Determination of ambroxol hydrochloride in pure solutions and some of its pharmaceutical preparations under batch and FIA conditions

Nour T. Abdel-Ghani *, Salwa H. Hussein

Faculty of Science, Chemistry Department, Cairo University, Giza, Egypt

Abstract

New ambroxol (Amb) ion selective plastic membrane electrodes of both conventional and coated graphite types based on the ion-pair of ambroxolium tetraphenylborate (Amb–TPB) ion-pair are prepared. The conventional type electrode was fully characterized in terms of membrane composition, life span, pH, ionic strength and temperature. It was applied to potentiometric determination of ambroxol in pure solutions and pharmaceutical preparations under batch and flow injection conditions. The potentiometric determination was used in the determination of ambroxol in muco syrup in four batches of different expiry dates, also the amounts of ambroxol released after 1, 8 and 16 h from the muco sustained release type (S.R.) capsules were also assayed. The selectivity of the electrode toward a large number of excipient like inorganic cations, sugars and amino acids was tested. The solubility product of the ion-pair and the formation constant of the precipitation reaction leading to the ion-pair formation were determined conductimetrically.

1. Introduction

Ambroxol hydrochloride (*trans*-4-[(2-amino-3,5-di-bromobenzyl) amino] cyclohexanol hydrochloride) is reported [1] as mucolytic in acute and chronic bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. The content of ambroxol hydrochloride was determined in human plasma and pharmaceutical preparation by HPLC [2–5] capillary gas isotachophoresis [6], HPLC and UV detection [7,8] capillary electrophoresis and fluorescence detection [9], reverse phase HPLC [10,11], flow injection analysis into a fused-silica capillary [12], automatic extraction spectrophotometric method [13] and gas chromatography with electron capture detector [14],

no potentiometric method has been published for the determination of ambroxol hydrochloride.
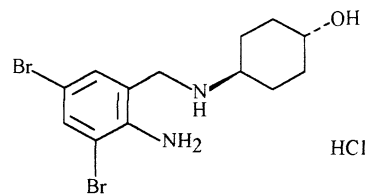
Ambroxol Hydrochloride

In the present work, a plastic membrane selective electrode for ambroxol ion (Amb) has been constructed; it is based on the incorporation of Amb–TPB ion exchanger in polyvinyl chloride (PVC) membrane plasticized with dioctyl phthalate (DOP). The electrode was fully characterized under batch conditions and then used to determine the drug both in batch and by applying flow injection technique, which is considered a very efficient way of improving the performance character-

* Corresponding author.
E-mail addresses: ntghani@chem-sci.cairo.eun.eg, noureta2002@yahoo.com (S.H. Hussein).

istics of ISEs for various reasons, among of them: (i) the permanent liquid stream has a conditioning effect on the sensor membrane, leading to a better sensitivity and stability and increasing reproducibility of the e.m.f. readings; (ii) the liquid junction and the streaming potentials are stable; (iii) the way in which the sample is presented to the ISE is more closely defined and reproducible under flow-through conditions than in static (batch) measurements; (iv) the streaming of solution is beneficial in that it reduces the diffusion layer thickness and hence shortens the response time; and (v) the sample is not influenced by the electrode itself as any release from the membrane (e.g. dissolution) is transported down stream [15].

2. Experimental

2.1. Reagents

All chemicals used for the preparation of solutions were of analytical—reagent grade. Double distilled water was used for preparing solutions and as flow stream in FIA measurements. The carrier and reagent solutions were de-gassed by means of vacuum suction. Sample solutions used for injection were freshly prepared directly prior to measurements. Pure grade Amb–HCl and the pharmaceutical preparations (muco syrup, 15 mg/5 ml and muco S.R. capsule, 75 mg/capsule) were provided by Rameda Co. Egypt, while, ambroxol tablets (30 mg/tablet) were provided by Glaxo Co. Egypt.

2.2. Apparatus of batch and FIA systems

Potentiometric measurements in the batch mode were carried out with multimeter (Sanwa, Japan). A Techne circulator thermostat Model C-100 (UK) was used to control the temperature of the test solution. Saturated calomel electrode (SCE) was used as external reference electrode.

The electrochemical system was as follows: Ag/AgCl/filling solution/membrane/test solution//KCl salt bridge//SCE.

The flow injection set-up was composed of a four channel peristaltic pump (ISM 827) (Ismatec, Zurich, Switzerland) and a Model 5020 injection valve with exchangeable sample loop from Rheodyne (Cotati, CA, USA).

