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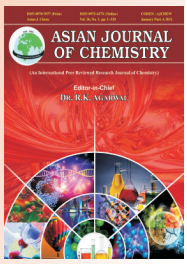

Comparative Potentiometric Study Using Modified 2-Hydroxypropyl β-Cyclodextrin and Modified Carbon Nanotubes Sensors for Determination of Ambroxol Hydrochloride

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A novel and sensitive electrochemical method for potentiometric determination of ambroxol hydrochloride was proposed. The proposed method was based on the fabrication of four ambroxol sensors using *o*-nitrophenyloctyl ether as plasticizer in a polymeric matrix polyvinyl chloride. Sensors I and II were conventional plastic membrane type and prepared from ambroxol-phosphomolybdate as ion pair, while sensor II was modified using 2-hydroxypropyl β-cyclodextrin as an ionophore. Sensors III and IV were carbon paste type and fabricated using ambroxol-phosphotungstate as ion pair while, sensor IV was modified using carbon nanotubes. The fabricated sensors were carefully investigated and characterized. They displayed Nernstian responses of (54.50 ± 0.6, 56.29 ± 0.3, 57.95 ± 0.8 and 58.32 ± 0.2 mV decade^{-1}) for sensors I, II, III and IV, respectively at 25 °C over drug concentration ranges of $1.0 \times 10^{-5} - 1.0 \times 10^{-2}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-7} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-8} - 1.0 \times 10^{-2}$ mol/L with lower detection limits of 5.0×10^{-6}, 4.9×10^{-7}, 4.8×10^{-8} and 5.0×10^{-9} mol/L for the four mentioned sensors, respectively. The effect of possible interfering species, pharmaceutical additives and some related pharmacological action drugs was investigated using separate solution method. No interference was found. The investigated drug was subjected to forced degradation and the stability indicating of the drug was also studied. The standard addition method was used for determination of the investigated drug in its pharmaceutical dosage forms and biological fluids. The developed sensors gave satisfactory results and these results were validated and statistically analyzed by recoveries studies and compared with those from previously reported methods.

Keywords: Ambroxol hydrochloride, Carbon paste sensor, 2-Hydroxypropyl β-Cyclodextrin.

INTRODUCTION

Recently, carbon nanotubes (CNTs) have attracted intensive attention because of their promising application in electrochemical sensors[1]. Various advantages of carbon nanotubes as sensor materials have been shown for analysis of diversified chemicals of food quality, clinical and environmental interest. High thermal conductivity, remarkable mechanical properties, chemically stability and high surface to volume ratio of carbon nanotubes is very appealing for sensing applications[2].

Cyclodextrins are a group of structurally related natural product formed during bacterial digestion of cellulose. These cyclic oligosaccharides consists of (∝ -1,4)-linked ∝-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. Water-soluble cyclo-dextrin derivatives of commercial interest included the hydroxypropyl derivatives (Fig. 1a) were used in sensors modification[3]. They have been previously applied as sensor ionophores for potentiometric determinations[4].

Ambroxol hydrochloride (AM) (Fig. 1b) is chemically known as cyclohexanol, 4-[N-(2-amino-3,5-dibromo benzenyl]-amino)hydrochloride. It is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It is a drug that is normally used to treat a wide variety of respiratory problems. It alleviates chest conditions and it is normally used by people who suffer from bronchitis, pneumonia and bronchospasm asthma[5].

Several methods have been reported for determination of ambroxol hydrochloride in its pure form and biological fluids. These methods are high performance liquid chromatography[6-9], liquid chromatography coupled with mass spectrometry[10,11], thin layer chromatography[12,13], capillary gas-liquid chromatography mass spectrometry[14,15] and capillary zone electrophoresis[16]. Furthermore, there are other analytical techniques concerned with the determination of ambroxol hydrochloride such as spectrophotometry[17-19], voltammetry[20] and potentiomerty[21].

The object of the present study is the fabrication of four types of sensors plastic membrane type sensor I and modified β-cyclodextrin sensor II using ambroxol-phosphomolybdate (AM-PM) as electroactive material. Also, carbon paste sensor

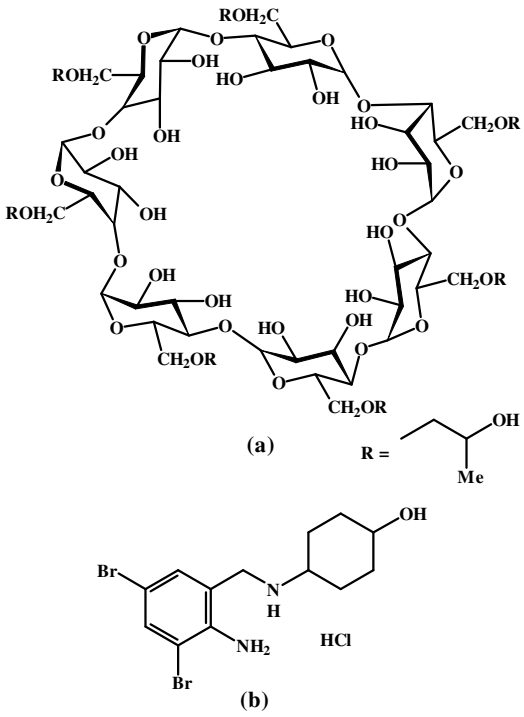

Fig. 1. (a) Chemical structure of 2-hydroxypropyl β-cyclodextrin (b) chemical structure of ambroxol hydrochloride

III and modified carbon nanotubes carbon paste sensor IV using ambroxol-phosphotungstate as electroactive material. The fabricated sensors were used for determination of ambroxol hydrochloride in its pure form, dosage forms and biological fluids. The optimization and performance characterization were tested. Stability indicated studies were investigated and the drug was determined in the presence of its degradations. The proposed method was validated according to ICH guidelines[22].

