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RESEARCH ARTICLE

New potentiometric membrane sensors for determination of alverine citrate in pharmaceutical compounds and biological fluids

M. M. Khalil [1,*], Y. M. Issa[2], S. I. M. Zayed [3,4], F. Q. Ali[1]

1. Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt
2.Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt
3. Faculty of Industrial Education, Beni-Suef University, Beni-Suef, Egypt
4.Chemistry Department, Faculty of Science, Taif University, Taif, KSA

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Corresponding Author

M. M. Khalil

Abstract

The construction and performance characteristics of novel alverine citrate (Alv) plastic membrane electrodes based on incorporation of alverine citrate with the ion exchangers, sodium tetraphenylborate (Alv-TPB), ammonium reineckate (Alv-Rt) and a mixture of both (Alv-TPB/Rt) into plasticized poly(vinyl chloride) membrane are described. The sensors showed a near-Nernstian slope of 57.24, 56.31 and 57.01 mV decade^{-1} at 25±0.1°C within the concentration range 1.00×10^{-5}–1.00×10^{-2}, 1.99 $\times10^{-5}$-1.00×10^{-2} and 3.98×10^{-5}-1.00×10^{-2} M Alv, with detection limits of 4.07×10^{-6}, 3.98×10^{-6}, 9.33×10^{-6} M Alv for Alv-TPB, Alv-Rt, Alv-TPB/Rt electrodes, respectively. The membrane electrodes performed satisfactorily over pH ranges of 1.48–7.56, 1.52–7.97 and 1.56–7.82 for the three electrodes, respectively. The selectivity coefficients of the developed sensors indicated excellent selectivity for Alv. Surface analysis using scanning electron microscopy was used to determine the cause of the limited life span of plastic membrane electrode. The proposed sensors displayed useful analytical characteristics for the determination of Alv in pharmaceutical preparation and biological fluids such as plasma and urine samples.

1. Introduction

Alverine citrate (Figure 1), N-methyl-N-(3-phenylpropyl)benzenepropanamine citrate, is a commonly used smooth muscle relaxant agent [1]. It has been used in the treatment of irritable bowel syndrome [2]. It decreases the sensitivity of the intestinal mechanoreceptors in response to chemical stimulation in anaesthetized cats [3] and reduces 5-hydroxytryptamine 1A receptor-mediatd rectal hypersensitivity in the rat [4]. Hayase et al. investigated the mechanisms underlying the paradoxical ability of Alverine to enhance spontaneous activity in smooth muscles while suppressing evoked activity. They have, however, come to the conclusion that further research has to be carried out for assessing the therapeutic potential of ALV to treat detrusor smooth muscle dysfunction [5]. It was able to inhibit the spontaneous contractions and the nervous control of rabbit proximal colon [6]. It was also found to induce the hepatitis [7]. Recently, this drug has been determined by liquid chromatography tandem mass spectrometry [8,9]. This method requires prior derivatization or extraction step, and involves the use of expensive equipment. On the other hand, potentiometric sensors offer simple, selective and sensitive technique for drug analysis, therefore, has had wide applications in this field. However, no potentiometric membrane sensors have been developed and used for alverine determination yet.

In connection with our continuing research oriented towards construction and analytical applications of ISEs [10-15], the present work describes preparation and investigation of performance characteristics of new ISEs based on PVC for the determination of alveine citrate in pure solutions, pharmaceutical preparations and biological fluids (spiked urine and plasma).

2. Experimental

2. 1. Reagents and materials

All chemicals were of analytical grade. Double distilled water was used throughout all experiments. Pure grade alverine citrate and the pharmaceutical preparation meteospasmyl capsules (60 mg/capsule) were provided by farco Pharmaceutical Co., Alexandria City, Egypt. Sodium tetraphenylborate (NaTPB), ammonium reineckate (Rt), poly(vinyl chloride) of high molecular weight (PVC), dioctyl sebacate (DOS), and tricresyl phosphate (TCP) were obtained from Fluka (U.S.A.). Tetrahydrofuran (THF), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) were purchased from Merck (Germany). The metal salts were provided by BDH as nitrates or chlorides. Stock solutions of the metal salts were prepared in bidistilled water and standardized when-ever necessary. In the analysis of biological fluids, human urine and plasma were used, plasma was obtained from National Blood Transfusion Services, Beni-Suef, Egypt.

2. 2. Apparatus

Potentiometric and pH-measurements were carried out using 702 titroprocessor equipped with a 665 dosimat (Switzerland) made by Metrohm. A mLw W20 circulator thermostat was used to control the temperature of the test solutions. A saturated calomel electrode (SCE) was used as the external reference, while a Ag/AgCl electrode was used as an internal reference. The electrochemical system may be represented as follows: Ag/AgCl/filling solution/membrane/test solution//KCl salt bridge//saturated calomel electrode.

2. 3. Preparation of the ion pair

The ion pairs, Alv-TPB and Alv-Rt were prepared by mixing 100 mL 10^{-2} M alverine citrate solution with 100 ml of 10^{-2} M of sodium tetraphenylborate or ammonium reineckate. The formed precipitates were filtered, washed thoroughly with bidistilled water and dried at room temperature. The composition of the ion-pair was found to be 1 : 1 both in case of Alv-TPB and Alv-reineckate as confirmed by elemental analysis data. The percentage values found are 77.99, 6.69 and 2.24 and the calculated values are 77.73, 6.73 and 2.05 for C, H and N, respectively, in case of Alv-TPB, while in case of Alv-Rt the percentage values found are 41.56, 5.57 and 16.08 and the calculated values are 41.35, 5.78 and 16.06 for C, H and N, respectively.

2. 4. Conductometric measurements

Conductometric titrations were followed with a Jenway conductivity meter. 50 mL of 1.0×10^{-3} M Alv solution was titrated against a 1.0×10^{-2} M NaTPB or Rt solution. The conductance of the solution was measured after each addition of the titrant . The titration plot (conductance versus molar ratio) showed a break which corresponds to the stoichiometry of the complexes.