The electrode was connected to a WTW pMX 2000 microprocessor pH/ion meter and interfaced to a Model BD111 strip chart recorder from Kipp and Zonn (Deflt, The Netherlands).

The flow injection measurements were carried out in a two-line system as shown in Fig. 1, the sample was injected into a distilled water stream, which then merged with another stream of distilled water. In both lines the same tubing size was used, offering the same flow rate. The connector of the two streams was linked to the detector by a 50-cm tube of 0.4-mm internal diameter.

A wall-jet cell, providing a low dead volume, fast response, good wash characteristics, ease of construction and compatibility with electrodes of different shape and size, was used in flow measurements, where a Perspex cup with axially positioned inlet polypropylene tubing was mounted at the sensing surface of the electrode body. The optimized distance between the nozzle and the sensing surface of the electrode was 5 mm; this provides the minimum thickness of the diffusion layer and consequently a fast response [16]. The ISE with flow cup, reference electrode (SCE) and the outlet tubes were placed in a beaker, where the level of solution was kept 1 cm above the electrode surface.
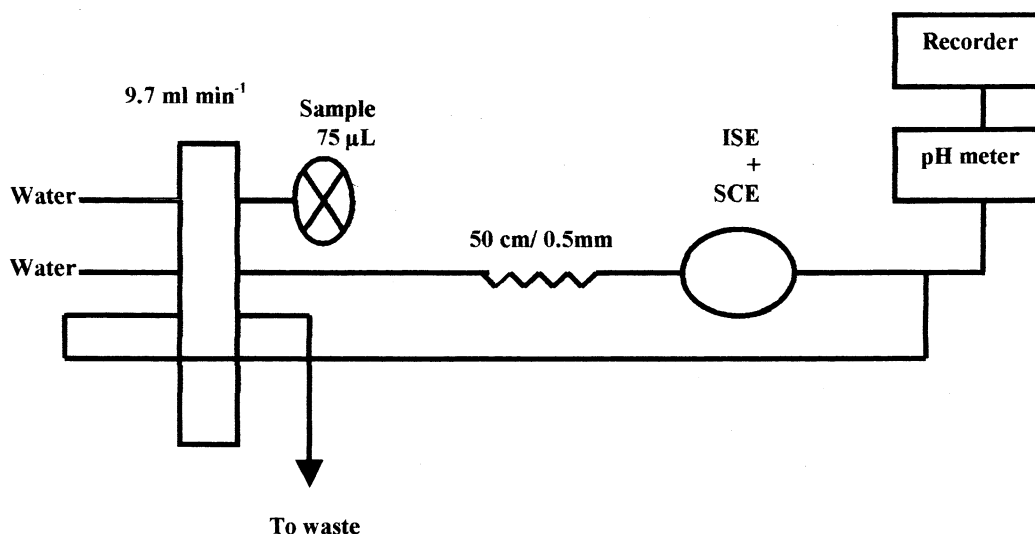

Fig. 1. Schematic diagram of the flow injection system used in measurements.

Table 1
Composition of different Amb–TPB responsive membrane and the slope of corresponding calibration plot

Membrane	Composition (%)			Slope (mV/decade)	RSD [a] (%)
	IP [b]	DOP	PVC		
I	2.0	49.0	49.0	55.50	0.53
II	3.0	48.5	48.5	56.50	0.73
III	5.0	47.5	47.5	55.35	0.72
IV	7.0	46.5	46.5	54.70	1.30

[a] RSD is the relative S.D. ($n = 4$).
[b] IP is the ion-pair percent.

2.3. Preparation of the ion exchanger

The ion exchanger, ambroxolium tetraphenylborate (Amb–TPB) was prepared by mixing 100 ml of 10^{-2} mol/l of ambroxol hydrochloride with 100 ml of 10^{-2} mol/l of sodium tetraphenylborate. The white precipitate formed, after digestion over night, was filtered, washed by double distilled water until chloride free and dried at room temperature. The product was subjected to elemental analysis for C, H, and N at the Microanalytical Center Cairo University, Egypt, the result showed good agreement with the calculated values for a 1:1 (Amb–TPB) ion-pair. The found values were 60.8, 5.2 and 3.5 where the calculated values were 63.6, 5.6 and 4 for C, H and N%, respectively.

