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Novel Polymeric Membrane and Coated Wire Electrodes for Determination of Alverine Citrate in Pharmaceutical Formulations and Biological Fluids

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Selective and sensitive alverine electrodes have been fabricated by constructing a polymeric membrane (PME) and coated wire (CWE) electrodes. These are based on the ion-pair between phosphotungstic acid (PME_1, CWE_1) and phosphomolybdic acid (PME_2, CWE_2) in a poly (vinyl chloride) matrix, plasticized with DBP. The influence of membrane composition and pH on the potentiometric responses of electrodes was investigated. The response characteristics of the membrane were compared with polymeric membrane electrode (PME) as well as with coated wire electrode (CWE). The electrodes exhibit Nernstian slope of 59.1±0.4, 59.3±0.6, 59.2±1.1 and 59.5±1.1 mV decade^{-1} with limits of detection of 7.6×10^{-6}, 8.3×10^{-6}, 6.6×10^{-6} and 8.1×10^{-6} mol L^{-1} alverine citrate for PME_1, PME_2, CWE_1 and CWE_2, respectively. Furthermore, the electrodes generated constant potentials in the pH range of 1.44-8.05 and 1.42-8.31 for PME_1 and PME_2, respectively. Selectivity coefficients for alverine citrate relative to numbers of potential interfering substances were investigated. The electrodes were highly selective for alverine over a large number of similar compounds. The proposed electrodes displayed useful analytical characteristics for the determination of alverine citrate in bulk powder, pharmaceutical formulation, and biological fluids (urine and plasma). The practical utility of the proposed electrodes has also been demonstrated by thier usage as indicator electrodes in potentiometric titration of phosphotungstic acid with alverine cation solution.

Keywords: Alverine citrate; potentiometry, polymeric membrane electrode; coated wire electrode; biological fluids.

1. INTRODUCTION

Alverine citrate, N-methyl-N-(3-phenylpropyl) benzenepropanamine citrate, is a commonly used smooth muscle relaxant agent [1]. It is able to inhibit the spontaneous contractions and the

nervous control of rabbit proximal colon [2], and induce the hepatitis [3]. Hayase et al. [4] 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 alverine citrate to treat detrusor smooth muscle dysfunction. It has been used in the treatment of irritable bowel syndrome [4]. It decreases the sensitivity of the intestinal mechanoreceptors in response to chemical stimulation in anaesthetized cats [5] and reduces 5-hydroxytryptamine 1A receptor-mediatd rectal hypersensitivity in the rat [6]. Recently, this drug has been determined by liquid chromatography tandem mass spectrometry [7,8]. However, this method requires prior derivatization or extraction step, and involves the use of expensive equipment. Selective analytical methodologies, which are easily operated and involve harmless reagents, cost effective equipment, have therefore been proposed as alternative to standard methods. Potentiometric sensors based on ion-selective electrodes are especially suited for such determination because they offer advantages such as selectivity, sensitivity, good precision, simplicity and low cost [9], wide linear range, simple instrumentation, relatively fast response, rapid determination of a variety of ions in different types of sample, and applicability to colored and turbid solutions providing possible interfacing with automated and computerized systems. These characteristics have inevitably led to the preparation of numerous sensors for several ionic species, and the list of available electrodes has grown substantially over the last few years [10-15]. Various types of electrodes have been suggested for determination of ionic species, polymeric membrane and coated wire electrodes. The size of polymeric membrane ISEs can be reduced by eliminating the internal reference solution in construction of coated wire electrodes (CWEs) involving miniaturization of ISE [16,17]. The aim of the present work is to devise the polymeric membrane electrode (PME) and coated wire electrode (CWE) as alverine selective electrodes based on two ion-pairs between phosphotungstic acid (Alv-PT) and phosphomolybdic acid (Alv-PM) as an electroactive material and dibutyl phthalate (DBP) as an anion excluder in PVC matrix.

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. Phosphotungstic acid (PT), Phosphomolybdic acid (PM), poly (vinyl chloride) of high molecular weight (PVC), dioctyl sebacate (DOS), and tricresyl phosphate (TCP) were obtained from Fluka (USA). Tetrahydrofuran (THF), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) were purchased from Merck (Germany). The metal salts were provided by BDH company (UK) 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 Regional Blood Transfusion Center, Beni-Suef, Egypt and used within 24 h.

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 systems may be represented as follow:

Ag/AgCl//internal solution/membrane/test solution//SCE, and

Graphite rod or silver wire//membrane/test solution//SCE

2.3. Preparation of the ion pair

The ion-pairs, Alv-PT and Alv-PM were prepared by mixing 150 mL 10^{-2} mol L^{-1} alverine citrate solution with 50 mL of 10^{-2} mol L^{-1} of phosphotungstic acid or phosphomolybdic acid. 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 3:1 both in case of Alv-PT and Alv-PM as confirmed by elemental analysis data.