2. 5. Electrode preparation

The electrode was constructed as described previously [14,16]. The membranes were prepared by dissolving varying amounts of the ion pair and PVC in 10 mL THF. To these, solvent mediators, viz. DBP, DOS, TCP and DOP were added to get membranes of different compositions. The mixture was stirred with a glass rod. When the solution became viscous it was poured into a 6.0 cm Petri dish. The solution was then allowed to evaporate for 24 h at room temperature. Transparent membranes of about 0.2 mm thickness were obtained. A 12 mm diameter disk was cut out from the prepared membrane and glued using PVC–THF paste to the polished end of a plastic cap attached to a glass tube. The electrode body was filled with a solution of 1×10^{-1} M NaCl and 1×10^{-4} M Alv citrate. The electrode was preconditioned before use by soaking in a 1.0×10^{-3} M Alv citrate solution for 0.5 h. The ratio of membrane ingredients, time of contact and concentration of conditioning solution were optimized so that the potentials recorded were reproducible and stable.

2. 6. Electrodes calibration

The conditioned electrodes were calibrated by separately transferring 50 mL aliquots of solutions (10^{-6} to 10^{-2} M) of Alv into a series of 100-mL beakers. The membrane electrodes, in conjunction with saturated calomel electrode, were immersed in the above test solutions and allowed to equilibrate while stirring. The potential was recorded after stabilising to ±1 mV, and the potential was plotted as a function of the negative logarithm of Alv concentration.

2. 7. Effect of temperature

The effect of temperature on the performance of the potentiometric sensors was evaluated by construction of calibration curves at different temperatures (25-55 °C).

2. 8. Potentiometric Determination of Alv citrate

Alverine citrate has been determined potentiometrically using the investigated electrodes by the standard addition method [17] and by potentiometric titration with a standard solution of NaTPB.

2. 9. Determination of Alv citrate in Meteospasmyl capsules

The required amount from the capsules gel was dissolved in 30 mL bidistilled water and filtered in 50 mL measuring flask. The residue was washed three times with bidistilled water, and the volume was completed to the

mark by the same solvent. The contents of the measuring flask were transferred into a 100 mL titration cell and subjected to potentiometric determination of Alv citrate.

2. 10. Determination of Alv citrate in spiked urine and plasma samples

Different amounts of Alv citrate and 5 mL urine or plasma of a healthy person were transferred to 50-mL measuring flask and completed to the mark by bidistilled water. The contents of the measuring flask were transferred to a 100-mL beaker, and subjected to potentiometric determination of Alv citrate by the standard addition method.

2. 11. Scanning electron microscopy (SEM)

JEOL scanning electron microscope (JSM - 5610 LA) is used to investigate the morphology of the surfaces of freshly prepared and expired electrode membranes.

3. Results and discussion

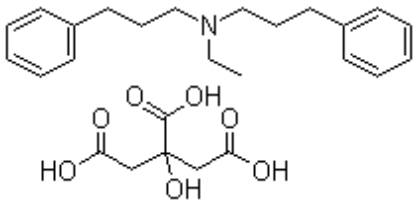


Fig. 1. Structure of alverine citrate

3.1. Optimizaion of membrane composition

Alverine cation was found to form 1:1 water insoluble ion-pair complex with each of sodium tetraphenylborate and ammonium reineckate as indicated by elemental analysis data and ascertained using conductometric titration (Fig. 2). The prepared ion-pairs were identified and examined as ion exchange sites in PVC membrane sensors responsive for alverine cation.

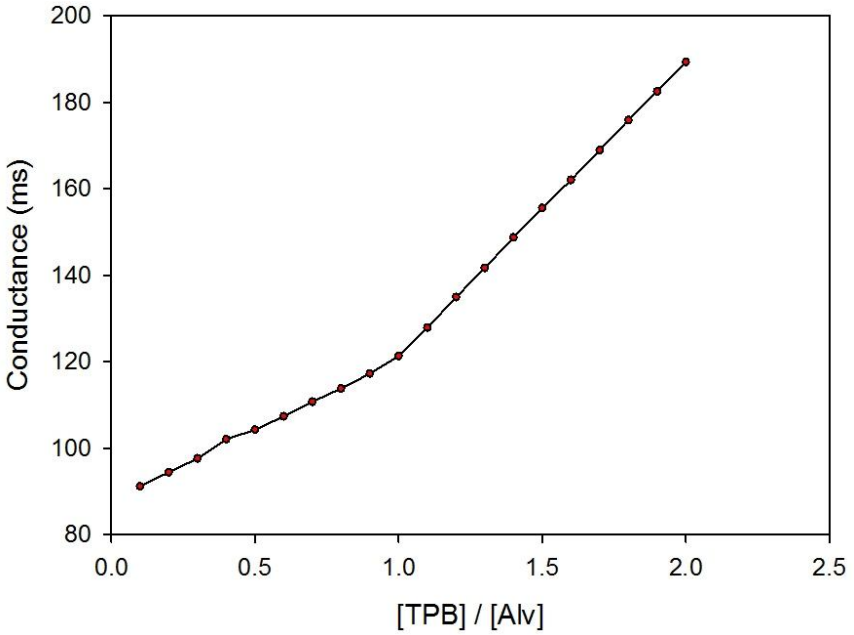


Fig. 2. Conductometric titration curve of 1.0 x 10^{-2} M Alverine citrate against 1.0 x 10^{-2} M NaTPB

The selectivity and sensitivity of ion-selective elecrodes (ISEs) obtained for a given membrane depend significantly on the membrane ingredients, nature of plasticizers and additive used [18–20]. Thus, different aspects of membrane preparation based on the ion-exchangers (Alv-Rt and Alv-TPB) were optimized and the results are given in Table1.

