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Preparation of a diclofenac potentiometric sensor and its application to pharmaceutical analysis and to drug recovery from biological fluids

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Abstract

A novel diclofenac ion-selective electrode is prepared, characterized and used in pharmaceutical analysis. The diclofenac complex with hexadecylpyridinium bromide is obtained in situ by soaking the PVC-membranes in a 1×10^{-2} M diclofenac solution. Among four different solvent mediators tested, dibutyl phthalate (DBP) exhibited a proper behavior including 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 1.0×10^{-5} to 1.0×10^{-2} M with a limit of detection of 4.0×10^{-6} M. The electrode displays a good selectivity for diclofenac with respect to a number of common inorganic and organic species. It can be used in a pH range of 6.0–9.0. The membrane sensor was successfully applied to the determination of diclofenac in its tablets as well as for its recovery from blood serum and urine samples.
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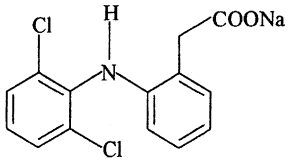
Keywords: Diclofenac-selective electrode; Potentiometry; Pharmaceutical analysis

1. Introduction

Diclofenac, 2-(2′,6′-dicholoroanilino)phenylacetic acid, is a non-steroidal anti-inflammatory drug (NSAID) used for treatment of different diseases such as rheumatoid arthritis, ankyllosing spondylitis, osteoarthritis and sport injuries [1].

Although its mechanism of action has not been clearly established, many of the diclofenac effects appear to be associated principally with inhibition of prostaglandin synthesis [2,3]. Due to the vital importance of the assay of diclofenac (or diclofenac sodium) for pharmaceutical formulations and biological fluids, several analytical methods including colorimetry [4], chromatography [5], spectrophotometry [6], spectrofluorimetry [7] and voltammetry [8] have been reported for the determination of the drug in its pure and dosage forms. However, some of these methods need expensive equipment and/or are time-consuming.

In recent years, there has been a growing need for constructing chemical sensors for the fast and economical monitoring of pharmaceutical compounds [9–13]. In this paper, we report a simple potentiometric PVC-membrane sensor for the determination of diclofenac in pharmaceutical preparations. The membrane electrode proposed in this study was made from plasticized-PVC using a water-insoluble hexadecylpyridinium–diclofenac ion pair complex as an ion-exchanger.



Diclofenac sodium

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Table 1
Effect of solvent mediator on the response of diclofenac electrode

Membrane number	(%) Composition				
	PVC	Plasticizer	HDPB	Slope (mV decade^{-1})	Linear range (M)
1	32	NPOE, 66	2	−52.5	1.0×10^{-5}–3.0×10^{-2}
2	32	DBP, 66	2	−59.0	1.0×10^{-5}–1.2×10^{-2}
3	32	DOS, 66	2	−57.0	2.0×10^{-5}–1.0×10^{-2}
4	32	AP, 66	2	−31.0	1.0×10^{-5}–1.0×10^{-2}

2. Experimental

2.1. Reagents

Analytical-reagent grade dibutyl phthalate (DBP), acetophenone (AP), *o*-nitrophenyl octyl ether (NPOE), hexadecylpyridinium bromide (HDPB), high molecular weight PVC and tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany). The diclofenac sodium (from Merck), β-cyclodextrins (from Fluka, Buchs, Switzerland) and nitrate salts of the cations used (all from Merck) were of the highest purity available and used without any further purification expect for vacuum drying. Doubly distilled deionized water was used throughout.

2.2. Preparation of electrodes

The general procedure to prepare the membrane electrodes was to mix thoroughly 32 mg of powdered PVC and 2 mg of hexadecylpyridinium bromide with 66 mg of DBP as solvent mediator in 5 ml THF. The resulting mixture was transferred into a glass dish of 2 cm diameter. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A polyethylene tube of 5 mm i.d. was dipped into the mixture for about 10 s so that a transparent membrane of about 0.3 mm thickness was formed. After removing the tube from the mixture it was kept at room temperature for about 1 h. The tube was filled with internal filling solution (1.0×10^{-2} M diclofenac). The electrode was finally conditioned for 1 h by soaking in 1.0×10^{-2} M solution of diclofenac sodium. A silver/silver chloride electrode was used as an internal reference electrode. The working diclofenac solutions were prepared by appropriate dilution of a 0.1 M stock standard diclofenac sodium solution.

2.3. Emf measurements

All emf measurements were carried out with the following cell assembly:

Ag–AgCl|KCl (3 M)|internal solution, 1.0×10^{-2} M diclofenac|PVC membrane|test solution|Hg–Hg_2Cl_2, KCl (sat'd).

A Metrohm ion analyzer pH/mV meter was used for potential measurements at 25.0 ± 0.1 °C. Activities were calculated according to the Debye–Huckel procedure [14].

3. Results and discussion

3.1. Membrane material and composition

Diclofenac reacts with hexadecylpyridinium bromide (HDPB) to form a stable water-insoluble ion-pair complex. In this work, the ion-pair complex of diclofenac was obtained in situ by soaking the PVC membranes containing HDPB in a 0.01 M diclofenac sodium solution for 1 h.

It is well-known that sensitivity and selectivity of ion-selective electrodes depend not only on the nature of ionophore used, but also significantly on the membrane composition and the properties of the plasticizer employed. A study of the influence of solvent mediators on the potentiometric response characteristics of the diclofenac ion-selective electrode based on drug–hexadecylpyridinium bromide ion-pair complex were investigated and the results are summarized in Table 1 and the corresponding emf responses are shown in Fig. 1. As seen, among the four different plasticizer used, the use of 66% DBP in the presence 2% HDPB (no. 2, Table 1) results in the best sensitivity (with a Nernstian slope of −59.0 mV decade^{-1}) and the widest linear range. The influence is due to the polarity of the plasticizer, which can be estimated from the interaction of charged species with a continuum of given dielectric constant [15,16]. As seen, the use of AP reduces both the sensitivity and linear range of the electrode because it produces a glassy fragile membrane with a high ohmic resistance.

