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Design and Application of a Levamisole-Selective Membrane Sensor

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Abstract- The interaction of organic cation levamisole (Lev) with tropeoline was investigated with the help of thermal studies and IR spectroscopy. These slightly soluble associates were used as electrode active substances (EAS) in plasticized polyvinyl chloride (PVC) membranes of ion-selective electrodes (ISE), sensitive to organic cation levamisole. Based on the experimental data, a new method of levamisole determination in industrial products by direct potentiometry with the help of membrane sensor was developed.

Keywords- Electrode active substance, Levamisole, Membrane sensor, Direct potentiometry, IR spectroscopy

1. INTRODUCTION

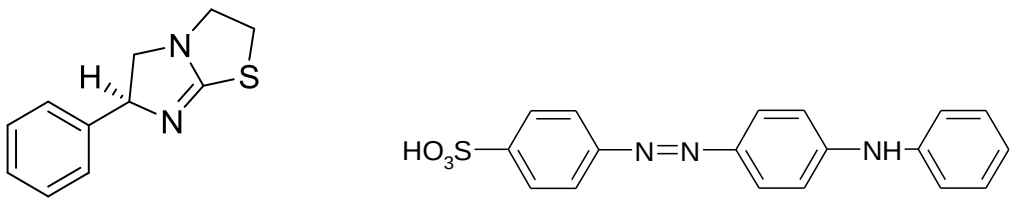
A rapid increase in the development and production of sensors during the last three decades signifies a fundamental change in chemical analysis tools. The most numerous group of these chemical sensors is composed of potentiometric sensors that include ion-selective electrodes (ISEs) [1–3]. Active development of ionometry began with the studies of new chemical sensors and their promising use in pharmaceutical analysis [4]. Currently a wide

assortment of commercially available ISEs is being enriched with new developments and improvements in the known types of sensors.

Levamisole (Fig. 1 (a)), (S)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole is a potent broad-spectrum antihelmintic drug which is widely used in veterinary medicine for the treatment of gastrointestinal parasites in cattle, sheep and pigs. It is normally administered orally, by pour-on or by subcutaneous or intramuscular injection. It has been used as an immunostimulant in humans. Levamisole hydrochloride is used in the treatment of a variety of immune and autoimmune diseases. Levamisole hydrochloride liniments have been developed for the administration of this drug, which can treat skin type immune therapy effectively. It is a white to off-white (light yellow) crystalline powder, soluble in water, methanol, slightly soluble in ethanol, very slightly soluble in chloroform, insoluble in acetone; stable under ordinary conditions [5,6].

Various techniques have been used for the determination of levamisole in biological fluids. They include gas chromatography with nitrogen-phosphorus detection (GC-NPD) [7] and flame ionization (GC-FID) [8] and liquid chromatography (LC) with ultraviolet (UV) detection [9-11]. Several methods have been developed for levamisole quantification such as polarography [12], gas-liquid chromatography [13] and high performance liquid chromatography (HPLC) [11,14,15]. There is only one report on determination of levamisole hydrochloride using a PVC membrane ion selective electrode (ISE) based on levamisole - tetraphenyl borate as an active material [16].

Considering large amount of the pharmaceutical production by various manufacturers and wide use of Lev in medical practice, the need for the preliminary and continuous control at each stage of their use and storage is manifest. The task of analytical chemistry in this challenge is the development and the implementation of efficient methods of the quality control of the medicines.



a b

Fig. 1. Chemical structure of levamisole (a) and tropeoline 00 (b)

Therefore, the development of new potentiometric sensors using ion associates is of current interest. The simultaneous study of chemical–analytical characteristics of isolated solid ion associates, the effect of the electrode active substances (EAS) used, pH of the analyzed solution, properties of the membrane plasticizer, etc. are also of importance. The

combination of those factors which define the electrochemical properties of ISEs and the determination and consideration of the relations between certain parameters would permit, in our opinion, the improvement of the electrochemical characteristics of electrodes and would predict the properties of other ISEs in their development for new classes of substances.

