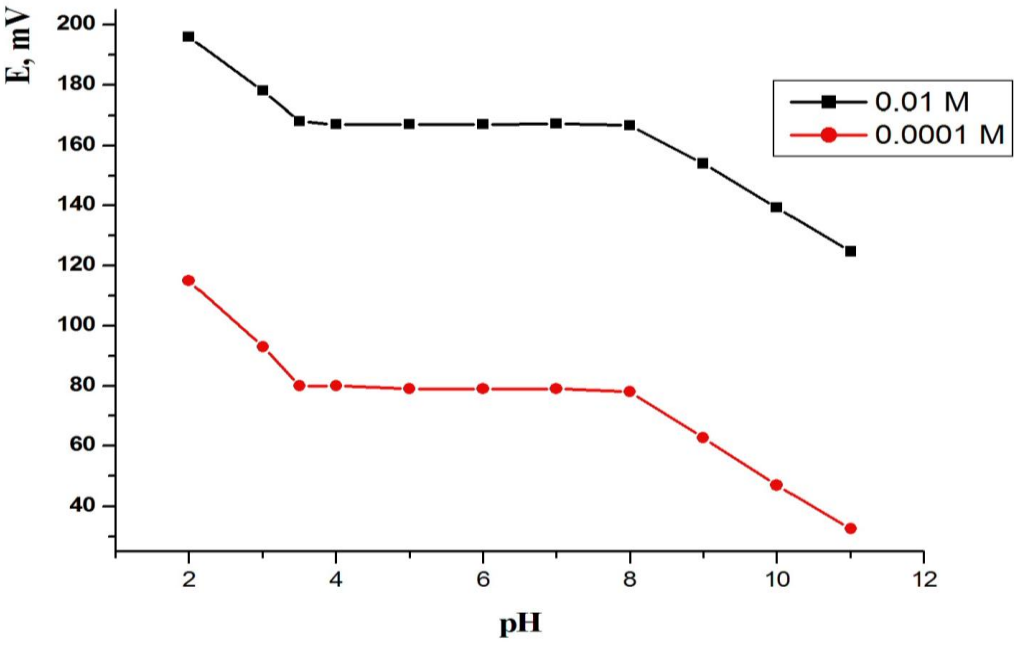
Figure 2. Nernstian slope using (a) CPE, (b) PVC membrane and (c) SPE.

Table 2. Effect of soaking time on the CPE, PVC and SPE performance in the potentiometric titration of 3 mL of 10^{-2} mol L^{-1} OphC with 10^{-2} mol L^{-1} NaTPB.

Time of soaking	CPE		PVC		SPE	
	Potential break at the end point, mV	ΔE/ΔV (mV/mL)	Potential break at the end point, mV	ΔE/ΔV (mV/mL)	Potential break at the end point, mV	ΔE/ΔV (mV/mL)
With out	197	497.5	120	305	83	212.5
5 min	151	382.5	41	107.5	46	120
15 min	161	407.5	56	145	77	197.5
30 min	191	482.5	70	180	164.5	426.25
1 hr	220	555	133	335	226	575
2 hr	218	550	113	285	181.5	468.75
12 hr	159	402.5	83	210	65	167.5
24 h	185	467.5	79	200	40	105

3.3. The pH effect

The influence of the hydrogen ion towards the EMF of the electrodes was tested at 1.0×10^{-2} and 1.0×10^{-4} mol L^{-1} of the drug solution by varying the pH from 2.0 to 11.0 with diluted HCl or NaOH. It is clear from Figure (3) that the electrodes have stable readings in the pH range 3.5-8.0, 3.5-7.5 and 3.0-8.0 for CPE, PVC membrane and SPE, respectively. The change at higher pHs could be the result of hydroxide precipitate formation, while in the low pH range, competitive proton binding is probably behind the decreased potential values [33].



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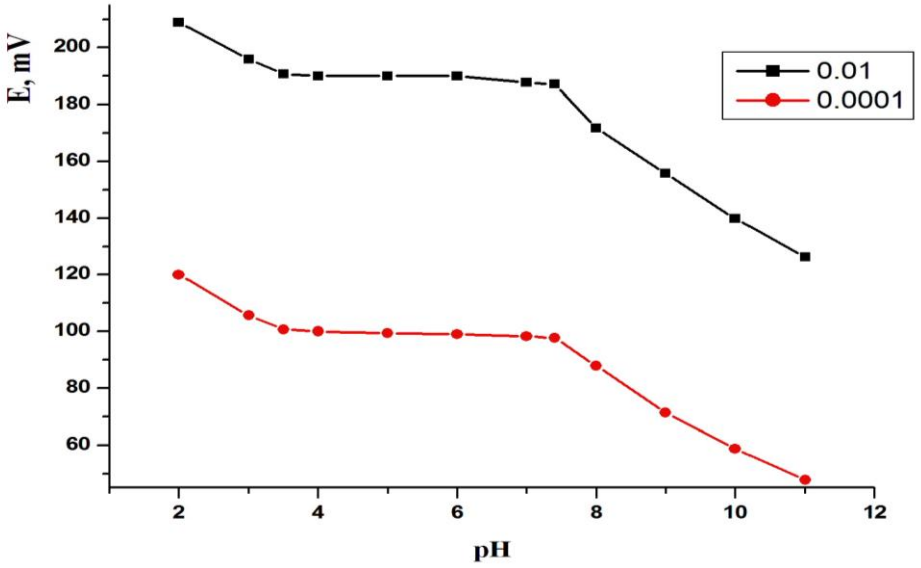

