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RESEARCH ARTICLE

Novel In Situ Modified PVC Membrane Potentiometric Sensors for Determination of Levamisole Hydrochloride in Human Plasma, Urine and Bovine Milk

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Abstract

This paper is focused on construction and investigating the characteristics of novel levamisole (LVM) in situ modified polymeric membrane sensors. Two sensors were prepared based on the cation exchangers phosphotungstic acid and phosphomolybdic acid (PME_1 and PME_2, respectively) using dibutyl phthalate as plasticizing solvent. These sensors showed Nernstian slopes of 59.2±0.4 and 58.31±0.4 mV decade^{-1} in the concentration ranges ($1.0x10^{-6}$-$1.0x10^{-2}$ and $1.0x10^{-5}$-$1.0x10^{-2}$ mol L^{-1}) with low detection limits ($1.0x10^{-6}$ and $2.8x10^{-6}$mol L^{-1}) for PME1and PME2, respectively. The sensors were found to be very selective and usable within a wide pH range (2-8). They exhibited fast response times (< 10 s), good stability and long life span (40 and 37 days), respectively. LVM is determined successfully in pure solutions, pharmaceutical preparations and biological fluids (human plasma, urine and bovine milk) using the standard addition and potentiometric titration methods with high accuracy and precision.

Introduction

Levamisole (LVM) (2,3,5,6-tetrahydro-6- Phenyl imidazole [2,1-b] thiazole) (Fig. 1) [1] ($C_{11}H_{13}N_2SCl$) belongs to synthetic imidazothiazole derivatives. It is a white to almost white crystalline powder, which is almost odorless and is freely soluble in water. It is quite stable in acid aqueous media but hydrolyzes in alkaline or neutral solutions.

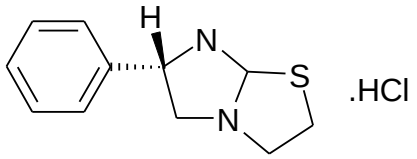

Fig 1. The chemical structure of levamisole hydrochloride

This drug is a broad spectrum anthelminthic drug widely used to control internal parasites in large livestock and occasionally in human medicine [2]. Because LVM acts as an inhibitor of lipid peroxidation, it is also a radio-protectant drug [3]. Also, levamisole is an immunomodulator in different cancer cells including colorectal, breast cancer, melanoma, and leukemia [4]. Besides, it has been shown that levamisole has anti-cancer activity in combination with fluorouracil (5-FU) as adjuvant therapy for colon carcinoma [5].

Levamisole gained forensic interest after the increase of its use as an adulterant in illicit cocaine samples; as a result, levamisole is now found in the majority of cocaine seized worldwide, linked to debilitating and eventually fatal immunologic effects in cocaine abusers [6]. Excess use of levamisole was found to have serious adverse side effects such as agranulocytosis, cutaneous vasculopathy and leukoencephalopathy [7]. The presence of levamisole determines an additional health threat due to aminorex, one of its main metabolites which was found to cause pulmonary hypertension [8-10].

Therefore, its quantification is necessary in different biological samples and bulk formulations, as well as in different finished product dosage forms. A variety of methods have been reported for analysis of LVM. These methods include HPLC determination in biological samples and tablets [11-15]. Liquid chromatography–mass spectrometry (LC–MS) and LC–MS/MS were used for its determination [16-19]. Some other methods such as gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), thin-layer chromatography (TLC), capillary electrophoresis, atomic absorption, amperometric flow-injection methods and spectrophotometry were also reported for LVM analysis [20-27]. However, these methods need expensive instruments as well as laborious and time-consuming extraction procedures.

Thus, there is critical need for the development of selective, portable, inexpensive diagnostic tools for the determination of this analyte. Analytical methods based on potentiometric detection with ion-selective electrodes (ISEs) can be considered as an advantageous alternative because they are eco-friendly techniques, provide easy construction and manipulation, present good selectivity in a wide concentration range, a relatively low detection limit, show fast response and perform non-destructive analysis. Ion-selective electrodes (ISEs) with polymeric membranes are the most commonly-used potentiometric sensors. Under a variety of membrane types, solvent polymeric membranes have proved to be especially suited for clinical analysis since they can easily be manufactured in different sizes and shapes and are less affected by the response of biological substrate such as protein, enzyme and antibody [28-30].

This has led to increasing interest by our research group in the development and application of ion-selective electrodes using various ion-pairs for the determination of some selected drugs [31-39]. With this intent, we used PTA and PMA as ion exchangers, for the development of novel in situ modified PVC membrane sensors for determination of LVM. Performance characteristics of novel electrodes reveal low detection limit, high sensitivity, good selectivity, widen the pH range, broaden the concentration range, fast response, long life span and application for accurate determination of LVM in pure form, pharmaceutical preparations and biological fluids.

Experimental

Reagents and materials

All the chemicals used were of analytical grade. Bidistilled water was used throughout all experiments. Tricresyl phosphate (TCP), dioctyl adipate (DOA), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) were purchased from Merck (Germany). Phosphotungstic acid (PTA) and phosphomolybdic acid (PMA) were obtained from Fluka (USA), polyvinyl chloride (PVC) of high molecular weight and tetrahydrofuran (THF) were purchased from Aldrich chemical Company (USA). 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 whenever 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. Bovine milk was purchased from the local market.

