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A New Diclofenac Membrane Sensor Based on Its Ion Associate with Crystal Violet. Application to Diclofenac Determination in Urine and Pharmaceuticals

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A novel diclofenac ion-selective electrode has been prepared and used in pharmaceutical analysis. The ion-associate of diclofenac with basic dye (crystal violet) was used as the membrane carrier. Among the four different solvent mediators tested, dibutylphthalate (DBP) exhibited proper behavior including the Nernstian slope of the calibration curve, fast response time and good reproducibility of the emf values. The electrode exhibits a Nernstian slope of 59 ± 1 mV decade^{-1} for diclofenac in the concentration range 5.0×10^{-5}-5.0×10^{-2} M with the limit of detection of 2.5×10^{-5} M. The electrode displays good sensitivity with the respect to a number of common inorganic and organic species. It can be used in a pH range of 6-11. The membrane sensor was successfully applied to the determination of diclofenac in capsules and for its recovery from urine samples.

Keywords: Diclofenac, Ion-selective membrane electrode, Potentiometry, Pharmaceutical analysis

INTRODUCTION

With increasing regulatory pressures on the pharmaceutical industry, there is a growing need for robust sensor systems that allow rapid and reliable determinations, particularly in quality control analysis.

Diclofenac 2-[(2,6-dichlorophenyl) amino] benzene-acetic acid monosodium salt (Fig. 1) is an extensively used, non-steroidal, anti-inflammatory drug with analgesic and antipyretic properties. It is used to relieve the symptoms of many diseases such as rheumatoid arthritis, osteoarthritis, non-articular rheumatism and sport injuries [1]. Several analytical methods have been developed for the quantitative determination of these drugs both in pharmaceuticals and

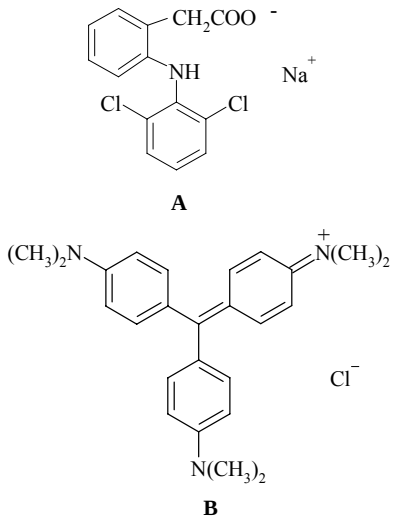

Fig. 1. The chemical structure of sodium diclofenac (**A**) and crystal violet (**B**).

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biological samples. These methodologies include spectrophotometry [2-5], fluorimetry [6-8], high-performance liquid chromatography (HPLC) [9], gravimetric analysis [10] and others [11-14].

Ion-selective electrodes are often used for detecting ionic species in aqueous solutions. They are usually highly sensitive and very easy to use [15-17]. The present communication describes further search for a satisfactory PVC electrode for determination of diclofenac. The electrode based on ion-associate diclofenac and crystal violet was found to give useful results for determination of diclofenac in pharmaceuticals and urine samples.

EXPERIMENTAL

Reagents

All chemicals were of analytical-reagent grade. Distilled water was used to prepare all solutions and in all experiments. Dibutylphtalate (BDP), dibutylsebacenat (DBS), dioctyl-phtalate (DOP), dinonilphtalate (DNP), cyclohexanone (CHN), tetrahydrofuran (THF), high molecular weight PVC were obtained from Sigma-Aldrich. The 0.04 M buffer solutions of pH 2.5-11.5 ranges were freshly prepared.

The freshly prepared aqueous standard solutions (1.0×10^{-7}-5.0×10^{-2} M) of diclofenac were prepared in 0.04 M of a buffer solution (for the study of pH effect) for analytical purposes. Buffer solutions (pH 2.5-11.5*)* were prepared by mixing 0.04 M H_3BO_3, 0.04 M CH_3COOH, 0.04 M H_3PO_4 and 0.2 M NaOH. The ionic strength was adjusted with 0.1 M KCl.

The following commercial dosage forms were analyzed the with new ion-selective electrode: Naclofen injectable ampoule (Slovenia) labeled to contain 75.0 mg × 3 ml^{-1} of diclofenac (sodium salt), Naclofen capsules (Slovenia) labeled to contain 75.0 mg × 3 ml^{-1} of diclofenac (sodium salt) and Diclofenac retard (Ukraine) labeled to contain 75.0 mg of diclofenac (sodium salt) per capsule**.**

Electrode Preparation and Conditions

An ion-associate of diclofenac with crystal violet was prepared by mixing equal quantities of 1.0×10^{-2} M diclofenac and 1.0×10^{-2} M of basic the dye. The solution settled for 2 h and the sediment of ion associate was filtered using quantitative rapid filter paper). This residue was treated with 50 ml of cold distilled water. The filter paper containing the precipitate was dried for 24 h at room temperature. This ion associate was used as an electrode-active substance for preparing of ion-selective electrode that was used for diclofenac determination.