Table 1. Optimization of membrane composition (w/w %) for Alverine electrodes

	Composition % w/w				Slope	Linear range	LOD	*RSD %*
	Ion-pair	PVC	DBP	KTPB	mV/decade	(M)	(M)	
Electrode no.	Alv-TPB electrodes							
1	3	48.5	48.5	--	53.39	1.99×10^{-5} –1.00×10^{-2}	4.07×10^{-6}	0.33
2	5	47.5	47.5	--	57.24	1.00×10^{-5} – 1.00×10^{-2}	4.07×10^{-6}	0.10
3	7	46.5	46.5	--	54.21	1.99×10^{-5} – 1.00×10^{-2}	4.36×10^{-6}	0.77
4	9	45.5	45.5	--	54.52	1.00×10^{-5} – 1.00×10^{-2}	5.37×10^{-6}	0.41
	Alv-Rt electrodes							
5	0.5	49.75	49.75	--	54.56	1.99×10^{-5} – 1.00×10^{-2}	6.02×10^{-6}	0.24
6	1	49.5	49.5	--	51.33	1.99×10^{-5} –1.00×10^{-2}	7.94×10^{-6}	0.25
7	2	49.0	49.0	--	49.09	3.98×10^{-5} – 1.00×10^{-2}	1.58×10^{-5}	1.29
8	3	48.5	48.5	--	48.13	6.30×10^{-5} – 1.00×10^{-2}	2.51×10^{-5}	0.48
9	0.5	49.70	49.70	0.10	53.68	1.99×10^{-5} – 1.00×10^{-2}	7.07×10^{-6}	1.42
10	0.5	49.65	49.65	0.20	54.78	1.99×10^{-5} – 1.00×10^{-2}	9.33×10^{-6}	0.42
11	0.5	49.60	49.60	0.30	56.31	1.99×10^{-5} – 1.00×10^{-2}	3.98×10^{-6}	0.04
12	0.5	49.55	49.55	0.40	55.26	1.99×10^{-5} – 1.00×10^{-2}	2.51×10^{-5}	0.76
	Alv-TPB/Rt electrodes							
13	0.5 : 0.5	49.5	49.5	--	57.01	3.98×10^{-5} – 1.00×10^{-2}	9.33×10^{-6}	0.34
14	0.5 : 1	45.5	45.5	--	56.63	3.98×10^{-5} – 1.00×10^{-2}	7.94×10^{-6}	0.28

RSD : relative standard deviation (four determinations)

3.1.1. Effect of ion pair concentration

The effect of membrane composition on the potentiometric response of the electrodes was investigated by varying the percentages of the ion pair, while keeping the percentages of the PVC and the plasticizer equal 1:1 (Table 1). The results showed that the electrodes made of membrane with 5% Alv-TPB (electrode no. 2), 0.5% Alv-Rt+0.3% KTPB (electrode no. 11) and 0.5% Alv-TPB:0.5% Alv-Rt (electrode no. 13) exhibit the best performance characteristics [slope 57.24, 56.31 and 57.01 mV decade^{-1} at 25±0.1°C and detection limit 4.07×10^{-6}, 3.98×10^{-6},9.33×10^{-6} M Alv], respectively. Typical calibration plots for electrodes are shown in Fig.3. Electrodes containing the above mentioned percentages of the ion pairs were used for further studies.

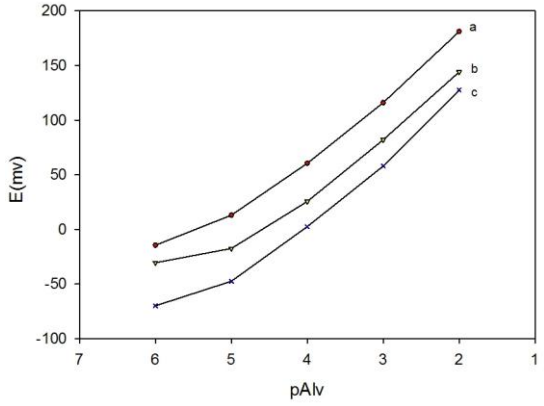


Fig. 3. Calibration curves for alverine electrodes: (a) Alv–Rt+0.3% KTPB; (b) Alv–TPB/Rt; (c) Alv–TPB

3.1.2. Effect of plasticizer

The plasticizer mainly acts as a fluidizer, allowing homogeneous dissolution and diffusion mobility of the ion-pair inside the membrane. The nature of the plasticizer must be properly controlled in order to minimize the electrical asymmetry of the membrane and to limit fouling of the sensor. The nature of the plasticizer has a marked

influence on the response slope, linear domain and also on the selectivity of the PVC membrane electrodes. The influence of the plasticizer type on the characteristics of the Alv-sensors was investigated by using four plasticizers with different polarities including DBP , DOP , DOS , and TCP as shown in Table 2. The results indicate that DBP is the best plasticizer tested. Poor sensitivities for the elecrodes plasticized using DOP, DOS and TCP are due to low solubilities or low distributions of Alv-Rt and Alv-TPB ion pairs in these solvents [21]. The electrodes using DBP as a plasticizer provide higher Nernstian slope, wide response range, more stable potential reading and lower limit of detection due to the better extraction of the drug in the organic layer of the membrane [22,23].

Table 2. Effect of the plasticizers on the alverine responsive electrodes

Composition % w/w			Slope	Linear range	LOD	*RSD*
Ion-pair	PVC	plasticizer	mV/ decade	(M)	(M)	*%*
Alv-TPB electrodes						
5	47.5	TCP	53.56	1.99×10^{-5} – 1.00×10^{-2}	4.36×10^{-6}	0.09
5	47.5	DBP	57.24	1.00×10^{-5} – 1.00×10^{-2}	4.07×10^{-6}	0.10
5	47.5	DOS	52.85	1.99×10^{-5} – 1.00×10^{-2}	4.78×10^{-6}	0.21
5	47.5	DOP	53.05	1.99×10^{-5} – 1.00×10^{-2}	4.78×10^{-6}	0.28
Alv-Rt electrodes						
0.5	49.75	TCP	44.38	1.00×10^{-5} – 1.00×10^{-2}	3.98×10^{-6}	0.96
0.5	49.75	DBP	54.56	1.99×10^{-5} – 1.00×10^{-2}	6.02×10^{-6}	0.24
0.5	49.75	DOS	46.84	1.99×10^{-5} – 1.00×10^{-2}	6.02×10^{-6}	0.44
0.5	49.75	DOP	40.82	7.94×10^{-6} – 1.00×10^{-2}	3.16×10^{-6}	0.64

3.1.3. Effect of addition of anionic additive

The main function of the addition of lipophilic anions in cation selective membrane elecrodes is to introduce perm selectivity and to improve the selectivity of an ion sensor by decreasing the membrane resistance and alverine cation interference [24]. The influence and concentration of membrane additives was also investigated by incorporating lipophilic additives like KTPB (Table 1). As is obvious from Table 1 (electrodes no. 5 and 11), the use of 1 mg KTPB for Alv-Rt ion pair significantly improves the performance characteristics of the membrane sensor and increases the life time of the membrane sensor.