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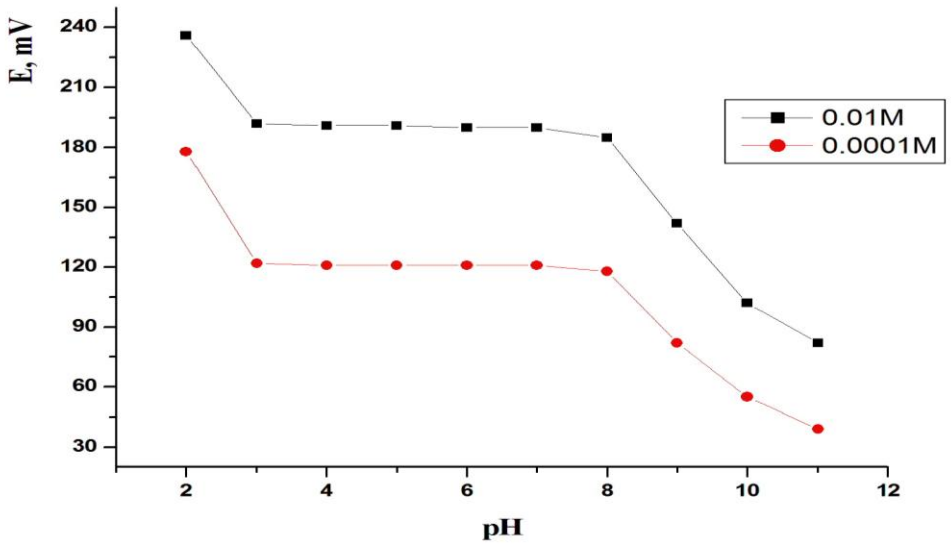

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Figure 3. Effect of pH on the electrodes performance using (a) CPE, (b) PVC membrane and (c) SPE.

3.4. Selectivity coefficients

Potentiometric selectivity coefficients can be measured with different methods that fall into two main groups, namely (1) mixed solution methods, and (2) separate solution methods, Selectivity coefficient (K_{ij}) for every interfering secondary ion was determined by the separate solutions method [31, 32, 34]. Separate drug primary ion (i) and interfering secondary ion (j) solutions were prepared having equal concentrations (1.0×10^{-3} mol L^{-1}). Their potentials E_i and E_j were measured using SPE, CPE and PVC electrodes plasticized with *o*-NPOE. Selectivity coefficients were calculated using the following equations:

$$\log K_{ij} = (E_j - E_i) / S \qquad (1)$$

$$\log K_{ij} = [(E_j - E_i) / S] + [1 + (Z_i / Z_j)] \log[i] \quad (2)$$

Equation (1) is used for monovalent secondary ions whereas equation (2) is used for divalent or higher ones. Z_i and Z_j are the charges on the primary and secondary ions, respectively.

While the selectivity coefficients for of many nitrogenous compounds such as starch, sugars and glycine were obtained by the matched method which is totally independent of the Nicolsky equation. The following equation is applied:

