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ARTICLES

A Clotrimazole-Selective Electrode and its Application to Pharmaceutical Analysis

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Abstract—The effect of measurement conditions and the nature of the plasticizer of an ion-selective electrode membrane (*ortho*-nitrophenyloctyl ether, dinonyl adipate, dibutyl phthalate) on the main performance characteristics of clotrimazole-selective electrodes with polyvinyl chloride ion-exchange membranes containing potassium tetrakis(4-chlorophenyl)borate as an ion exchanger was studied. The introduction of small (up to 20%) ethanol additives into the aqueous solution leads to a significant improvement of the potential stability and expands the working range of the electrodes. All of the studied electrodes have high selectivity to clotrimazole with respect to both inorganic and organic cations, slope of the electrode function close to the theoretical value, and low limits of detection (5.0×10^{-8}–7.5×10^{-8} M). An electrode with a membrane plasticized with *ortho*-nitrophenyloctyl ether was the best for the combination of characteristics. It was demonstrated that clotrimazole can be determined in model solutions and various dosage forms (spray, cream, suppositories, tablets) using direct potentiometry and potentiometric titration with sodium tetraphenylborate. The proposed procedures are rapid (10–15 min) and characterized by excellent reproducibility (the relative standard deviation does not exceed 1.2%).

Keywords: clotrimazole, potentiometry, ion-selective electrode, drug analysis
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Clotrimazole (1-[(2-chlorophenyl)diphenylmethyl]-1*H*-imidazole) is a synthetic pharmaceutical preparation with antifungal activity; it is produced as vaginal tablets, suppositories, spray, ointment, and cream [1]. Clotrimazole is a derivative of imidazole; it has a broad spectrum of action. It is used for the local treatment of candidiasis and other mycoses; it is also effective against dermatophytes, yeast and mold fungi, pathogens of multi-colored lichen and erythrasma, and gram-negative and gram-positive bacteria.

A pharmacopoeial method for determining clotrimazole in a substance is the nonaqueous titration with $HClO_4$ in glacial acetic acid; β-naphthobenzene is used as an indicator. Chromatographic methods are widely used in drug analysis [2–4]. The listed methods of analysis have some disadvantages. Nonaqueous titration is nonselective and requires the use of toxic solvents (glacial acetic acid, acetic anhydride). Chromatographic methods involve the use of expensive equipment and consumables and a relatively long and labor-consuming sample preparation associated with the need in the separation of the matrix components of the test sample, which can disable the chromatographic column.

A convenient alternative to these methods is potentiometry using ion-selective electrodes (ISEs). The real advantages of the potentiometric method include the low cost of instrumentation, rapidness due to the simplicity of sample preparation, selectivity, sufficient sensitivity, and a possibility of work in turbid and colored media. The prospect of using the potentiometric method in the pharmaceutical analysis is also due to the relative simplicity of the development of appropriate ISEs, at least for the determination of sufficiently hydrophobic organic cations and anions. Their high affinity to the membrane phase, caused by their lipophilicity, determines the rather high selectivity to these ions relatively inorganic ingredients (three orders of magnitude and higher). There are vast possibilities for controlling their selectivity by means of a purposeful selection of an ion exchanger and plasticizer and the introduction of specific solvating additives into the membrane [5–7]. Hundreds of articles were devoted to the application of the potentiometric method in the pharmaceutical analysis; these papers are partially generalized in [8–10]. Potentiometry is included in the state pharmacopeias of many leading countries as an official method.

Clotrimazole is a highly hydrophobic amine (the logarithm of the distribution constant between water

and *n*-octanol is 6.1) [11], which makes possible the development of highly selective electrodes for its determination. Clotrimazole-selective electrodes with plasticized polyvinyl chloride and cellulose hydrate membranes containing clotrimazole molybdophosphate as an ion exchanger and dibutyl phthalate or *ortho*-nitrophenyloctyl ether as a plasticizer were described in both the conventional (with internal liquid filling) [12, 13] and solid-contact [14] versions. The disadvantages of all the described electrodes include a high limit of detection (1×10^{-5} M) and low selectivity to inorganic cations ($K^{Pot}_{Clot^+, Na^+} = 1 \times 10^{-3}$) [13], which is due to the insufficient lipophilicity of the phosphomolybdate anion, on the one hand, and its relatively high affinity to inorganic cations, on the other hand. Electrodes with polyvinyl chloride membranes showed better properties than those with cellulose hydrate membranes, and liquid-filled electrodes possessed higher stability of the potential and a higher slope of the electrode function compared to solid-contact ones [12, 14].

