

# РОЗДІЛ I

## Аналітична хімія

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### Potentiometric Determination of Levamisole in Pharmaceutical Formulations by Membrane Sensors Based on Ion-Pair

The characteristics, performance and application of membrane sensor based on ion-pair Levamisole Picrate are described. The sensor's response to the levamisole ion has the sensitivity of (38–52) mV/pC over the range of  $1 \cdot 10^{-5}$ – $1 \cdot 10^{-1}$  mol/L, and the detection limit is  $2,5 \cdot 10^{-6}$  mol/L at pH 3,0–8,0. The sensor is easily assembled at a relatively low cost and has fast response time (5–10 s). The proposed sensor displayed good selectivity for levamisole ion in various substances. It was used to determine levamisole hydrochloride in pharmaceuticals.

**Key words:** Levamisole; PVC Membrane sensors; Potentiometry; Pharmaceutical analysis.

**Introduction.** Levamisole, (S)-6-Phenyl-2,3,5,6-tetrahydroimidazo-[2,1-b][1,3]-thiazole (Fig. 1) is a potent broad-spectrum antihelmintic drug, which is widely used in veterinary medicine for the control of gastrointestinal parasites in cattle, sheep and pigs. It is normally administered orally, by pour-on or by subcutaneous or intramuscular injection. The recommended dose is 8 mg/kg body weight. It has been used as an immunostimulant in humans [1]. Levamisole hydrochloride is used in the treatment of a variety of immune diseases and autoimmune diseases; levamisole hydrochloride liniments have been developed for the administration of this drug which can treat skin type immune therapy effectively. Light yellow crystalline powder, it is soluble in water, methanol, slightly soluble in ethanol, very slightly soluble in chloroform, insoluble in acetone; stable under ordinary conditions [16].

A great deal of work has been done by the researchers on the current application and future possibilities for altering the drug activities and evaluation with new method development by instrumental methods.

Various techniques have been used for the determination of levamisole in biological fluids. They include gas chromatography with nitrogen-phosphorus detection (GC-NPD) [24] and flame ionization (GC-FID) [18] and liquid chromatography (LC) with ultraviolet (UV) detection [2; 17; 23]. The methods developed for levamisole quantification include polarography [8], gas-liquid chromatography [7] and high performance liquid chromatography (HPLC) [2; 6; 17]. There is only one report on determination of levamisole hydrochloride using a PVC membrane ion selective electrode (ISE) based on levamisole hydrochloride-tetraphenyl borate as an active material [22].

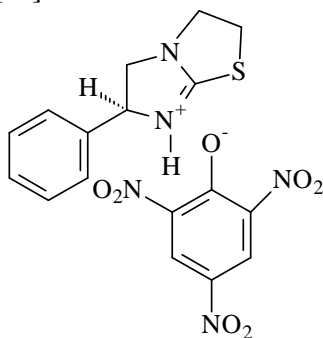


Fig. 1. The chemical structure of levamisole picrate

The potentiometric sensors remain an important and promising field of analytical chemistry. This is related to the advantages of potentiometry (simplicity, fast response, sensitivity, selectivity, analysis in muddy and colored media etc.) over other analysis methods [22]. A research of new electrode-active substances is important. Due to the functional properties of ion-pair (IP) to donate both a cation and an anion as potential-determining ions, they are the most universal ionophores. It is likely that solubility is the main restriction for their use as ionophores. The solubility of IPs should be sufficiently low in aqueous phase, otherwise they will be washed out from the membranes; and at the same time, high in the membrane phase, otherwise it will be difficult to obtain a homogeneous membrane. Previously, we analyzed the applicability of ISEs based on ion-pairs as electrode-active substances in the analysis of pharmaceuticals [3–5; 9–15; 19].

**The aim of this work is** to study a simple and inexpensive method for the assaying of levamisole in pharmaceutical forms. The method is based on the ion-pair of levamisole picrate (Fig. 1) and using this ion-pair as an electrode active substance for a membrane potentiometric sensor.

