Flow injection potentiometric determination of amantadine HCl

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Abstract

New amantadine (Am) ion selective plastic membrane electrodes of both conventional and coated graphite types based on the ion-pair of amantadinium tetraphenylborate (Am-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 amantadine in pure state and pharmaceutical preparation under batch and flow injection conditions. The selectivity of the electrode toward a large number of 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. © 2002 Published by Elsevier Science B.V.

1. Introduction

Amantadine hydrochloride (Am-HCl) (tricyclo[3.3.1.1]decan-1-amine, hydrochloride) [665-66-7] is reported [1] as a synthetic antiviral agent that inhibits the penetration and the replication of the virus, the action is directed mainly to the virus and not to the host cell and prevent the release of the viral infectious nucleic acid to the host cell. The content of Am-HCl was determined in tablets by acid-dye spectrophotometry [2], by potentiometric titration of organic cations with sodium tetraphenylborate (TPB) using a liquid membrane tetraphenylborate ion selective electrode [3], in pure solution and pharmaceutical preparation by oscillopolarography [4], in plasma and urine with automated derivatization [5], in human plasma by capillary gas chromatography [6], by HPLC [7,8], in human plasma and urine by gas chromatography [9,10] and by flow injection analysis with using an amantadine flow through electrode [11]. Two ion selective electrodes have been constructed for the determination of amantadine, one of them involves amantadine–dipicrylamine as ion-pair and the other comprised a membrane of 32% of polyvinyl chloride (PVC), 64% of 2-nitro-

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phenyl octyl ether and 4% of dinonylnaphthalene sulphonic acid [12]. In the present work, a plastic membrane selective electrode for amantadinium ion (Am) has been constructed, it is based on the incorporation of amantadinium tetraphenylborate (Am-TPB) ion exchanger in PVC membrane plasticized with dioctylphethalate (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 characteristics of ISEs for various reasons, including the following: (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 [13]. Am-HCl under investigation is represented by the following formula.
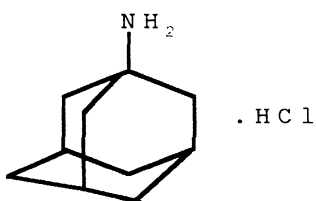
Amantadine Hydrochloride

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 prior to measurements. Pure grade Am-HCl and the pharmaceutical preparation (Adamine capsule, 100 mg per capsule) were provided by Rameda 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.

The electrochemical system was as follows:

Ag/AgCl/filling solution/membrane/test solution/ /KCl salt bridge//SCE.

The potentials of the ISE were measured against SCE as external reference electrode under open circuit conditions at the experimental temperature.

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).

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 [14]. 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.

2.3. Preparation of the ion exchanger

The ion exchanger, Am-TPB was prepared by mixing 100 ml of 10^{-2} mol 1^{-1} of Am-HCl with 100 ml of 10^{-2} mol 1^{-1} of sodium TPB. The white precipitate formed, after digestion by standing the precipitate in dark place 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 (Am-TPB) ion-pair. The values found are 83.1, 7.9 and 3.0 and the calculated values are 86.79, 8.14 and 2.97 for C, H and N%, respectively.

2.4. Preparation of conventional and coated graphite types electrodes

The conventional electrode was constructed as previously described [15]. The membrane composition was studied by varying the percentages (w/w) of the ion exchanger, PVC and DOP until optimum characteristics are reached.

The membranes were prepared by dissolving the required amount of ion exchanger, DOP and PVC in about 10 ml tetrahydrofuran, the solution mixture was poured into a 7.5 cm Petridish 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 1^{-1} in NaCl and 10^{-3} mol 1^{-1} in Am-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 a graphite bar (7 cm length, 1.2 cm width and 2 mm thickness). One of the two ends of the bar is used for the connection while the other, about 1 cm length, was dipped in a solution of the same optimum membrane composi-

tion 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 bar.