2.4. Electrode preparation

2.4.1. Polymeric membrane electrode

The electrode was constructed as described previously [18]. 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 and 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} mol L^{-1} NaCl and 1×10^{-4} mol L^{-1} alverine citrate. The electrode was preconditioned before use by soaking in a 1.0×10^{-3} mol L^{-1} alverine citrate solution for 30 min.

2.4.2. Coated wire electrode

A pure graphite rod (4 mm diameter) or silver wire (1 mm diameter) was insulated by tight polyethylene tube, leaving 1 cm at one end for coating and 1 cm at the other end for connection. The end of polished wires were momentarily dipped in the coating solution (a solution of the same optimum membrane composition used for the conventional type) and allowed to dry in air for about 1 min to make a film of the coating material. The process was repeated until a membrane film nearly 1 mm thick was formed (about 8 times). The prepared electrode was preconditioned by soaking for 30 min in 10^{-3} mol L^{-1} alverine citrate solution.

2.5. Electrodes calibration

The conditioned electrodes were calibrated by separately transferring 50 mL aliquots of solutions (10^{-6} to 10^{-2} mol L^{-1}) of alverine citrate 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 stabilizing to ±1 mV, and the potential was plotted as a function of the negative logarithm of Alv concentration.

2.6. Selectivity

The modified separate solution and the matched potential methods (MPM) [19-21] were employed to determine the selectivity coefficients, $K_{Alv,j}^{pot}$, of the potentiometric sensors towards different species. In the modified separate solution method, the potential of a cell comprising a working electrode and a reference electrode is measured in two separate solutions, where, E_1 is the potential measured in 1×10^{-3} mol L^{-1} alverine citrate, E_2 the potential measured in 1×10^{-3} mol L^{-1} of the interfering compound, z is the charge of interfering specie and S is slope of the electrode calibration plot. The selectivity coefficients were determined by the modified separate solution method using the rearranged Nicolsky equation:

$$K_{Alv,j}^{pot} = (E_2 - E_1)/S + \log[Alv] - \log[J^{z+}]^{1/z}$$

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 the expression:

$$K_{A,B}^{pot} = (á_A - a_A) / a_B$$

In the present studies a_A and $á_A$ were kept at 1.0×10^{-4} and 1.19×10^{-4} mol L^{-1} Alv citrate and a_B was experimentally determined.

2.7. Potentiometric Determination of alverine citrate

Alverine citrate has been determined potentiometrically using the investigated electrodes by the standard addition method [22] and by potentiometric titration with a standard solution of PTA.

2 8. Determination of alverine 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 alverine citrate.

2.9. Determination of alverine citrate in spiked urine and plasma samples

Different amounts of alverine 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 alverine citrate by the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Optimization of membrane composition

The potential response of the ion-selective electrode obtained for a given membrane depends significantly on the incorporation of additional membrane components. Therefore, the variation in potential with different amounts of ion-pairs was examined as shown in Table 1. In this case of carrier type ion-selective electrodes, the extraction equilibrium in the vicinity of the interface between the membrane and aqueous layer affects the potentiometric response of membrane. A carrier content of 12% Alv-PT (electrode No. 4) (PME_1) and 9% Alv-PM (electrode No. 11) (PME_2) [slope 59.1±0.4 and 59.3±0.6 mV decade^{-1} at 25±0.1°C and detection limit 7.6×10^{-6}, 8.3×10^{-6} mol L^{-1} alverine citrate, respectively] were chosen as the optimum ion-pair concentration, because the surface conditions of the PVC membrane deteriorated on decreasing and increasing the carrier content (Fig. 1a, b). The electrode Nos. 1, 2, 3, 5, 9, 10, and 12 showed a potentiometric response with slopes of 54.1, 54.5, 58.5, 57.6, 56.8, 57.6 and 57.3 mV decade^{-1}, respectively.

Table 1. Optimization of membrane compositions and their potentiometric response for polymeric membrane alverine selective electrodes