2. EXPERIMENTAL

All EMF measurements were carried out with the following cell assembly. An I-160 M (Gomel, Belarus) model pH/mV meter with a Ag–AgCl saturated reference electrode was used for the measurements of potential difference at 25.0±1.0 °C.

All chemicals were of analytical-reagent grade. Distilled water was used to prepare all solution and in all experiments.

The modeling of the membrane composition of the ion-selective sensors utilized high molecular weight polyvinylchloride (PVC) (Sigma-Aldrich), dibutyl phtalate (DBP) (South African Republic), dibutyl sebacate (DBS) (Merck), dioctyl phtalate (DOP) (Merck), dinonyl phtalate (DNP) (Merck), tricresyl phosphate (TCP) (Acros organics, USA), diethyl phtalate (DEP) (Merck), cyclohexanone (CHN) (Shostka, Ukraine) and tetrahydrofurane (THF) (Labscan Ltd, Ireland) .

The ionic strength of the solutions was adjusted with 0.2 mol/l LiCl solution. The pH value of solutions was maintained with the use of a buffer mixture (0.04 mol/l CH_3COOH, H_3BO_3, H_3PO_4, and a 0.2 mol/l NaOH solution) and monitored by potentiometry with a glass electrode.

Stock solution (0.01 mol/l) of levamisole (Farmaton, Ukraine) was prepared as follows: accurately measured portion of the injection solution equivalent to 118 mg/ml of the levamisole was added to 5 ml of buffer solution with pH 4.0 and diluted to 50 ml with 0.1 mol/l solution of LiCl. It was further diluted to produce working standard solutions with the concentration range 1×10^{-1}–1×10^{-7} mol/l.

An ion-pair of levamisole tropeoline 00 was prepared by mixing equimolar quantities of 1×10^{-2} mol/l levamisole and anionic dye tropeoline 00 (Shostka, Ukraine) (Fig. 1 (b)). The solution was settled for 2 h and the IP sediment was filtered (quantitative rapid filter paper). This residue was treated with 50 ml of cold distilled water. The precipitate was dried and used as an electrode active substance for preparing the levamisole-sensitive sensor.

The generally accepted technique of preparing a plasticized membrane consists of thorough mixing of the electrode-active substance with PVC dissolved in cyclohexanone or tetrahydrofuran followed by the evaporation of the solvent in a glass ring. PVC requires plasticization and places a constraint on the choice of mediator. Plasticized PVC membranes were prepared according to the recommendations [17]. The sensing membrane was prepared by mixing 70 mg of PVC powder and 15 mg of IP with 0.12 ml of a plasticizer. The mixture was stirred until the PVC was well moistened, and then the mixture was dispersed in 0.5 ml

THF or CHN. The resulting mixture was transferred into a glass dish of 25 mm diameter. The solvent was evaporated slowly at room temperature until a solid membrane of about 0.3 mm thickness was formed. A desired piece of the membrane was cut and then was attached to an end of polyethylene tube using viscous solution of PVC as an adhesive. The resulting sensor was then filled with an internal solution of 1.0×10^{-2} mol/l levamisole hydrochloride and conditioned for 15 min. Then a copper wire was immersed into the tube.
The electrode was conditioned by soaking the prepared electrode in 1×10^{-2} mol/l levamisole solution for 2 h.

The thermal studies (TG, DTG and DTA) were carried out on an apparatus for complex dynamic thermal analysis under the following conditions: temperature range 20–700°C, heating rate 5 K/min, sample weight 27.3 mg, static air medium, channel sensitivities: DTA – 150 mV, TG–50 mV.

The IR spectra were measured on an AVATAR 330 FT-IR instrument (Thermo Nicolet).
Aliquots (5 ml) of 1×10^{-1}–1×10^{-7} mol/l aqueous levamisole in 0.04 mol/l acetate buffer of pH 4.0 were transferred into 50 ml beakers. The levamisole sensitive membrane sensors (indicator electrode) together with the single junction Ag/AgCl reference electrode were immersed in the Lev solution. The solution was stirred and the potential reading was recorded after stabilization to ±0.3 mV. The electromotive force (e.m.f.) was plotted as a function of levamisole concentration. The calibration plot was used for measuring samples under the same conditions.