3.2. Effect of internal soluion

The potential response of the polymeric membrane electrodes for alverine cation based on Alv-Rt and Alv-TPB ion pairs were studied at different concentrations of internal solution (1.0×10^{-2} to 1.0×10^{-5} M Alv). It was found that the best results in terms of slope and working concentration range have been obtained with internal solution of concentration 1.0×10^{-4} M. Thus, 1.0×10^{-4} M concentration of the reference solution was quite appropriate for the smooth functioning of the proposed electrodes.

3.3. Effect of soaking and life time of the electrodes

Freshly prepared electrode must be soaked to activate the surface of the membrane to form an infinitesimally thin gel layer at which ion exchange occurs. This preconditioning process requires different times depending on diffusion and equilibration at the electrode test solution interface; a fast establishment of equilibrium is certainly a condition for a fast potential response [25]. For all of the electrodes, the presoaking time was 0.5 h. The lifetimes of the electrodes were determined by soaking the electrodes in 1.0×10^{-3} M alverine solution for interval ranging till the electrode lose its Nernstian behavior. This behavior established that the loss of plasticizer, ionic site from the polymeric film due to leaching into the bathing solution is a primary reason for the limited lifetimes of the electrodes. The response of the electrodes has been measured by recording the calibration graph at 25 °C at different intervals. The results indicate that, in case of electrode no. 5 during the first 2 h of soaking, the slope remains constant at about 54.56 mV concentration decade^{-1} at 25 °C, then decreased, reaching to about 49.57 mV concentration decade^{-1} after 4 h. This poor performance of electrode no. 5 may be attributed to the low lipophilicity of the Alv-Rt ion pair that negatively affects the phase equilibrium state of Alv at the immediate vicinity of the membrane surface. In case of electrode no. 2 during the first 6 days of soaking, the slope remains constant at about 57.24 mV concentration decade^{-1} at 25 °C, then slightly decreased, reaching to about 55.70 and 52.45 mV concentration decade^{-1} after 9 and 17 days, respectively then decreases reaching 47.61 after 21 days. In case of electrode no. 11 during the first 6 days, the slope remains constant at about 56.25 mV concentration decade^{-1} then slightly decreases, reaching about 53.37 mV concentration decade^{-1} after 8 days and reaching 47.16 mV

concentration decade^{-1} after 10 days. In case of electrode no. 13 during the first 2 days, the slope remains constant at about 57.01 mV concentration decade^{-1} at 25 °C, then decreases reaching 51.36 and 46.73 after 5 and 7 days, respectively.

Table 3. Response characteristics for different alverine- plastic membrane electrodes

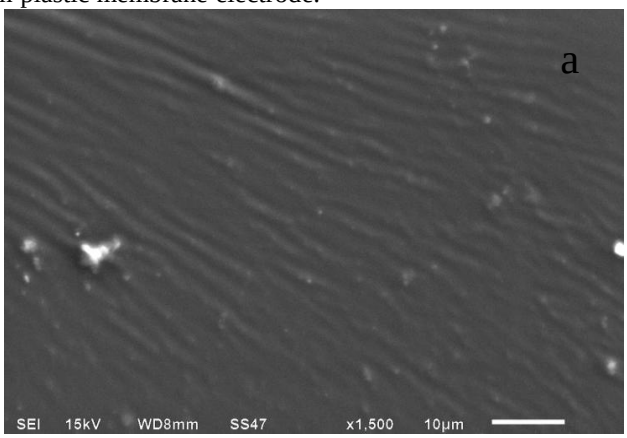
Parameters	Alv-TPB	Alv-Rt+0.3% KTPB	Alv-TPB/ Rt
Electrode-composition (w/w)% (ion-pair/PVC/plasticizer)	(5/ 47.50/ 47.50 DBP)	(0.5/ 49.60/ 49.60 DBP)	(0.5:0.5/ 49.5/ 49.5 DBP)
Slope (mV/decade)	57.24	56.31	57.01
Limit of detection (M)	4.07×10^{-6}	3.98×10^{-6}	9.90×10^{-6}
Limit of quantitation (M)	1.35×10^{-5}	1.32×10^{-5}	3.29×10^{-5}
Linear range (M)	1.00×10^{-5}-1.00×10^{-2}	1.99×10^{-5} – 1.00×10^{-2}	1.00×10^{-5} – 1.00×10^{-2}
Correlation coefficient (r^2)	0.9996	0.9994	0.9998
Response time (s)	8-10	10-12	10-13
Life span (days)	17	8	6

3. 4. Regeneration of the electrodes

The previously mentioned discussion reveals that soaking of the electrodes in the alverine solution for a long time has a negative effect on the response of the membrane. The same effect appears after working with electrodes for a long time. The regeneration of the electrodes was tried simply by reformation of the ion-pairs on the external gel layer of membrane as reported previously [25]. The regeneration of the alverine membrane was successfully achieved by soaking the expired electrodes for 24 h in a solution that was $1.0\text{x}10^{-2}$ M sodium tetraphenylborate, ammonium reineckate or a mixture of them, followed by soaking for 3 h in $1.0\text{x}10^{-2}$ M alverine. The exhausted electrodes showed a Nernstian slopes 47.61, 47.16 and 46.73 mV decade^{-1} for electrodes no.2, 11 and 13, respectively, and for the same electrodes after regeneration the slopes were 54.35, 52.17 and 51.46 mV decade^{-1}, respectively. It was found that the lifespan of the regenerated electrodes is limited to 4 h due to the ease of leaching of the lipophilic salts from the gel layer at the surface of the electrode compared with those that are attached homogeneously to the PVC network through the solvent mediator.

3.5. Effect of soaking on the morphology of the membrane's surface

The electron microscopy images of surfaces of fresh and expired electrodes were obtained by tracing the secondary electrons emitted from the membrane surface. Drastic changes in the morphology of the surfaces which can be attributed to prolonged soaking of the electrodes have been observed. Images for a membrane including Alv-Rt ion pair is taken as a representative (Fig. 4). These morphological changes are ascribed to some sort of solvent/gel layer interaction leading ultimately to shrinking of the polymeric network [Fig. 4b]. The harm of these surface changes is that they generate unequal strains at the deformed areas and consequently produce asymmetry potentials [26]. These potentials interfere with the phase boundary equilibrium of Alv at the membrane surface, and negatively affect the performance of the electrode. This study was examined recently [27] for tetradecyltrimethylammonium plastic membrane electrode.