Sensors No.	Composition of membrane% (w/w; mg)				Slope mV/decade	Linear concentration range (mol L^{-1})	LOD (mol L^{-1})	LOQ (mol L^{-1})	RSD %
	Alv-PT	Alv-PM	PVC	Plasticizer					
1	5	-	47.5	47.5 DBP	54.07	1.00×10^{-5} –1.00×10^{-2}	5.12×10^{-6}	1.70×10^{-5}	0.45
2	7	-	46.5	46.5	54.47	5.00×10^{-5} – 1.00×10^{-2}	7.76×10^{-6}	2.58×10^{-5}	1.98
3	9	-	45.5	45.5	58.53	1.00×10^{-5} – 1.00×10^{-2}	7.24×10^{-6}	2.41×10^{-5}	0.85
4	12	-	44.0	44.0	59.08	1.00×10^{-5} –1.00×10^{-2}	7.58×10^{-6}	2.52×10^{-5}	0.41
5	15	-	42.5	42.5	57.61	1.00×10^{-5} –1.00×10^{-2}	9.33×10^{-6}	3.11×10^{-5}	0.83
6	12	-	45.5	44.0 DOP	21.15	5.01×10^{-5} –1.00×10^{-2}	1.86×10^{-5}	6.19×10^{-5}	2.57
7	12	-	45.5	44.0 DOS	30.80	5.01×10^{-5} –1.00×10^{-2}	7.76×10^{-6}	2.58×10^{-5}	0.68
8	12	-	45.5	44.0 TCP	27.66	1.00×10^{-5} –1.00×10^{-2}	7.41×10^{-6}	2.46×10^{-5}	2.45
9	-	5	47.5	47.5 DBP	56.80	5.00×10^{-5} –1.00×10^{-2}	1.99×10^{-5}	6.62×10^{-5}	1.77
10	-	7	46.5	46.5	57.58	1.00×10^{-5} –1.00×10^{-2}	7.94×10^{-6}	2.64×10^{-5}	0.42
11	-	9	45.5	45.5	59.34	1.00×10^{-5} –1.00×10^{-2}	8.31×10^{-6}	2.76×10^{-5}	0.64
12	-	12	44.0	44.0	57.27	1.00×10^{-5} –1.00×10^{-2}	9.12×10^{-6}	3.03×10^{-5}	2.32
13	-	9	46.5	44.5 DOP	26.25	1.00×10^{-5} –1.00×10^{-2}	7.94×10^{-6}	2.64×10^{-5}	2.13
14	-	9	46.5	45.5 DOS	43.77	5.00×10^{-5} –1.00×10^{-2}	2.48×10^{-5}	8.25×10^{-5}	1.66
15	-	9	46.5	45.5 TCP	38.53	5.00×10^{-5} –1.00×10^{-2}	1.31×10^{-5}	4.36×10^{-5}	2.75

LOD: limit of detection
LOQ: Limit of quantitation
RSD: Relative standard deviation (four determinations)

Table 2. Optimization of membrane compositions and their potentiometric response for coated wire alverine selective electrodes

Sensors No.	Composition of membrane% (w/w; mg)					Slope mV/decade	Linear concentration range (mol L^{-1})	LOD (mol L^{-1})	LOQ (mol L^{-1})	RSD %
	Alv-PT	Alv-PM	PVC	DBP	Electrode bed					
16	12	-	44.0	44.0	Graphite	59.27	1.00×10^{-5} –1.00×10^{-2}	6.65×10^{-6}	2.21×10^{-5}	1.14
17	12	-	44.0	44.0	Silver	57.58	5.00×10^{-5} –1.00×10^{-2}	1.31×10^{-5}	4.36×10^{-5}	2.48
18	12	-	44.0	44.0	Platinum	61.59	1.00×10^{-5} –1.00×10^{-2}	9.54×10^{-6}	3.17×10^{-5}	2.56
19	12	-	44.0	44.0	Copper	54.85	5.00×10^{-5} –1.00×10^{-2}	1.20×10^{-5}	3.99×10^{-5}	2.41
20	-	9	45.5	45.5	Graphite	56.36	5.00×10^{-5} –1.00×10^{-2}	3.54×10^{-5}	1.17×10^{-4}	2.21
21	-	9	45.5	45.5	Silver	59.52	1.00×10^{-5} –1.00×10^{-2}	8.12×10^{-6}	2.70×10^{-5}	1.13
22	-	9	45.5	45.5	Platinum	57.13	5.00×10^{-5} –1.00×10^{-2}	1.65×10^{-5}	5.49×10^{-5}	1.07
23	-	9	45.5	45.5	Copper	62.85	5.00×10^{-5} –1.00×10^{-2}	1.86×10^{-5}	6.19×10^{-5}	1.53

However, they still have sufficient sensitivity with good linear range and can thus be used for the determination of alverine in solution. Since PME_1 and PME_2 have the highest sensitivity among all studied compositions, it was studied in detail as alverine selective electrodes and all further investigations were carried out with these particular membranes. Furthermore, these membrane compositions were used for the preparation of the coated wire electrodes.

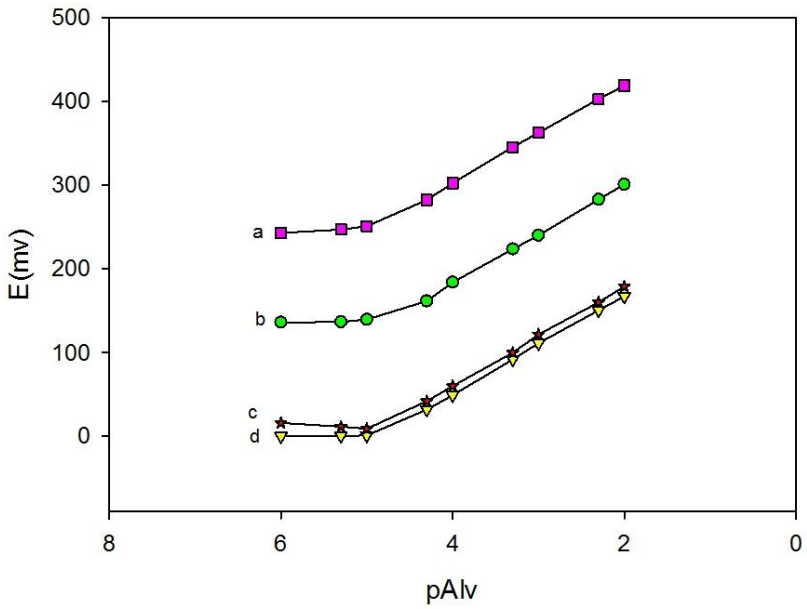

Figure 1. Calibration curves for alverine electrodes: (a) CWE_1; (b) CWE_2; (c) PME_2; (d) PME_1