EXPERIMENTAL

All chemicals used throughout the experimental analysis were of analytical grade. Distilled water was used throughout all measurements. Phosphomolybdic acid (PMA) 99.9 %, phosphotungstic acid (PTA) 99.1 %, high purity graphite powder (1-2 μm), high molecular weight of poly vinyl chloride (PVC) and multi-wall carbon nanotubes powder (carbon > 95.0 %, O.D. × L 6-9 nm × 5 μm), 2-hydroxypropyl β-cyclodextrin were purchased from Sigma-Aldrich, Germany. Di-octylphthalate (DOP) 99.5 %, di-butyl phthalate (DBP) 99.0 %, di-butyl sebacate (DBS) ≥ 97.0 %, di-octyl sebacate (DOS) ≥ 97.0 %, *o*-nitrophenyloctyl ether (*o*-NPOE) 99.0 % and tetrahydrofuran (THF) were provided by Fluka, Switzerland. Sodium hydroxide 98.0 %, zinc sulphate ≥ 99.0 % and hydrochloric acid 36.5 % were purchased from BDH laboratory supplies (England). Pure grade ambroxol hydrochloride was kindly supplied from Sedico Co. (Egypt). The pharmaceutical preparation (Ambroxol® 30 mg/tablet) was purchased from local drug stores. Phosphate buffer of pH 6.0 was prepared by mixing 13.2 mL of 1.0 mol/L K_2HPO_4 (dibasic) with 86.8 mL of 1.0 mol/L KH_2PO_4 (monobasic) and the pH was adjusted to the exact value of pH 6.0. Urine samples were obtained from healthy volunteers and serum samples (Multi-Serum Normal, Randox Laboratories UK) were obtained from commercial sources.

Potentiometric and pH measurements were carried out using HANNA instrument model-211 microprocessor pH-meter. Ag/AgCl electrode was used as an external reference electrode. AREX heating magnetic stirrer connected with a circulator thermostat was used to control the temperature of the test solutions. Ivyman distiller system -AC-L8 was used for distilled water. Scanning electron microscope (SEM), JEOL JSM-6060 LV-(Japan) was used for surface structure studies of carbon nanotubes sensor.

Preparation of analytical solutions

Standard drug solution: Stock ambroxol hydrochloride solution 0.1 mol/L was freshly prepared daily by dissolving 1.036 g in 25 mL distilled water. Working solutions ($1.0 \times 10^{-8} - 1.0 \times 10^{-1}$ mol/L) were prepared by appropriate dilution with distilled water.

Preparation of ambroxol hydrochloride tablet solution: Twenty tablets (Ambroxol® 30 mg/tablet) were finally powdered and weighed. An accurately weighed portion containing 0.4 g ambroxol hydrochloride was dissolved in 5 mL distilled water then sonicated for 20 min. The resulting solution was filtered through filter paper No. 2. The filtered solution was completed to 10 mL with distilled water to obtain 0.1 mol/L. Working solutions $1.0 \times 10^{-5} - 1.0 \times 10^{-2}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-7} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-8} - 1.0 \times 10^{-2}$ mol/L for sensors I, II, III and IV, respectively were prepared by appropriate dilution with distilled water.

Preparation of spiked serum and urine solutions: Spiking technique method was used for determination of ambroxol hydrochloride in human serum and urine. The human serum and urine samples were adjusted at pH 6 using phosphate buffer. Accurately measured aliquots of ambroxol hydrochloride solutions were spiked in centrifugation tubes containing 1 mL of previously adjusted human serum or 5 mL of urine. Then vortex was done for 5 min. For human serum 1 mL acetonitrile, 0.1 mL of NaOH (0.1 mol/L), 1 mL of $ZnSO_4 \cdot 7H_2O$ (5.0 % w/v) were added, where most of the interfering species (mainly proteins) were removed by precipitation[23]. After centrifugation for 30 min at 3500 rpm, the clear supernatant layer was filtered through 0.5 μm Milli-pore filter. For human urine no further treatment was done and working solutions were then prepared by serial dilution to obtain ambroxol hydrochloride concentration ranges of $(1.0 \times 10^{-5} - 1.0 \times 10^{-2}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, 1.0×10^{-7} - 1.0×10^{-2} and 1.0×10^{-8} - 1.0×10^{-2} mol/L for sensors I, II, III and IV, respectively. Ambroxol hydrochloride was determined using AM-PM and AM-PT fabricated sensors.

Preparation of ambroxol-phosphomolybdate and phosphotungstate ion pairs: To prepare AM-PM and AM-PT ion pairs, 150 mL of 1.0×10^{-2} mol/L of ambroxol hydrochloride was mixed with 50 mL of 1.0×10^{-2} mol/L of phosphomolybdic acid or phosphotungstic acid. The obtained precipitate was left over night and filtered using a Whatman filter paper No. 2, washed with distilled water, dried at room temperature for 24 h and ground to a fine powder. The composition of the ion pairs were $[C_{13}H_{19}Br_2ClN_2O]_3$ $[P(Mo_3O_{10})_4]$ and $[C_{13}H_{19}Br_2ClN_2O]_3[P(W_3O_{10})_4]$. They confirmed by elemental analysis data which revealed that the composition to be is 3:1 for (AM:PM) and (AM:PT). The calculated percentages of C,

H and N are 15.27, 1.87, 2.74, 11.36, 1.39 and 2.04 % while the found percentages are 15.32, 1.91, 2.95, 11.39, 1.42 and 2.09 % for AM-PM and AM-PT, respectively.

Also IR spectrum analysis was performed to investigate the prepared AM-PM and AM-PT ion-pairs. From IR spectrum (Figs. 2a and 2b), it was found that the observed bands at 3755 and 3754 cm^{-1} indicate O-H stretching aliphatic, two bands at 2958 and 2859 cm^{-1} indicate the presence of N-H stretching group. Also the observed bands at 1622 and 1621 cm^{-1} indicate the presence of C=C group, the presence of bands at 1457 and 1456 cm^{-1} indicate the presence of C-H ring stretching. Additionally, the bands at 607 and 594 cm^{-1} indicate the presence of C-Br. There was no absorption band in case of PMA and PTA reagents. This indicated the formation of ion-pairs AM-PM and AM-PT.