2.4. Preparation of conventional and coated graphite types electrodes

The membranes were prepared by dissolving the required amount of ion exchanger, DOP and PVC (Table 1) in about 10 ml tetrahydrofuran, the solution mixture was poured into a 7.5 cm Petri-dish and left to dry in air. A 12-mm diameter disk was cut out from the membrane and glued to the polished end of a plastic cap attached to a glass tube. The electrode body was filled with a solution that is 10^{-1} mol/l in NaCl and 10^{-3} mol/l in Amb–HCl. A Ag/AgCl wire is immersed in the internal solution of the electrode to act as internal reference.

The coated graphite electrode was prepared using graphite rode (5-cm length and 4-mm diameter). One of the two ends of the rode is used for connection while the other, about 1 cm length, was dipped in a solution of the same optimum membrane composition used for the conventional type and left to dry in air. The process was repeated several times till a layer of proper thickness was formed covering the terminal of the graphite rode.

2.5. Conductimetric determination of the solubility product and the formation constant of Amb–TPB ion-pair

The solubility product and the formation constant of Amb–TPB ion-pair were conductimetrically determined as previously described [17]. For this purpose a series of solutions of different concentration ($C = 10^{-4}$ to 10^{-2} mol/l) was prepared for each Amb–HCl and Na–TPB. The measured conductivities of these solutions at 25 °C were used to calculate the specific conductivities (K), corrected for the effects of the solvent and dilution, then the equivalent conductivities (λ) of the solutions, ($\lambda = 1000\ K/C$) were obtained. Straight lines plots of λ versus ($C^{1/2}$) were constructed and the equivalent conductance at infinite dilution (λ_0) values were determined for both Amb–HCl ($\lambda_{0\ Amb–HCl}$) and Na–TPB ($\lambda_{0\ Na–TPB}$) from the intercept of respective lines with the λ-axis. The activity coefficients of the ions employed were taken as unity because all the solutions were sufficiently dilute; moreover all the ions under study were univalent and, consequently, they were less affected by changes in the ionic strength of the solution [18]. $\lambda_{0\ Amb–TPB}$ was calculated from Kohlrausch's law of independent migration of ions using $\lambda_{0\ Amb–HCl}$ and $\lambda_{0\ Na–TPB}$ values. The solubility (S) and solubility product (K_{sp}) of ion-pair were obtained applying the following equations:

$$S = K_s \times 1000/(\lambda_{0\ Amb–TPB}) \text{ and } K_{sp} = S^2$$

where K_s is the specific conductivity of a saturated solution of Amb–TPB determined at 25 °C and corrected for the effect of the solvent. Stirring a suspension of the ion-pair of the saturated solution precipitate in distilled water for 3 h made the saturated solution. The conductivities were measured using Engineered System and Designs conductometer, Model 72. [Conductance/TDS](Jenway).

2.6. Preparation of sample solutions and recommended procedure for determination of Amb–HCl

Amb–HCl was determined potentiometrically using the investigated electrode under batch conditions by applying standard addition methods. The standard addition method was carried out by adding small portion of 5×10^{-2} mol/l standard Amb–HCl solution to 50 ml water containing different amounts (2.0–200.0 mg) of pure compound or the pharmaceutical preparations (muco syrup and ambroxol tablet). The change in millivolt readings was recorded after each addition and

used to calculate the concentration of Amb–HCl sample solutions. In case of S.R. capsule the content of the capsule was put in a permeable cup to prevent the insoluble ingredient to pass to the solution and allowed to be released in distilled water at 37 °C with stirring at 50 r.p.m. to simulate the release of the active ingredient (Amb–HCl) inside the human body. The produced solution was taken after 1, 8 and 16 h then the standard addition methods were applied on these solutions and the result was compared with the theoretical reported results obtained using USP (1) basket apparatus at 50 r.p.m. in 900 ml water as a medium to the release [19](25–35% after 1 h, 55–65% after 8 h and 75–100% after 16 h of the content of the capsule 75 mg/capsule).

For sampling of ambroxol tablet (30 mg/tablet), 20 tablets were weighed and ground and the required weight for the preparation of different concentration was taken and dissolved, filtered and completed to 50 ml with distilled water. Where in case of syrup the content of three bottles mixed thoroughly and the required volume was taken and diluted to 50 ml with distilled water. The standard addition methods were used for determination of ambroxol hydrochloride in four batches of muco syrup (15 mg/5 ml) as a trial to prove that the method can be used for verifying the validity of the drug.