Pure-grade levamisole hydrochloride (LVM, Mwt = 240.75 $g.mol^{-1}$) was supplied by KAHIRA Pharm. & Chem. Ind. Co., Egypt. The pharmaceutical preparation was Katrex® (levamisole hydrochloride, 40 mg/tablets,) andpurchased from local drug stores. Standard solution of 10^{-2} mol L^{-1} levamisole hydrochloride was freshly prepared by dissolving the accurately weighed amount in bidistilled water. Working solutions of the drug (1.0×10^{-7}–1.0×10^{-2} mol L^{-1}) were prepared by suitable dilution from the standard solution with bidistilled water.

Stock solutions of 10^{-2} mol L^{-1} PTA or PMA were prepared by dissolving the accurately weighed amount of the pure solid in bidistilled water. Solutions of sodium hydroxide and hydrochloric acid of concentrations within the range (0.1–1.0) mol L^{-1} were used for adjusting the pH of the medium.

Apparatus

The electrochemical system of potentiometric sensors may be represented as follows:

Ag/AgCl/internal solution/membrane/test solution/Ag/AgCl double-junction reference electrode.

An Ag/AgCl double-junction reference electrode (Metrohm 6.0222.100) was used as the external reference. Potentiometric and pH-measurements were carried out using 702 titroprocessor equipped with a 665 dosimat by (Metrohm, Switzerland). A mLw W20 circulator thermostat was used to control temperature of the test solutions.

Electrodes construction

Membranes of different compositions were prepared as indicated in Table 1. The general procedure to prepare the polymeric membrane was as follows: different percentages of each ion-pairing agent (cover the range of 3–9%), PVC and plasticizer with equal percentages were dissolved in minimum volume of tetrahydrofuran (THF), and the resulting mixture was transferred into a Petri dish of 5 cm diameter. The total weight of constituents in each batch was fixed at 0.35 g. The Petri dish was then covered with a filter paper and left to dry in air. To obtain a uniform

membrane thickness, the amount of THF was kept constant, and its evaporation was fixed for 24 h. Thickness of the membrane is about 0.2 mm. A 12 mm diameter disk was cut out from the prepared membrane and glued to one end of a Pyrex glass tube using PVC-THF paste. Ratio of membrane ingredients, time of contact and concentration of conditioning solution were optimized; so that the potentials recorded were reproducible and stable within the standard deviation [31].

Membrane to membrane reproducibility was assured by carefully following the optimum condition of fabrication. The membrane that gave reproducible results and showed best performance was selected for further studies. The optimized electrode body was filled with a solution of 1.0×10^{-1} mol L^{-1} NaCl and 1.0×10^{-2} mol L^{-1} LVM. The electrode was preconditioned before use by soaking in a 1.0×10^{-2} mol L^{-1} LVM solution for 15 minutes and storing in the same solution when not in use.

Construction of calibration curves

The conditioned electrodes were immersed in conjunction with the Ag/AgCl double-junction reference electrode in solutions of levamisole hydrochloride in the range of 1.0×10^{-7}- 1.0×10^{-2} mol L^{-1}. They were allowed to equilibrate whilst stirring and recording the e.m.f. readings within ±1 mV. The mV concentration profiles were plotted. The regression equations for the linear part of the curves were computed and used for subsequent determination of unknown concentrations of levamisole hydrochloride.

Effect of pH

The effect of pH on the response of the investigated electrodes was studied using 10^{-2}, 10^{-3} and 10^{-4} mol L^{-1} LVM solutions over the pH range of 1–11. This is done by immersing the electrodes in the drug solution. The pH was gradually increased or decreased by addition of very small volumes of dilute NaOH or HCl solutions, respectively. The potential obtained at each pH was recorded.

Selectivity coefficient determination

The separate solution method [40, 41] and the matched potential method (MPM) [42, 43] were employed to determine the selectivity coefficients, $\log K^{pot}_{LVM,\,J^{z+}}$, of the potentiometric sensors towards different species. In the 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 1.0×10^{-3} mol L^{-1} LVM, E_2 the potential measured in 1.0×10^{-3} mol L^{-1} of the interfering compound, z_1 and z_2 are the charges of LVM and interfering species, respectively, and S is slope of the electrode calibration plot. The selectivity coefficients were determined by the separate solution method using the rearranged Nikolsky equation [41]:

$$\log K^{pot}_{LVM,\,J^{z+}} = ((E_1 - E_2)/S) + (1 + (z_1/z_2)) \log a$$

In 1995, IUPAC recommendation [42] prescribes the MPM [43, 44] as the method of choice for ions of different charge. MPM is considered [42] as a purely operational method, not relying on any theoretical or empirical model equation. The quantity used to express the extent of interference is the ratio of the primary ion concentration increment to the interfering ion concentration that gives the same potential change in a constant initial backgroundof primary ion. 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:

$$K^{pot}_{A,B} = (´a_A - a_A)/a_B$$

In the present study a_A and á$_A$ were kept at 1.0×10^{-4} and 1.2×10^{-4} mol L^{-1} levamisole hydrochloride and a_B was experimentally determined.