$$\log K^{pot}_{D,B} = (a_D' - a_D) / a_B$$

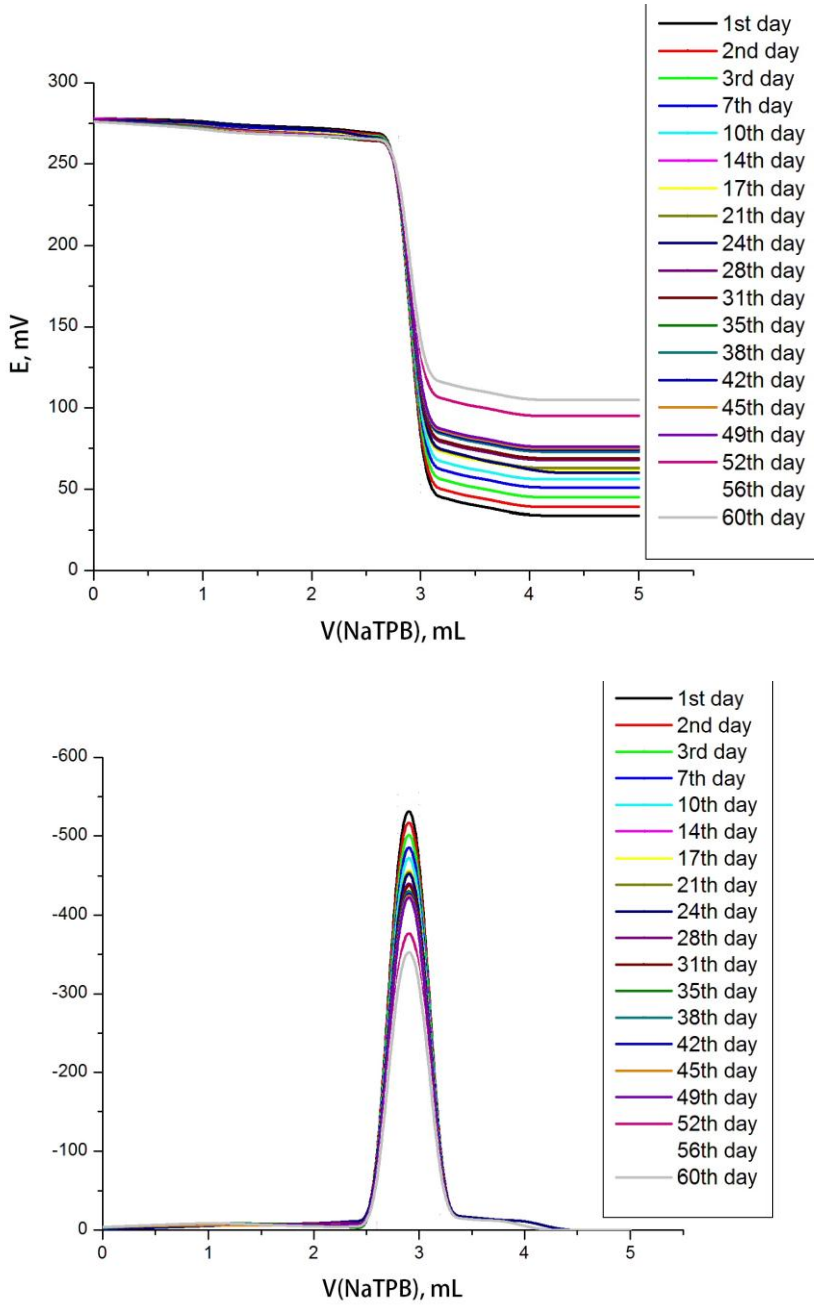
The influence of some inorganic cations, anions, sugars and glycine on the OphC-electrodes was investigated (Table 3). The selectivity coefficients values of the SPE, CPE and PVC membrane electrodes reflect a very high selectivity of the investigated electrodes for the orphenadrine cation (Oph^+). The inorganic cations do not interfere owing to the differences in ionic size, and consequently their mobilities and permeability, as compared with those of Oph^+ (Table 3). Also, the smaller the energy of hydration of the cation, the greater the response of the membrane. In the case of sugars and glycine, the high selectivity is mainly attributed to the difference in polarity and lipophilic character of their molecules relative to OphC [35].

Table 3. Potentiometric selectivity coefficients of the OphC sensors.

Interfering ions (B)	$\log K^{pot}_{D,B}$					
	CPE		PVC		SPE	
	Matched method	SSM	Matched method	SSM	Matched method	SSM
Glucose	2.748	-----	3.376	-----	2.611	-----
Lactose	2.258	-----	3.552	-----	2.436	-----
Fructose	2.136	-----	3.112	-----	3.224	-----
Maltose	2.066	-----	2.746	-----	3.574	-----
Starch	2.171	-----	2.472	-----	2.681	-----
Sucrose	2.363	-----	4.647	-----	3.084	-----
Glycine	3.185	-----	2.464	-----	3.066	-----
Co^{2+}	-----	1.475	-----	2.281	-----	1.531
Ca^{2+}	-----	2.139	-----	3.935	-----	2.005
NH_4^+	-----	1.063	-----	1.323	-----	1.073
Na^+	-----	1.216	-----	1.675	-----	1.318
Cd^{2+}	-----	1.073	-----	2.316	-----	1.724
Ni^{2+}	-----	1.685	-----	2.298	-----	1.636
K^+	-----	1.374	-----	1.576	-----	1.160
Cl^-	-----	5.573	-----	6.798	-----	6.504
F^-	-----	3.520	-----	3.230	-----	3.440

3.5 Lifetime

The aim of these tests was to compare lifetime of different electrodes related to their preparation mode. In overall, lifetime taken into account (CPE, PVC membrane and SPE plasticized with *o*-NOPE) show relatively good performance. After this time the total potential change and the potential break at the end point of the sensors will decrease where the electrodes were used extensively (twenty times per day). It is well established that the loss of plasticizer, carrier, or ionic site from the polymeric film due to leaching into the sample is a primary reason for limited lifetimes of the sensors [28, 29]. Figures (4a-c) shows the comparison between CPE, PVC membrane and SPE life time, respectively.