A plasticizer is an important constituent and influences the detection limit, selectivity and sensitivity of the electrode [23] for it has a dual function: it acts both as a plasticizing agent, enabling the homogenous solubilization of the membrane ion-pair and modifying the distribution constant of the ion-pair used. The proportion of the plasticizer used must be optimized in order to minimize the electrical asymmetry of the membrane, to keep the sensor as clean as possible, and to stop leaching of

the active ingredients to the bathing solution [24]. Furthermore, the nature of the plasticizer affects both the dielectric constant of the membrane and the mobility of the ion-pair [25]. Therefore, the influence of the plasticizer type on the characteristics of the alverine-electrodes was investigated by using four plasticizers with different polarities including DBP, DOP, DOS, and TCP as shown in Table 1. The results indicate that DBP is the best plasticizer tested, meanwhile, poor sensitivities for the elecrodes plasticized using DOP, DOS and TCP are due to low solubilities or low distributions of Alv-PT and Alv-PM ion pairs in these solvents [26]. 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 [27,28].

The effect of the bed nature on the efficiency of the coated wire electrodes was investigated. The optimized coating mixture was used for preparation of electrodes with different conductive beds, namely silver, copper, graphite and platinum. After conditioning, each electrode was examined in the concentration range from 10^{-6}-10^{-2} mol L^{-1} alverine citrate. The dynamic range of concentration and the limit of detection of the electrodes were evaluated according to the IUPAC recommendations [29]. As is obvious from Table 2, the graphite (electrode No. 16) (CWE_1) and silver (electrode No. 21) (CWE_2) wires give the best response towards alverinium cation [slope 59.2±1.1 and 59.5±1.1 mV $decade^{-1}$ at 25±0.1°C and detection limit 6.6×10^{-6}, 8.1×10^{-6} mol L^{-1} alverine citrate, respectively] for Alv-PT and Alv-PM ion pairs, respectively (Fig. 1c, d). Therefore, graphite and silver wires were used as the inner solid contact for the electrodes in this study.

3.2. Effect of internal solution

The polymeric membrane electrodes for alverinium cation based on Alv-PT and Alv-PM ion-pairs were investigated at different concentrations of internal solution (1.0×10^{-2} to 1.0×10^{-5} mol L^{-1} alverine citrate) and the potential response of the electrodes was observed. 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} mol L^{-1}. Thus, 1.0×10^{-4} mol L^{-1} concentration of the reference solution was quite appropriate for the smooth functioning of the proposed electrodes.

3.3. Effect of soaking

The lifetime of the electrodes was determined by soaking both PME and CWE in 1.0×10^{-3} mol L^{-1} alverine citrate solution for different intervals till the electrode lost its Nernstian behaviour. This behavior is attributed to the decomposition of the ion-pair and loss of other components in the membrane phase that was in contact with aqueous test solution containing alverinium cation. The response of the electrodes has been measured by recording the calibration graph at 25°C at different intervals. The results showed that the lifetime measured in this way was found to be 14, 10, 4 and 3 days for PME_1, PME_2, CWE_1 and CWE_2, respectively. It was observed that the life-time of the CWE was shorter than that of the membrane electrode although both electrodes were prepared using the

same cocktail. This would be due to the formation of water film at the interface between the membrane and the electronic conductor and interaction of the membrane species with the graphite[30].

3.4. Response time, reversibility and reproducibility

Response time is an important factor for any ion-selective electrode. Thus, in the case of all electrodes, the average response time required for the electrodes to reach a potential response within ±1 mV of final equilibrium values after successive immersion in a series of solutions each having a 10-fold difference in concentration was measured (Fig. 2). To evaluate the reversibility of the electrode, a similar procedure in the opposite direction was adopted with measurements performed in the sequence of high-to-low sample concentrations.

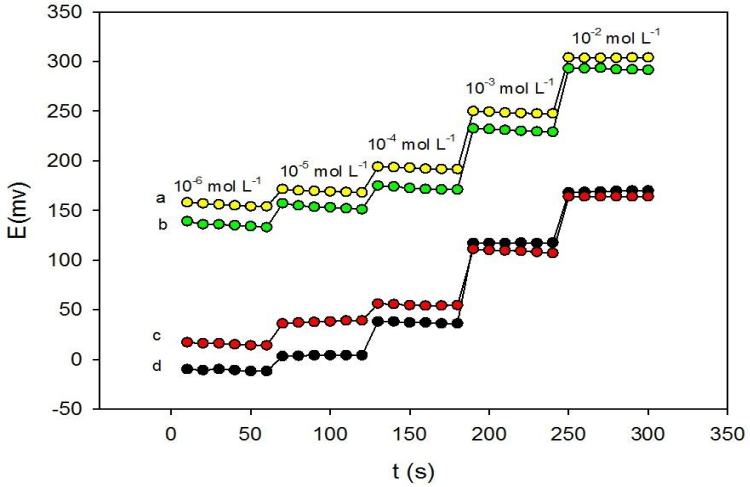

Figure 2. Dynamic response time for alverine electrodes: a) CWE_2; (b) CWE_1; (c) PME_2; (d) PME_1.