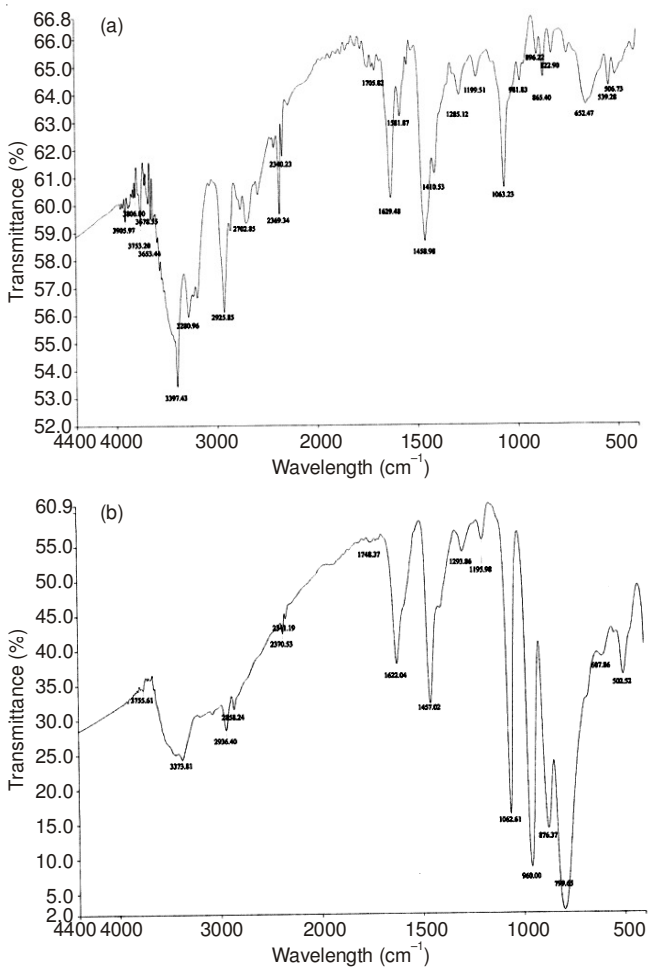

Fig. 2. (a) IR spectrum of ambroxol-phosphomolybdate (b) IR spectrum of ambroxol-phosphotungstate

Preparation of forced degradation products of ambroxol hydrochloride: The fabricated sensors were used for determination of the investigated drug in its forced degradation products. The preparation of forced degradation products of ambroxol hydrochloride was carried out by adding 1 mL of 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide or/and 3.0 % hydrogen peroxide to about 5 mg of ambroxol hydrochloride. The samples were refluxed separately at 80 °C for 12 h[12]. The solution was then cooled, neutralized and then diluted with methanol. The degradation process completeness was tested on silica gel 60 F_{254} using K_2HPO_4 pH (6.5): acetonitrile (68:32 v/v) as mobile phase. The degradation product was filtered and washed and then left to dry in air for 3 days.

Sensor construction

Preparation of PVC membrane and modified β-cyclodextrin sensors: The optimum performance characteristics were achieved by mixing 190 mg polyvinyl chloride PVC with 10 mg of AM-PM ion pair and 0.4 mL *o*-nitrophenyloctyl ether (*o*-NPOE) as plasticizer. The electroactive material AM-PM was dissolved in 5 mL THF as solvent mediator. The solution mixture was poured into 8 cm petri dish and left to dry in air. To obtain the uniform membrane thickness, the amount of THF was kept constant and its evaporation was fixed for 24 h. The modified β-cyclodextrin membrane was prepared by adding 50 mg 2-hydroxypropyl β-cyclodextrin to the previously mentioned procedure.

A circular membrane was attached to a polyethylene tube (8 mm diameter) using THF. An internal solution containing mixture of equal volumes of 1×10^{-3} mol/L ambroxol hydrochloride and 1×10^{-3} mol/L potassium chloride was used. The constructed sensor was preconditioned by soaking the sensor in 1×10^{-3} mol/L ambroxol hydrochloride for 24 h and stored in the same solution. All potentiometric measurements were performed using the following cell assembly: Ag/AgCl/internal solution/membrane/test solution//KCl salt bridge//Ag/AgCl.

Preparation of carbon paste and modified carbon nanotubes sensors: To fabricate carbon paste sensor, 60 % of pure graphite powder (1-2 μm) was mixed with 30 % *o*-NPOE as liquid plasticizer and 10 % ion pair (AM-PT) in an agate mortar. The mixture was mixed well to obtain homogenized paste. Teflon holder (3 mm in diameter) was used for packing the paste carefully. Electrical contact was made with a copper rod through the centre of the sensor holder. To obtain shiny, smooth and reproducible surface, filter paper was used for surface polishing. The sensor was conditioned by dipping it in 1×10^{-3} mol/L ambroxol hydrochloride solution for 10 h. All potentiometric measurements were performed using the following cell assembly: carbon paste/test solution/Ag/AgCl reference electrode.

The modification of carbon paste was carried out by adding a small amount of multi-wall carbon nanotubes particles and the paste was homogenously mixed. The fabricated paste was carefully packed in sensor holder and was left to dry for one day. The shiny and smooth surface was obtained by polishing the sensor surface using filter paper. The sensor was conditioned by dipping it in 1.0×10^{-3} mol/L ambroxol hydrochloride solution for 10 h. All potentiometric measurements were performed using the following cell assembly: Modified carbon nanotubes/test solution/Ag/AgCl reference electrode. The surface structure of carbon paste sensors was examined using scanning electron microscope as shown in Figs. 3a and 3b.

Sensor calibration: Working solutions containing 1.0×10^{-8} – 1.0×10^{-1} mol/L of ambroxol hydrochloride were employed to study sensor calibration. The calibration graphs were plotted for all potentiometric measurements using the fabricated sensors in conjunction with Ag/AgCl reference electrode. The potential of each sensor was recorded and plotted against the logarithm of drug concentration.

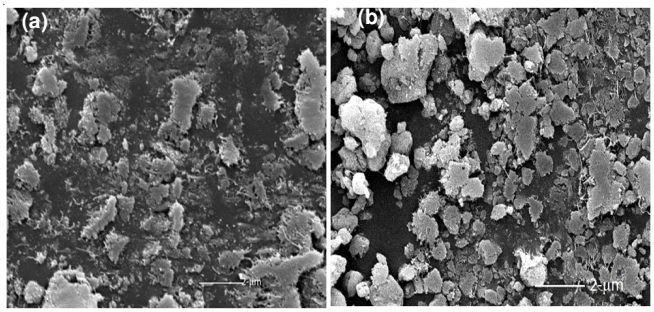

Fig. 3. Scanning electron micrographs of carbon paste sensor surface (a) carbon paste, (b) modified carbon nanotube carbon paste

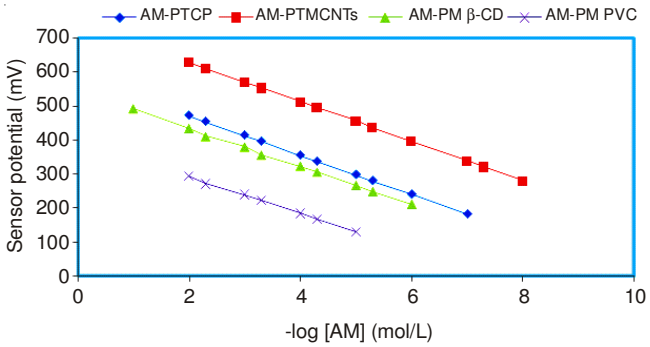

Fig. 4. Typical calibration graph of AM-PM plastic membrane and modified 2-hydroxypropyl β-CD, AM-PT Carbon paste and modified carbon nanotubes sensors

Standard addition method: Standard addition method was used for determination of ambroxol hydrochloride in its dosage forms. The method was based on adding small increments of the investigated drug test solution *vs*. the sensor potential. Each fabricated sensor was immersed into 50 mL drug test solution with unknown concentration and the equilibrium potential of E_1 was recorded. Then 0.1 mL of standard drug solution was added into the testing solution and the equilibrium potential E_2 was recorded. The concentration of the testing sample can be obtained from the change of potential ($\Delta E = E_2 - E_1$).