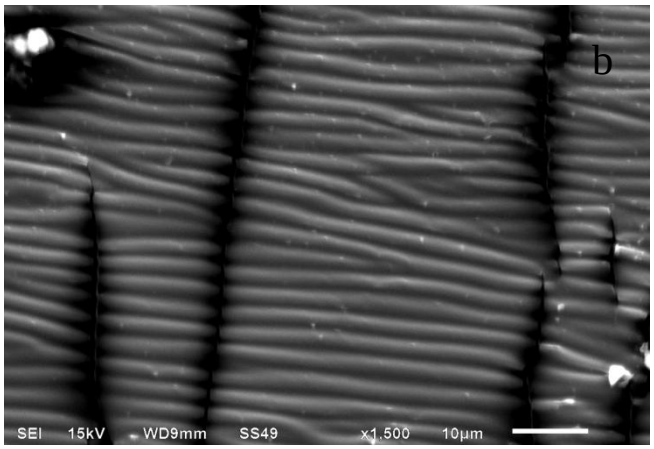


Fig. 4. High-resolution electron micrographs of a membrane surfaces containing Alv-Rt ion pair. (a) Fresh membrane (1500-fold magnification), and (b) expired membrane (3500-fold magnification)

3.6. Response time of the electrode

The conventional IUPAC definition of response time for ISE is the time which elapses between the instant when an ISE and a reference electrode are brought into contact with the sample solution [28]. The response time obviously is dependent on concentration change. In this study, practical response time of the proposed electrodes was examined by recording the potential readings at time intervals of 10 s over 1 min. The relation between potential reading and response time was plotted for 5.0×10^{-5}-10^{-2} M Alv. The required time for the electrodes to reach values within ±1 mV of the final equilibrium potential was less than 10 s as shown in Fig. 5.

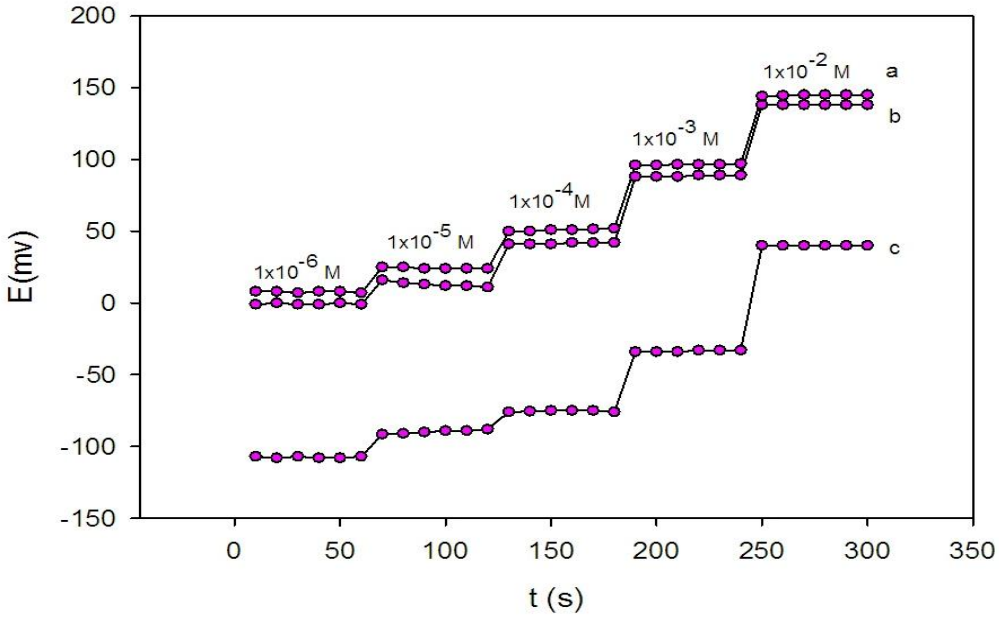


Fig. 5. Dynamic response time for alverine electrodes: (a) Alv–Rt +0.3% KTPB; (b) Alv-TPB/Rt; (c) Alv-TPB

3.7. Effect of pH and electrolytes

The effect of pH on the electrode potential was investigated by recording the e.m.f values of the drug-selective electrode in 10^{-3} and 10^{-4} M alverine solution. The pH of this solution was adjusted by introducing very small drops of hydrochloric acid solution (0.10-1.0 M) and/or sodium hydroxide solution (0.10-1.0 M). Representative curves for the Alv electrodes are shown in Fig. 6. The results showed that the potential of the electrode remains constant in

pH ranges 1.48–7.56, 1.52–7.97 and 1.56–7.82 for electrodes no. 2, 11 and 13, respectively. In these ranges, the electrodes can be applied for the determination of alverine. The considerable decrease of the potential observed at pH levels higher than 7.56, 7.97 or 7.82 probably originating from the precipitation of the free basic drug.The electrodes response was checked in bidistilled water, 0.04 M Britton Robinson buffer pH 4.07, 0.1 M acetate buffer pH 4.00 and 0.1 M phathalate buffer pH 4.00 at 25 **ºC**. The results indicate that, for samples containing bufferes, the electrodes exhibited low Nernstian slopes and unstable potential reading. However, the best results were obtained in bidistilled water.