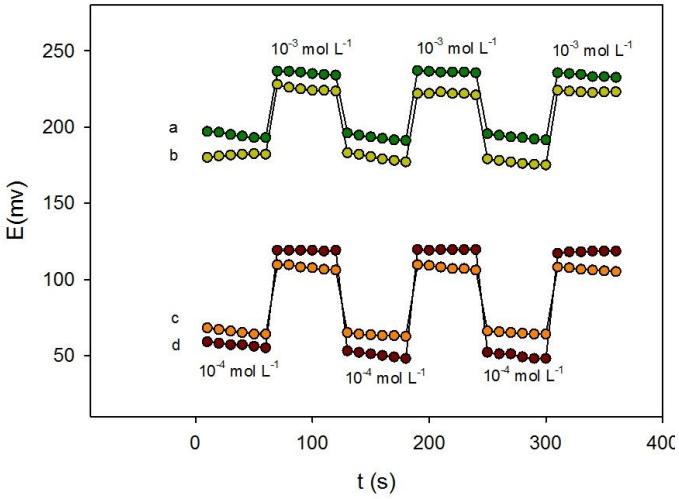

Figure 3. Dynamic response time of alverine electrodes: a) CWE_2; (b) CWE_1; (c) PME_2; (d) PME_1 for several high-to-low sample cycles.

The results, depicted in Fig. 3, clearly indicate that the potentiometric response of the electrode is reversible. The S.D. of 10 replicate measurements at 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} Alv citrate were ±0.58 and ±1.6 mV for PME_1, ±0.57 and ±1.25 mV for PME_2, ±1.25 and ±1.0 mV for CWE_1 and ±0.5 and ±1.25 mV for CWE_2, respectively. This indicates the excellent repeatability of the potential response of the electrodes.

3.5. pH dependence

Since pK_a of alverine is 10.44, therefore at pH 7.98 alverine is nearly completely in the cationic form. The concentration distribution diagram for alverine citrate species is constructed using SPECIES program [31] (Fig. 4).

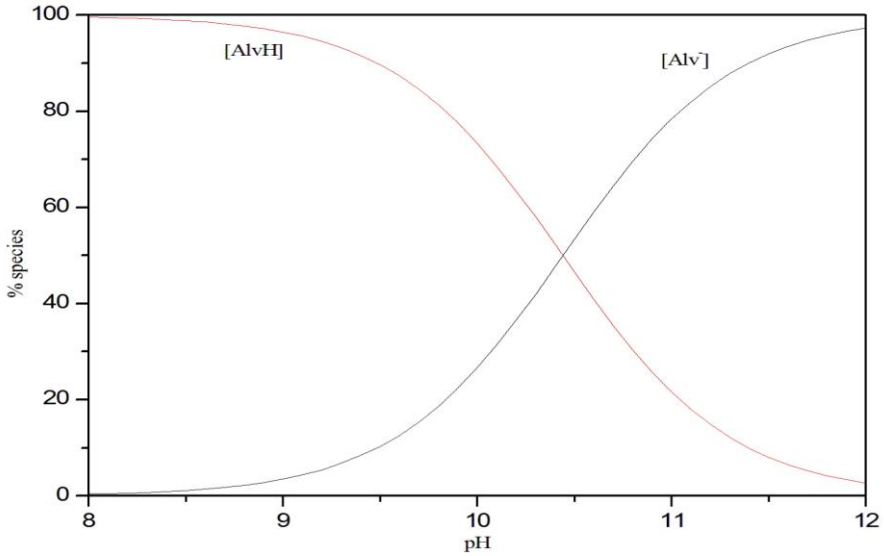

Figure 4. Representative concentration distribution diagram for alverine citrate species

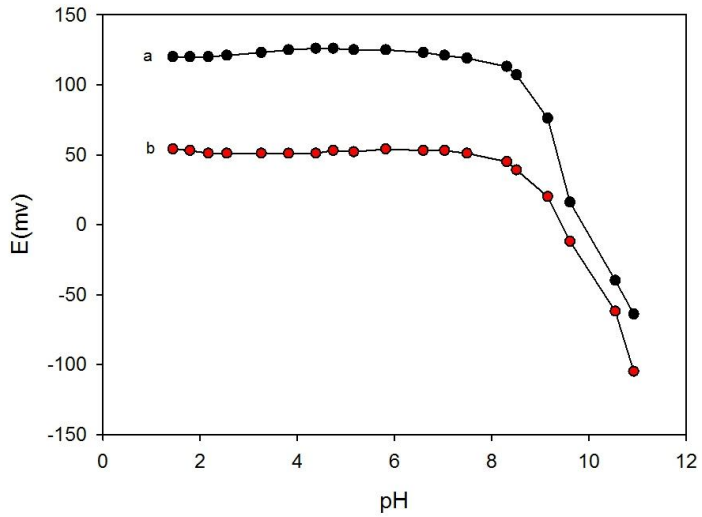

Figure 5. Effect of pH of the test solution on the potential response of the PME_2 electrode: (a) 1.0 x 10^{-3} mol L^{-1} alverine citrate; (b) 1.0 x 10^{-4} mol L^{-1} alverine citrate.