RESULTS AND DISCUSSION

Nature and response characteristics of ambroxol sensors: The fabricated ambroxol hydrochloride sensors were based on the reaction of ambroxol hydrochloride with phosphomolybdic acid and phosphotungstic acid to form AM-PM and AM-PT as electroactive materials. The prepared electroactive materials were water insoluble but readily soluble in organic solvents such as THF. The nature and performance characteristics of the fabricated sensors were investigated. Fig. 4 showed that the investigated sensors displayed Nernstian responses (54.50 ± 0.6, 56.29 ± 0.3, 57.95 ± 0.8 and 58.32 ± 0.2 mV decade^{-1}) at 25 °C over drug concentration ranges $1.0 \times 10^{-5} - 1.0 \times 10^{-2}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-7} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-8} - 1.0 \times 10^{-2}$ mol/L with lower detection limits of 5.0×10^{-6}, 4.9×10^{-7}, 4.8×10^{-8} and 5.0×10^{-9} mol/L for sensors I, II, III and IV, respectively. As shown in Table-1, the obtained results revealed better performance characteristics for modified β-cyclodextrin (sensor II) than the conventional plastic membrane (sensor I). The use of 2-hydroxypropyl β-cyclodextrin as ionophore provided high stability to the complex formed between the cationic drug and the chelating agent which can be attributed to the presence of high donation (OH-groups), thus the membrane selectivity and sensitivity were substantially enhanced[4].

In case of carbon paste sensor III and modified carbon nanotubes carbon paste sensor IV, it was found that the addition of 1.0-5.0 w % carbon nanotubes to modify carbon paste sensor played an important role in the enhancement of sensor response. The improvement effect of carbon nanotubes on performance of the modified sensor can be attributed to their chemical stability and good electric conductivity properties of carbon nanotubes. Also, their porous surface structure and large surface area facilitate a better electrolyte sensor interface that improves the wetting property with solvents.

Effect of plasticizers: The effect of plasticizers were carefully studied using five different kinds of plasticizers such as DOP with dielectric constant (ε = 5.1), DBP (ε = 6.4), DBS (ε = 4.5), DOS (ε = 4.0) and *o*-NPOE (ε = 24.0). The use of each plasticizer content ratio 45, 48, 50, 55 and 60 w % was investigated. Table-2 showed the effect of plasticizers on the slopes of AM-PM and AM-PT sensors. It was cleared that the use of *o*-NPOE as plasticizer provided good performance characteristics of sensors. This can be attributed to the higher dielectric constant (ε = 24.0) of *o*-NPOE than other plasticizers.

TABLE-1
ELECTROCHEMICAL RESPONSE CHARACTERISTICS OF AM-PM AND AM-PT SENSORS

Parameter[a]	AM-PM (sensor I) plastic membrane	AM-PM (sensor II) Modified β-cyclodextrin	AM-PT (sensor III) carbon paste	AM-PT (sensor IV) modified carbon nanotubes
Slope (mV/decade)	54.50 ± 0.6	56.29±0.3	57.95 ± 0.8	58.32 ± 0.2
Intercept	402.04	547.67	587.35	746.95
Regression equations	$E_{mV} = (54.5 \pm 0.6) \log [AM] + 402.0$	$E_{mV} = (56.3 \pm 0.3) \log [AM] + 547.7$	$E_{mV} = (57.9 \pm 0.8) \log [AM] + 587.4$	$E_{mV} = (58.3 \pm 0.2) \log [AM] + 746.9$
Correlation coefficient (r)	0.9997	0.9998	0.9999	0.9999
Linear range (mol/L)	1.0×10^{-5}-1.0×10^{-2}	1.0×10^{-6}-1.0×10^{-1}	1.0×10^{-7}-1.0×10^{-2}	1.0×10^{-8}-1.0×10^{-2}
Lower limit of detection	5.0×10^{-6}	4.9×10^{-7}	4.8×10^{-8}	5.0×10^{-9}
Response time (s)	45	30	20	15
Working pH range	3-8	3-8	3-8	3-8
Life time (day)	50	60	45	70
Temperature (°C)	25	25	25	25
Accuracy (%)	98.9 ± 0.93	99.1 ± 0.12	99.4 ± 0.52	99.6 ± 0.17
Robustness[b]	98.9 ± 0.82	99.3 ± 0.16	99.6 ± 0.23	99.7 ± 0.18
Ruggedness[c]	98.6 ± 0.15	99.4 ± 0.62	99.5 ± 0.17	99.8 ± 0.28

[a]Mean of six measurements; [b]Small variation in method parameters were carried out as pH of phosphate buffer (pH 8.0 ± 1); [c]Comparing the results by those obtained by different sensors assemblies using (Jenway 3510 pH meter)

TABLE-2
EFFCT OF PLASTICIZERS ON THE SLOPES OF THE FABRICATED AM-PM AND AM-PT SENSORS

Plasticizer	Slope (mV decade^{-1})			
	AM-PM plastic membrane sensor I	AM-PM modified β-cyclodextrin sensor II	AM-PT carbon paste sensor III	AM-PT modified carbon nanotubes sensor IV
Di-octyl sebacate	49.9	52.3	53.3	55.2
Di-butyl sebacate	51.4	53.7	54.2	56.4
Di-butyl phthalate	52.0	55.2	55.9	57.2
Di-octylphthalate	53.6	55.8	56.5	57.6
o-Nitrophenyloctyl ether	54.5*	56.3*	57.9*	58.3*

*The optimum value for the studied sensors

From the obtained results it can be concluded that the use of plasticizers in the fabrication of sensors plays an important role in the improvement of their performance characteristics. They give some permeable properties to the sensors and improve their mechanical stability. Also, in case of carbon paste sensors the selection of suitable plasticizer will improve the physical properties of these sensors and promote the binding between their carbon particles.