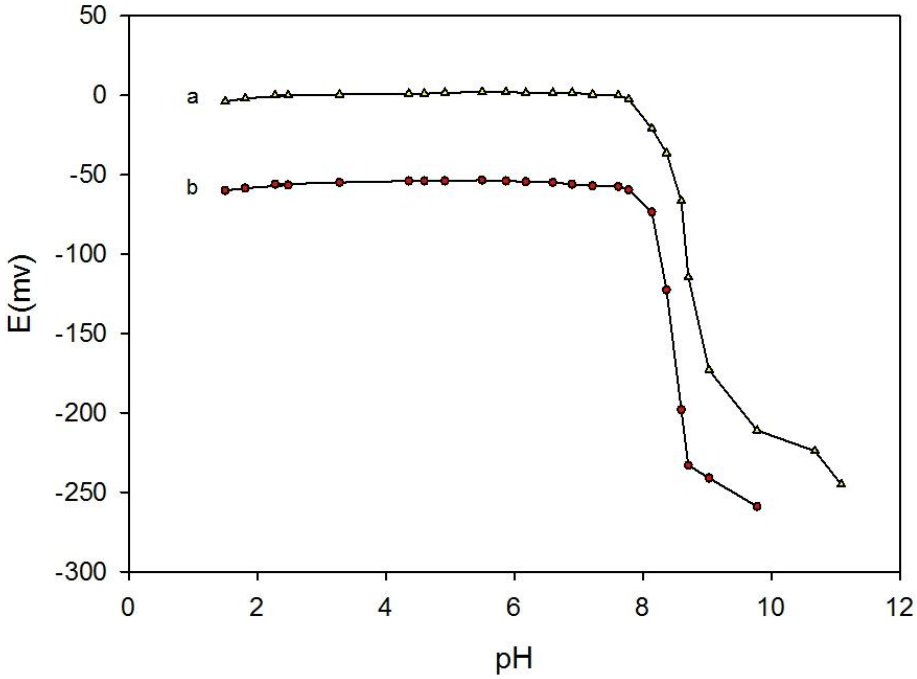


Fig. 6. Effect of pH of the test solution on the potential response of the Alv-TPB electrode: (a) 1.0 x 10^{-3} M Alv; (b) 1.0 x 10^{-4} M Alv

3.8. Selectivity

Selectivity, which describes an ion-selective electrode's specificity toward the target ion in the presence of some inorganic cations, sugars, amino acids, vitamins and urea, is the most important characteristic of these devices. The selectivity of an ion-pair based membrane electrode depends on the physico-chemical characteristics of the ion-exchange process at the membrane. For example, sample solution interface, mobility of the respective ions in the matrix of the membrane and on the hydrophobic interactions between the primary ions and the PVC membrane [29]. The selectivity of the alverine membrane electrode is related to the free energy of transfer of the alverine cation between aqueous and membrane phases.

The potentiometric selectivity coefficients, which reflect the relative response of the membrane sensor for the primary ion over the interfering species that present in solution were determined using two methods, the separate solution method (SSM) [30] in case of inorganic cations and the matched potential method (MPM) [31] in case of other neutral species. In the separate solution method, the Nicolsky Eisenman equation [32] was used:

$$\log K^{pot}_{Alv,J^{z+}} = (E2 - E1)/S + \log[Alv] - \log[J^{z+}]^{1/z}$$

where E_1 and E_2 are the electrode potentials in a 1.0×10^{-3} M solutions of Alverine and J^{Z+} interfering ions, respectively, and S is the slope of the calibration graphs in mV concentration decade^{-1}.

In the matched potential method, the selectivity coefficient was determined by measuring the change in potential upon increasing the primary ion activity from an initial value of a_A to $á_A$ and a_B represents the activity of interfering ion added to the reference solution of primary ion of activity a_A which also brings the same potential change. It is given by expression:

$$K^{pot}_{A,\mathbf{B}} = (á_A - a_A) / a_B$$

In the present studies a_A and $á_A$ were kept at 1.0×10^{-4} and 1.19×10^{-4} M alverine and a_B was experimentally determined. None of the investigated species interferes, as shown by the very small values of the selectivity coefficient (Table 4). This reflects a very high selectivity of the investigated electrodes towards Alv. The inorganic cations do not interfere because of the difference in their mobility and permeability as compared to alverine cation. In case of sugars and amino acids, the high selectivity is related to the difference in polarity and lipophilic nature of their molecules relative to alverine cation.

Table 4. Selectivity coefficient values of the alverine- plastic membrane electrodes

	$K^{pot}_{Alv,J^{z+}}$					
	Alv-TPB		Alv-Rt+ 0.3% KTPB		Alv-TPB/ Rt	
Interferent	SSM	MPM	SSM	MPM	SSM	MPM
Na^+	5.99×10^{-3}	----	4.85×10^{-4}	----	5.92×10^{-3}	----
K^+	1.43×10^{-2}	----	2.74×10^{-3}	----	2.52×10^{-2}	----
NH_4^+	1.15×10^{-2}	----	4.46×10^{-3}	----	9.73×10^{-3}	----
Li^+	4.22×10^{-3}	----	2.10×10^{-3}	----	5.76×10^{-2}	----
Fe^{2+}	5.38×10^{-4}	----	7.28×10^{-4}	----	1.36×10^{-3}	----
Ca^{2+}	5.87×10^{-4}	----	1.13×10^{-4}	----	8.65×10^{-4}	----
Mg^{2+}	1.02×10^{-5}	----	1.76×10^{-4}	----	2.30×10^{-4}	----
Mn^{2+}	7.30×10^{-4}	----	6.10×10^{-4}	----	2.53×10^{-3}	----
Cu^{2+}	7.60×10^{-3}	----	1.54×10^{-3}	----	2.06×10^{-3}	----
Co^{2+}	6.71×10^{-4}	----	1.61×10^{-3}	----	1.82×10^{-3}	----
Vitamine B1	----	4.25×10^{-2}	----	1.25×10^{-2}	----	5.53×10^{-2}
Vitamine B6	----	2.69×10^{-2}	----	6.11×10^{-2}	----	4.68×10^{-2}
Glucose	----	1.41×10^{-3}	----	1.36×10^{-3}	----	5.63×10^{-4}
Fructose	----	1.60×10^{-3}	----	1.30×10^{-3}	----	9.57×10^{-4}
Lactose	----	1.51×10^{-3}	----	1.17×10^{-3}	----	9.03×10^{-4}
Maltose	----	1.09×10^{-3}	----	1.44×10^{-3}	----	8.35×10^{-4}
Urea	----	1.18×10^{-3}	----	1.47×10^{-3}	----	8.56×10^{-4}
Glycine	----	1.13×10^{-3}	----	1.41×10^{-3}	----	9.29×10^{-4}
β-alanine	----	1.46×10^{-3}	----	3.2×10^{-3}	----	1.43×10^{-3}

3.9. Effect of temperature of the test solution

For studying the effect of temperature on the response of electrodes utilizied, the potential of 1.0×10^{-6} - 1.0×10^{-2} M drug solution were measured at (25, 35, 45, 55°C) and the calibration curves were constructed. The standard electrode potentials (E°_{elec}) were obtained from the calibration curves, corresponding to each temperature. It is observed that the electrodes gave a high response in the temperature range 25-55 °C; the isothermal coefficients values were calculated amounting to 0.643, 0.663 and 0.525 mV/°C for electrode no. 2, 11 and 13, respectively. This confirms a fairly high thermal stability of the examined electrodes.