Under FIA conditions, a series of solutions of different concentrations was prepared from tablet and the peak heights were measured at three flow rates (5.35, 9.7 and 17.85 ml/min), and then compared with those obtained by injecting a standard solution of Amb–HCl of the same concentration.

The released amount of Amb–HCl from S.R. capsule was determined under FIA conditions by injecting the solution produced after 1, 8 and 16 h, the peak heights were measured and compared by those obtained by injecting standard solution (75 mg Amb–HCl).

3. Result and discussion

3.1. Optimization of the ISE response in batch and coated graphite type

3.1.1. Composition of the membrane

Several membrane compositions were investigated in which the content of ion exchanger ranged from 2.0 to 7.0% of Amb–TPB. For each composition, the electrodes were repeatedly prepared four times. The preparation process was highly reproducible, as revealed from the low relative standard deviation (RSD) values of the slopes obtained employing the prepared membrane, Table 1. The best performances were obtained by using composition that is 3% of the ion-pair (II), resulting in slopes of 56.5 and 55.8 mV/concentration decade, at 25 °C after minimum pre-soak time of 2 and 0.5 h for

the conventional and coated graphite types, respectively. The usable concentration range was found to be 2×10^{-5} to 5×10^{-2} mol/l Amb–HCl in case of batch and coated graphite type. In all subsequent studies, electrodes made using composition (II) were used.

3.1.2. Effect of soaking

Freshly prepared electrodes can be used after pre soaking in 10^{-3} mol/l Amb–HCl where the mean slopes are 55.5, 56.5, 55.35, 54.7 and 55.8 (mV/concentration decade) for composition I, II, III, IV and coated graphite type of composition II, respectively. It was previously known that the continuous soaking affect negatively on the response of the electrode due to the leaching of the active ingredients (ion-exchanger and plasticizer) to the bathing solution [20].

In the present work the effect of soaking was examined on the four compositions to determine which composition provide the electrode of the longest life-time. It was found that for conventional type electrodes the soaking process firstly increase the slopes where they reached 57.0 after 8 days, 58 after 12 days, 56.5 after 10 days and 55.5 mV/concentration decade after 7 days for compositions I, II, III, IV, respectively, that may be attributed to the enhancement of the mobility of the ion at the surface of the electrode, then slopes become constant for about 10 and 20 days for compositions I and II, respectively and 42 days for compositions III and IV, the slopes then decrease to reach 50 mV/concentration decade after 15, 28, 58, and 55 days for compositions I, II, III, IV, respectively. In case of coated graphite type the electrode showed a relatively short lifetime as the slope decreased to reach 51.5 mV/concentration decade after 24 h. The lifetime of the coated graphite type can be enhanced to 5 days by keeping the electrode in dry closed vessel at room temperature.

3.1.3. Solubility product of the Amb–TPB ion-pair

The determination of the solubility product of the ion-pair is important since its reciprocal is approximately equal to the formation constant of the ion-pair, which is tightly related to the degree of hydrophobicity of ion-pair. So, as the hydrophobicity of ion-pair (Amb–TPB) increases, the leaching process of it to the aqueous bathing solution, which is the main determining factor in the lifetime of the electrode membrane, decreases. The solubility product of the Amb–TPB was determined conductimetrically as described in the experimental part and was found to be 1.06×10^{-7} indicating a very low solubility of the ion-pair (3.25×10^{-4} mol/l). Consequently, the formation constant of the reaction Amb + TPB = Amb–TPB, was 9.4×10^{6}, revealing that the degree of completeness of the reaction is more than 99.9%. In the above equilibrium, the solubility of the undissociated ion-pair in water (i.e.

the intrinsic solubility) was omitted as it provides too small contribution to the total solubility.

3.1.4. Effect of temperature of the test solution

Calibration graphs were constructed for composition (II) electrode at different test solution temperatures (25, 30, 35, 40, 45, 50, 55, 60 and 70 °C). For the determination of the isothermal coefficient (dE°/dt) of the electrode, the standard electrode potentials of the test electrode (E°) with respect to normal hydrogen electrode at each temperatures were obtained from calibration graphs as the intercepts at pAmb = 0 (after subtracting the values of the standard electrode potential of the calomel electrode at these temperatures) and were plotted versus (t-25), where t is the temperature of the test solution in °C.