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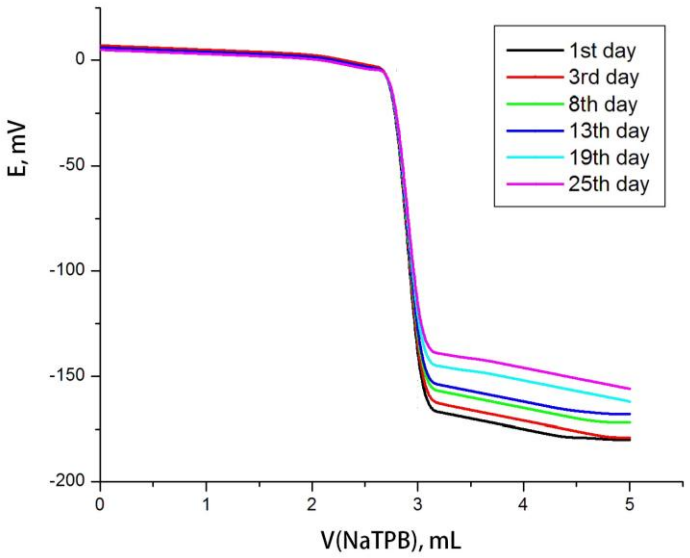
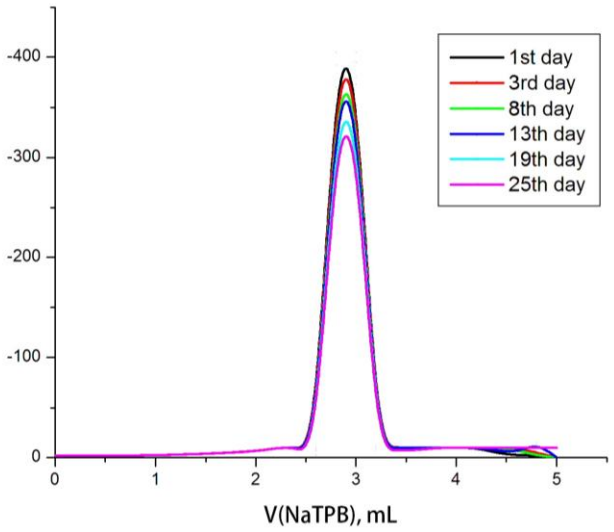
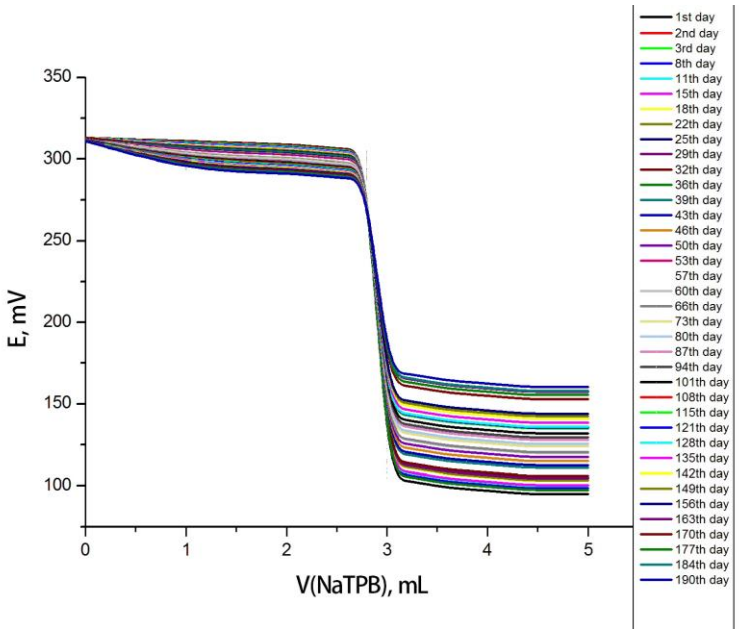

E, mV
V(NaTPB), mL
1st day
3rd day
8th day
13th day
19th day
25th day


V(NaTPB), mL
1st day
3rd day
8th day
13th day
19th day
25th day

B


E, mV
V(NaTPB), mL
1st day
2nd day
3rd day
8th day
11th day
15th day
18th day
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32th day
36th day
39th day
43th day
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94th day
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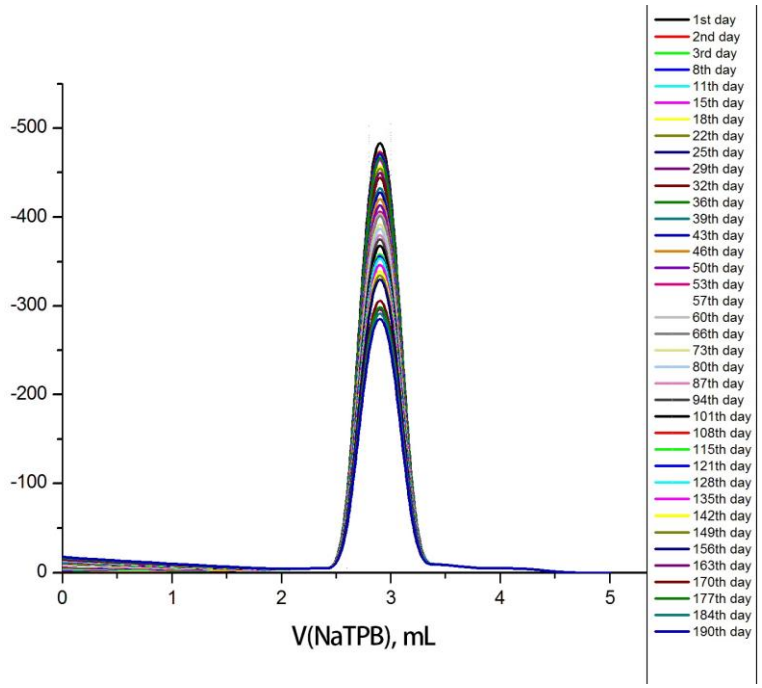

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Figure 4. The lifetime of (a) CPE, (b) PVC membrane and (c) SPE using o-NPOE plasticizer.