Table 1. Data of DTA curve of the ion associate $Tr00^{-}Lev^{+}$ and decomposition products

Ion pair	**Temperature range, °C**	**Loss of mass, %**		**Product**
		Theoretical	**Experimental**	
$C_{29}H_{26}N_5O_3S_2$	200–250(-220)	11.51	11.5	SO_2
	250–280	5.51	5.2	CO
	280–410	10.06	10.0	$2N_2$
		5.76	6.0	S
	410–480	5.04	5.0	C_2H_4
	480–600	30.40	30.5	$NH(C_6H_5)_2$
	600–700	32.20	32.0	(14C+11H)
Total loss of mass		100.00	100.0	

3. RESULTS AND DISCUSSION

Thermal behavior of obtained IP was investigated by differential thermal analysis that would show the thermal stability and the character of the decomposition of the complex. The thermolysis of $Tr00^{-}Lev^{+}$ IP undergoes three stages that fit a theoretical interpretation. The thermogram is shown in Fig. 2 and its computation is presented in Table 1.

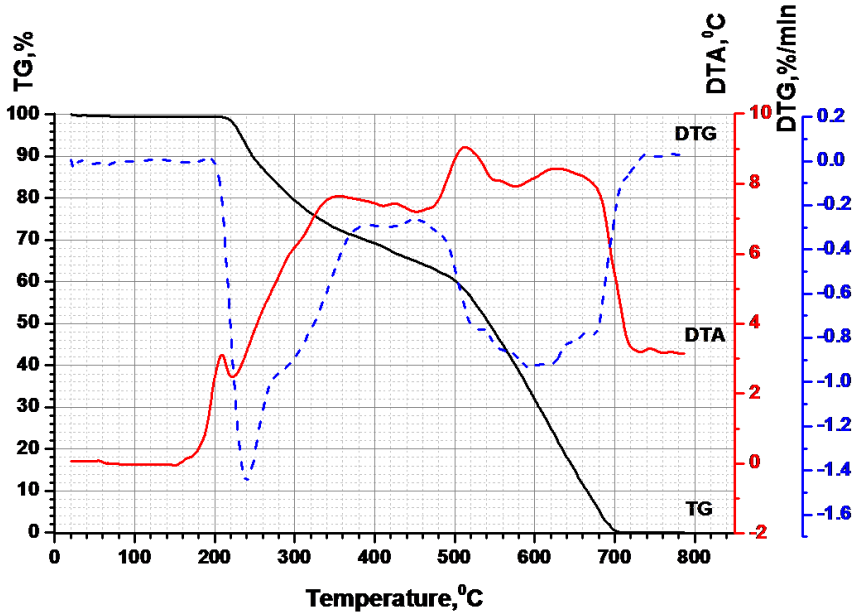


Fig. 2. Simultaneous TG, DTA curves of the Tr00·Lev^{+}

Table 2. Observed bands of levamisole, potassium picrate and IA Lev-tropeoline 00

Wave number, cm^{-1}	**Assignment**	**Wave number, cm^{-1}**	**Assignment**	**Wave number, cm^{-1}**	**Assignment**
tropeoline 00		Lev-tropeoline		levamisole	
3404	ν(N–H)	2688	ν(C–H)	3430	ν(N–H)
				3099	ν(C–H)
				3056	ν(C–H)
				2950	ν(C–H)
1668	ν(N=N)				
1585-1486	ν(C–C)	1596	Ar.ring	1583	Ar.ring
		1506	Ar.ring	1525	Ar.ring
				1496	ν(C–N)
				1445	$\nu(C–H)_{def.}$
				1345	ν(C–N)
1317	ν(C=N)	1306	ν(C=N)	1050	$\nu(C–H)_{tiof}$
1198	ν_{as} SO_2	1166-1106	ν(S=O)		
1140	ν_{s} SO_2				
1095	δ(C–H)	1030	$\nu(C–H)_{tiof}$	737	$\nu(C–H)_{tiof}$
				703	$\nu(C–H)_{tiof}$
625	ν(S–O)				