A straight line plot is obtained according to Antropov's equation [21]:

$$E° = E°_{(25)} + (dE°/dt)(t-25)$$

where $E°_{(25)}$ is the standard electrode potential at 25 °C, the slope of the straight line obtained represents the isothermal coefficient of the electrode. The obtained isothermal coefficient (0.00099 V/°C) reflects a fairly high thermal stability of the electrode within the investigated temperature range. The investigated electrode was found to be usable up to 70 °C without noticeable deviation from the Nernstian behavior.

3.1.5. Effect of ionic strength

Sodium chloride was found to be a good ionic strength adjustor. So, the performance characteristic for the Amb–TPB electrode was studied at different sodium chloride concentrations in order to throw some light about the effect of such ionic strength adjustor on the performance of the electrode.

It was found that the slope of the electrode increased from 56.87 (mV/concentration decade) in distilled water to reach its maximum value 58.26 (mV/concentration decade) in 10^{-4} mol/l of NaCl and then decreased gradually in higher concentrations of NaCl and reach 54.00 mV/concentration decade in 5×10^{-2} mol/l NaCl and this may be attributed to the interference of Na$^+$ ions. So, it is recommended to use a solution that is 10^{-4} mol/l of NaCl when it is necessary to adjust the ionic strength to achieve the nearest Nernstian behavior for the electrode.

3.2. Optimization of FIA response

The response of the electrode under FIA conditions has been optimized by studying the following factors:

(i) *Injection volume:* Samples of different volumes (20–500 μl) were injected. In general the higher the sample volume, the higher are the peak heights and residence time of the sample at the electrode surface,
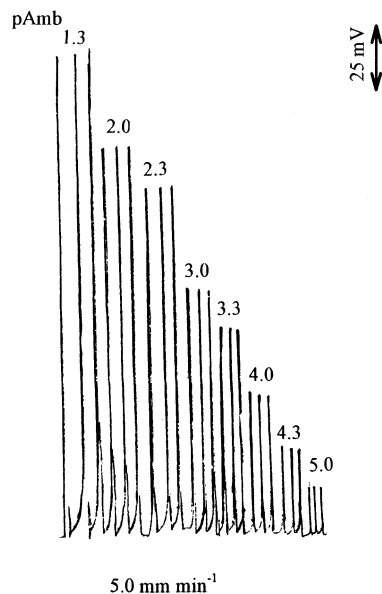

Fig. 2. Recording for Amb–TPB electrode under optimum FIA conditions.

requiring a longer time to reach a steady state and greater consumption of sample. A sample loop of size 75 μl was used through out this work giving about 95% of the maximum peak height obtained by a 500 μl loop but with a shorter time to reach the baseline and less reagents consumption.

(ii) *Effect of flow rate:* The dependence of the peak heights and time to reach the baseline on the flow rate were studied and the response of the electrode to a 10^{-2} mol/l solution at different flow rates (4.15, 5.35, 7.50, 9.7, 12.5, 17.85, 23.25, 25.00, 27.00 and 30.00 ml/min) was monitored. It was found previously that with a constant injection volume, the residence time of the sample was inversely proportional to the flow rate and the recovery time increased linearly with residence time of the sample at the active membrane surface. In the present study it was found that, as the flow rate increased, the peaks become sharper until a flow rate of 25.00 ml/min was reached, above which the peaks were nearly the same. A flow rate of 9.7 ml/min was chosen for further investigations, where about 99% of the maximum peak height obtained within short time to reach the baseline and less consumption of the carrier. Fig. 2 shows typical recordings obtained from Amb–TPB electrode under optimum FIA conditions.

Under the above optimum conditions of injection volume (75 μl) and flow rate (9.7 ml/min), the dispersion coefficient was found to be 1.23 i.e limited dispersion that aids to reach the optimum sensitivity and fast response of the electrode [22].

3.2.1. Electrode response in FIA

In potentiometric measurements using FIA, the electrode potential depends on the activity of the main

ion sensed, this is considered to be the principle advantage of the device; also in flow measurements the dependence is semi-logarithmic over a wide analyte activity range according to the Nickolsky–Eisenman equation. However, the main unfavorable feature of this detection method is the slow response of electrode potential to concentration change, especially when low concentrations are measured, and the response depends mainly on the state of the membrane surface at the interface with the measured solution. This slow response is a fairly good reason for the super-Nernstian sensitivities obtained in FIA measurements using the investigated electrode at different flow rates [23]. An increase in the slope of the calibration plots was observed compared with batch measurements, where potential is measured under conditions very close to the equilibrium at the membrane-solution interface [24] so, the slope of the calibration graphs ranged from 70 to 74.3 mV/concentration decade compared with 56.5 mV/concentration decade under batch condition. The usable concentration range of the electrode in FIA is 5×10^{-5} to 5×10^{-2} mol/l.