The influence of pH on the response of both PME_1 and PME_2 was studied at 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} alverine citrate solution. The pH of the solution was varied by addition of small volumes of 0.1 mol L^{-1} solution of either HCl or NaOH and the results are shown in Fig. 5. The potential pH profile obtained indicates that the responses of the two electrodes were fairly constant over the pH range 1.44-8.05 and 1.42-8.31 for PME_1 and PME_2, respectively. The observed potential drift at higher pH values could be due to the formation of non-protonated alverine. The electrodes response was checked in bidistilled water, 0.04 mol L^{-1} Britton Robinson buffer pH 4.07, 0.1 mol L^{-1} acetate buffer pH 4.00 and 0.1 mol L^{-1} phathalate buffer pH 4.00 at 25**ºC**. The results indicate that, for samples containing buffers, the electrodes exhibited low Nernstian slopes and unstable potential reading. However, the best results were obtained in bidistilled water.

3.6. Selectivity of the electrode

The selectivity behavior is obviously one of the most important characteristics of an ion-selective electrode, determining whether a reliable measurement in the target sample is possible. The selectivity coefficients for alverine cation with respect to a variety of inorganic cations, sugars, amino acids, vitamins and urea were determined by the modified separate solution method (MSSM) and the matched potential method (MPM). The selectivity coefficient values for the proposed electrodes are listed in Table 3. From the data given in Table 3, it is immediately obvious that the proposed electrodes are high selective towards alverine citrate. The inorganic cations do not interfere because of the difference in their mobility and permeability as compared to alverine cation. The selectivity sequence significantly differs from the so called Hofmeister selectivity sequence [32] (i.e. selectivity solely based on lipophilicity of 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. The mechanism of selectivity is mainly based on the stereospecificity and electrostatic environment, and is dependent on how much fitting is present between the locations of the lipophilicity sites in two competing species in the bathing solution side and those present in the receptor of the ion-exchanger [33,34]. The data given in Table 3 revealed that, in most cases, the selectivity coefficients obtained for the CWEs are lower than those for the polymeric membrane electrode, emphasizing the superiority of the former electrode in this respect as well [35, 36].

Table 3. Selectivity coefficient values of the alverine- selective electrodes

	$K^{pot}_{Alv,j}$							
Interferent	PME_1		PME_2		CWE_1		CWE_2	
	MSSM	MPM	MSSM	MPM	MSSM	MPM	MSSM	MPM
Na^+	1.22×10^{-3}	----	9.60×10^{-3}	----	1.11×10^{-3}	----	7.60×10^{-3}	----
K^+	9.49×10^{-3}	----	9.22×10^{-3}	----	1.21×10^{-3}	----	8.55×10^{-3}	----
NH_4^+	7.79×10^{-3}	----	8.51×10^{-3}	----	7.27×10^{-3}	----	5.56×10^{-3}	----
Li^+	4.52×10^{-3}	----	3.94×10^{-3}	----	2.72×10^{-4}	----	2.75×10^{-3}	----
Fe^{2+}	4.54×10^{-4}	----	9.60×10^{-3}	----	2.39×10^{-4}	----	7.75×10^{-4}	----

Ca^{2+}	6.92×10^{-4}	----	1.69×10^{-3}	----	3.29×10^{-4}	----	1.44×10^{-4}	----
Mg^{2+}	3.41×10^{-4}	----	1.27×10^{-3}	----	3.70×10^{-4}	----	1.34×10^{-4}	----
Mn^{2+}	2.75×10^{-3}	----	4.83×10^{-3}	----	1.32×10^{-3}	----	4.49×10^{-4}	----
Cu^{2+}	9.49×10^{-4}	----	1.04×10^{-3}	----	5.29×10^{-4}	----	2.22×10^{-4}	----
Co^{2+}	2.01×10^{-3}	----	5.03×10^{-3}	----	1.12×10^{-3}	----	3.69×10^{-4}	----
Vitamine B1	----	2.17×10^{-2}	----	2.74×10^{-2}	----	2.04×10^{-2}	----	9.24×10^{-3}
Vitamine B6	----	3.56×10^{-2}	----	1.49×10^{-2}	----	1.30×10^{-2}	----	9.62×10^{-3}
Glucose	----	2.17×10^{-3}	----	1.12×10^{-3}	----	1.72×10^{-3}	----	4.15×10^{-3}
Fructose	----	2.67×10^{-3}	----	3.02×10^{-3}	----	1.29×10^{-3}	----	1.72×10^{-3}
Lactose	----	1.37×10^{-3}	----	4.15×10^{-3}	----	2.39×10^{-3}	----	1.34×10^{-3}
Maltose	----	1.43×10^{-3}	----	2.39×10^{-3}	----	1.99×10^{-3}	----	1.18×10^{-3}
Urea	----	4.50×10^{-3}	----	3.49×10^{-3}	----	1.93×10^{-3}	----	1.29×10^{-3}
Glycine	----	1.21×10^{-3}	----	1.24×10^{-3}	----	1.29×10^{-3}	----	1.36×10^{-3}
β-alanine		5.14×10^{-3}	----	4.01×10^{-3}	----	3.29×10^{-3}	----	1.41×10^{-3}