**Material and methods.** All chemicals were of analytical-reagent grade. The modeling of the membrane composition of the potentiometric sensor used high molecular weight PVC, dibutylphthalate (DBP), dioctylphthalate (DOP), dinonylphthalate (DNP), diethylphthalate (DEP), tricresylphosphate (TCP) and tetrahydrofuran (THF), which were obtained from Sigma-Aldrich. Distilled water was used to prepare all solution in all experiments.

Standard solutions and buffers were prepared freshly with deionized water. Buffer solution were prepared by mixing corresponding amounts of 0,04 mol/L  $H_3BO_3$ , 0,04 mol/L  $CH_3COOH$ , 0,04 mol/L  $H_3PO_4$  and 0,2 mol/L NaOH.

An ion-pair of levamisole picrate was prepared by mixing equal quantities of  $1 \cdot 10^{-2}$  mol/L levamisole and picric acid. The solution was settled for 2 h and the ion-pair sediment was filtered (quantitative rapid filter paper). This residue was treated with 50 ml of cold distilled water. The precipitate was dried as an electrode-active substance for preparing the levamisole-sensitive sensor.

The sensing membrane was prepared by mixing 70 mg of PVC powder and 15 mg of levamisole picrate with 0,12 mL of a plasticizer – TCP. The mixture was stirred until the PVC was well moistened, and then the mixture was dispersed in 0,5 mL THF. The resulting mixture was transferred into a glass dish of 25 mm diameter. The solvent 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 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  $1,0 \cdot 10^{-2}$  mol/L levamisole hydrochloride and conditioned for 15 min. Then, a copper wire was immersed into the tube.

The selective sensor was calibrated under a conventional working regime; the solutions for the calibration were prepared by the consecutive dilution of 0,1 mol/L levamisole hydrochloride with bidistilled water. The potential of the electrochemical cell was measured in standard solutions with progressively increasing concentration under constant stirring. The performance of the sensor was investigated by measuring the emf values of various levamisole hydrochloride solutions.

An IA–123 model ionometer with Ag/AgCl reference electrode was used for the measurements of potential difference at  $(25,0 \pm 0,1)$  °C.

**Results and discussion.** Because the sensitivity and selectivity of any membrane sensor are significantly affected by the composition of the levamisole-sensitive membrane and the nature and amount of the solvent mediators and additives used, we decided to study such effects on the behavior of the proposed sensor. The effect of the nature of the plasticizer and the amount of ion-pair of levamisole picrate on the potential response of the proposed levamisole-selective electrode were investigated. The obtained results are provided in Table 1. Evidently, TCP is a more effective solvent mediator than all others in the preparation of the levamisole-selective sensor.

The influence of the membrane thickness on the potential responses of the levamisole sensor was investigated. The optimum thickness of the membrane is 0,15–0,35 mm.

The concentration of internal solution does not cause any significant difference in the potential response of the sensor except for the change in the intercept of the resulting Nernstian plots.

The effect of pH of the test solution on the response of the levamisole-sensitive membrane was examined for  $5 \cdot 10^{-3}$  mol/L and  $5 \cdot 10^{-4}$  mol/L concentrations. The pH effect of the test solution on the electrochemical behavior of the sensor was studied under a constant concentration of levamisole hydrochloride and

varying the content of the hydrogen ions in the pH range 3,0–10,5 which was adjusted with HCl or NaOH solution. The results are illustrated in Fig. 2. The potential remains constant in the pH range of 3,0–8,0.

The response time of the sensor was measured after successive immersion in a series of levamisole solutions with tenfold increase of the levamisole ion concentration each, from  $1 \cdot 10^{-7}$  mol/L to  $1 \cdot 10^{-1}$  mol/L. The static response time thus obtained was 3–5 s for  $1 \cdot 10^{-4}$ – $1 \cdot 10^{-1}$  mol/L levamisole ion concentration. At lower concentrations ( $1 \cdot 10^{-7}$ – $1 \cdot 10^{-5}$  mol/L), however, the response time approaches 5–15 s.