3.2.2. Effect of pH

In batch measurements the variation in potential with pH change was followed by the addition of small volumes of HCl and NaOH (0.1–1.0 mol/l) to the test solution (1×10^{-2}, 5×10^{-3} and 5×10^{-4} mol/l Amb–HCl), whereas in FIA, a series of 10^{-2} mol/l Amb–HCl solutions of different pH values ranging from 1.0 to 7.5 (solutions of higher pH values cannot be prepared due to the precipitation of ambroxol as a base) were injected in the flow stream and the peak heights, representing variation of potential response with pH, were measured.
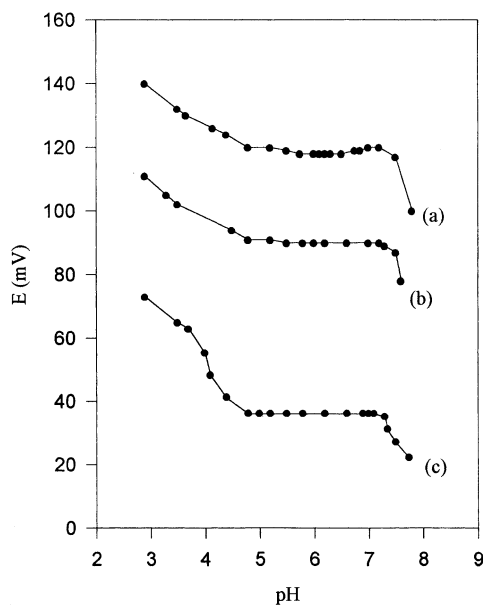

Fig. 3. The effect of pH of the test solution (a) 1×10^{-2}; (b) 5×10^{-3}; (c) 5×10^{-4} mol/l Amb–HCl on the potential response of Amb–TPB electrode.

It is evident that the electrode does not respond to pH changes in the range 4.8–7.3 in both batch and FIA conditions. The increase in potential at pH < 4.8, may be due to the penetration of H^+ into the membrane surface, while the decrease in potential at pH > 7.3 can be attributed to the precipitation of ambroxol. Representative curves are given in Fig. 3.

3.2.3. Selectivity of the electrode

It was shown earlier for solid-state membrane electrodes that the apparent selectivity coefficient K^{pot}_{Amb, j^+} measured under transient flow injection conditions might differ significantly from that measured under batch conditions [25,26]. This is interpreted by the difference in the time of interaction of interferents with the membrane surface. This difference increases with increase in the interaction of the interferent with the membrane in comparison with the main sensed ion, and also the interference process is highly dependent on the rate of diffusion and the exchange reaction of the interfering ion. Therefore, in FIA measurements, where the sample remains in contact with the electrode for a short period of time, the apparent selectivity is expected to be different from that found in batch conditions.

The influence of some inorganic cations, sugars, and amino acids on the Amb electrode was investigated. Under FIA conditions, the values of selectivity coefficients were calculated based on potential values corresponding to the peak heights for the same concentrations of the drug and the interferent (j^+), whereas under batch conditions the separate solution method was applied by measuring the potentials of 10^{-2} mol/l of both Amb–HCl (E_1) and the interferent ion (E_2) separately and the selectivity coefficients were calculated using the slope of the calibration graph (S) by applying the following equation:

$$\text{Log } K^{pot}_{Amb, \ j^+} = \frac{E_2 - E_1}{S} + \log [\text{Amb}] - \log [j^{z+}]^{1/z}$$

This method considered the simplest way to evaluate the degree of interference that might be taking place and is used to perform measurements in important biological samples such as blood.

In this work, for sugars and amino acids, the tolerance for using the electrode for determination of ambroxol without interference was determined; this is because the nature of charging of the interfering species is partially due to induced polarity inside the molecule. The mixed solution method, which is time consuming owing to prepare many solutions and to perform many steps, is used when $-\log K^{pot}_{Amb, \ j^+}$ was < 3.0 because in such cases the species investigated are relatively interfering, therefore additional method is required to confirm such interference.