The general procedure for preparing the membrane electrode was to mix thoroughly 0.1 g of powdered PVC and 0.04 g of the ion associate of diclofenac and crystal violet with 0.08 ml of DBP as a solvent mediator in 5 ml CHN (in some cases THF). The resulting mixture was transferred to a glass dish 2.5 cm in diameter. The solvent evaporated slowly at room temperature. The thickness of the membrane after drying was 0.5 mm. A diclofenac membrane 5 mm in diameter was cut out and glued to one end of a polyethylene tube using a 10% solution of PVC. A solution 1.0×10^{-2} M (in some cases 5.0×10^{-2} M) of diclofenac sodium was used as the internal reference solution.

Apparatus

All emf measurements were carried out with the following cell assembly. An I-160 M model pH/mV meter with an Ag-AgCl reference electrode was used for measuring the difference in potentials at 25.0 ± 0.1 ^{o}C. The Ukrainian State Pharmacopeia 2004 [1] reports a potentiometric method using 0.1 M HCl for the determination of diclofenac. The pH of the solutions was adjusted with an AKVILON pH meter (Model ES-11.7, Russia).

Determination of Diclofenac Ion in Pharmaceutical Formulations and Urine Samples

The analytical products were purchased locally or directly from the manufactures and all were tested prior to the listed expiration date. Three pharmaceutical formulations containing diclofenac salt and other components were analyzed with the diclofenac-sensitive electrode.

Diclofenac in pure form. A freshly prepared 5.0×10^{-2} M aqueous solution of diclofenac (standard substance) was used as the stock solution. Next solutions (50 ml) of diclofenac 1.0×10^{-7}-1.0×10^{-2} M were prepared by suitable dilution of the stock solution with water. The ion strength of the final solutions used for the potentiometric determination was kept constant at 0.1 M by the addition of potassium chloride.

Liquid samples. The contents of nine vials were mixed. An aliquot equivalent to three vials was transferred to a 50 ml volumetric flask and diluted to the mark with a proper potassium chloride solution (the ion strength of solution was 0.1 M KCl). Diclofenac content was determined using the proposed ion-selective electrode and the calibration graph method. The procedure was repeated five times and was validated by the potentiometric titration methods [1].

Solid samples. Fifteen tablets were weighed to calculate the average tablet weight. They were subsequently powdered and homogenized. A portion of the powdered mass equivalent to about 225.0 mg of diclofenac was accurately weighed and dissolved with 40 ml of water. The resulting mixture was filtered and the ionic strength was adjusted to 0.1 M with KCl. Finally, this solution was diluted to the mark with water in a 50 ml flask and analyzed using the same procedure as mentioned above for diclofenac in pure form. This procedure was repeated 5 times.

Urine samples. Urine samples were prepared as follows. Before breakfast 5 healthy volunteers each received a tablet (1.0 × 100 mg Dicloran® CP; India). Urine samples were collected in individual flasks after 5 h of drug administration and analyzed using the method of standard addition. Then 10, 30, 50 ml aliquots of urine samples were transferred to 100 ml volumetric flasks, 10 ml of a standard 1.0×10^{-2} M solution was added and diluted to the mark with a proper potassium chloride solution (the ion strength of solution was 0.1 M KCl). Finally, the proposed ion-selective electrode was used to determine diclofenac content in the solution.

RESULTS AND DISCUSSION

Electrode Response, Calibration Curve, and Life Time

The emf response of the membrane at varying concentration of diclofenac (Fig. 2) indicates a rectilinear range from 5.0×10^{-5}-5.0×10^{-2} M. The slope of the calibration curve was 59 ± 1 mV decade^{-1} of diclofenac sodium concentration. The limit of detection, as determined from the intersection of the two linear segments of the calibration graph, was 2.5×10^{-5} M. The prepared membrane electrode was found to have a very fast potential response. The static response time obtained for the electrode is only about 2-3 s over the entire concentration range. The prepared membrane electrode can be used for at least 3 months without any measurable divergence in potential.

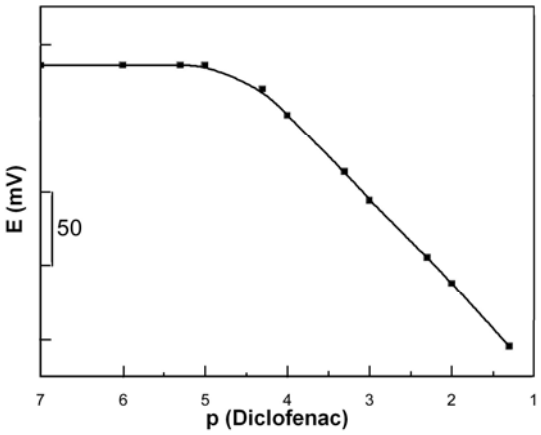

Fig. 2. The calibration curve for the proposed diclofenac-selective electrode (E = -78.41 + 57.81 pC).