3.6. Effect of titrants

It is obvious from these data that NaTPB is the most suitable titrant on the performance of electrodes. Table (4) shows the total potential change and the abrupt change in the potential at the end point obtained for the titration of OphC with different titrants using CPE, PVC membrane and SPE.

Table 4. Potentiometric titration of 3 mL of 10^{-2} mol L^{-1} OphC with different titrants: a) 1×10^{-2} mol L^{-1} NaTPB, b) 1×10^{-2} mol L^{-1} RN, c) 3.3×10^{-3} mol L^{-1} PMA, d) 3.3×10^{-3} mol L^{-1} PTA, using SPE, CPE and PVC membrane electrodes.

Titrants	Total potential change, mV			Potential break at the end point, mV			ΔE/ΔV, (mV/mL)		
	CPE	PVC	SPE	CPE	PVC	SPE	CPE	PVC	SPE
NaTPB	238	201	241	220	182	226	555	467.5	575
PMA	32	20	40	16	6	21	47.5	17.5	55
RN	105	48	126	87	34	111	230	87.5	285
PTA	58	30	69	41	16	50	110	42.5	130

3.7. Effect of temperature

To study the effect of temperature on the response of electrodes utilized, the potential of 1.0×10^{-6} - 1.0×10^{-2} mol L^{-1} OphC solutions were determined in (10, 20, 30, 40, 50 and 60 °C) and the calibration graph were constructed, the standard electrode potentials ($E°_{elec.}$) (obtained from the calibration plots) corresponding to each temperature. From the table, it is obvious that the electrode gave a good Nernstian response in the temperature range 10–50 °C. For the determination of the isothermal coefficient ($dE°/dT$) of the electrode, the standard electrode potential ($E°_{elec.}$) at different temperatures was plotted vs. (t – 25), where t is the temperature of the test solution. A straight-line plot was obtained according to the following equation [35]:

$$E° = E°_{(25)} + (dE° / dT)(t - 25)$$

Isothermal coefficients are found to be 0.024, 0.030 and 0.039 V/C° for CPE, PVC membrane and SPE, respectively.

3.8. Effect of plasticizer type:

The role of plasticizer may be considered analogous to that of the organic solvent in liquid membrane electrodes and it influences both the selectivity and sensitivity of the electrode. When these electrodes are used to monitor the potentiometric titration based on ion pair formation, the magnitude of both the potential break and sharpness at the inflexion point of the titration curve is predetermined by the plasticizer polarity (dielecterical constants, ε) as a result of higher extractability of the ion pair into the plasticizer [36].

The influence of the plasticizer choice on the electrode performances has been studied as the electrode plasticized with *o*-NPOE is compared with those plasticized with DBP, DOP, DOS, or TCP (Figure 5a-c) for CPE, PVC membrane and SPE, respectively. From the all tested plasticizers, *o*-NPOE shows the highest total potential change (238, 156 and 241 mV) and the highest potential break at the end point (220, 133 and 226 mV) for CPE, PVC membrane and SPE, respectively (Figure 5).

No electrode preconditioning is needed before applying in the potentiometric titration and excellent titration curves can be achieved from the second titration process, while electrodes fabricated using other plasticizers need either to operate the titration process at least 5-7 times or to soak the electrode in the aqueous solution of the ion pair for more than one hour before using these electrodes in the titration process.

Also the electrode plasticized with DBP showed the shortest response time compared with other electrodes plasticized with the rest of plasticizers which is reflected on the total time required to achieve stable potential readings and the titration time.