Response time: The effect of response time of fabricated sensors was carefully investigated. The response time of sensors is the time required for sensors to reach a stable potential reading. The evaluation of response time of the fabricated sensors ambroxol hydrochloride standard solutions in the range of 1.0×10^{-8}– 1.0×10^{-1} mol/L were used. It was found that the dynamic response time for sensors I and II was 45 and 30 s while in the case of sensors III and IV the response time was 20 and 15 s for periods of 30, 45, 60 and 70 days for sensors I, II, III and IV, respectively without significant change in the sensor parameters.

Effect of pH: The effect of pH on the potential of the fabricated sensors was studied using 1.0×10^{-3} mol/L ambroxol hydrochloride solution. The test solution was firstly acidified using 0.1 mol/L hydrochloric acid and the potential readings were recorded then the pH was gradually increased using 0.1 mol/L sodium hydroxide. The recorded potential was plotted *vs*. pH. As cleared form Fig. 5 the fabricated AM-PM and AM-PT sensors displayed safe pH range at 3-8. It was noticed that below pH 3 the potential reading was decreased due to the high acidity and the interference of H^+. While, at higher pH more than 8 the sensors showed sharp decrease in potential due to the effect of OH^- on the test solution.

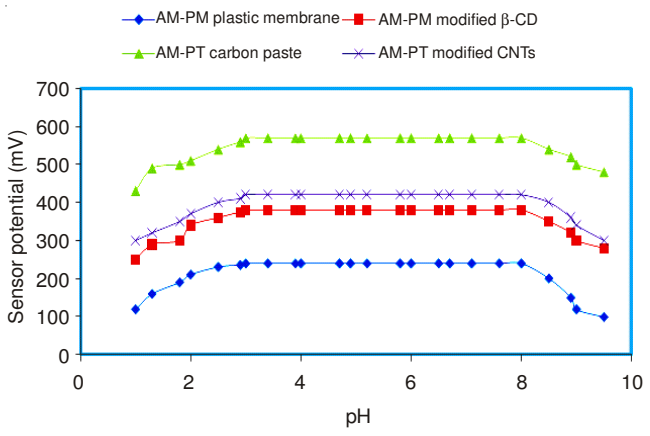

Fig. 5. Effect of pH on AM-PM and AM-PT sensors using (1×10^{-3} mol/L) ambroxol hydrochloride

Selectivity of sensors: To study the selectivity coefficients of AM-PM and AM-PT sensors, the selectivity of fabricated sensors was tested towards different common cations, sugars, amino acids and some additive substances using separate solution method[24]. Also the influence of some related pharmacological action drugs were examined. The following equation was applied for the selectivity coefficients of the proposed sensors:

$$\log K^{Pot}_{AM} J^{z+} = (E_2 - E_1)/S + \log [AM] - \log [J^{z+}]^{1/z}$$

where, E_1 is the electrode potential in 1.0×10^{-3} mol/L ambroxol hydrochloride solution, E_2 is the potential of the electrode in 1.0×10^{-3} mol/L solution of the interferent ion J^{z+} and S is the slope of the calibration plot. The results reported in Table-3 clarified the high selectivity of the fabricated sensors. The main mechanism of selectivity is dependent on the matching between the locations of lipophilic sites in the two competing species in the bathing solution side and those present in the receptor of ion pair. The inorganic cations, sugars and some amino acids showed insignificant interferent effect during the determination of ambroxol hydrochloride. Also, the effect of some related pharmacological action drugs such as bromhexine hydrochloride was examined. The obtained results showed no interferent effect during the determination of ambroxol hydrochloride.

Effect of temperature on the sensors performance: The effect of temperature of the test solution on sensors performance was studied. This was carried out by plotting the calibration graphs (sensor potential *vs*. p^{AM}) at different test solution temperatures (25, 30, 40, 50, 60 and 70 °C) for all sensors. Table-4 summarized the slopes, usable concentration ranges and the standard sensor potential (E°) at each temperature.

To determine the isothermal coefficients (dE°/dt) of the sensors, the standard sensor potentials (E°) against the normal hydrogen electrode at different temperatures were measured. This can be carried out by plotting the calibration graphs as the intercepts at $p^{AM} = 0$ (after subtracting the values of the standard sensor potential of Ag/AgCl electrode at these temperatures) and plotting *vs*., (t-25) where t is the temperature of test solution in °C. The obtained straight line was according to the following equation[25]:

$$E° = E°(25) + (dE°/dt)\ (t-25)$$

The isothermal coefficients were represented from the slopes of the straight lines obtained. They were amounted to 0.00182, 0.00031, 0.00095 and 0.00974 V° C^{-1} for sensors I, II, III and IV, respectively. These low values were revealed that the AM-PM and AM-PT sensors have high thermal stability within the studied temperature range.

TABLE-3
SELECTIVITY COEFFICIENTS ($K^{Pot}_{AM^+}$) FOR AM-PM AND AM-PT SENSORS USING SEPARATE SOLUTION METHOD (1.0×10^{-3} MOL/L AMBROXOL HYDROCHLORIDE)