3.7. Analytical applications

The proposed electrodes were found to work well under laboratory conditions. It can be seen that the amount of alverine cation can be accurately determined using the proposed electrodes. To assess the applicability of the proposed electrodes, alverine cation was measured in pure solutions, pharmaceutical preparations (Meteospasmyl capsules, 60 mg/capsule), spiked urine and plasma samples, by applying the standard addition method. The obtained average recovery and relative standard deviation values are summarized in (Tables 4 and 5), which reflect the high accuracy and precision of the electrodes. The optimized alverine selective electrodes were successfully applied as indicator electrodes in the potentiometric titration of alverine citrate solution with PTA solution (Table 4). The well-defined potential jumps of titration curves (Fig. 6) corresponds to formation of a Alv–PTA salt of 3:1 stoichiometry indicating the high sensitivity of the electrodes.

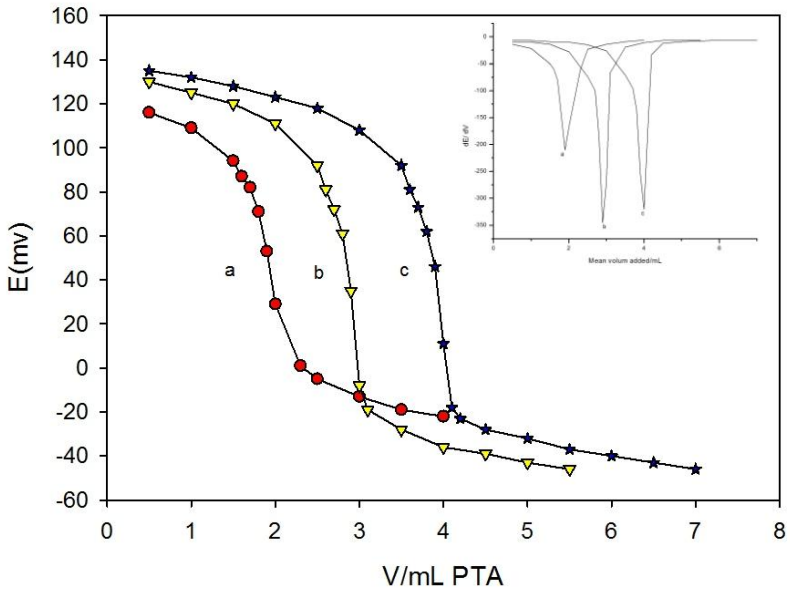

Figure 6. Potentiometric titration curves and its first derivative of (a) 6, (b) 9 and (c) 12 mL of 1.0 x 10^{-2} mol L^{-1} alverine citrate using PME_1 electrode and 1.0 x 10^{-2} mol L^{-1} PTA as titrant.

Obviously, the two methods, standard addition and potentiometric titration, can be applied to the determination of alverine citrate in bulk powder and in pharmaceutical formulations or in biological fluids without interference by the excipients expected to be present in capsules or the constituents of body fluids.

3.8. Statistical analysis and validity of the proposed method

The linearity, limit of detection, precision, accuracy, and ruggedness/robustness were the parameters used for the method validation. As mentioned before, the measuring range of the alverine electrodes is between 1×10^{-5} and 1×10^{-2} mol L^{-1} alverine citrate.

3.8.1. Precision and accuracy of the method

The precision and accuracy of the method are expressed as RSD and % of deviation of the measured concentration (recovery %), also reproducibility (day to day) were investigated, and the relative standard deviations for the proposed electrodes were obtained. The limit of detection of the studied sensors (LOD) was calculated, defined as the alverine citrate concentration corresponding to the intersection of the extrapolation of the linear part of the calibration curve with the tangent of the curved part; these were 7.6×10^{-6}, 8.3×10^{-6}, 6.6×10^{-6} and 8.1×10^{-6} mol L^{-1} alverine citrate for PME_1, PME_2, CWE_1 and CWE_2, respectively.

3.8.2. Ruggedness

For ruggedness of the method a comparison was performed between the intra- and inter-day assay results for alverine obtained by two Ph.D. candidates. 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 1.98% which indicates that the method is capable of producing results with high precision.