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

The solubility product and the formation constant of Am-TPB ion-pair were conductimetrically determined as previously described [16]. For this purpose, a series of solutions of different concentration ($C = 10^{-4}$–10^{-2}) mol 1^{-1} was prepared for each Am-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, ($\lambda = 1000K/C$) were obtained. Straight line plots of λ versus ($C^{1/2}$) were constructed and the equivalent conductance at infinite dilution (λ_0) values were determined for both Am-HCl ($\lambda_{0 \text{ Am-HCl}}$) and Na–TPB($\lambda_{0 \text{ Na–TPB}}$) from the intercept of respective line 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 [17]. $\lambda_{0 \text{ Am-TPB}}$ was calculated from Kohlrausch's law of independent migration of ions using λ_0 Am-HCl and $\lambda_{0 \text{ Na–TPB}}$ values. The solubility (S) and solubility product (K_{sp}) of ion-pair were obtained applying the following equations:

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

where K_s is the specific conductivity of a saturated solution of Am-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 & Designs [ESD] conductometer, Model 72. [Conductance/TDS](Jenway).

3. Results and Discussion

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

3.1.1. Composition of the membrane

Four different membrane compositions that contain 2 (I), 5 (II), 6 (III) and 10% (IV) of the ion-pair were investigated. The slopes of their calibration plots were found to be 55.5, 54.7, 56.75 and 56.25 with relative standard deviation (RSD) 1.30, 0.50, 2.00 and 1.90 for compositions I, II, III, VI, respectively. The preparation process is fairly reproducible as indicated by the small values of the RSDs. Electrodes made by using membrane composition III show the nearest performance characteristic to the nernstian behaviour (slope $= 56.75$ and 56.35mV per concentration decade, at 25 °C for batch and coated graphite type, respectively).

The usable concentration range was found to be 2×10^{-5}–5×10^{-2} and 2×10^{-4}–5×10^{-2} mol l^{-1} Am-HCl in case of conventional and coated graphite type, respectively, the smaller concentration range in case of coated graphite type may be attributed to the difference in the physical and mechanical parameters of the membrane of the conventional type and those of the layer on the coated graphite type, in addition of the absence of the internal solution in the coated electrode which might increase the sensitivity of the membrane. In all subsequent studies electrodes made of membrane III were used.

3.1.2. Effect of soaking

Freshly prepared electrodes can be used without preconditioning where the mean slopes of the calibration graphs constructed in distilled water are 55.5, 54.7, 56.75, 56.25 and 56.35 mV per concentration decade for composition I, II, III, IV and coated graphite type of composition III, respectively. The electrode then soaked at room temperature in 10^{-3} mol l^{-1} Am-HCl. It was found 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 [18]. It was noticed that the conventional type electrodes worked in nearly Nernestian behaviour in the first 20 days then

decrease gradually to reach 50 (mV per concentration decade) for composition I after 30 days, and 51 (mV per concentration decade) for composition II and III after 32 days, but in case of composition VI it decreased to reach 51.4 after 7 days only. While in case of coated graphite type the electrode showed a relatively short lifetime as the slope decreased to reach 52.3 after 24 h. Naturally, it was noted that in all cases, electrode which had been kept dry in a closed vessel and stored in a refrigerator showed nearly constant values and the same response properties for several months in case of conventional type, but in case of coated graphite type the life time can be enhanced only to 1 week by keeping the electrode in the refrigerator. Hence it is recommended that unused electrodes should be kept dry in closed vessel in a refrigerator in order to extend their life spans substantially.

3.2. Effect of temperature of the test solution

Calibration graphs [electrode potential (E_{elec}) versus pAm] were constructed at different test solution temperatures (25, 30, 35, 45, 55, 60 and 70 °C). For the determination of the isothermal coefficient ($dE°/dt$) of the electrode, the standard electrode potentials ($E°$) against the normal hydrogen electrode at the different temperatures were obtained from calibration graphs as the intercepts at pAm $= 0$ (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 [19]:

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

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 (0.00084 V/°C). The value of the obtained isothermal coefficient of the electrode indicates that the electrode has a fairly high thermal stability within the investigated temperature range. The investigated electrode was found to be usable up to 70 °C without noticeable deviation from the nearnestian behaviour.

3.3. Effect of ionic strength

Calibration graphs were constructed at different concentrations of NaCl (10^{-3}–10^{-1} mol 1^{-1}). It was found that the slope of the electrode increased gradually from 55.8 mV per concentration decade in distilled water to reach its maximum value 57.5 mV per concentration decade in 5×10^{-3} mol 1^{-1} of NaCl and then decreased in higher concentration of NaCl to reach 52 mV per concentration decade in 10^{-1} mol 1^{-1} NaCl which may be attributed to the interference of high concentration of Na^+ ions.