Interferent	$K^{Pot}_{AM^+}$			
	AM-PM plastic membrane sensor I	AM-PM modified β-cyclodextrin sensor II	AM-PT carbon paste sensor III	AM-PT modified carbon nanotubes sensor IV
Na^+	1.5×10^{-3}	1.1×10^{-4}	2.2×10^{-4}	1.5×10^{-4}
K^+	6.0×10^{-3}	2.4×10^{-4}	4.9×10^{-4}	4.8×10^{-4}
NH_4^+	2.3×10^{-3}	1.5×10^{-4}	1.5×10^{-4}	1.7×10^{-4}
Ca^{2+}	9.4×10^{-3}	4.8×10^{-4}	6.2×10^{-4}	3.3×10^{-4}
Mg^{2+}	7.5×10^{-4}	7.4×10^{-4}	8.8×10^{-4}	1.8×10^{-4}
Zn^{2+}	8.6×10^{-4}	7.3×10^{-4}	4.5×10^{-4}	7.7×10^{-4}
Cu^{2+}	5.2×10^{-3}	3.6×10^{-3}	3.3×10^{-4}	3.5×10^{-4}
Fe^{3+}	1.2×10^{-4}	4.5×10^{-4}	1.2×10^{-4}	2.4×10^{-4}
Al^{3+}	8.9×10^{-3}	4.9×10^{-3}	4.4×10^{-4}	6.3×10^{-4}
Glucose	1.4×10^{-3}	4.7×10^{-3}	1.1×10^{-3}	1.4×10^{-4}
Lactose	5.4×10^{-3}	1.4×10^{-3}	4.0×10^{-3}	2.2×10^{-4}
Sucrose	2.1×10^{-5}	5.5×10^{-5}	2.4×10^{-5}	8.5×10^{-4}
Starch	3.8×10^{-4}	1.8×10^{-4}	5.5×10^{-4}	7.4×10^{-4}
Serine	7.1×10^{-3}	5.7×10^{-3}	9.4×10^{-3}	5.9×10^{-5}
Glycine	4.6×10^{-3}	3.4×10^{-3}	3.3×10^{-3}	4.0×10^{-5}
Histadine	8.4×10^{-4}	7.1×10^{-4}	5.1×10^{-4}	5.2×10^{-5}
Thymine	1.3×10^{-4}	1.2×10^{-4}	1.9×10^{-4}	1.7×10^{-4}
Ornithine	6.8×10^{-3}	3.3×10^{-3}	2.3×10^{-3}	2.7×10^{-4}
Glutamine	4.9×10^{-4}	7.1×10^{-5}	1.7×10^{-4}	1.4×10^{-5}
Bromhexine hydrochloride	8.7×10^{-4}	9.8×10^{-5}	9.9×10^{-4}	9.8×10^{-5}

TABL-4
PERFORMANCE CHARACTERISTICS OF AM-PM AND AM-PT SENSORS AT DIFFERENT TEMPERATURES

Type of sensor	Temperature (°C)	Slope (mV) decade^{-1}	Usable concentration range	E° (mV[a])
AM-PM plastic membrane sensor I	25	54.5	1.0×10^{-5}-1.0×10^{-2}	150
	30	56.2	1.0×10^{-5}-1.0×10^{-2}	158
	40	59.0	1.0×10^{-5}-1.0×10^{-2}	170
	50	61.4	1.0×10^{-5}-1.0×10^{-2}	190
	60	63.8	1.0×10^{-5}-1.0×10^{-2}	212
	70	65.6	1.0×10^{-4}-1.0×10^{-2}	218
AM-PM modified β-cyclodextrin sensor II	25	56.3	1.0×10^{-6}-1.0×10^{-1}	320
	30	58.7	1.0×10^{-5}-1.0×10^{-1}	326
	40	60.2	1.0×10^{-5}-1.0×10^{-2}	340
	50	62.5	1.0×10^{-5}-1.0×10^{-2}	354
	60	64.8	1.0×10^{-5}-1.0×10^{-2}	366
	70	66.2	1.0×10^{-4}-1.0×10^{-2}	378
AM-PT carbon paste sensor III	25	57.9	1.0×10^{-7}-1.0×10^{-2}	430
	30	59.4	1.0×10^{-6}-1.0×10^{-2}	437
	40	60.6	5.0×10^{-6}-1.0×10^{-2}	451
	50	63.3	5.0×10^{-6}-1.0×10^{-2}	467
	60	65.2	1.0×10^{-5}-1.0×10^{-2}	482
	70	67.4	1.0×10^{-5}-1.0×10^{-2}	497
AM-PT modified carbon nanotubes sensor IV	25	58.3	1.0×10^{-8}-1.0×10^{-2}	560
	30	59.8	5.0×10^{-7}-1.0×10^{-2}	563
	40	61.2	5.0×10^{-6}-1.0×10^{-2}	569
	50	63.5	5.0×10^{-6}-1.0×10^{-2}	577
	60	64.8	5.0×10^{-6}-1.0×10^{-2}	584
	70	66.3	1.0×10^{-5}-1.0×10^{-2}	589

[a]E°; Standard sensor potential against normal hydrogen electrode (NHE)

Effect of soaking: The fabricated AM-PM and AM-PT sensors were examined in terms relevant to soaking time. The effect of soaking on the potential of the fabricated sensors was studied by soaking each sensor in 1.0×10^{-3} mol/L drug solution for 24 h. It was found that the optimum soaking time was 24 and 10 h for sensors I, II and III, IV, respectively. The slopes of the calibration curves were 54.50 ± 0.6, 56.29 ± 0.3, 57.95 ± 0.8 and 58.32 ± 0.2 mV decade^{-1} for sensors I, II, III and IV, respectively. After prolonged soaking upon different intervals 7, 15, 25, 30, 35, 40, 50, 60 and 70 days the slopes were slightly decreased to be 51.6, 54.3, 56.4 and 57.0 mV decade^{-1} after 35 days, while continuous soaking for 70 days caused sharp decrease in sensors potential to reach 49.3 and 52.8 mV decade^{-1} after 50 and 60 days for AM-PM plastic membrane and modified b-cyclodextrin sensors, 52.7 and 54.3 mV decade^{-1} after 45 and 70 days for AM-PT carbon paste sensor and modified carbon nanotubes sensor. The obtained results revealed that the conventional AM-PM plastic membrane

sensor has longer lifespan than carbon paste, while the use of modified β-cyclodextrin and carbon nanotubes sensors improved the performance characteristics of the sensors.

The regeneration of AM-PM and AM-PT sensors was successfully carried out by soaking the exhausted sensor for 24 h in 1.0×10^{-2} mol/L PMA or PTA, followed by 3 h in 1.0×10^{-2} mol/L ambroxol hydrochloride solution. After regeneration the fabricated AM-PM and AM-PT sensors displayed a potential response of 50.7, 54.5, 55.8 and 56.9 mV decade^{-1} for sensors I, II, III and IV, respectively. It was found that the lifespan of the regenerated sensors is limited to 2, 6, 5 and 8 h for the previously mentioned sensors.

Analytical applications

Quantification of ambroxol hydrochloride: Ambroxol hydrochloride was quantified directly in its pure form using AM-PM and AM-PT sensors. The mean % recoveries were recorded. The obtained results were 99.3 ± 0.69, 99.5 ± 0.64, 99.8 ± 0.48 and 99.9 ± 0.60 for sensors I, II, III and IV, respectively. Furthermore, the results obtained were encouraging, so the proposed method was applied for the determination of ambroxol hydrochloride in its pharmaceutical preparations; the results were 99.2 ± 0.49, 99.4 ± 0.44, 99.7 ± 0.71 and 99.7 ± 0.41 for the above mentioned sensors.