Potentiometric determination of levamisole hydrochloride

The standard addition method was applied [33,45], in which small increments of the standard solution 10^{-2} mol L^{-1} of LVM hydrochloride were added to 50 mL aliquot samples of various concentrations from pure drug or pharmaceutical preparations. The change in millivolt reading was recorded for each increment and used to calculate the concentration of LVM hydrochloride sample solution using the following equation:

$$C_x = C_s\left(\frac{V_s}{V_x+V_s}\right)\left(10^{n(\Delta E/S)} - \frac{V_x}{V_s+V_x}\right)^{-1}$$

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

Potentiometric titration of levamisole hydrochloride

Aliquots of 1.0×10^{-2} mol L^{-1} drug solution (pure or tablet) were transferred into 50-mL volumetric flasks and made up to the mark with bidistilled water. Different concentrations of LVM hydrochloride were prepared, then titrated potentiometrically with a standard solution of 1.0×10^{-2} mol L^{-1} PTA. The volume of the titrant at equivalence point was obtained using the conventional S-shaped curves.

Determination of levamisole hydrochloride in pharmaceutical preparations

An accurate weight of Katrex® (40 mg/tablet) tablets ground and finely powdered in a mortar was dissolved in the bidistilled water up to 30 mL by stirring for 1 h. The solution was filtered in a 50-mL measuring flask. The residue was washed three times with bidistilled water; the volume was completed to the mark by the same solvent to form 1.0×10^{-2} mol L^{-1} solution. The resulting potentials of the drug solution were directly measured using its corresponding ion-selective electrode.

Determination of levamisole hydrochloride in biological fluids

Different amounts of LVM hydrochloride and 5 mL of plasma or urine of a healthy person or milk of healthy bovine were transferred to a 50 mL measuring flask and completed to the mark using bidistilled water. The contents of the measuring flask were transferred to a 100-mL beaker, and then subjected to standard addition method.

Results and Discussion

Optimization of membrane composition

Levamisole cation was found to form 3:1 stable water insoluble ion-pair complex with each of phosphotungstic acid and phosphomolybdic acid as indicated conductometricaly (Fig. 2).

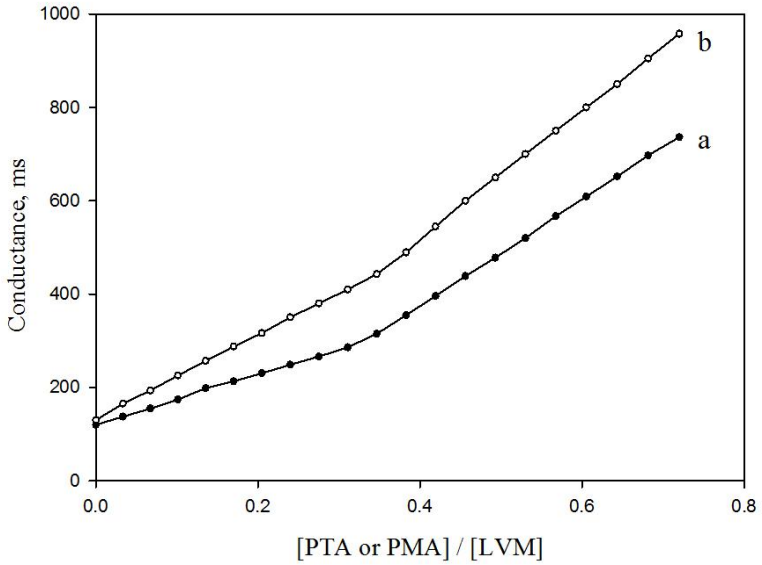

Fig. 2. Conductometric titration curves of 1.0×10^{-2} mol L^{-1} LVM against 1.0×10^{-2} mol L^{-1} a) PTA and b) PMA

The electroanalytical performance and electrode potential of an ISE are dependent upon the selective extraction of the target ion which creates the electrochemical phase boundary potential due to thermodynamic equilibria at the sample/electrode interface. Using of a suitable ion pairing agent in the electrode matrix has the advantage of reducing the time required for the electrode preparation where there is no need for ion pair (IP) preparation as well as expansion of the application of ion selective electrodes (ISEs) for the determination of drugs that cannot be precipitated as suitable IPs.

In order to determine the suitable content of ion-pairing agents (PTA and PMA), several electrodes of a varying nature and ratio of ion pairing agent were prepared from the systematic investigation of each electrode composition. Experimental trials proved that a certain percentage of each ion pairing agents was optimum, indicated by the Nernstian behavior of the electrodes. However, further increase of the ion pairing agents over this percentage resulted in a diminished response slope of the electrode, most probably due to some inhomogenities and possible saturation of the membrane [46]. The results given in Table 1 show that the optimum ion pairing agent percentage is 5% for PTA and PMA that gave the highest slope values of 59.2±0.36, 58.3±0.37 mV decade^{-1} for PME1and PME2, respectively.