Table 1

**Characteristics of different electrode active substances with different solvent mediators of levamisole ion sensitive sensor**

Amount of the ion-pair, %	Plasticizer, 65 %	Slope (mV)	Linear range, mol/L	Detection limit, mol/L
1	TCP	$45,0 \pm 1,0$	$1,0 \cdot 10^{-4}$ – $1,0 \cdot 10^{-1}$	$1,0 \cdot 10^{-4}$
3	TCP	$52,0 \pm 1,2$	$2,5 \cdot 10^{-5}$ – $1,0 \cdot 10^{-1}$	$3,2 \cdot 10^{-5}$
5	TCP	$41,0 \pm 1,0$	$1,5 \cdot 10^{-4}$ – $1,0 \cdot 10^{-1}$	$1,0 \cdot 10^{-4}$
7	TCP	$42,0 \pm 1,0$	$1,0 \cdot 10^{-5}$ – $1,0 \cdot 10^{-1}$	$3,2 \cdot 10^{-6}$
7	DNP	$46,0 \pm 1,1$	$2,5 \cdot 10^{-5}$ – $1,0 \cdot 10^{-1}$	$7,9 \cdot 10^{-6}$
7	DOP	$46,0 \pm 1,1$	$2,5 \cdot 10^{-5}$ – $1,0 \cdot 10^{-1}$	$6,3 \cdot 10^{-6}$
7	DBP	$41,0 \pm 1,0$	$3,2 \cdot 10^{-5}$ – $1,0 \cdot 10^{-2}$	$1,0 \cdot 10^{-5}$
7	DEP	$40,0 \pm 1,2$	$1,0 \cdot 10^{-5}$ – $1,0 \cdot 10^{-1}$	$6,3 \cdot 10^{-6}$
10	TCP	$38,0 \pm 1,0$	$1,0 \cdot 10^{-4}$ – $1,0 \cdot 10^{-1}$	$1,0 \cdot 10^{-4}$
15	TCP	$40,0 \pm 1,1$	$3,0 \cdot 10^{-4}$ – $1,0 \cdot 10^{-1}$	$3,0 \cdot 10^{-4}$

An important characteristic of any potentiometric sensor is its response to the primary ion in the presence of others ions solution, which is expressed in terms of the potentiometric selectivity coefficients.

The potentiometric selectivity coefficient for membrane levamisole sensitive sensor  $-\lg K_{Lev,I}^{pot}$  was determined for a number of cations by the matched potential methods [25]. No interference was noted for most of the compounds of solution such as  $NH_4^+$  (>4),  $Cu^{2+}$  (>4),  $K^+$  (>4),  $Na^+$  (>4),  $Co^{2+}$  (>4),  $Ba^{2+}$  (>4),  $Ca^{2+}$  (>4),  $Mg^{2+}$  (>4), tetramethylammonium bromide (>4), benzyltrimethylammonium chloride (>4). A comparison with reported electrodes indicates that the selectivity coefficients of the proposed sensor are better than those reported for other levamisole ion-selective electrodes.

Consequently, cation interactions with the membrane phase, and thus membrane transport selectivity, are dictated primarily by the partition coefficients of given cation between the aqueous test solution and the solvent or plasticizer used to prepare the polymer film.

The proposed membrane sensor based on ion-pair of levamisole picrate was found to work well under laboratory conditions. It was applied for the determination of levamisole hydrochloride in pharmaceutical forms (Table 2).

Table 2

**Recovery data for levamisole acid amounts in pharmaceutical forms ( $F_{tabl.} = 5,05$ ;  $t_{tabl.} = 2,78$ )**

Sample	Label amount, mg/tablet	Found by proposed sensor			Found by potentiometric titration			F-test	t-Test
		mg	$S^2$	RSD (%)	mg	$S^2$	RSD (%)		
Decaris, Richter Gedeon Ltd	150	$150,8 \pm 0,86$	0,48	0,46	$150,0 \pm 0,9$	0,56	0,5	0,86	1,79
Decaris, Richter Gedeon Ltd	50	$51,1 \pm 1,0$	0,41	1,57	$50,2 \pm 0,8$	0,39	1,24	1,59	2,00
Levamisole-Zdorovye, Kharkiv Ukraine	150	$150,6 \pm 1,0$	0,62	0,52	$150,2 \pm 1,2$	0,87	0,62	0,71	0,77

The sensor is easy to assemble at a relatively low cost and can be used for a period up to 3-5 months without any considerable divergence in potential.