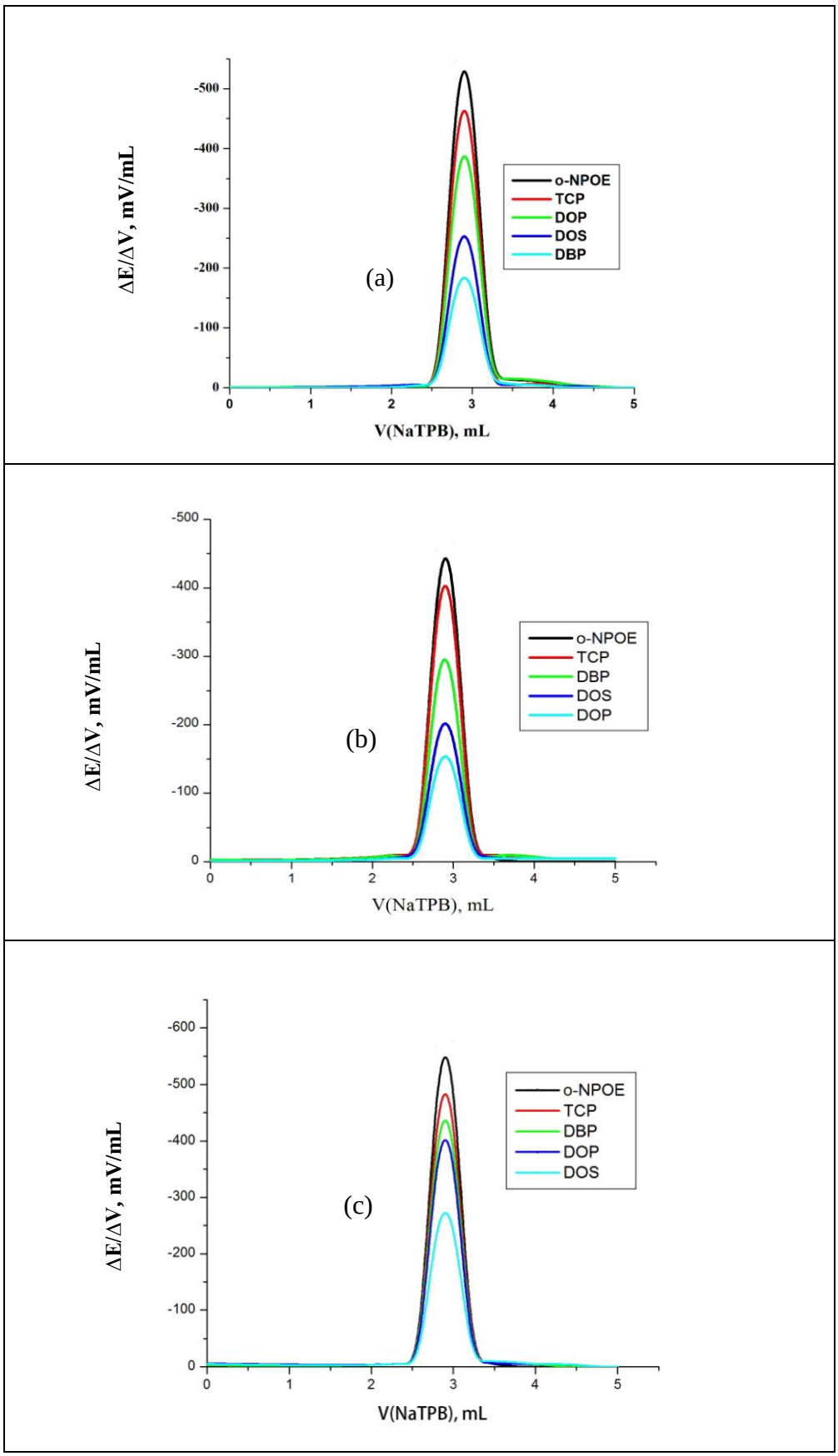

Figure 5. The effect of plasticizer type using: a) CPE, b) PVC membrane and c) SPE.

3.9. Response time:

For analytical applications, the response time of a fabricated sensor is of critical importance. The average time required for the electrode to reach a steady potential response within ±1 mV of the final equilibrium value after successive immersion of a series of OphC solutions, each having a 10-fold difference in concentration, is investigated [37]. The electrode response time is found to be 9, 13 and 7 s for CPE, PVC membrane and SPE, respectively, Figure (6) which is much shorter than any previously mentioned drug electrode [38-43] and the equilibrium potentials essentially remained constant for over 10 min. This fast and stable potential reading is reflected on the time needed for complete titration process as it is only about 3-5 min.