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

Electrode	Composition % w/w			Slope	Linear range	LOD	RSD	r^2
No.	IPA	PVC	DBP	mV/decade	mol L^{-1}	mol L^{-1}	(%)	
PME_1	**PTA**							
1	3.0	48.5	48.5	52.7±0.97	1.0x10^{-5}-1.0x10^{-2}	7.9x10^{-6}	1.84	0.9995
2*	**5.0**	**47.5**	**47.5**	**59.2±0.36**	**1.0x10^{-6}-1.0x10^{-2}**	**1.0x10^{-6}**	**0.61**	**0.9997**
3	7.0	46.5	46.5	54.4±0.88	1.0x10^{-5}-1.0x10^{-2}	7.4x10^{-6}	1.61	0.9999
4	9.0	45.5	45.5	50.9±0.45	1.0x10^{-5}-1.0x10^{-2}	8.3x10^{-6}	0.88	0.9986
PME_2	**PMA**							
5	3.0	48.5	48.5	54.6±0.83	1.0x10^{-5}-1.0x10^{-2}	7.1x10^{-6}	1.52	0.9990
6*	**5.0**	**47.5**	**47.5**	**58.3±0.37**	**1.0x10^{-5}-1.0x10^{-2}**	**2.8x10^{-6}**	**0.63**	**0.9996**
7	7.0	46.5	46.5	56.3±0.53	1.0x10^{-5}-1.0x10^{-2}	4.3x10^{-6}	0.95	0.9999
8	9.0	45.5	45.5	51.6±0.25	1.0x10^{-5}-1.0x10^{-2}	8.5x10^{-6}	0.48	0.9943

IPA: ion-pair agent
LOD: limit of detection
RSD: relative standard deviation (four determinations)

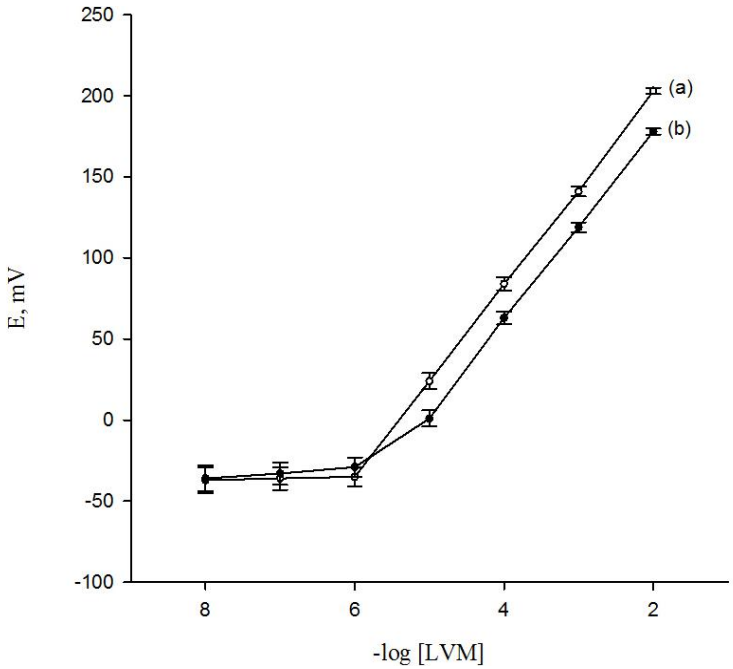

Fig. 3. Calibration graphs for a) PME_1 and b) PME_2 at optimum membrane composition

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. In exploration for a suitable plasticizer for constructing these electrodes, four plasticizers with different polarities including DBP, DOP, DOA and TCP were used as shown in Table 2. The results revealed that DBP was the best plasticizer tested. Poor sensitivities for the electrodes plasticized using DOP, DOA and TCP are due to low solubilities or low distributions of PTA and PMA ion-pairing agents in these solvents [47]. 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 [48-50].

Table 2. Effect of the plasticizers type on the levamisole responsive electrodes

Electrode	Composition % w/w			Slope	Linear range	LOD	RSD	r^2
No.	IPA	PVC	plasticizer	mV/ decade	mol L^{-1}	mol L^{-1}	(%)	
PME_1	**PTA**							
1*	**5.0**	**48.5**	**48.5 DBP**	**59.2±0.36**	**$1.0x10^{-6}$-$1.0x10^{-2}$**	**$1.0x10^{-6}$**	**0.61**	**0.9997**
2	5.0	48.5	48.5 DOA	54.8±0.35	$1.0x10^{-5}$-$1.0x10^{-2}$	$7.9x10^{-6}$	0.65	0.9998
3	5.0	48.5	48.5 DOP	50.7±0.62	$1.0x10^{-5}$-$1.0x10^{-2}$	$9.3x10^{-6}$	1.23	0.9985
4	5.0	48.5	48.5 TCP	54.5±0.99	$1.0x10^{-5}$-$1.0x10^{-2}$	$8.5x10^{-6}$	1.82	0.9994
PME_2	**PMA**							
5*	**5.0**	**48.5**	**48.5 DBP**	**58.3±0.37**	**$1.0x10^{-5}$-$1.0x10^{-2}$**	**$2.8x10^{-6}$**	**0.63**	**0.9996**
6	5.0	48.5	48.5 DOA	55.2±0.96	$1.0x10^{-5}$-$1.0x10^{-2}$	$6.3x10^{-6}$	1.74	0.9998
7	5.0	48.5	48.5 DOP	54.3±0.79	$1.0x10^{-5}$-$1.0x10^{-2}$	$6.6x10^{-6}$	1.45	0.997
8	5.0	48.5	48.5 TCP	53.1±0.43	$1.0x10^{-5}$-$1.0x10^{-2}$	$5.5x10^{-6}$	0.80	0.998

IPA: ion-pair agent
LOD: limit of detection
RSD: relative standard deviation (four determinations).