Thermal decomposition starts above 200°C and is accompanied by the loss of mass and the discharge of the decomposition products. This is followed by carbonization and combustion of the IP, which is shown at the thermogram by small exo effects at 205°C, endo

effects at 240 °C and a significant loss of mass. There is no NH_3 in the decomposition products. At higher temperature we can see a significant loss of mass and few thermo effects at 350, 505, 630 °C. A large amount of amorphous carbon remained as black plaque at the wall of the crucible in which the thermolysis reaction took place.

The vibration spectra analysis was performed by infrared spectroscopy in the range 400 – 4000 cm^{-1}. The obtained infrared spectra of the ion pair of levamisole-tropeoline. In addition, the infrared band assignments are given in Table 2 [18]. These assignments are based on the comparison of the spectra of the formed ion associates and with the spectra of the free reactants (Table 2, Fig. 3).

The IR spectra of tropeoline 00 features the –C=C– stretching observed at 1600, 1500 cm^{-1}, the –S=O stretching at 1198–1140 cm^{-1}, the –C=N stretching at 1317 cm^{-1}, the –N–H stretching at 3404 cm^{-1}. In the IR spectra of the IP, the aromatic –C–H stretch was observed at 3026 cm^{-1}, the –C=C– stretching at 1594, 1506 cm^{-1}, the –C–H stretching at 2688 cm^{-1}.

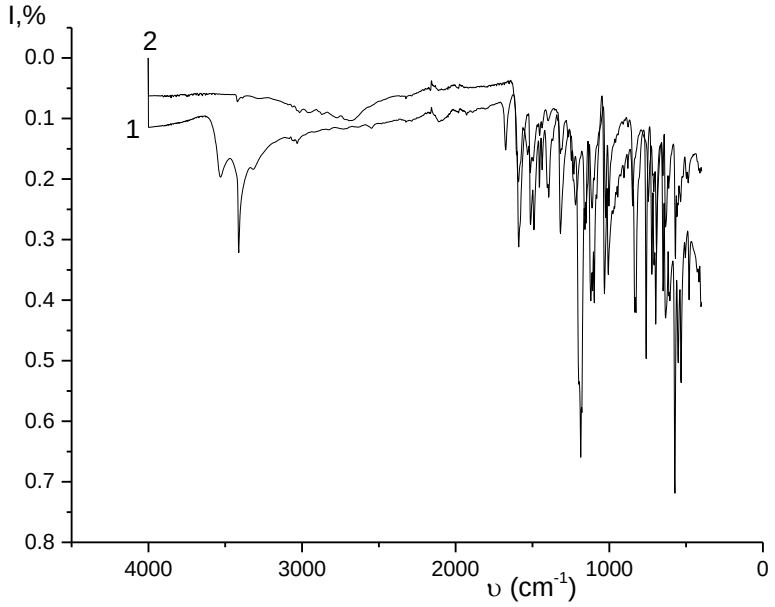


Fig. 3. IR spectra of tropeoline 00 (1) and ion pair (2)

The present work exhibit new membrane sensor levamisole–tropeoline 00 in which PVC was used as a polymeric matrix. The developed sensor exhibit a good selectivity with respect to related substances, additives in dosage forms and heavy metals. The conventional design was prepared, characterized and compared according to IUPAC recommendations.