The goal of this work was to develop a clotrimazole-selective electrode with a low limit of detection and high selectivity, to justify the selection of conditions for its practical application, and to clarify the possibilities of its use for determining clotrimazole in various dosage forms (spray, cream, suppository, tablets).

EXPERIMENTAL

Reagents and solutions. We used the following Fluka reagents to prepare the membranes: polyvinyl chloride as a polymer matrix; potassium tetrakis(4-chlorophenyl)borate as an ion exchanger; and *ortho*-nitrophenyloctyl ether (*ortho*-NPOE), dinonyl adipate (DNA), and dibutyl phthalate (DBP) as plasticizers. Sodium tetraphenylborate (Applichem) was used as a titrant in precipitation potentiometric titration.

For the preparation of solutions, a clotrimazole substance of pharmacopoeial purity, salts of inorganic cations of analytical grade, and salts of physiologically active amines of pharmacopoeial purity were used. Klotrimazol tablets produced by Pharmland (Belarus), Klotrimazol suppositories manufactured by Farmaprim (Moldova), Clotrimazole cream produced by Hyperion (Romania), and Klotrimazol spray from Pharmtechnology (Belarus) were purchased in the pharmacy network of the city of Minsk.

A RADWAG AS 220/C/2 analytical balance was used at all stages of work. Volumes of up to 1 mL were sampled with a Lenpipet single-channel microdoser (100–1000 μL; Russia). Volumes of 5, 10, and 20 mL were sampled with appropriate Mohr pipettes. Other volumes of ethanol were sampled using volumetric glass pipettes.

Preparation of ISEs and potentiometric measurements. The ISE membranes were prepared by the procedure [15] and adhered to the electrode bodies with a solution of polyvinyl chloride in tetrahydrofuran. The membrane compositions of all the ISEs studied were the same and contained 33% of polyvinyl chloride, 1% of potassium tetrakis(4-chlorophenyl)borate, and 66% of the corresponding plasticizer:*ortho*-NPOE, DNA, or DBP.

The measurements were carried out with I-160 and I-160MP potentiometers; an EVL-1M3.1 silver–silver chloride saturated electrode was used as a reference electrode. A BAT-15.2MP automatic titration unit was used for potentiometric titration.

The selectivity coefficients were determined by a modified separate solution method [16]. A series of aqueous solutions of organic and inorganic cations were prepared: Na^+ and K^+ solutions were 0.1 and 1 M, and solutions of organic cations were 10^{-3} and 10^{-4} M. The produced ISEs were soaked in a 1 M NaCl solution for 24 h. A 0.1 M NaCl solution was used as the internal reference solution. The value of the ISE potential in the prepared solutions was then determined, beginning with the least hydrophobic cation. Upon transition to each new cation, the ISE was soaked for 20–30 min in a more concentrated solution of this cation. The selectivity coefficients were calculated by equation

$$K_{ij}^{Pot} = \exp\left(\frac{(E_j - E_i)F}{RT}\right)\frac{a_i}{a_j},$$

where E_j and E_i are the measured values of the potentials in solutions of the foreign and main ions; F is the Faraday constant; R is the universal gas constant; T is temperature, K; a_i and a_j are the activities of the corresponding ions.

A 10^{-5} M solution of clotrimazole was placed inside the ISE, and the electrode was soaked in a 10^{-4} M solution of clotrimazole (both solutions were prepared in 0.01 M HCl). The limits of detection and the slopes of the E–log$c$ dependences were determined from the electrode functions obtained by the method of successive two-fold dilution. The dependence of the ISE potential on pH was determined by adding small amounts of NaOH (1×10^{-3} to 6 M) to 100 mL of a 10^{-4} M clotrimazole solution in 0.1 M HCl. The pH value was controlled with an ESL-43-07 glass electrode.

Since potassium tetraphenylborate is insoluble, a saturated solution of potassium chloride in the reference electrode was replaced with a mixture of sodium sulfate and sodium chloride for the potentiometric titration of clotrimazole.