Table 2
Selectivity coefficients for the ambroxol electrode

Interferent	$-\log K_{Amb, j^+}^{pot}$		
	Batch		FIA
	SSM [a]	MSM [b]	
Ag^{+} [c]	2.13	–	1.73
Cu^{2+}	2.78	3.15	2.84
NH_4^+	2.85	3.15	2.28
K^+	2.90	3.25	2.42
Pb^{2+}	2.96	3.85	2.93
Hg^{2+}	3.15	3.60	3.17
Al^{3+}	3.10	–	3.53
Na^+	3.20	–	1.78
Fe^{3+}	3.20	–	3.13
Ba^{2+}	3.53	–	2.80
Ni^{2+}	3.60	–	3.13
Co^{2+}	3.60	–	3.25
Mn^{2+}	3.74	–	2.93
Sr^{2+}	4.04	–	3.22
Mg^{2+}	4.16	–	3.33
Ca^{2+}	4.21	–	3.27
Zn^{2+}	4.26	–	3.25

[a] SSM, separate solution method.
[b] MSM, mixed solution method.
[c] K_{Amb,j^+}^{pot} for Ag^+ cannot be determined by mixed method due to the precipitation of AgCl.

The determined selectivity coefficients of the electrode, Table 2, reflect a very high selectivity of the investigated electrode for the ambroxol cation under both FIA and batch conditions. Also the results obtained in case of amino acids and sugars under FIA, using peak height comparison, come in good agreement with those under batch conditions, where it was found that the electrode could be used safely without interference in presence of glucose, maltose, lactose, fructose and glycine up to 1080, 480, 540, 480 and 420 folds of ambroxol, respectively.

3.3. Analytical application

The results of the standard addition, which are given in Tables 3 and 4, show that the mean recovery of the amounts taken (2.0–200.0 mg) in the proposed method ranged from 99.38 to 101.5, 97.88 to 100.6 and 98.57 to 101.25% with RSDs of 0.9 to 2.65, 1.01 to 2.50 and 0.5 to 2.15 for pure solutions, ambroxol tablet (30 mg/tablet) and muco syrup (15 mg/5 ml) respectively. It is worthy to mention that the results obtained for muco syrup batches of different expiry dates, Table 3, show that the concentration of ambroxol hydrochloride was not affected by time except after 6 months from the expiration date where the concentration begin to decrease and the recoveries were ranged from 88.6 to 91.0 with RSD = 0.55–2.25.

Also, the results of muco S.R. capsule (75 mg/capsule) obtained by applying standard addition method showed good agreement with the theoretical values of the percent of the amounts released which were 34.58, 64.7 and 84.6% with RSDs 1.48, 0.73 and 1.6% after 1, 8 and 16 h respectively, Table 5.

The FIA results represented in Table 6 showed that the flow rates did not affect the recovery values except for a rate of 17.85 ml/min, where the electrode takes a shorter time to attain the baseline, and the recoveries were higher than those obtained under batch conditions especially at higher concentrations. The mean recoveries for the amount taken (2.0–200 mg) ranged from 96.9 to 99.00, 99.00 to 100.67 and 101 to 104.8% with RSDs of 0.52–2.37, 0.5–3.1 and 0.72–2.5% for flow rates of 5.35, 9.87 and 17.85 ml/min, respectively.

The obtained results for the determination of The amount of Amb–HCl released from S.R. capsule (Table 5) were in a good agreement with both the theoretical reported values and those obtained by applying the standard addition methods; the amount released was 34.15, 64.81 and 83.26% with RSDs of 1.77, 1.6 and 0.7% after 1, 8 and 16 h, respectively.

Muco syrup gives a very low recovery under FIA conditions specially at high concentrations and that may be attributed to the relatively high viscosity of the syrup that impede its flow-through the tubes of the FIA system which in turn leads to longer time to achieve the base line; moreover the sensitivity of the membrane

Table 3
Determination of ambroxol hydrochloride in muco syrup (15 mg/5 ml) at different expiry date by applying the standard addition method under batch conditions

Taken (mg)	Newly manufactured batch		One month before its expiration date		One month after its expiration date		Six months after its expiration date	
	Recovery (%)	RSD [a] (%)	Recovery (%)	RSD [a] (%)	Recovery (%)	RSD [a] (%)	Recovery (%)	RSD [a] (%)
2	101.25	0.52	101.1	1.25	100.28	2.67	90.6	1.55
4	101	1.93	101.75	1.75	100.5	1.8	90.5	0.55
20	98.57	1.08	100.9	1.42	100.27	1.26	91	1.36
40	99.8	1.88	99.8	1.89	99.03	1.96	88.6	2.25
200	99.36	2.15	100.5	1.36	98.95	1.62	91	1.5

[a] Four determinations.