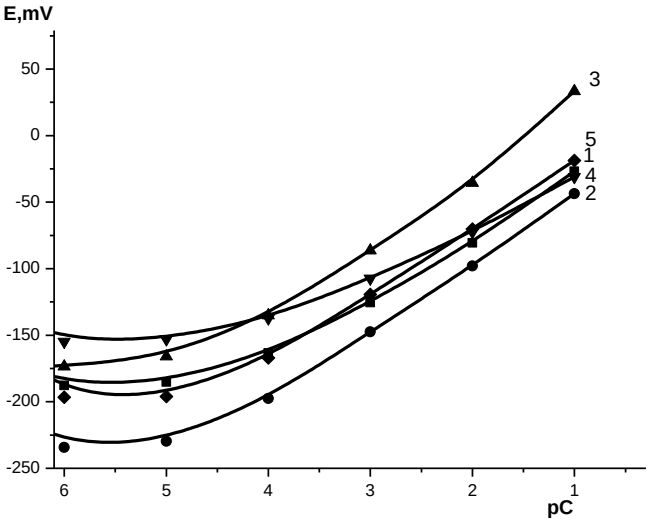


Fig. 4. Graphical dependence of the ion-selective electrode potential on the Lev concentration E=f(pC). EAS content: 1–3%; 2–5%; 3–7%; 4–10%; 5–13%.

Table 3. Effect of EAS content and plasticizer nature on main electroanalytical characteristics of the developed sensors

EAS content, %	**Plasticizer, content, %**	**Slope, mVpC^{-1}**	**Linearity range, mol/l**	**Low detection limit, mol/l**
3	DBS, 60	49±1	5×10^{-3}–1×10^{-1}	1.4×10^{-4}
5		54±1	1×10^{-4}–1×10^{-1}	7.9×10^{-5}
10		51±1	5.8×10^{-5}–1×10^{-1}	3.0×10^{-5}
13		50±1	1×10^{-4}–1×10^{-1}	6.3×10^{-5}
5	DBS, 60	54±1	1×10^{-4}–1×10^{-1}	7.9×10^{-5}
	DNP, 60	24±1	5×10^{-3}–1×10^{-1}	2.5×10^{-3}
	DBP, 60	12±1	5×10^{-3}–1×10^{-1}	2.5×10^{-3}
	TCP, 60	30±1	1×10^{-4}–1×10^{-1}	1.3×10^{-4}
	DOP, 60	26±1	1×10^{-4}–1×10^{-1}	6.3×10^{-5}
	DEP, 60	47±1	1×10^{-4}–1×10^{-1}	6.3×10^{-5}

Generally, the sensitivity, selectivity, working range, and stability of an ion-selective electrode depend not only on nature of the IP, but are also strongly influenced by the nature and amount of the plasticizer and additives.

To assess the effect of the plasticizer nature, uniform membranes were prepared with DBP, DOP, DBS, DNP, DEP and TCP. It was established that the best plasticizers for the membranes based on IA (Lev^+)($Tr00^-$) are DBS and DEP with electrode function slope 53.8

and 47.2 mV/pC and the detection limit 7.9×10^{-5} mol/l and 6.3×10^{-5} mol/l respectively (Table 3, Fig. 4).

The response time was measured that is necessary for the potential of the membrane electrode to reach the value within ±1 mV of the final equilibrium value after immersing the electrode in the solution of levamisole with 10-fold difference in concentration. The sensors showed rapid response within 15 seconds for drug solutions $\geq1\times10^{-3}$ mol/l and 25 seconds for 1×10^{-5} mol/l. The lifetime of the sensors were examined by re-calibration every 2 days. There was no noticeable deterioration in the sensor performance in terms of detection limit, calibration curve slope and response time over a period of 30 days (Fig. 5).