3.4. Optimization of FIA response

The flow injection measurements were carried out in a two-line system; 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 stabilization of the base line potential has been achieved by using both water and buffer electrolyte as sample carrying solution but the latter show shorter peak heights. The connector of the two streams was linked to the detector by a 50 cm tube of 0.4 mm internal diameter. Fig. 1 shows, the configuration of the system used in the measurements. The dispersion coefficient was found to be 1.23, i.e. limited dispersion that aids optimum sensitivity and fast response of the electrode [20].

3.4.1. 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, requiring a longer time to reach a steady state and greater consumption of sample [21]. A sample loop of size 75 μl was used through out this work giving about 98% of the maximum peak height obtained by a 500 μl loop but with a shorter time to reach the baseline and less reagents consumption.

3.4.2. Effect of flow rate

The dependence of the peak heights and time to reach the baseline on the flow rate were studied, the response of the electrode to a solution that is 10^{-2} mol 1^{-1} being studied 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^{-1}). It was found previ-
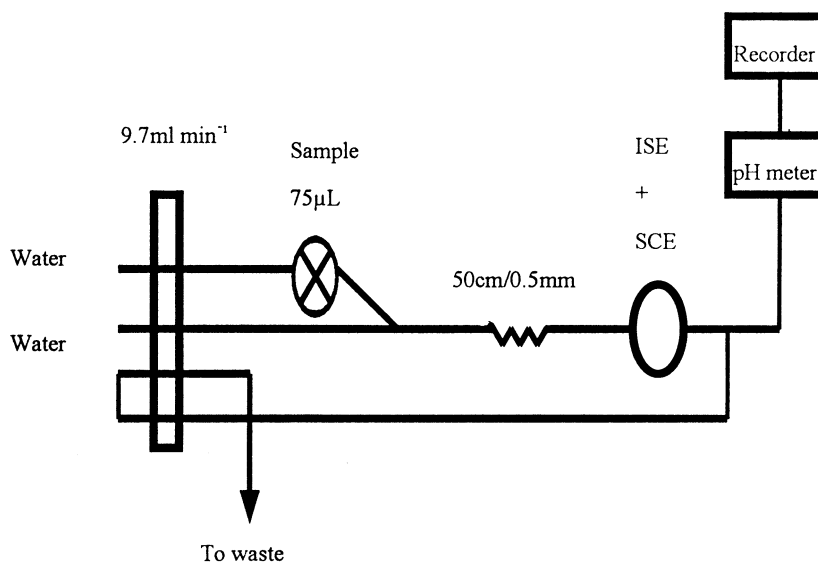

Fig. 1. Schematic diagram of the flow injection system used in the measurements.
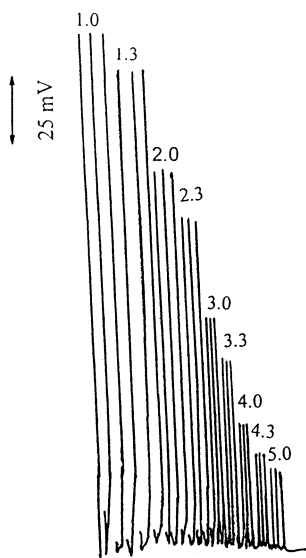
Fig. 2. Recording for Am-TPB electrode under optimum FIA conditions.

ously [22] 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^{-1} was reached, above which the peaks were nearly the same. A flow rate of 9.7 ml min^{-1} 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 Am-TPB electrode under optimum FIA conditions.

3.5. 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 a 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 unfavourable 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 [23,24]. 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 [25]. So, the slopes of the calibration graphs were 56.00, 62.86, 66.55, 66.61, 65.40, 64.00, 65.00, 63.00, 64.90 and 61.51 at 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^{-1}, respectively compared with 56.75 mV per concentration decade under batch condition. The usable concentration range of the electrode in FIA is 5×10^{-5}–1×10^{-1} mol l^{-1}.