Table 4
Determination of ambroxol hydrochloride in pure solutions and in ambroxol tablet (30 mg/tablet) by applying standard addition method under batch conditions

Taken (mg)	Pure solutions			Ambroxol tablet (30 mg/tablet)		
	Found (mg)	Recovery (%)	RSD [a] (%)	Found (mg)	Recovery (%)	RSD [a] (%)
2.00	1.99	99.50	1.20	1.99	99.49	1.22
4.00	4.05	100.11	2.65	4.02	100.60	2.05
20.00	19.88	99.38	1.07	20.46	102.32	2.50
40.00	40.12	100.29	2.41	39.99	99.98	1.01
200.00	203.00	101.50	0.90	195.76	97.88	1.29

[a] Four determinations.

Table 5
Determination of the released amount of ambroxol hydrochloride after 1, 8 and 16 h under FIA and batch conditions

Time of the release	Theoretical values (%)	FIA		Batch	
		Found (%)	RSD [a] (%)	Found (%)	RSD [b] (%)
After 1 h	25–35	34.15	1.77	34.58	1.48
After 8 h	55–65	64.81	1.60	64.70	0.73
After 16 h	75–100	83.26	0.70	84.60	1.60

[a] Three determinations.
[b] Four determinations.

Table 6
Determination of ambroxol hydrochloride in ambroxol tablet (30 mg/tablet) under FIA conditions

Taken (mg)	Flow rate 5.35 ml/min		Flow rate 9.7 ml/min		Flow rate 17.85 ml/min	
	Recovery (%)	RSD [a] (%)	Recovery (%)	RSD [a] (%)	Recovery (%)	RSD [a] (%)
2.00	96.90	2.37	100.46	3.10	102.45	2.50
4.00	99.00	1.75	99.00	1.75	1010	1.73
20.00	98.65	0.70	100.67	1.15	104.7	1.13
40.00	97.30	0.52	100.50	0.80	104.8	0.86
200.00	96.54	0.52	99.01	0.50	103.10	0.73

[a] Three determinations.

Table 7
Statistical treatment of data obtained for the determination of ambroxol–HCl using Amb electrode in comparison with reference method

	Reference method	Pure solution	Muco syrup	Ambroxol tablet	
		Batch	Batch	Batch	FIA
Mean recovery	99.05	100.15	99.99	100.05	99.93
S.D.	1.15	1.64	1.51	1.61	1.46
Probability		0.05	0.05	0.05	0.05
$F^{3.5}$ value(5.41)		2.03	1.72	1.96	1.61
t-value (2.365)		0.89	0.84	0.68	0.63
Intercept [a]		0.24	0.03	0.46	0.23
Slope [a]		1.01	0.99	0.98	0.99

[a] Intercepts and slopes of the regression lines (found versus taken).

will be less than the excepted to the concentration changes, hence a very low recovery will be produced.

The results were subjected to linear regression analysis (found values versus taken), using the computer pro- gram sigma plot-2 in order to establish whether the investigated electrode exhibits any fixed or proportional bias. The slopes and intercepts of the regression lines did not differ significantly from the ideal values, revealing

the absence of a systematic error during the measurements within the investigated concentration range. Also, they were compared with the results obtained from the reference method [6] by applying F- and t-tests. The values obtained (Table 7) show that the present methods are in comparable precision to the reference method and there is no significant difference between the mean values obtained by the two methods. In addition, the proposed methods are easy, cheap and do not need any sophisticated or expensive instruments.

4. Conclusion

The previous results showed that the proposed method for the determination of ambroxol hydrochloride in its pure solutions and pharmaceutical preparations is characterized by a high degree of precision, selectivity and accuracy when compared with the reference method, within wide concentration, temperature and pH ranges as shown by the F- and t-values, also the method can be used as a shelf stability test, that was proved by measuring the ambroxol content in muco syrup at different expiration dates and the amount of ambroxol released from the muco S.R. capsule at different time intervals. In addition the FIA conditions shortened the time needed for the determination when compared with the previous published methods of determination of Ambroxol such as HPLC, Electrophoresis and spectrophotometry which need many reagents and equipments (carrier gas, derivatizing agent and special detectors); in addition, these methods take longer time for measuring one sample depending on the retention time of Ambroxol, derivatization procedure or method of extraction. Therefore, the proposed method is considered very simple in application and of low expenses without any need of sophisticated instrument in comparison with the previously published methods of determinations.

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