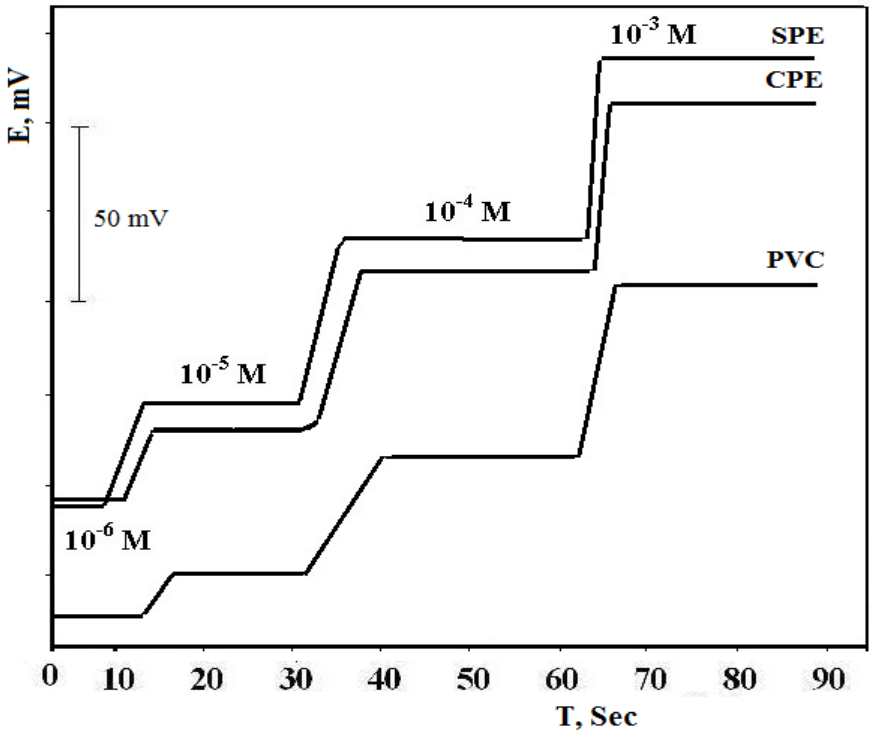

Figure 6. Dynamic response of OphC sensors: a) 1×10^{-6}, b) 1×10^{-5}, c) 1×10^{-4}, d) 1×10^{-3}, e)1×10^{-2} mol L^{-1} OphC.

3.11. Application on pharmaceutical and official method:

The designed sensors were utilized to determine OphC in pharmaceutical preparations (Norflex tablet) using the proposed potentiometric method. The results obtained were compared to the official method [7] and the data obtained are summarized in Table (5). There were no significant differences between the calculated and comparative values indicating that the electrodes can be used for potentiometric determinations of OphC in such samples. Statistical evaluation of the results of analysis

of pure OphC by the proposed electrodes and the official method [7] showed that there is no significant difference between the proposed and reported method in terms of accuracy and precision Table (5).

Table 5. Determination of orphenadrine citrate in pure solutions and pharmaceutical preparation applying the proposed and official methods.

Electrode (Plasticizer type)	Chemicals	Proposed [Drug] mg mL^{-1}		Official [Drug] µg mL^{-1}		Recovery%		SD* (RSD* (%))	SD** (RSD** (%))
		Taken	Found	Taken	Found	Proposed	Official		
CPE (o-NPOE)	Pure solution	3.83 10.00	3.72 9.85	100.0	98.50	97.13 98.50	98.50	0.10 (2.69) 0.25 (2.54)	0.29 (3.19)
	Norflex tablet	3.83 10.00	3.77 9.89			98.43 98.90		0.12 (3.18) 0.19 (1.92)	
PVC (o-NPOE)	Pure solution	3.83 10.00	3.78 9.75			98.69 97.50		0.06 (1.59) 0.12 (1.23)	
	Norflex tablet	3.83 10.00	3.74 9.90			97.65 99.00		0.07 (1.87) 0.28 (2.83)	
SPE (o-NPOE)	Pure solution	3.83 10.0	3.80 9.94			99.22 99.40		0.08 (2.11) 0.14 (1.41)	
	Norflex tablet	3.83 10.00	3.79 10.05			98.96 100.5		0.09 (2.37) 0.34 (3.38)	

* Proposed method ** Official method

3.11. Determination of OphC using potentiometric titration and standard addition method (SAM):

In the proposed potentiometric method, OphC is determined in norflex tablets using the potentiometric titration and standard addition method (Table 6).

Table 6. Determination of OphC in pharmaceutical sample by direct potentiometric titration and standard addition methods using CPE, PVC membrane and SPE.

Electrode utilized	[OphC] Taken (mg mL^{-1})	[OphC] Found (mg mL^{-1})		Recovery (%)a		SD (RSD, %)a	
		Titration	SAM[b]	Titration	SAM	Titration	SAM
CPE	5.40 8.43	5.33 8.44	5.37 8.34	98.70 100.1	99.44 98.93	0.16 (2.10) 0.28 (2.61)	0.10 (1.90) 0.19 (2.33)
PVC	5.40 8.43	5.29 8.50	5.31 8.39	97.96 100.8	98.33 99.88	0.19 (2.50) 0.31 (2.87)	0.14 (2.69) 0.20 (2.34)
SPE	5.40 8.43	5.43 8.41	5.52 8.48	100.6 99.76	102.2 100.6	0.14 (1.81) 0.25 (2.33)	0.1 (1.77) 0.21 (2.45)

[a] Mean of four determinations.

[b] SAM = Standard addition method

The concentration of the OphC analyte was determined by SAM which depends on the application of the following equation to each volume of the standard concentrated solution added to the unknown concentration.

$$C_x = C_s[V_s)/(V_x - V_s)]x10^{n(\Delta E/s)} - (V_x)/(V_s - V_x)]$$

where C_x and V_x are the concentration and the volume of the unknown, respectively. Cs and Vs are the concentration and the volume of the standard, respectively, S the slope of the calibration graph, and E is the change in millivolt due to the addition of the standard.

Results of the analysis in the proposed system revealed that weight of the taken amount was 5.40 and 8.43 mg mL^{-1} and the weight found for CPE, PVC membrane and SPE were tabulated in Table (6).