Effect of pH

The influence of pH of the test solution on the potential response of the membrane (for a 6.0×10^{-3} M diclofenac solution) was tested in the range 2.5-11.5 (adjusted with buffer solutions). The results are shown in Fig. 3. As can be seen, the potential remains constant from pH 5.0-11.0, beyond which the potential changes considerably; this can be taken as the working pH range of the electrode.

Effect of Solvent Mediators

Sensitivity and selectivity of ion-selective electrodes depend not only on the nature of the electrode-active substance used, but also on its quantities and the properties of the solvent mediators employed. The percentage ($\omega(IA)$, %) of ion associate in the membrane was calculated as follows:

$$\varpi(IA),\% = \frac{m(IA)}{m(IA) + m(PVC) + m(sol.mediator)} \cdot 100\%$$

The influence of solvent mediators on the potentiometric response characteristics of the diclofenac selective electrode based on the diclofenac ion associate with crystal violet was investigated. The results are summarized in Table 1 and

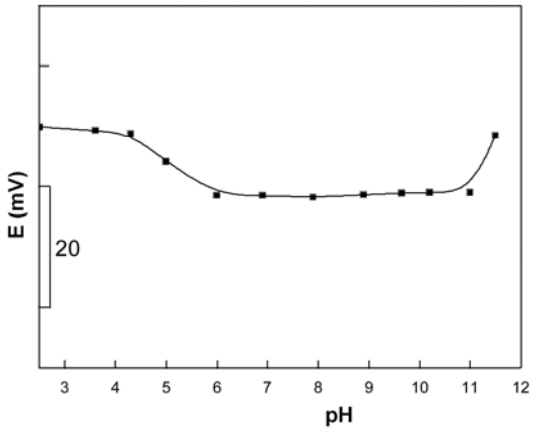

Fig. 3. Effect of pH on the response of the proposed diclofenac-selective electrode.

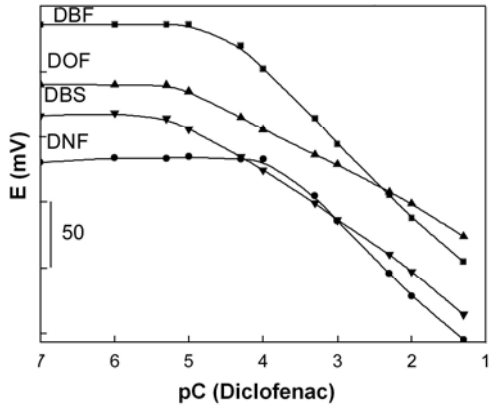

Fig. 4. Effect of the solvent mediator on the response of the proposed electrode.

Table 1. Effect of the Solvent Mediator and Ion Associate Content on the Response of the Diclofenac Electrode[a]

Amount of IA (%)	Solvent mediator	Solvent	Concentration of internal reference solution (M)	Slope (mV)	Linear range (M)	Detection limit (M)
25	DBP	CHN	1×10^{-2}	59.0 ± 1.0	5×10^{-4} -5×10^{-2}	2.5×10^{-5}
25	DBS	CHN	1×10^{-2}	43.1 ± 1.2	5×10^{-4}-5×10^{-2}	4.0×10^{-5}
25	DOF	CHN	1×10^{-2}	32.3 ± 1.3	1×10^{-5} -5×10^{-2}	7.9×10^{-6}
25	DNF	CHN	1×10^{-2}	56.6 ± 1.1	5×10^{-3}-5×10^{-2}	1.3×10^{-4}
25	DBP	CHN	5×10^{-2}	58.2 ± 1.0	5×10^{-4} -5×10^{-2}	2.5×10^{-5}
25	DBP	THF	1×10^{-2}	57.3 ± 1.2	1×10^{-4} -5×10^{-2}	5.0×10^{-5}
25	DBP	THF	5×10^{-2}	58.4 ± 1.1	1×10^{-4}-5×10^{-2}	5.0×10^{-5}
43	DBP	CHN	1×10^{-2}	56.0 ± 1.0	5×10^{-4}-5×10^{-2}	2.5×10^{-4}
60	DBP	CHN	1×10^{-2}	51.2 ± 1.2	5×10^{-4}-5×10^{-2}	2.5×10^{-4}

[a]Average of five determinations; response times 2-3 s.

the corresponding emf responses are shown in Fig. 4. As can be seen, among the four different solvent mediators used, some significant differences in the potential response of the electrodes was found. Consequently, the electrode prepared using DBP was selected for the remaining studies.