The proposed method was successfully applied for the determination of ambroxol hydrochloride in biological fluids. The results obtained for determination of ambroxol hydrochloride in urine were 99.1 ± 0.41, 99.0 ± 0.64, 99.6 ± 0.19 and 99.7 ± 0.41 while for human serum the recorded results were 98.9 ± 0.26, 99.3 ± 0.43, 99.1 ± 0.33 and 99.3 ± 0.49 for sensors I, II, III and IV, respectively (Table-5).

The evaluation of the proposed method was carried out by applying statistical analysis for the obtained results using student's t- and F- tests at 95 % confidence level[26]. Table-6 showed that the obtained results were in good agreement with those obtained from a reported spectrophotometric method[19] (which was based on diazotisation of ambroxol hydrochloride with sodium nitrite in presence of acetic acid then coupled with β-naphthol in alkaline medium and measuring the absorbance at λ_{max} 425 nm).

Content uniformity assay of the tablets: The content uniformity assay of ambroxol hydrochloride in its tablets ambroxol® (30 mg/tablet) was determined using the fabricated AM-PM and AM-PT sensors. To study the content uniformity assay of ambroxol hydrochloride tablets, ten individual tablets were placed in separate 100 mL beakers and dissolved in 100 mL distilled water. The fabricated sensors were used directly to measure the investigated drug. The mean potential was used to evaluate the content uniformity from the calibration graph. The obtained results obtained as % recoveries and standard deviations were 98.2 ± 0.42, 98.4 ± 0.26, 99.5 ± 0.28 and 99.8 ± 0.12 for sensors I, II, III and IV, respectively.

Determination of ambroxol hydrochloride in its degradation products: The fabricated AM-PM and AM-PT sensors were used for determination of ambroxol hydrochloride in the presence of its degradation products. The potential readings for the fabricated sensors using 1.0×10^{-3} mol/L in the presence of (20, 40, 60, 80 and 100 %) were compared with those obtained by 1.0×10^{-3} mol/L pure ambroxol hydrochloride solution. It was found that the mean % recoveries were 98.6 ± 0.24, 98.8 ± 0.14, 99.3 ± 0.25 and 99.5 ± 0.58 for sensors I, II, III and IV, respectively, indicating good selectivity and high sensitivity of the fabricated sensors for determination of ambroxol hydrochloride even in the presence of its degradation products.

Method validation: Validation parameters were studied according to ICH guidelines[22] to assess the performance of the proposed method. Linearity, lower limit of detection, accuracy, precision, robustness and ruggedness were investigated.

Linearity and lower limit of detection: The linearity of the proposed method was tested using ambroxol hydrochloride test solutions ranging from $1.0 \times 10^{-8} - 1.0 \times 10^{-1}$ mol/L. The drug test solutions were subjected to AM-PM and AM-PT sensors detection systems. The linearity was determined by plotting the calibration graphs against -log concentration of ambroxol hydrochloride. The results obtained clarified that the constructed sensors displayed Nernstian response over linear concentration ranges of $1.0 \times 10^{-5} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$ mol/L, $1.0 \times 10^{-7} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-8} - 1.0 \times 10^{-2}$ mol/L for sensors I, II, III and IV, respectively. From the obtained results, we can deduce that the use of 2-hydroxypropyl β-cyclodextrin increases the sensitivity of the membrane towards ambroxol cation and the linear concentration range was increased for sensor II more than sensor I. While, in case of carbon paste sensors the use of pours large surface area of modified carbon nanotubes sensor and due to its best sensor electrolyte interface, carbon nanotubes improved the performance of the sensor for the detection of small concentrations of ambroxol hydrochloride. The detection limit was calculated according to IUPAC recommendation which stated that the detection limit is the concentration at which the measured potential differs from that predicted by the linear regression by more than 18 mV. The detection limits were found to be about 5.0×10^{-6}, 4.9×10^{-7} and 4.8×10^{-8} and 5.0×10^{-9} mol/L for sensors I, II, III and IV, respectively.

Robustness and ruggedness: To evaluate the robustness of the proposed method phosphate buffer pH 8 was used to introduce small change in pH. The percentage recoveries were calculated for the investigated drug solutions at pH 8 ± 1. The obtained results were 98.9 ± 0.82, 99.3 ± 0.16, 99.6 ± 0.23 and 99.7 ± 0.18 for sensors I, II, III and IV, respectively. These results were closely in agreement with those obtained from standard drug solutions.

The ruggedness of the proposed method was tested using another pH-meter (Jenway 3510). The recorded results as % recoveries were 98.6 ± 0.15, 99.4 ± 0.62, 99.5 ± 0.17 and 99.8 ± 0.28 for the previously mentioned sensors, respectively.

Accuracy and precision: The accuracy of the proposed method was tested by determining the investigated drug in the presence of its coformulated substance lactose using standard addition method. The obtained results were calculated in terms of % recovery values. The calculated % recoveries were 98.9 ± 0.93, 99.1 ± 0.12, 99.4 ± 0.52 and 99.6 ± 0.17 for sensors I, II, III and IV, respectively.

To investigate the precision of the proposed method, intra-day and inter-day terms were used. The studies were performed by repeating the determination to nine replicates. The calculated % RSD values were 0.58, 0.37, 0.28 and 0.18 % for