The proposed membrane is very easy to prepare and shows high sensitivity and wide pH range. This approach is cost-effective and has other advantages, such as high accuracy, selectivity, and fast response for the determination of levamisole hydrochloride in dosage forms.

**Conclusions.** The results obtained in present work demonstrate that membrane potentiometric sensors based on the ion-pair of levamisole picrate with internal comparison solution were developed. The proposed sensors exhibit long lifetime, good stability, sensitivity, precision and selectivity. They are low cost and easy to both prepare and use.

Techniques with high metrological characteristic for the quantitative determination of levamisole hydrochloride in pharmaceuticals were developed. The results of this study show that a potentiometric sensor may provide an attractive alternative for levamisole determination.

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**Кормош Жолт, Зубеня Наталя. Потенціометричні визначення левамизолу у фармацевтичних препаратах мембранними сенсорами з використанням іонних асоціатів.** Описано характеристики, відтворюваність і застосування мембранного сенсора на основі іонного асоціату Левамизол Пікрату. Відгук сенсора на левамизол-іон має чутливість (38–52) мВ/рС у діапазоні від  $1 \cdot 10^{-5}$ – $1 \cdot 10^{-1}$  моль/л, а межа виявлення –  $2,5 \cdot 10^{-6}$  моль/л при рН 3,0–8,0. Сенсор легко виготовляється при відносно низькій вартості і має малий час відгуку (5–10 с). Пропонований сенсор показує хорошу селективність левамизолу до різних речовин. Він був використаний для визначення левамизол гідрохлориду у фармацевтичних препаратах.

**Ключові слова:** левамизол, ПВХ-мембранний сенсор, потенціометрія, фармацевтичний аналіз.

**Кормош Жолт, Зубеня Наталя. Потенциометрические определения левамизола в фармацевтических препаратах мембранными сенсорами с использованием ионных ассоциатов.** Описаны характеристики, воспроизводимость и применение мембранного сенсора на основе ионного ассоциата Левамизол Пикрата. Отклик сенсора на левамизол-иона имеет чувствительность (38–52) мВ/рС в диапазоне от  $1 \cdot 10^{-5}$ – $1 \cdot 10^{-1}$  моль/л, а предел обнаружения –  $2,5 \cdot 10^{-6}$  моль/л при рН 3,0–8,0. Сенсор легко изготавливается при относительно низкой стоимости и имеет малое время отклика (5–10 с). Предлагаемый сенсор показывает хорошую селективность левамизола к различным веществам. Он был использован для определения левамизол гидрохлорида в фармацевтических препаратах.

**Ключевые слова:** левамизол, ПВХ-мембранный сенсор, потенциометрия, фармацевтический анализ.

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Валерій Гаєвський

### Калібрування кондуктометричного датчика

Розроблено спосіб калібрування кондуктометричних датчиків у водних розчинах вуглекислоти. Спосіб базується на теоретичних розрахунках карбонатної водної системи й одночасних кондуктометрично-потенціометричних вимірюваннях. Результати метрологічних досліджень показали, що відносна похибка калібрування не перевищує  $\pm 1\%$ .

**Ключові слова:** кондуктометрія, електропровідність, потенціометрія, карбонатна водна система, константи дисоціації.

**Постановка наукової проблеми та її значення.** Електропровідність водних середовищ – важлива фізико-хімічна характеристика, яка нормується майже у всіх нормативно-технологічних документах, тому метрологічні дослідження кондуктометричних датчиків, особливо таких, що самостійно виготовляються в дослідницьких лабораторіях, є важливим етапом його підготовки до вимірювань.

Для досліджень використали аналізатор якості водних систем (АЯВС) [1], який одночасно вимірює вісім фізико-хімічних параметрів, три з яких дають можливість розробити нову методику калі-