Effect of internal solution

Varying the composition of internal reference solution could considerably improve the linear range and limit of detection [51, 52], therefore, the response of the electrode in relation to the variation of internal solution was investigated using the optimum membrane composition. Three different concentrations of LVM.HCl (1.0×10^{-2}, 1.0×10^{-3}, and 1.0×10^{-4} mol L^{-1}) or with 1.0×10^{-1} mol L^{-1} NaCl were used. 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^{-2} mol L^{-1} LVM.HCl and 1.0×10^{-1} mol L^{-1} NaCl for PME_1 and PME_2 electrodes.

Performance characteristics of the constructed electrodes

The final performances of the constructed electrodes were investigated according to IUPAC recommendations [53].

Effect of soaking and lifetime 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 [54]. The lifetimes of the electrodes were determined for intervals till the electrode loses 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 ^{o}C at different intervals. Lifetimes of the electrodes were found to be 40 and 37 days for PME_1 and PME_2 electrodes, respectively during which the electrodes showed a slight gradual decrease in the slope and an increase in the detection limit. Effect of paste duration on the response characteristic of the proposed electrodes is shown in Table 3.

After preparation of the proposed electrodes, they were kept at 4^{o}C and directly used for potentiometric measurements.

Table 3. Response characterization of the proposed electrodes

Parameters	PME_1	PME_2
Slope (mV/decade)	59.2	58.31
Correlation coefficient (r^2)	0.9997	0.9996
Limitof detection (mol L^{-1})	$1.00x10^{-6}$	$2.75x10^{-6}$
Response time	≤ 10 s	≤ 10 s
Working pH range	2-8	2-8
Life time (days)	40	37
Linear range (mol L^{-1})	$10^{-6} – 10^{-2}$	$10^{-5} – 10^{-2}$
SD	0.36	0.37
RSD (%)	0.61	0.63
Thermal coefficient (V/^oC)	0.00042	0.0034

Dynamic response time and repeatability of the electrode

The dynamic response time [53], is defined as the time which elapses between the instant at which an ion-selective electrode and a reference electrode (ISE cell) are brought into contact with a sample solution. The dynamic response time of the electrode was tested by measuring the time required to achieve a steady-state potential (within ±1 mV) after successive immersions of the electrode in a series of drug solutions, each having a 10-fold increase in concentration from 1.0×10^{-6} to 1.0×10^{-2} and 1.0×10^{-5} to 1.0×10^{-2} mol L^{-1} for PME_1 and PME_2, respectively. The electrodes yielded a steady potential within 10 s as shown in Fig. 4.

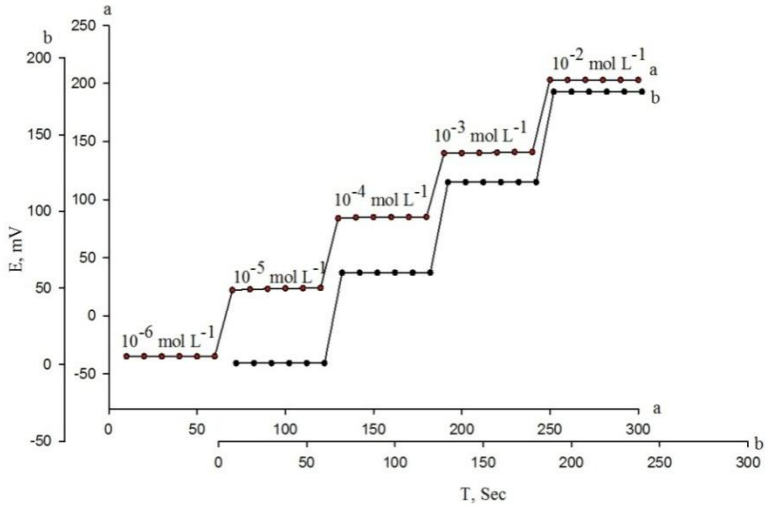

Fig. 4. Dynamic response time for a) PME_1 and b) PME_2 for step changes in concentrations of LVM from low to high

The repeatability of the potentials readings for each electrode was examined by subsequent measurement in 1.0×10^{-3} mol L^{-1} levamisole hydrochloride solution immediately after measuring in 1.0×10^{-2} mol L^{-1} levamisole hydrochloride solution (Fig. 5). An insignificant difference in potential readings was obtained, indicating good repeatability of the constructed electrode.