RESULTS AND DISCUSSION

Clotrimazole (see chemical structure Scheme 1) is a weak base; its constant in a water–ethanol (1 : 1) mixture is $pK_a = 4.7$ [17]. It occurs in the cationic form

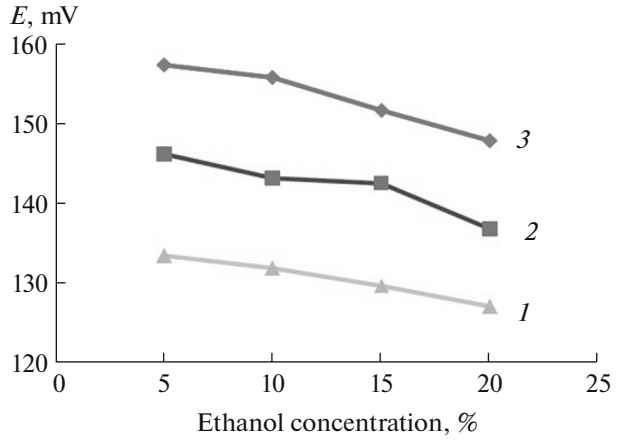


Fig. 1. Dependence of the potentials of ISEs in a 2.5×10^{-5} M clotrimazole solution against 0.01 M HCl on the alcohol concentration: (*1*) clotrimazole–SE-1 (*ortho*-NPOE), (*2*) clotrimazole–SE-2 (DBP), and (*3*) clotrimazole–SE-3 (DNA).

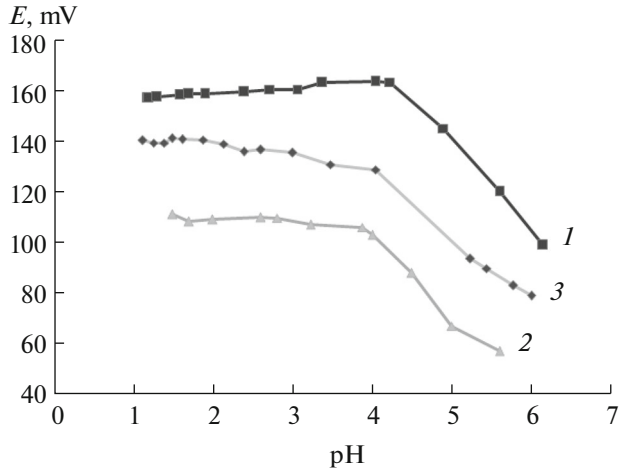
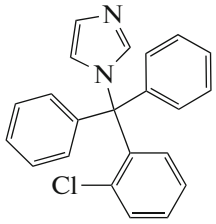


Fig. 2. Dependence of the potentials of ISEs in a 1×10^{-4} M clotrimazole solution on pH in a water–ethanol (4 : 1) mixture: (*1*) clotrimazole–SE-1 (*ortho*-NPOE), (*2*) clotrimazole–SE-2 (DBP), and (*3*) clotrimazole–SE-3 (DNA).

only in a sufficiently acidic medium (pH 1–4). Because of the high hydrophobicity of clotrimazole, its molecular form is slightly soluble in water (according to different data, from 0.21 to 0.49 mg/L [17, 18]); therefore, extraction with ethanol is commonly used to recover clotrimazole substance from various dosage forms (tablets, suppositories, ointments). To exclude the evaporation procedure and simplify the sample preparation, and because of the limited solubility of clotrimazole in water and the instability of its aqueous solutions, we evaluated a possibility of determining clotrimazole directly in aqueous alcoholic media.



Scheme 1. Chemical structure of clotrimazole.

Figure 1 shows the dependence of the ISE potential on the volume fraction of alcohol in a 1×10^{-4} M clotrimazole solution in 0.01 M HCl. An increase in the volume fraction of alcohol in the solution from 5 to 20% leads to an insignificant (by ~10 mV) decrease in the potential, while the magnitude of the effect is practically independent of the nature of the plasticizer. The nature of the change in potential as a function of the volume fraction of alcohol is caused by the superposition of two oppositely directed factors: on the one hand, a decrease in the hydration energy of the protonated amino group, which would lead to an increase in the affinity of the clotrimazole cation to the membrane phase and increase the potential, and on the other hand, a weakening of the effect of the hydrophobic interaction and, as a result, a decrease in the affinity to the membrane phase and a decrease in the potential. It is seen from the experimental data that the second factor dominates, but the overall effect is small, so that even in the mixed aqueous–alcoholic medium, the high affinity of the clotrimazole cation to the membrane phase is preserved, which supposes high selectivity with respect to more hydrophilic cations and a low limit of detection under these conditions.

The pH dependences of the potential of the investigated ISEs in a 10^{-4} M solution of clotrimazole in a binary water–alcohol mixture (4 : 1) are presented in Fig. 2. At pH > 4, a decrease in the potential is observed for all electrodes, while the electrode with the membrane plasticized with *ortho*-NPOE is characterized by the lowest dependence of the potential on pH in the range of 1–4.