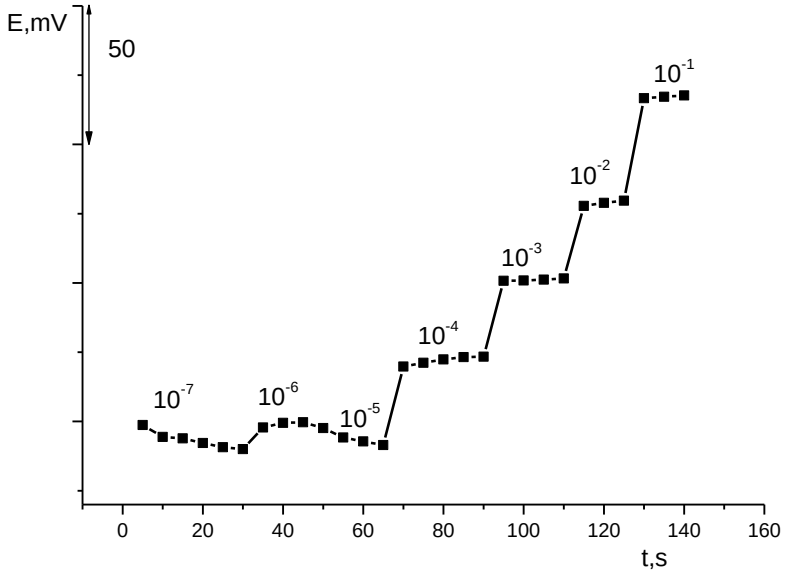


Fig. 5. Sensor response time for various levamisole concentrations

The sensor lifetime is mainly determined by the frequency of its use and averages 10 months from the manufacture date.

Table 4. Dependence of ISE electrode characteristics on the ionic strength of solution

Solvent	**Ionic strength**	**S, mV**	**Range of linearity, mol/l**	**C_{min}, mol/l**
DBS, 60%	0.05	34.3	1×10^{-1}–7.8×10^{-4}	2.6×10^{-4}
	0.1	33.2	1×10^{-1}–6.1×10^{-4}	5.5×10^{-4}
	0.5	54.0	1×10^{-1}–1.0×10^{-4}	7.9×10^{-5}
	1.0	23.1	1×10^{-1}–6.3×10^{-5}	3.2×10^{-5}

Main characteristics of the membrane-based electrode with tropeoline 00 EAS according to the ionic strength of solution are shown in Table 4. According to the experimental data, the

best concentration of LiCl in solution is 0.5 mol/l. Generally, the nature and concentration of the electrolyte are very important in the measurement of the electrode potential. The cation must not complete with the potential-defining cation for the place in the membrane [19].

The pH effect of the tested solution on the electrochemical behavior of the sensor was studied at a constant concentration of levamisole hydrochloride and varying the content of the hydrogen ions in the pH range of 2.0–11.0 which was adjusted with HCl or NaOH solution. The results are illustrated in Fig. 6. The potential remains constant in the range of 3.0–9.0. The observed potential drift at lower pH values may be attributed to the membrane response to H^+, and at higher pH values (pH>9) could be due to formation of levamisole in an ionic form (pK_a=9.5).

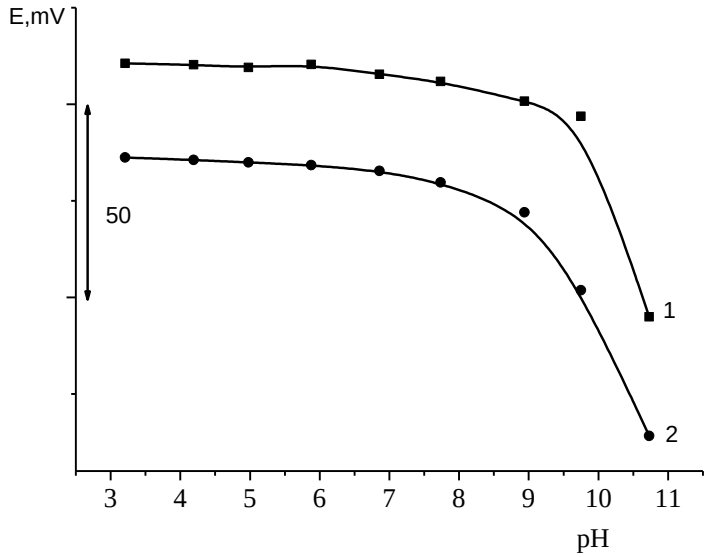


Fig. 6. The effect of the solution pH on the electrode potential at levamisole pC=2 (1), pC=3 (2)