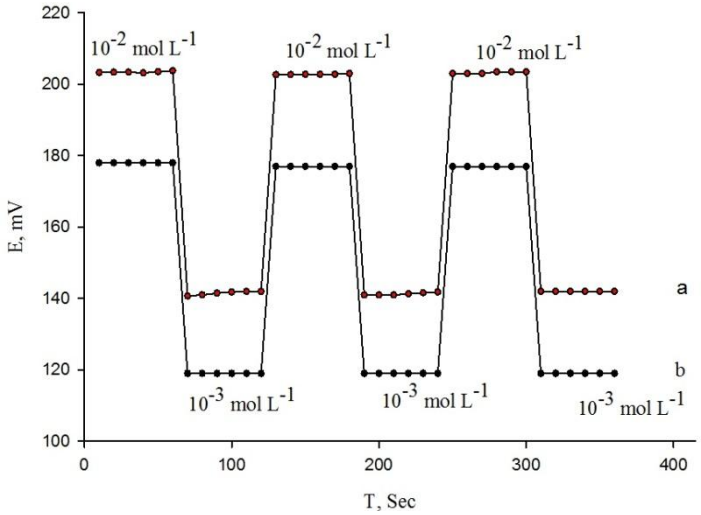

Fig. 5. Dynamic response of a) PME_1 and b) PME_2 for several high-to-low sample cycles

Effect of pH

Since pKa of levamisole is 8.0 [55], therefore, at pH 6.20 levamisole is nearly completely ionized, i.e. levamisole will be in the cationic form. The concentration distribution diagram for levamisole hydrochloride species is constructed using SPECIES program [56] (Fig. 6).

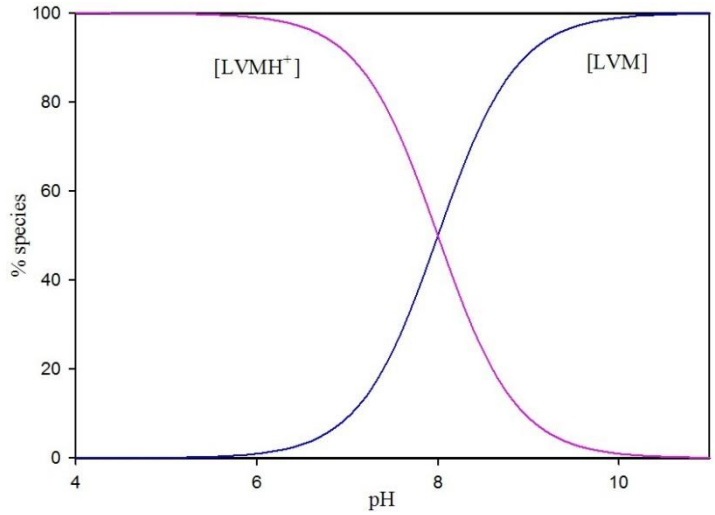

Fig. 6. Representative concentration distribution diagram for levamisole hydrochloride species

The effect of pH of solution on response of proposed electrodes was studied for three concentrations of LVM (1.0×10^{-2}, 1.0×10^{-3} and 1.0×1.0^{-4} mol L^{-1}) in pH range of 1.5–11.0. The pH was adjusted with (0.1–1.0 mol L^{-1}) solutions of hydrochloric acid or sodium hydroxide. The results showed that potential response remained almost constant over the pH range 2–8 for the two investigated sensors as shown in Fig. 7. At higher pH values, the potential showed a sharp decrease; due to the formation of nonprotonated LVM, leading to a decrease in concentration of LVM^+. However, at lower pH values, the decrease in potential may be attributed to interference of hydronium ion.

The sensors response was checked with bidistilled water, 0.1 mol L^{-1} acetate buffer pH 5.0 or 0.1 mol L^{-1} phthalate buffer pH 5.0. The best results were achieved in bidistilled water; because it provided not only a higher Nernstian slope but also a stable potential reading. Therefore, bidistilled water was used for all the constructed sensors.

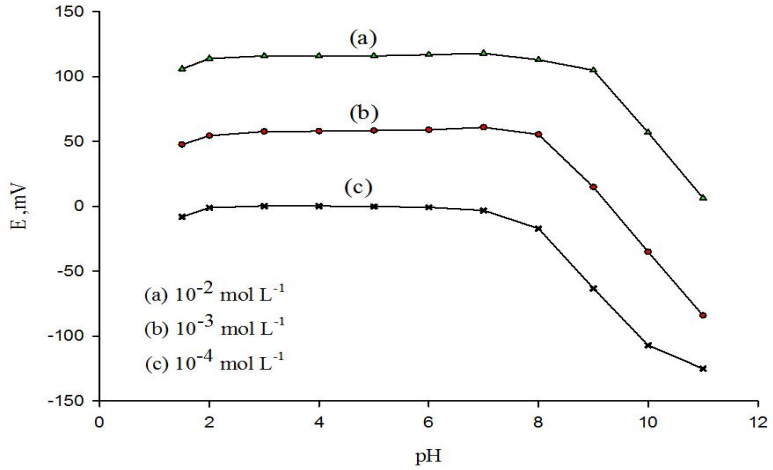

Fig. 7. Effect of pH at different LVM concentrations on emf values for PME_1

Effect of temperature

The thermal coefficients of the investigated sensors were determined as mentioned recently [31]. The values were found to be 0.00042 and 0.0034 V/^{o}C for PME_1 and PME_2, respectively; indicating that the sensors had high thermal stabilities within the studied temperature range and PME_1 sensor is more thermally stable than PME_2 sensor.