3.8.3. Robustness

The robustness was examined while the parameter values (pH of the medium 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.

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 [37]. The results (Table 6), show that the calculated F- and t-values did not exceed the theoretical values, reflecting the accuracy and precision of the applied method.

Table 4. Determination of alverine citrate in pure solutions and pharmaceutical preparations applying the standard addition and the potentiometric titration methods

Sample	Standard addition method			Potentiometric titration method		
	Taken (mg)	Recovery (%)	RSD (%)	Taken (mg)	Recovery (%)	RSD (%)
PME_1						
Pure solutions						
	1.184	101.18	0.68	28.41	100.00	0.87
	1.894	100.63	0.46	42.62	100.55	1.33
	2.368	98.98	1.76	56.83	98.75	0.48
Meteospasmyl (60 mg/capsule)						
	1.184	99.24	1.41	28.41	97.50	1.74
	1.894	98.25	0.89	42.62	98.33	0.35
	2.368	100.76	1.14	56.83	101.25	0.66
CWE_1						
Pure solutions						
	1.184	99.83	1.33	28.41	98.33	0.64
	1.894	100.26	1.06	42.62	97.50	1.95
	2.368	100.97	0.56	56.83	100.83	1.13
Meteospasmyl (60 mg/capsule)						
	1.184	101.35	1.12	28.41	102.50	0.94
	1.894	100.26	1.22	42.62	100.55	0.56
	2.368	102.19	0.93	56.83	99.58	1.43
PME_2						
Pure solutions						
	1.184	100.92	0.97	28.41	100.78	0.81
	1.894	99.73	0.31	42.62	101.66	1.38
	2.368	100.16	1.55	56.83	100.78	1.17
Meteospasmyl (60 mg/capsule)						
	1.184	98.80	1.74	28.41	98.33	0.72
	1.894	98.78	0.62	42.62	99.44	1.88
	2.368	99.19	0.88	56.83	99.58	0.68
CWE_2						
Pure solutions						
	1.184	101.18	1.86	28.41	100.73	1.29
	1.894	100.47	0.25	42.62	99.99	1.36
	2.368	99.78	0.15	56.83	100.66	0.46
Meteospasmyl (60 mg/capsule)						
	1.184	98.98	0.47	28.41	97.50	0.51
	1.894	100.16	0.33	42.62	99.33	1.76
	2.368	98.35	1.38	56.83	98.75	1.08

Table 5. Determination of alverine citrate in spiked plasma and urine samples applying the standard addition method

Sample	Spiked plasma			Spiked urine		
	Taken (mg)	Recovery (%)	RSD (%)	Taken (mg)	Recovery (%)	RSD (%)
PME_1						
	1.184	100.76	0.49	1.184	98.56	0.82
	1.894	100.26	0.40	1.894	99.47	0.89
	2.368	98.94	0.76	2.368	98.99	1.98
CWE_1						
	1.184	100.16	0.64	1.184	98.39	1.14
	1.894	99.63	0.91	1.894	100.16	1.02
	2.368	99.49	0.47	2.368	102.07	0.97
PME_2						
	1.184	102.12	0.69	1.184	100.08	1.13
	1.894	101.03	0.87	1.894	102.01	0.39
	2.368	101.95	0.97	2.368	101.98	1.97
CWE_2						
	1.184	100.76	0.59	1.184	99.41	0.12
	1.894	99.63	0.36	1.894	100.47	0.26
	2.368	98.85	0.21	2.368	99.87	0.15

Table 6. Statistical comparison between the results of an analysis of a pharmaceutical preparation applying the standard addition and potentiometric titration methods

Parameters	Standard addition method	Potentiometric titration method
PME_1		
Mean recovery (%)	99.41 [a]	99.02 [b]
SD	1.26	1.96
RSD (%)	1.27	1.98
F-ratio	2.42 (9.55)[c]	
t-test	0.32 (2.57)[d]	
CWE_1		
Mean recovery (%)	101.26 [a]	100.87 [b]
SD	0.96	1.48
RSD (%)	0.95	1.47
F-ratio	2.38 (9.55)[c]	
t-test	0.42 (2.57)[d]	
PME_2		
Mean recovery (%)	98.59 [a]	99.11 [b]
SD	0.71	0.68
RSD (%)	0.72	0.69
F-ratio	1.09 (19.20)[c]	
t-test	0.97 (2.57)[d]	
CWE_2		
Mean recovery (%)	[a]99.16	98.52 [b]
SD	0.91	0.93
RSD (%)	0.92	0.94
F-ratio	1.04 (9.55) [c]	
t-test	0.91 (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

Two kinds of potentiometric (PME and CWE) electrodes were constructed for determination of alverine cation. From the results obtained with our electrodes, it is clear that the prepared electrodes exhibited good operating characteristics including reasonable detection limit, relatively high selectivity, wide dynamic range and fast response for alverine citrate determination. These characteristics and the typical applications presented in this paper make the electrodes suitable for measuring alverine citrate content in pharmaceutical samples without a significant interaction from concomitant substances.

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