TABLE-5
STATISTICAL TREATMENT OF DATA OBTAINED BY DETERMINATION OF AMBROXOL HYDROCHLORIDE USING AM-PM AND AM-PT SENSORS

Sample	AM-PM plastic membrane sensor I			AM-PM modified β-cyclodextrin sensor II			AM-PT carbon paste sensor III			AM-PT modified carbon nanotubes sensor IV		
	Taken (mol/L)	Found	Recovery (%)	Taken (mol/L)	Found	Recovery (%)	Taken (mol/L)	Found	Recovery (%)	Taken (mol/L)	Found	Recovery (%)
Pure drug	5.0	4.99	98.0	6.0	6.01	100.2	7.0	7.02	100.3	8.0	8.01	100.1
	4.3	4.28	99.5	5.0	4.98	99.6	6.0	5.99	99.8	7.0	7.02	100.3
	4.0	3.98	99.5	4.0	3.96	99.0	5.0	4.99	99.8	6.0	5.98	99.7
	3.3	3.30	100.0	3.0	2.99	99.7	4.0	3.99	99.8	5.0	4.99	99.8
	3.0	2.97	99.0	2.0	1.97	98.5	3.0	3.01	100.3	4.0	3.97	99.3
	2.0	1.99	99.5	1.0	1.00	100.0	2.0	1.98	99.0	3.0	2.98	99.3
										2.0	2.02	101.0
*%Mean		99.3			99.5			99.8			99.9	
SD		0.69			0.64			0.48			0.60	
n		6			6			6			7	
Varianc		0.48			0.41			0.23			0.36	
% SE		0.28			0.26			0.19			0.23	
% RSD		0.69			0.64			0.48			0.60	
Ambroxol® 30 mg/tablet	5.0	4.95	99.0	6.0	5.99	99.8	7.0	6.99	99.9	8.0	7.99	99.9
	4.3	4.26	99.0	5.0	4.99	99.8	6.0	5.97	99.5	7.0	7.01	100.1
	4.0	3.97	99.2	4.0	3.98	99.5	5.0	4.96	99.2	6.0	6.01	100.2
	3.3	3.25	98.5	3.0	2.96	98.7	4.0	3.97	99.3	5.0	5.01	100.2
	3.0	2.97	99.0	2.0	1.99	99.5	3.0	2.97	99.0	4.0	3.98	99.5
	2.0	1.97	98.5	1.0	0.99	99.0	2.0	2.02	101.0	3.0	2.98	99.3
										2.0	1.96	98.0
*% Mean		98.9			99.4			99.6			99.7	
SD		0.29			0.44			0.73			0.78	
n		6			6			6			7	
Variance		0.08			0.19			0.53			0.60	
% SE		0.11			0.18			0.29			0.29	
% RSD		0.29			0.44			0.73			0.78	
Urine sample	5.0	4.96	99.2	6.0	5.98	99.7	7.0	6.97	99.6	8.0	7.95	99.4
	4.3	4.25	98.8	5.0	4.96	99.2	6.0	5.97	99.5	7.0	6.99	99.8
	4.0	3.99	99.7	4.0	3.95	98.8	5.0	4.96	99.2	6.0	6.01	100.2
	3.3	3.27	99.1	3.0	2.99	99.7	4.0	3.98	99.0	5.0	5.00	100.0
	3.0	2.98	99.3	2.0	1.96	98.0	3.0	2.96	98.7	4.0	3.99	99.8
	2.0	1.97	98.5	1.0	0.99	99.0	2.0	1.98	99.0	3.0	2.97	99.0
										2.0	2.00	100.0
*% Mean		99.1			99.0			99.2			99.7	
SD		0.41			0.64			0.34			0.41	
n		6			6			6			7	
Varian		0.17			0.41			0.12			0.17	
% SE		0.17			0.26			0.14			0.15	
% RSD		0.41			0.64			0.34			0.41	
Serum sample	5.0	4.96	99.2	6.0	5.99	99.8	7.0	6.95	99.2	8.0	7.96	99.5
	4.3	4.25	98.8	5.0	4.97	99.4	6.0	5.96	99.3	7.0	6.96	99.4
	4.0	3.97	99.3	4.0	3.98	99.5	5.0	4.98	99.6	6.0	5.99	99.8
	3.3	3.26	98.7	3.0	2.96	98.6	4.0	3.95	98.8	5.0	4.97	99.4
	3.0	2.96	98.7	2.0	1.99	99.5	3.0	2.96	98.7	4.0	3.98	99.5
	2.0	1.98	99.0	1.0	0.99	99.0	2.0	1.98	99.0	3.0	2.96	98.7
										2.0	1.99	99.5
* % Mean		98.9			99.3			99.1			99.4	
SD		0.26			0.43			0.33			0.34	
n		6			6			6			7	
Variance		0.07			0.18			0.11			0.12	
% SE		0.11			0.18			0.13			0.13	
% RSD		0.26			0.43			0.33			0.34	

*Mean of six measurements

TABLE-6
STATISTICAL TREATMENT OF DATA OBTAINED FOR THE DETERMINATION OF AM IN AMBROXOL ®30 mg/TABLET BY PROPOSED AND REPORTED METHODS[19] USING STANDARD ADDITION METHOD

Type of sensor	Taken (mol/L)	Mean % ± SD	n	Variance	SE	% RSD	t-test	F-test
AM-PM plastic membrane	1.0×10^{-5}-1.0×10^{-2}	99.2 ± 0.49	6	0.24	0.20	0.49	1.63 (2.228)*	2.38(5.05)*
AM-PM modified β-cyclodextrin	1.0×10^{-6}-1.0×10^{-1}	99.4 ± 0.44	6	0.19	0.18	0.44	0.12 (2.228)*	2.78 (5.05)*
AM-PT carbon paste	1.0×10^{-7}-1.0×10^{-2}	99.6 ± 0.73	6	0.53	0.29	0.73	0.47 (2.228)*	1.08 (5.05)*
AM-PT modified carbon nanotubes	1.0×10^{-8}-1.0×10^{-2}	99.7 ± 0.78	7	0.60	0.29	0.78	0.24 (2.201)*	1.05 (4.39)*
Reported method	1.0×10^{-6}-1.0×10^{-2}	99.8 ± 0.76	6	0.57	0.31	0.76	–	–

*Figures in parentheses are the tabulated values of t-and F- testes at 95 % confidence limit[26]

determination of ambroxol hydrochloride in (Ambroxol® 30 mg/tablet) using AM-PM and AM-PT sensors I, II, III and IV, respectively. The above % RSD values are less than 2 % indicating good precision.

Conclusion

A novel sensitive and accurate electrochemical method for determination of ambroxol hydrochloride was proposed. The proposed method was based on the fabrication of four different sensors. Sensors I and II were plastic membrane type and fabricated using ambroxol-phosphomolybdate as ion pair while sensor II was modified using 2-hydroxypropyl β-cyclodextrin. Sensors III and IV were carbon paste type and fabricated using ambroxol-phosphotungstate as ion pair while sensor IV was modified using carbon nanotubes. The described sensors were sufficiently simple and selective for the quantitative determination of ambroxol hydrochloride in pure form, its pharmaceutical formulations and biological fluids. The use of 2-hydroxy propyl β-cyclodextrin as ionophore increased the membrane sensitivity and selectivity of sensor II more than sensor I. Furthermore, the use of modified carbon nanotubes carbon paste in sensor IV for determination of the investigated drug provided higher sensitivity, lower limit of detection, faster dynamic response time and wider concentration range than sensor III and other fabricated types. The statistical data analysis proved successful agreement with those obtained from a reported method.

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