3.6. Effect of pH

The effect of pH of the test solutions on the electrode potentials was studied in batch and FIA measurements. 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^{-1}) to the test solution (1×10^{-2}, 7.5×10^{-3} and 5×10^{-3} mol l^{-1} Am-HCl), whereas in FIA, a series of of 10^{-2} mol l^{-1} Am-HCl solutions of different pH values ranging from 1.0 to 12.0 were injected in the flow stream and the peak heights, representing variation of potential response with pH, were measured.

It is evident that the electrode does not respond to pH changes in the range 2.5–8.0 in both batch and FIA conditions. Nevertheless, at pH values lower than these values, the potential decreased gradually, which can be related to interference of hydronium ion, while at pH > 8.0 the increase that takes place can most probably attributed to the changes in liquid-junction potential as a result of the penetration of hydroxyl ions into the gel layer of the membrane. Representative curves are given in Fig. 3.

3.7. Selectivity of the electrode

It was shown earlier for solid state membrane electrodes that the apparent selectivity coefficient $K_{Am, jz+}^{pot}$ measured under transient flow injection conditions may differ significantly from that measured under batch conditions [26–29]. 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 [30]. 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 Am 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^{z+}), whereas under batch conditions

Fig. 3. The effect pf pH of the test solution: (a) 10^{-2}; (b) 7.5×10^{-3}; (c) 5×10^{-3} mol 1^{-1} Am-HCl on the potential response of Am-TPB electrode.

the separate solution method was applied by measuring the potentials of 10^{-2} mol 1^{-1} of both Am-HCl (E_1) and the interferent (E_2) ion separately and the selectivity coefficients $K_{Am, j+}^{pot}$ were calculated using the slope of the calibration graph of the test electrode (S) by applying the following equation:

$$\text{Log } K_{Am, j+}^{pot} = \frac{E_2 - E_1}{S} + \log[Am] - \log[j^{z+}]^{1/z}.$$

A high concentration of the interferent ion was used (10^{-2} mol 1^{-1}) to ensure that there is no interference if lower concentrations than this are present under the same experimental conditions in each of batch and FIA methodologies. This method is 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 [31].

In this work, for sugars and amino acids, the tolerance for using the electrode for determination of amantadine 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 only as a confirmation when $-\log K_{Am, jz+}^{pot}$ was < 3.0.

The determined selectivity coefficients of the electrode, Table 1, reflect a very high selectivity of the investigated electrode for the amantadine cation under both FIA and batch conditions. Also the results obtained in case of amino acids and sugars under FIA come in good agreement with those under batch conditions, where it was found that the electrode can be used safely without interference in presence of glucose, maltose, lactose, fructose and glycine up to 45.7-, 314.3-, 114.3-, 114.3- and 200-folds of amantadine, respectively.

3.8. Analytical application

Am-HCl was determined potentiometrically using the investigated electrode under batch conditions by both potentiometric titration and standard addition methods [32]. In the potentio-
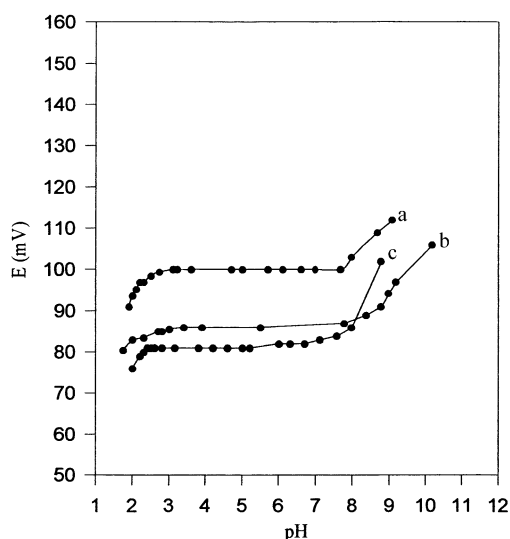
Table 1
Selectivity coefficients for the amantadine electrode

Interferent	$-\log K_{\mathrm{Am}, jz}^{\mathrm{pot}}$		
	Batch		FIA
	SSM[a]	MSM[b]	
Na^+	2.15	3.12	1.82
K^+	2.05	3.00	1.80
NH_4^+	2.15	2.50	2.05
Hg^{2+}	2.70	4.00	1.85
Ag^{+c}	2.56	–	2.40
Pb^{2+}	3.00	–	3.00
Ni^{2+}	3.33	–	3.20
Co^{2+}	3.62	–	3.23
Sr^{2+}	3.73	–	3.00
Ba^{2+}	4.00	–	3.10
Mn^{2+}	4.10	–	2.70
Zn^{2+}	4.16	–	3.13
Al^{3+}	4.23	–	3.66
Mg^{2+}	4.35	–	3.23
Ca^{2+}	4.41	–	2.75
Fe^{3+}	4.59	–	3.47
Cu^{2+}	4.68	–	3.00