Typical electrode functions are shown in Fig. 3. The experimentally determined analytical characteristics of the ISEs (slope of electrode function, working concentration range, selectivity coefficients (K_{ij}^{Pot}), and pH range of functioning) are presented in Table 1.

The slope of the electrode function of the ISE based on *ortho*-NPOE is close to the theoretical value; the ISEs based on DNA and DBP exhibit slightly lower slopes. The linear range of operation is approximately the same for all the electrodes studied, and in the region of high concentrations, it is limited by the low solubility of clotrimazole. The limit of detection is minimal in the case of ISE based on *ortho*-NPOE.

The main interfering cation in pharmaceuticals based on clotrimazole is the sodium cation, which is usually included in their composition as sodium phosphate. The selectivity coefficients for the Na^+

Table 1. Performance characteristics of ion-selective electrodes

Characteristic	Conditions	*o*–NPOE	DBP	DNA
pH range	pH 1–6.3	1–4.1	1–4	1–4
S, mV	0.01 M HCl, water : ethanol (4 : 1)	57.2	51.7	53.4
Limit of detection, M		5.0×10^{-8}	6.1×10^{-8}	7.5×10^{-8}
Working concentration range (p*c*)		4.0–6.6	4.0–6.7	4.0–6.7
Repeatability of potential, mV ($n = 10$, $P = 0.95$, 0.01 M HCl, water : ethanol (4 : 1))	1×10^{-4} M	±0.7	±0.2	±0.4
	1×10^{-5} M	±0.8	±0.3	±0.4
Repeatability of potential, mV ($n = 10$, $P = 0.95$, water, 0.01 M HCl)	1×10^{-4} M	±1.3	±0.9	±0.9
	1×10^{-5} M	±1.5	±1.1	±1.2
K_{ij}^{Pot}	Na^+ 0.1 M	4.7×10^{-11}	1.5×10^{-8}	2.5×10^{-8}
	K^+ 0.1 M	1.86×10^{-9}	6.16×10^{-8}	7.9×10^{-8}
	Diphenylhydramine 1×10^{-3} M	2.6×10^{-2}	1.2×10^{-2}	9.2×10^{-3}
	Naphazoline 1×10^{-3} M	1.8×10^{-3}	1.3×10^{-3}	1.5×10^{-3}
	Tropisetron 1×10^{-3} M	5.8×10^{-4}	6.2×10^{-4}	4.7×10^{-4}

and K^+ cations are markedly increased in the series of *ortho*-NPOE < DBP < DNA, in accordance with the increase in the basicity of the plasticizers and their solvating ability with respect to small cations. As for organic cations, the effect of the nature of the plasticizer on selectivity is insignificant. The reproducibility of the potential for the electrode with the membrane plasticized with *ortho*-NPOE is somewhat lower than for the other two electrodes but is at an acceptable level. In water–alcohol solutions, the reproducibility of the potential is higher than in the absence of alcohol. The electrodes remain operative for at least three months. On the strength of all the data, the ISE based on *ortho*-NPOE exhibits the best analytical characteristics. This electrode was used to develop procedures for the potentiometric determination of clotrimazole in medicinal preparations.

Table 2. Composition of pharmaceutical preparations

Product (manufacturer)	Composition
Klotrimazol, 100 mg, tablets (Pharmland, Belarus)	Clotrimazole 100 mg, lactose monohydrate, potato starch, crospovidone, copovidone, magnesium stearate, adipic acid
Klotrimazol, 100 mg, vaginal suppositories (Farmaprim, Moldova)	Clotrimazole 100 mg, semisynthetic glycerides (Suppocyr or Witepsol) up to 2.0 g
Clotrimazole, 1% cream for external use (Hyperion, Romania)	Clotrimazole, cetyl alcohol, petrolatum, glycerol, Tween 80, nipagin, distilled water
Klotrimazol, 10 mg/mL spray for topical use (Pharmtechnology, Belarus)	Clotrimazole, isopropyl myristate, ethanol

We studied a possibility of determining clotrimazole in various dosage forms: tablets, suppositories, ointments, and spray. The composition of the analyzed preparations is shown in Table 2. The following unified sample preparation procedure is proposed based on the concentration of clotrimazole in dosage forms, its solubility in alcohol and water, and the electrode performance.