3.10. Analytical application

The accuracy and applicability of the proposed electrodes were evaluated by its application for the determination of alverine citrate in pure solutions and in pharmaceutical preparations (Meteospasmyl capsules) by both the standard addition and the potentiometric titration methods . Representive potentiometric titration curves are shown in Figs. 7 and 8. The obtained average recovery and relative standard deviation values are summarized in (Tables 5, 6), which reflect the high accuracy and precision of the electrodes. Determination of alverine citrate in spiked urine and plasma samples was also carried at four different levels of concentration using the standard addition technique (Tables 7, 8). The proposed electrodes can therefore be applied to the determination of Alv citrate in pure solutions, in pharmaceutical preparations, in spiked urine and plasma samples without fear of interference caused by the excipients expected to be present in tablets or in the consitituents of the body fluids. The results obtained from the standard addition method of the drug were compared with those obtained from the potentiometric titration method by applying F-and t-tests [33]. The results (Table 9), show that the calculated F- and t-values did not exceed the theoretical values, reflecting the accuracy and precision of the applied method.

For ruggedness of the method a comparison was performed between the intra- and inter-day assay results for alverine obtained by two Ph.D. students. The RSD values for the intra- and inter-day assays of alverine in the cited formulations performed in the same laboratory by the two analysts did not exceed 2.17%. On the other hand, the robustness was examined while the parameter values (pH of the eluent and the laboratory temperature) were being

deliberatly slightly changed. Alverine recovery percentages were good under most conditions, not showing any significant change when the critical parameters were modified.

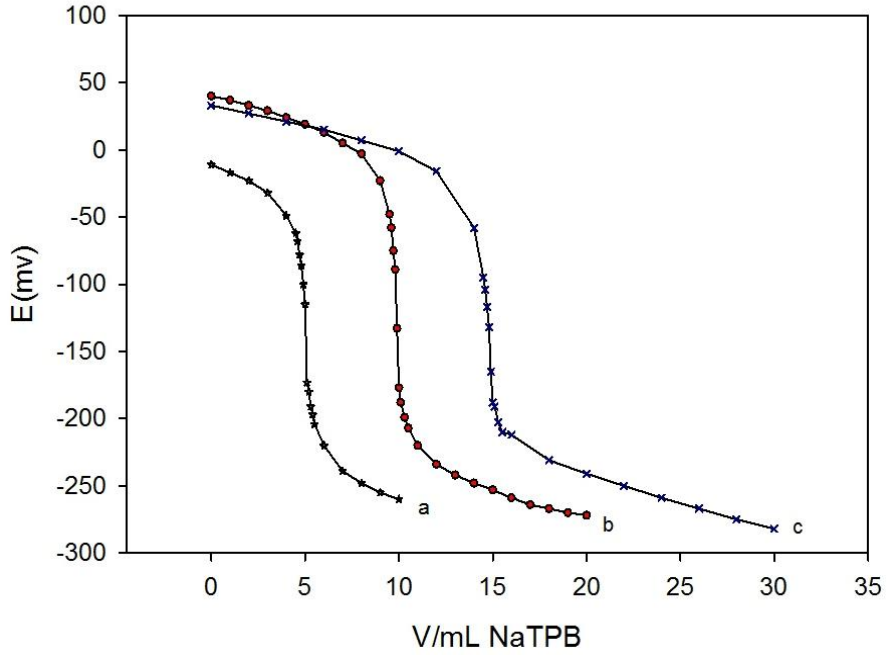


Fig. 7. Potentiometric titration curves of (a) 5, (b) 10 and (c) 15 mL of 10^{-2} M Alv using Alv-Rt+ 0.3% KTPB electrode and 10^{-2} M NaTPB as titrant

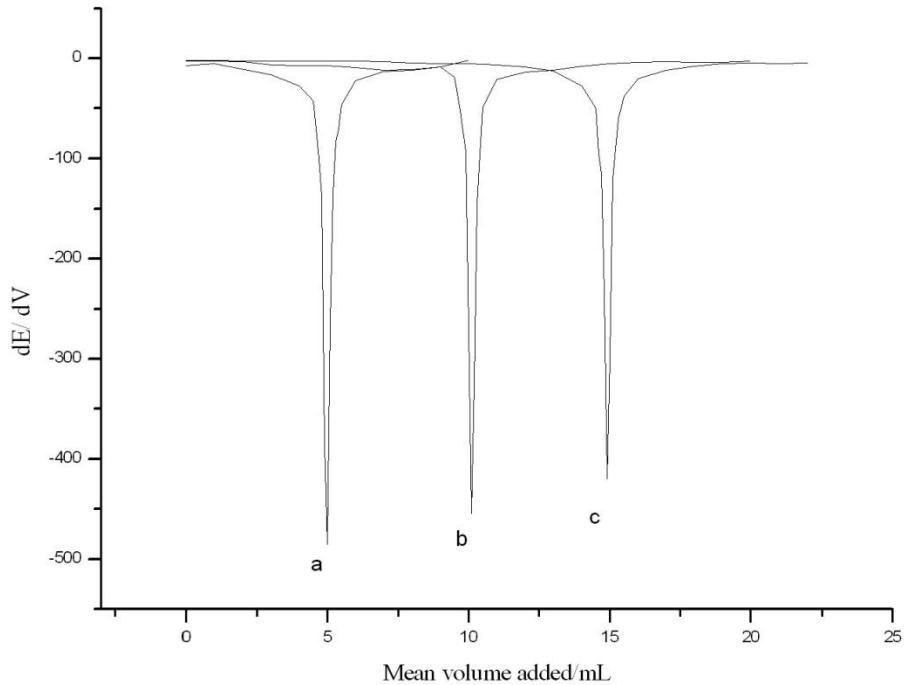


Fig. 8. Differential curves for potentiometric determination of (a) 5, (b) 10 and (c) 15 mL 10^{-2} M Alv against using Alv-TPB/Rt electrode and 10^{-2} M NaTPB as titrant

Table 5. Determination of alverine citrate in pure solutions and pharmaceutical preparations applying the standard addition method