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

metric titration method different volumes of 10^{-2} mol l^{-1} of pure Am-HCl or its pharmaceutical preparation was taken (2, 5, 10, 15 ml) and completed to 50 ml with distilled water then titrated against TPB, Table 2, the end point was determined by plotting E (mV) versus V (ml).

The standard addition method was applied by adding small portion of 10^{-1} mol l^{-1} standard Am-HCl solution to 50 ml water containing different amounts of pure compound or the pharmaceutical preparation (1.0–200.0 mg). The change in millivolt readings was recorded after each addition and used to calculate the concentration of Am-HCl sample solutions. The standard addition method was used for determination of Am-HCl in two batches of Adamine capsules (100 mg per capsule), a newly manufactured one and other one 6 months before its expiration date which is the proper allowed time to return the nearly expired capsule to the company.

For sampling of Adamine capsules (100 mg per capsule), the content of 20 capsules was dissolved in distilled water, the solution then filtered to get rid of the insoluble ingredient and completed to with distilled water and used as stock solution. The required volumes for the preparation of different concentration was taken and diluted to 50 ml with distilled water.

The results of the standard addition, which are given in Table 3, are in good agreement with those obtained from the reference method [2]. The later is based on determining amantadine spectrophotometry using bromocresol green acid-dye at 413 nm). The mean recovery of the amounts taken (1.0–200.0 mg) in the proposed method ranged from 98.2 to 102.1% with RSD = 0.29–2.3% for pure solutions and newly manufactured capsule, also it is worthy to mention that the result obtained for Adamine capsules 6 months before its expiration date prove that the active ingredient Am-HCl still valid with nearly the same concentration after 2-year of its manufacturing date where the mean recovery ranged from 96.8 to 100.9 with RSD = 0.2–1.9%.

Table 2
Determination of Am-HCl by applying the potentiometric titration method

Pure solutions				Adamine capsule (100 mg per tablet)			
Taken (mg)	Found (mg)	Recovery (%)	RSD[a] (%)	Taken (mg)	Found (mg)	Recovery (%)	RSD[a] (%)
7.5	7.67	102.3	6.0	7.5	7.73	103.1	4.3
18.7	19.63	105.0	0.9	18.7	19.5	104.3	1.7
37.5	37.95	101.2	1.1	37.5	38.25	102.0	2.1
56.3	58.27	103.5	0.56	56.3	55.17	98.0	1.1

[a] Four determinations.

Table 3
Determination of Am-HCl by applying the standard addition method under batch conditions

	Pure solution				Adamine capsule (100 mg per capsule) newly manufactured				Adamine capsule (100 mg per capsule) 6 months before its expiration date			
	Taken (mg)	Found (mg)	Recovery (%)	RSD[a] (%)	Taken (mg)	Found (mg)	Recovery (%)	RSD[a] (%)	Taken (mg)	Found (mg)	Recovery (%)	RSD[a] (%)
	1.0	1.00	100.9	0.67	1.0	1.02	102.10	0.6	1.0	1.01	100.10	0.8
	2.0	1.99	99.4	1.28	2.0	2.04	102.00	1.9	2.0	2.02	100.90	1.6
	10.0	9.82	98.2	0.16	10.0	9.99	99.99	1.3	10.0	9.68	96.80	0.2
	20.0	20.29	100.6	1.17	20.0	20.35	101.75	1.4	20.0	20.09	100.40	1.8
	100.0	100.3	100.3	0.67	100.0	100.9	100.90	1.6	100.0	98.50	98.50	1.8
	200.0	200.0	99.96	0.73	200.0	200.72	100.36	2.3	200.0	200.4	100.40	1.9

[a] Four determinations.