3.1. Sensor selectivity

The potentiometric selectivity coefficients **(**$K^{pot}_{Lev^+,I^+}$) of the levamisole sensors were measured by the separate solutions method. The influences of some inorganic cations were investigated by separate solution method in which the Nicolsky-Eisenman equation was used. The potentiometric coefficients in cases of species without charges were determined using the matched potential method. The potentiometric selectivity coefficients are defined in this method as the activity ratio of primary ions and interfering ions that give the same potential charge under identical conditions. The potentiometric selectivity coefficient of the levamisole based sensors depends on the selectivity of the ion-exchange process at the membrane-sample interference, the mobility of the respective ions in the membrane. The free energy transfer of the levamisole ion between the aqueous and the organic phase could also control the

selectivity of the proposed sensor. The results obtained are summarized in Table 4. No interference from ions such as NH_4^+, K^+, Na^+, Co^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, cetylpyridinium chloride, tetramethylammonium chloride, cetyltrimethylammonium bromide, tetrabutylammonium iodide, benzyltriethylammonium chloride etc. was recorded. The proposed sensors exhibited a high selectivity towards levamisole with respect to the test ion.

3.2. Determination of levamisole in pharmaceutical preparations and biological fluids

3.2.1. Tablets

Five tablets were accurately weighted and finely powdered. A portion of powder equivalent to one tablet of levamisole was accurately weighted, transferred to 50 ml volumetric flask, dissolved and shaken for 15 minutes with 5 ml of 0.04 mol/l acetate buffer of pH 4.0 and 5 ml of 0.5 mol/l solution LiCl. The solution was then diluted to the mark.

Table 5. Results of the determination of levamisole amounts in pharmaceuticals and biological fluids ($F_{tabl.}$=5.05; t_{tabl}=2.78)

Sample	Label amount, mg	Found by proposed sensor			Found by potentiometric titration		
		mg	S^2	RSD (%)	mg	S^2	RSD (%)
Decaris, Richter Gedeon Ltd	150	150.7±1.04	0.29	0.56	150.7±1.79	2.09	0.96
Decaris, Richter Gedeon Ltd	50	49.1±0.67	0.7	1.08	49.9±0.77	0.38	1.24
Levamisole-Zdorovye, Kharkiv Ukraine	150	150.6±1.67	1.82	0.90	150.9±1.35	1.18	0.72
Urine	59	58.9±0.47	0,14	0.64	59.6±0.77	0.39	1.06

3.2.2. Biological fluids (drugs in urine samples)

Aliquots of 5 ml urine were transferred to 100-ml measuring flasks, made up to the mark with the respective buffers (solution 1). Solution with levamisole concentration of 118 mg/ml was used. The aliquots of 1.0 ml, 2.0 ml of the drug solution were transferred into 50 ml

measuring flasks and made up to the mark with solution 1 and were analyzed by potentiometric determination with the developed drug electrode.

The e.m.f. of the solution was measured above and the corresponding concentration was determined using the calibration plot.

Levamisole in various drugs formulation was determined by direct potentiometric measurements using these sensors. The potentials measured by those sensors were recorded and compared with the calibration graph. The proposed membrane sensor based on ion pair of levamisole tropeoline was found to work well under laboratory conditions. It was applied for the determination of levamisole in pharmaceutical forms (Table 5).

4. CONCLUSION

New levamisole-sensitive sensor with PVC membrane was developed that contain ion pair of levamisole with tropeoline 00 as electrode-active substance. The proposed electrodes exhibit long lifetime, good stability, sensitivity, precision, accuracy and selectivity. They are low-cost, easy to prepare and to use.

We have shown on the basis of experimental results the successful applicability of the new membrane electrodes based on the ion pairs of levamisole with tropeoline 00 for the determination of levamisole in pharmaceutical forms. According to the results of the statistical analysis, the proposed techniques are uniformly precise and uniformly correct.

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