4. METHOD VALIDATION

The analytical method was validated according to the international conference for Harmonization (ICH) guidelines under the optimized experimental conditions: linearity, accuracy, precision, specificity and stability.

4.1. Linearity

The linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method and the data obtained are summarized in Table (1).

4.2. Limit of detection (LOD) and quantification (LOQ)

The limit of detection and quantification were calculated by LOD = 3δ/S and LOQ = 10δ/S, respectively, where S is the slope of the calibration curve and δ is the standard deviation of the response of the blank or the standard deviation of the intercepts of regression lines. The values listed previously in Table 1, indicate that the proposed CPE, PVC membrane and SPE sensors are sensitive to detection of low concentrations of OphC.

4.3. Accuracy

The accuracy of the proposed CPE, PVC membrane and SPE sensors for the determination of OphC is investigated by using standard addition and potentiometric titration methods. OphC is determined in norflex samples prepared from serial concentrations of OphC reference standards. The results summarized in Table 6, show that the proposed method is an accurate one, as indicated by the

percentage recovery values, for the determination of OphC in its pharmaceutical preparations without interferences from the coformulated adjuvants.

4.4. Precision

In order to determine the precision of the proposed methods, solutions containing three different concentrations of OphC were prepared and analyzed in four replicates and the analytical results are summarized in Table (7). The low values of the relative standard deviation (% RSD) also indicate the high precision and the good accuracy of the proposed methods. RSD (%) and SD values were obtained within the same day to evaluate repeatability (intra-day precision) and over five days to evaluate intermediate precision (inter-day precision).

4.4. Robustness and Ruggedness

The robustness of this proposed method was done by investigating to what extent the capacity of the method remains unaffected by a small but a deliberate variation in method parameters and hence provides an indication of its reliability during normal usage [44, 45]. The ruggedness of the proposed method was done by investigating the reproducibility of the results obtained by the analysis of the same samples under different conditions such as different instruments, laboratories and analysts. The results obtained using another model of pH-meter (HANNA 211, Romania) were compared with those obtained using Jenway 3505 pH-meter. The results obtained are close and also reveal validity of the method (Table 1).

Table 7. Intra- and Inter-days precision of the determination of OphC using the three types of electrodes with determination of pure and pharmaceutical tablet.

Drug	Electrode type (plasticizer used)	Taken, mg mL^{-1}	Intra day				Inter day			
			Found, mg mL^{-1}	Recovery %	SD	RSD%	Found, mg mL^{-1}	Recovery %	SD	RSD%
Pure form	CPE (o-NPOE)	5.80	5.70	98.27	0.050	0.884	5.74	98.96	0.045	0.783
		9.60	9.45	98.43	0.123	1.301	9.64	100.4	0.137	1.421
		15.90	15.58	97.98	0.256	1.643	15.78	99.24	0.081	0.513
	PVC (o-NPOE)	5.80	5.68	97.93	0.065	1.120	5.75	99.13	0.125	2.173
		9.60	9.45	98.43	0.190	2.010	9.48	98.75	0.088	0.928
		15.90	15.48	97.35	0.385	2.487	15.74	98.99	0.114	0.724
	SPE (o-NPOE)	5.80	5.78	99.65	0.054	0.934	5.77	99.48	0.125	2.166
		9.60	9.74	101.4	0.158	1.622	9.50	98.95	0.068	0.715
		15.90	15.70	98.74	0.145	0.923	15.84	99.62	0.142	0.896
Norflex tablet	CPE (o-NPOE)	7.45	7.38	99.06	0.090	1.219	7.50	100.6	0.095	1.266
		13.80	13.72	99.42	0.285	2.077	13.60	98.55	0.120	0.882
		20.22	20.09	99.35	0.130	0.647	20.05	99.15	0.264	1.316
	PVC (o-NPOE)	7.45	7.35	98.65	0.084	1.142	7.40	99.32	0.084	1.135
		13.80	13.60	98.55	0.180	1.323	13.77	99.78	0.093	0.675
		20.22	20.30	100.3	0.378	1.862	20.30	100.3	0.195	0.960
	SPE (o-NPOE)	7.45	7.40	99.32	0.088	1.189	7.52	100.9	0.107	1.423
		13.80	13.64	98.84	0.074	0.542	13.64	98.84	0.175	1.282
		20.22	20.28	100.2	0.235	1.158	20.35	100.6	0.234	1.149

5. CONCLUSION

The screen-printed carbon electrode is a promising tool for direct OphC determination and can be used for direct applications in real samples without any pretreatment. It was possible to determine the OphC contained in pharmaceutical samples following the standard addition and potentiometric titration methods using the PVC, CPE and screen-printed electrodes. A good analytical performance has been demonstrated. The proposed method shows a low detection limit, good sensitivity and excellent stability of the screen-printed carbon electrode over the CPE and PVC membrane. The carbon paste electrode, PVC and screen printed electrode have shown good Nernstian slope, rapid response time and relatively long term stability. Application of these electrodes for the potentiometric determination of this antihistamine drug in quality control department in drug sector and controller section is more economic and less time consuming compared to the most frequently used HPLC method.

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