One suppository, a crushed tablet, or a sample of cream weighing 1 g is transferred quantitatively to a 200-mL conical flask; 100 mL of ethanol is added, and the mixture is stirred with a magnetic stirrer for 5 min. In analyzing the suppository, the solution is heated to 40°C; otherwise, dissolution is carried out at room temperature. Then, 1 mL of the resulting solution is placed in a 100-mL volumetric flask; 19 mL of ethanol and 10 mL of a 0.01M HCl solution are added, and the mixture is brought to the mark with distilled water. When analyzing the spray, 10 mL of the sample to be analyzed is placed in a 100-mL volumetric flask; 10 mL of ethanol and 10 mL of a 0.01M HCl solution

Table 3. Determination of clotrimazole in model solutions and medicinal preparations* ($n = 3$, $P = 0.95$)

Object	Added/claimed	MLS		PT	
		Found	RSD, %	Found	RSD, %
Model solution	113.7 mg	114 ± 2 mg	0.61	114.0 ± 0.6 mg	0.22
Tablets	100 mg	101 ± 2 mg	0.72	–	–
Suppositories	100 mg	101.5 ± 0.5 mg	0.18	104.8 ± 0.5 mg	0.20
Cream	1%	1.08 ± 0.03%	1.1	1.08 ± 0.01%	0.39
Spray	10 mg/mL	10.3 ± 0.3 mg/mL	1.2	10.4 ± 0.2 mg/mL	0.78

* Medicinal preparation passes the test if the concentration of the active substance does not exceed 85–115% of the average concentration.

are added, and the mixture is adjusted to the mark with distilled water. Ultimately, solutions containing 1 mg of clotrimazole in 100 mL of a water–alcohol (4 : 1) mixture are obtained in all cases, regarding the concentration of the active ingredient claimed in the dosage forms.

Clotrimazole was determined by direct potentiometry using the method of limiting solutions (MLS) and by precipitation potentiometric titration (PT) with sodium tetraphenylborate. The limiting solutions containing 0.69 and 1.67 mg of clotrimazole in 100 mL of a water–ethanol (4 : 1) mixture adjusted to 0.001 M HCl were prepared by dissolving the clotrimazole substance in ethanol, followed by dilution and acidification. For this purpose, the corresponding weighed portions of clotrimazole substance, 0.0696 and 0.1687 g (w = 99.1%), were placed in 100-mL volumetric flasks and brought to the mark with ethanol. Aliquot portions of the resulting solutions (1 mL) were quantitatively transferred to 100-mL volumetric flasks; 19 mL of ethanol and 10 mL of 0.01 M HCl were added, and the mixtures were adjusted to the mark with distilled water.

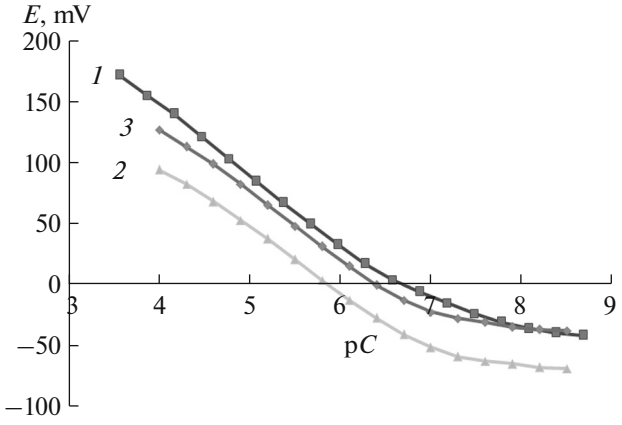


Fig. 3. Dependence of the potentials of ISEs on the clotrimazole concentration in a water–ethanol (4 : 1) mixture against 0.01 M HCl: (*1*) clotrimazole–SE-1 (*ortho*-NPOE), (*2*) clotrimazole–SE-2 (DBP), and (*3*) clotrimazole–SE-3 (DNA).

A 1×10^{-4} M solution of sodium tetraphenylborate, standardized using a 1×10^{-4} M solution of drotaverine hydrochloride, prepared by dissolving an accurately weighed portion, was used for potentiometric titration. The end-point was determined with the help of the Origin7 software package by the position of the maximum of the first derivative dE/dV. The results of clotrimazole determination in model solutions and in dosage forms are given in Table 3.

The results obtained by direct potentiometry and potentiometric titration are characterized by good reproducibility (the relative standard deviation (RSD) does not exceed 1.2%) and are in good agreement with each other as well as with the values claimed by manufacturers, which indirectly confirms the reliability of the results. Taking into account the simplicity of sample preparation, rapidness, and low cost, the method can be of interest for the determination of clotrimazole in the conditions of factory laboratories, primarily, at the stages of interoperational control of technological processes for obtaining the appropriate medicines.

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