Selectivity of the sensors

The selectivity behavior as one of the most important characteristics of ISEs, was studied for levamisole hydrochloride sensors with respect to a variety of ionic and nonionic species using separate solution method (SSM) and matched potential method (MPM), respectively [34]. The selectivity coefficients values of the sensors (Table 4) reflect their very high selectivity for the LVM cation. The high selectivity of sensors toward inorganic cations can be attributed to the differences in ionic size and consequently their mobilities and permeabilities as compared with LVM cation. In addition, the low interference of nonionic species may be ascribed to the difference in polarity and to the lipophilic nature of their molecules [32].

Table 4. Selectivity coefficient values $K^{pot}_{LVM,\,j}$ of various interfering species

$K^{pot}_{LVM,\,j}$				
	PME_1		PME_2	
Interferent	SSM	MPM	SSM	MPM
K^+	$3.0x10^{-3}$	–	$1.1x10^{-2}$	–
NH_4^+	$2.6x10^{-3}$	–	$7.2x10^{-3}$	–
Li^+	$1.9x10^{-3}$	–	$8.1x10^{-3}$	–
Ca^{2+}	$1.1x10^{-4}$	–	$2.2x10^{-4}$	–
Mg^{2+}	$7.9x10^{-5}$	–	$7.0x10^{-5}$	–
Co^{2+}	$8.9x10^{-5}$	–	$7.6x10^{-5}$	–
Cu^{2+}	$7.6x10^{-5}$	–	$8.9x10^{-5}$	–
Mn^{2+}	$9.2x10^{-5}$	–	$5.5x10^{-5}$	–
Ni^{2+}	$1.2x10^{-4}$	–	$6.7x10^{-5}$	–
Fe^{3+}	$1.4x10^{-4}$	–	$2.0x10^{-4}$	–
Vitamin C	$5.3x10^{-3}$	–	$7.8x10^{-3}$	–
Glucose	–	$6.8x10^{-3}$	–	$6.9x10^{-3}$
Fructose	–	$6.9x10^{-3}$	–	$4.7x10^{-3}$
Lactose	–	$6.2x10^{-3}$	–	$5.1x10^{-3}$
Maltose	–	$7.5x10^{-3}$	–	$6.7x10^{-3}$
Urea	–	$7.0x10^{-3}$	–	$7.6x10^{-3}$
Glycine	–	$7.2x10^{-3}$	–	$6.1x10^{-3}$
DL-alanine	–	$8.0x10^{-3}$	–	$5.9x10^{-3}$
L-hestidine	–	$1.7x10^{-2}$	–	$2.9x10^{-2}$

Potentiometric determination of levamisole

Analytical applicability of the investigated sensors was tested by applying potentiometric titration and standard addition methods in pure solutions and pharmaceutical preparations (Table 5, Fig. 8). The calculated F- and t-values, shown in Table 6 did not exceed the theoretical values, reflecting the precision and accuracy of the applied method.

Determination of LVM in spiked urine, plasma and bovine milk samples was carried using standard addition method; the mean recoveries obtained were in the range of 97-102 and 98.3–101.8% for PME_1 and PME_2 sensors, respectively (Table 7).

For ruggedness of the method a comparison was performed between the intra- and inter-day assay results for levamisole obtained by two M. Sc. candidates. The RSD values for the intra- and inter-day assays of levamisole in the cited formulations performed in the same laboratory by the two analysts did not exceed 2.43%. On the other hand, the robustness was examined while the parameter values (pH of the medium and the laboratory temperature) were being deliberately slightly changed. Levamisole recovery percentages were good under most conditions, not showing any significant change when the critical parameters were modified. This result indicates that the proposed sensors show a good reproducibility and stability.

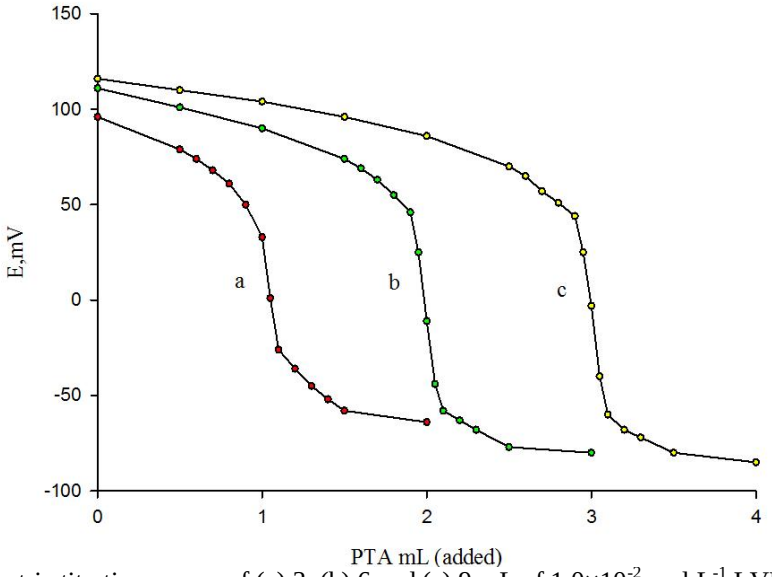

Fig. 8. (A) Potentiometric titration curves of (a) 3, (b) 6 and (c) 9 mL of 1.0×10^{-2} mol L^{-1} LVM using PME_2 and 1.0×10^{-2} mol L^{-1} PTA as titrant