Effect of Internal Reference Solution

The solutions 1.0×10^{-2} and 5.0×10^{-2} M of diclofenac sodium were used as internal reference solutions (Fig. 5). As it can be seen from Fig. 5, the concentration of internal reference solution does not significantly affect the potential response.

Interference Studies

One of the most important characteristics of many membrane sensors is their selectivity. Potentiometric selectivity coefficient defines the ability of an ion-selective electrode to distinguish between different ions in the same solution. It is not identical to the similar term used in separation process. The selectivity coefficient ($-\lg K^{pot}_{DICL,A}$) should preferably be evaluated by measuring the response of

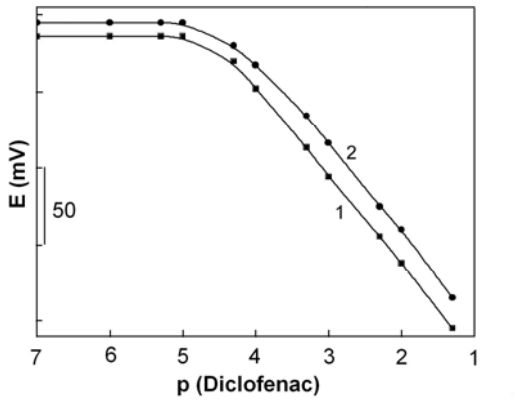

Fig. 5. The effect of the concentration of the internal reference solution on the response of the proposed diclofenac-selective electrode with DBP as a solvent mediator and 25% of ion-associate content (1.1×10^{-2} M, 2.5×10^{-2} M of diclofenac sodium).

Table 2. Selectivity Coefficients of Various Interfering Ions for the Diclofenac-Selective Electrode with DBP as a Solvent Mediator and 25% of Ion-Associate Content was Used)

Ion	$-lgK^{pot}_{DICL,A^-}$
Mg^{2+}	–
Ca^{2+}	–
Na^{+}	–
K^{+}	–
Cl^{-}	–
Br^{-}	2.0
I^{-}	–
NO_3^{-}	2.5
ClO_4^{-}	0.85
Benzoate	2.4
Glucose	–
Salicylate	1.0
Cl-salicylate	2.3
Aspirin	1.1
Tartrate	–
Glycine	–
Gistidine	–

"–" no interference

an ion-selective electrode in solutions of the primary ion, diclofenac, and the interfering ion, A^-. The selectivity coefficients of the electrode were determined using the separate solution method [18]. Table 2 lists the potentiometric selectivity coefficient data of the sensor for several diclofenac-related anions. The interfering effect of the ions is in the following order: Mg^{2+}, Ca^{2+}, K^+, Cl^-, Br^-, I^-, glucose, tartrate ion, glycine, gistidine > NO_3^- > benzoate ion > Cl-Sal > aspirin > ClO_4^-.

The results thus obtained clearly revealed that the proposed diclofenac selective electrode could be used over a wide pH range and in the presence of different interfering species.

Analytical Application

The proposed membrane diclofenac selective electrode was found to work under laboratory conditions. It was successfully applied to the determination of diclofenac in pharmaceuticals preparations (Table 3). As can be seen, the results, obtained using the standard method of potentiometric titration, are in satisfactory agreement with the labeled amounts.

Another analytical application of the proposed sensor is for the determination of diclofenac in urine samples. The diclofenac content in urine samples was determined with the help of the proposed electrode using the method of standard additions. The recovery percentage from five replicate measurements was found 3.4×10^{-3} M, RSD = 1.6% to be (99%).

CONCLUSIONS

The proposed electrode is very easy to prepare. The results of its analytical applications show that the electrode exhibits high sensitivity over a wide pH range. Due to its high diclofenac selectivity, the electrode is virtually unaffected by other anions present in samples, and is potentially useful for monitoring diclofenac concentration levels in pharmaceutical preparations, eliminating the need for preconcentration or pretreatment steps.

ACKNOWLEDGMENTS

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Table 3. Comparative Data for Diclofenac Determination in Pharmaceutical Preparations

Sample	Label amount, mg	Found by proposed electrode		Found by potentiometric titration [1]	
		mg	RSD (%) (n = 5)	mg	RSD (%) (n = 5)
Naclofen (Slovenia)	75.0 ampoule^{-1}	73.2 ± 0.8	0.9	74.4 ± 0.8	0.9
Naclofen (Slovenia)	75.0 capsule^{-1}	72.1 ± 1.2	1.3	73.8 ± 1.0	1.1
Diclofenac retard (Ukraine)	75.0 capsule^{-1}	73.9 ± 1.1	1.2	74.5 ± 0.7	0.8

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