Table 4
Determination of Am-HCl in capsules under FIA conditions

Taken (mg)	Flow rate 5.35 ml min⁻¹			Flow rate 9.7 ml min⁻¹			Flow rate 17.85 ml min⁻¹		
	Found (mg)	Recovery (%)	RSD (%)	Found (mg)	Recovery (%)	RSD (%)	Found (mg)	Recovery (%)	RSD (%)
1	0.94	94.0	1.64	0.98	98.0	1.6	1.02	102.0	1.5
2	1.92	95.8	1.3	1.96	97.93	1.9	2.00	100.0	0.63
10	9.45	94.5	1.83	9.95	99.49	1.7	9.94	99.4	1.5
20	18.41	92.07	1.5	20	100.0	0.24	19.84	99.21	1.2
100	98.95	98.95	1.8	99.47	99.47	0.78	99.47	99.47	0.78
200	197.71	98.85	0.69	197.7	98.85	0.84	199.53	99.77	0.83
900	893.65	99.29	0.53	893.65	99.30	0.53	900.00	100.0	1.05
1800	1782.35	99.01	0.99	1782.35	99.02	0.99	1801.76	100.09	0.98

The flow rates in Table 4 are given in units of ml min^{-1}.

Table 5
Statistical treatment of data obtained for the determination of amantadine using Am electrode in comparison with reference method

	Reference method	Pure solution		Adamine capsule (100 mg per capsule)	
		Batch	FIA	Batch	FIA
Mean recovery	100.6	99.89	98.76	101.3	98.94
S.D	0.603	0.78	1.04	1.5	1.08
Probability		0.05	0.05	0.05	0.05
$F^{3.3}$ value (9.27)		1.76	2.97	6.18	3.20
t-value (2.571)		1.28	2.31	0.65	2.01

Under FIA conditions, a series of solutions of different concentrations were prepared from capsules and the peak heights were measured at three flow rates (5.35, 9.7 and 17.85 ml min^{-1}), then compared with those obtained by injecting a standard solution of Am-HCl of the same concentration. From the results obtained, Table 4, it is clear that the flow rates did not affect the recovery values except for a rate of 5.35 ml min^{-1}, where the electrode needed a longer time to attain the baseline, and the recoveries were lower than those obtained under batch conditions for low concentrations, however for high concentrations, acceptable results were obtained. The mean recoveries for the amount taken (1.0–1800 mg) ranged from 92.07 to 99.29, 97.5 to 100.0 and 99.21 to 102.3% with RSDs of 0.53–1.83, 0.24–1.92 and 0.5–1.5% for flow rates of 5.35, 9.87 and 17.85 ml min^{-1}, respectively.

From the results obtained it is obvious that, higher concentrations under FIA conditions than those under batch conditions (standard additions method) can be determined, where the concentration using the standard addition method is calculated from the following equation:

$$C_x = C_s(V_S/V_x + V_s)[10^{n(\Delta E/S)} - (V_x/(V_x + V_s))]^{-1},$$

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

So the determination of the concentration depends mainly on ΔE hence to obtain noticeable ΔE we need to prepare higher concentration of standard which is very difficult and consume very large amount of the standard which is not needed under FIA conditions.

The results of the proposed method were compared with those that were previously obtained by Liu et al. [11], which applied to amantadine tablets with recoveries ranged from 94.3 to 99.6% and RSD of 1.1%, here it is obvious that the proposed method has higher recoveries with compatible RSDs.

The results were subjected to linear regression analysis (*found values versus taken*), using the computer program sigma plot-2. In order to establish whether the investigated electrode exhibit 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 [2] by applying F- and t-tests [33]. The values obtained in Table 5, 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 method is easy, cheap and does not need any sophisticated or expensive instruments.

3.9. Solubility product of the Am-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 (Am-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 Am-TPB was determined conductimetrically as described in Section 2 and was found to be 4.20×10^{-8} indicating a very low solubility of the ion-pair (2.05×10^{-4} mol l^{-1}). Consequently, the formation constant of the reaction Am + TPB = Am-TPB, was 2.38×10^7, 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 of a contribution to the total solubility.

4. Conclusion

The application of the proposed methods to the determination of Am-HCl in its pure solutions and pharmaceutical preparations is characterized by a high degree of precision and accuracy when compared with the official method. In addition the FIA conditions shortened the time needed for the determination and extended the limits of the determination to higher concentrations of the drug in its pure state or in its pharmaceutical preparation.

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