Table 5. Determination of levamisole hydrochloride in pure and pharmaceutical solutions 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 solution	0.963	102	1.28	7.221	99.33	1.16
	1.204	101.93	0.46	14.442	102.5	2.43
	6.019	98.78	1.83	21.663	100.55	0.956
	9.63	99.05	1.65			
katrex®	0.963	101.68	0.46	7.221	100.33	0.575
	1.204	100.28	1.69	14.442	101.83	1.13
	6.019	101.67	1.46	21.663	101.11	0.951
	9.63	97.82	1.60			
PME_2						
Pure solution	0.963	101.68	0.51	7.221	102.33	2.25
	1.204	98.74	0.85	14.442	100.83	1.43

	6.019		97.49	1.23	21.663		100.55	1.91
	9.63		97.82	1.60				
katrex®	0.963		98.76	1.96	7.221		100.0	0.00
	1.204		99.5	1.51	14.442		101.66	1.41
	6.019		100.94	2.29	21.663		101.11	1.90
	9.63		99.4	1.34				

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

Parameters	Standard addition method	Potentiometric titration method
PME_1		
Pure solution		
Mean recovery (%)	100.44 [a]	100.79 [b]
SD	1.76	1.59
RSD (%)	1.75	1.58
F-ratio	1.21 (9.55)[c]	
t-test	0.27 (2.57)[d]	
Tablets (katrex® tablets 40mg)		
Mean recovery (%)	100.36[a]	101.09 [b]
SD	1.818	0.75
RSD (%)	1.811	0.742
F-ratio	5.87 (9.55) [c]	
t-test	0.64 (2.57) [d]	
PME_2		
Pure solution		
Mean recovery (%)	98.93 [a]	99.75 [b]
SD	1.90	0.95
RSD (%)	1.92	0.94
F-ratio	3.96 (9.55)[c]	
t-test	1.89 (2.57)[d]	
Tablets (katrex® tablets 40mg)		
Mean recovery (%)	99.65[a]	100.92 [b]
SD	0.920	0.845
RSD (%)	0.923	0.837
F-ratio	1.18 (9.55) [c]	
t-test	1.87 (2.57) [d]	

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

Table 7. Determination of levamisole hydrochloride in spiked plasma, urine and milk samples applying the standard addition method

Electrode	Taken (mg)	Spiked plasma		Spiked urine		Spiked milk	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PME_1	0.963	100.32	1.43	102	1.12	101.4	1.80
	1.204	97.8	1.11	100.25	1.41	100.24	1.81
	6.019	98.71	1.64	99.34	1.96	97.29	1.03
	9.63	97.03	1.59	98.61	1.60	98.67	1.71
PME_2	0.963	101.5	1.06	99.41	1.94	99.27	1.65
	1.204	101.33	0.53	98.23	1.81	100.95	0.95
	6.019	101.87	0.72	98.32	1.24	100.94	1.83
	9.63	100.01	0.63	98.61	1.60	101.15	1.60

Comparison with reported methods

Although some performance characteristics of the reported methods [20,25,26] are better than those of the proposed sensors, our technique still shows superiority in many important ways. It is low coast, fast, more available and precise (RSD reaches 0.61%) as shown in Table 8, indicating the ability of the constructed sensors to face such automated methods.

Table 8. Comparison of the proposed levamisole sensors with published methods

Method	Linear range (mol L^{-1})	LOD (mol L^{-1})	r^2	RSD (%)	Ref.
Amperometric flow-injection method	$1.0 \times 10^{-8} – 5.0 \times 10^{-6}$	1.0×10^{-9}	0.9990	4.75 %	[25]
LC–MS/MS method	$4.1 \times 10^{-10} – 1.2 \times 10^{-7}$	1.2×10^{-10}	0.9997	11.4 %	[20]
Spectrophotometric method	$2.4 \times 10^{-5} – 9.9 \times 10^{-5}$	6.0×10^{-7}	0.9985	1.21 %	[26]
ISEs					
PME_1	$1.0 \times 10^{-6} – 1.0 \times 10^{-2}$	1.0×10^{-6}	0.9999	0.61 %	[P.W]
PME_2	$1.0 \times 10^{-5} – 1.0 \times 10^{-2}$	2.8×10^{-6}	0.9999	0.63 %	[P.W]

r^2: Correlation coefficient, P.W: Present work

Conclusion

The present work involves the preparation of novel PVC membrane sensors with in situ mode of modification. The described sensors are sufficiently selective for the quantitative determination of LVM in pure form, pharmaceutical dosage form, human urine, plasma and bovine milk. The sensors showed Nernstian slopes with low detection limit (1.0×10^{-6} mol L^{-1}) with fast response time (10 s) and long operational life time (40 days) in the concentration range 1 $.0 \times 10^{-6}$ to 1.0×10^{-2} mol L^{-1}. They also show high sensitivity, adequate selectivity, high thermal stability and applicability over a wide pH range (2-8) with no sample pretreatment.

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