Sample	Taken (mg)	Found (mg)	Recovery (%)	RSD (%)
Alv-TPB electrode				
Pure solutions				
	1.184	1.172	99.48	1.07
	1.894	1.895	100.17	1.98
	2.368	2.405	101.61	0.81
	4.736	4.643	98.05	1.17
Meteospasmyl (60 mg/capsule)				
	1.184	1.20	101.36	0.94
	1.894	1.892	99.91	1.19
	2.368	2.318	97.93	1.27
	4.736	4.733	99.95	1.82
Alv-RT+ 0.3% KTPB electrode				
Pure solutions				
	1.184	1.206	101.91	2.05
	1.894	1.911	100.90	1.64
	2.368	2.384	100.71	0.41
	4.736	4.726	99.79	0.21
Meteospasmyl (60 mg/capsule)				
	1.184	1.208	102.08	1.32
	1.894	1.899	100.29	1.33
	2.368	2.341	98.86	0.28
	4.736	4.716	99.59	0.90
Alv-TPB/Rt electrode				
Pure solutions				
	1.184	1.176	99.33	0.33
	1.894	1.858	98.07	2.01
	2.368	2.321	98.04	0.21
	4.736	4.631	97.80	1.31
Meteospasmyl (60 mg/capsule)				
	1.184	1.185	100.11	0.17
	1.894	1.891	99.78	0.91
	2.368	2.311	97.61	0.59
	4.736	4.601	97.16	0.64

Table 6. Determination of alverine citrate in pure solutions and pharmaceutical preparations applying the potentiometric titration method

Sample	Taken (mg)	Found (mg)	Recovery (%)	RSD (%)
Alv-TPB electrode				
Pure solutions				
	23.68	23.44	99.00	0.12
	47.36	47.36	100.00	0.31
	71.04	70.80	99.66	0.95
Meteospasmyl (60 mg/capsule)				
	23.68	22.96	96.95	0.74
	47.36	47.59	100.50	017
	71.04	70.32	99.00	1.26
Alv-RT+ 0.3% KTPB electrode				
Pure solutions				
	23.68	23.44	99.00	1.13
	47.36	46.88	98.98	0.59
	71.04	70.32	99.00	0.24

Meteospasmyl (60 mg/capsule)				
	23.68	23.91	100.97	1.06
	47.36	46.64	98.47	0.23
	71.04	70.80	99.66	0.85
Alv-TPB/Rt electrode				
Pure solutions				
	23.68	23.44	99.00	0.86
	47.36	46.64	98.50	1.36
	71.04	70.80	99.66	0.47
Meteospasmyl (60 mg/capsule)				
	23.68	23.91	101.00	0.27
	47.36	46.64	98.50	0.94
	71.04	69.85	98.3	1.76

Table 7. Determination of alverine citrate in spiked urine samples applying the standard addition method

Sample	Taken (mg)	Found (mg)	Recovery (%)	RSD (%)
Alv-TPB electrode				
	1.184	1.153	97.84	2.08
	1.894	1.881	99.39	0.84
	2.368	2.386	100.84	0.76
	4.736	4.743	100.18	0.91
Alv-RT+ 0.3% KTPB electrode				
	1.184	1.196	101.08	0.89
	1.894	1.913	100.98	0.98
	2.368	2.357	99.55	0.26
	4.736	4.784	101.03	0.84
Alv-TPB/Rt electrode				
	1.184	1.197	101.13	0.27
	1.894	1.915	101.12	0.26
	2.368	2.368	100.04	0.21
	4.736	4.705	99.39	0.91

Table 8. Determination of alverine citrate in plasma samples applying the standard addition method

Sample	Taken (mg)	Found (mg)	Recovery (%)	RSD (%)
Alv-TPB electrode				
	1.184	1.185	100.15	1.62
	1.894	1.854	97.90	2.12
	2.368	2.331	98.45	0.36
	4.736	4.684	98.90	1.94
Alv-RT+ 0.3% KTPB electrode				
	1.184	1.172	99.02	2.17
	1.894	1.900	100.33	0.37
	2.368	2.376	100.35	0.55
	4.736	4.611	97.37	0.24
Alv-TPB/Rt electrode				
	1.184	1.167	98.56	0.51
	1.894	1.913	101.02	0.97
	2.368	2.311	97.61	0.59
	4.736	4.600	97.13	1.99

Table 9. Statistical comparison between the results of an analysis of a pharmaceutical preparation Meteospasmyl capsules applying the standard addition and potentiometric titration methods

Parameters	standard addition method	potentiometric titration method
Alv-TPB electrode		
Mean recovery (%)	99.78 [a]	98.81 [b]
SD	1.410	1.782
RSD (%)	1.413	1.803
F-ratio	2.20 (9.55)[c]	
t-test	0.86 (2.57)[d]	
Alv-RT+ 0.3% KTPB electrode		
Mean recovery (%)	100.20 [a]	99.70 [b]
SD	1.379	1.250
RSD (%)	1.376	1.254
F-ratio	1.21 (19.20)[c]	
t-test	0.49 (2.57)[d]	
Alv-TPB/Rt electrode		
Mean recovery (%)	98.66	99.26
SD	1.495	1.504
RSD (%)	1.515	1.515
F-ratio	1.006 (9.55) [c]	
t-test	0.52 (2.57) [d]	

a: Average of four determinations
b: Average of three determinations
SD: standard deviation
RSD: relative standard deviation
c: Tabulated F-value at 95% confidence level
d:Tabulated t-value at 95% confidence level and six degrees of freedom

4. Conclusion

The proposed liquid membrane selective electrodes based on the Alv-TPB and Alv-Rt as sensing elements can be used as useful analytical tools for determination of alverine in vitro and in vivo. The lower values of the detection limits of these electrodes were found to be 4.07×10^{-6}, 3.98×10^{-6}, 9.33×10^{-6} M Alv for Alv-TPB, Alv-Rt, Alv-TPB/Rt electrodes, respectively. In addition, these electrodes showed a very good selectivity to alverine in the presence of many inorganic cations, sugars and amino acids. Therefore, these electrodes can be used as alternative analytical tools to chromatographic and spectrophotometric techniques, for the determination of this drug in pure solutions, pharmaceutical preparations and biological fluids (urine and plasma).

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