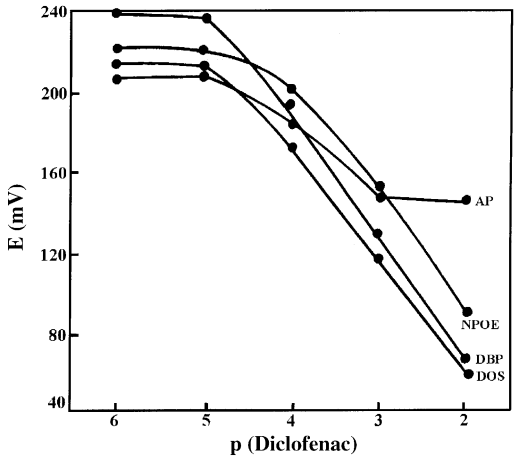

Fig. 1. Effect of solvent mediator on the response of the electrode proposed.

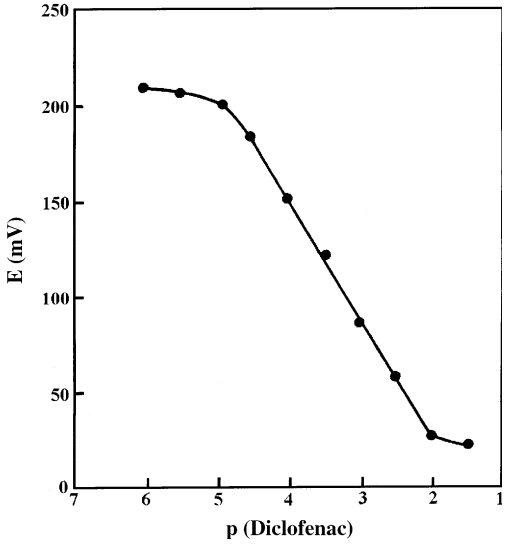

Fig. 2. Calibration curve for the proposed diclofenac-selective electrode.

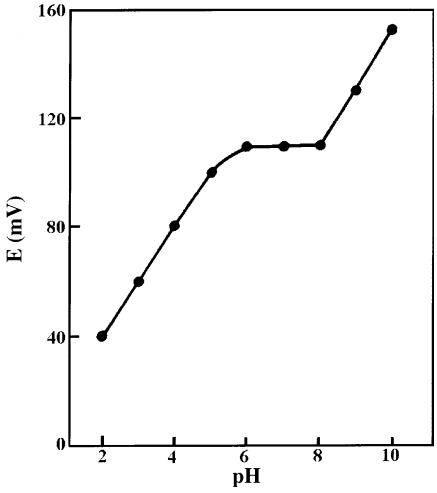

Fig. 3. Effect of pH on the response of the proposed diclofenac-selective electrode.

The proposed selective electrode was also examined at different concentrations of the inner reference solution. The concentration of the internal solution of diclofenac in the electrode was changed from 1.0×10^{-2} to 1.0×10^{-4} M and the potential response of the ion-selective electrode was measured. It was found that variation of the concentration of internal solution does not cause any significant difference in the potential response of the electrode. A 1.0×10^{-3} M concentration of internal solution is quite appropriate for proper functioning of the electrode.

The optimum conditioning time for the membrane electrode is 2 h. It then generates stable potentials when placed in contact with diclofenac solutions.

3.2. Calibration curve, response time and life time

The emf response of the membrane at varying concentration of diclofenac (Fig. 2) indicates a rectilinear range from 1.0×10^{-5} to 1.0×10^{-1} M. The slope of the calibration curve was -59.0 ± 1.0 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 4.0×10^{-6} M (1.3 ppm). The membrane electrode prepared was found to have a very fast potential response. The static response time obtained for the electrode is only about 10 s, over the entire concentration range. The membrane electrode prepared could be used for at least 3 months without any measurable divergence in potential.

3.3. Effect of pH

The influence of pH of the test solution on the potential response of the membrane (for a 1.0×10^{-3} M diclofenac solution) was tested in a pH range 3.0–11.0 (adjusted with either HCl or NaOH) and the results are shown in Fig. 3. As seen, potentials remain constant from pH 6.0–9.0 beyond which the potential changes considerably. Thus, the solution pH does not significantly modify and allows work without the need of using a buffer solution.

3.4. Interference studies

In order to investigate the selectivity of the proposed membrane ion selective electrode toward diclofenac with respect to various interfering ions, we used the fixed interference method [17,18]. The potentiometric selectivity coefficients ($K^{\text{pot}}_{\text{diclo}}$) were calculated graphically using the expression $\log K = a_k/(a_i)^{1/z}$, where a_k is the activity of diclofenac and a_i, that of interfering ion (1.0×10^{-2} M), and z is the charge of interfering ion. The resulting selectivity coefficients are summarized in Table 2. As is obvious from Table 2 none of the interfering species tested does significantly influence the potentiometric response of the proposed PVC-membrane electrode toward diclofenac ion. It is worth mentioning that the metabolites of the drug including 4′-hydroxy- and 5-hydroxy-diclofenac have no mea-

Table 2
Selectivity coefficient of various interfering ions for diclofenac electrode

Interference	K^{pot}
Cl^-	2.5×10^{-3}
Br^-	5.1×10^{-4}
NO_3^-	5.1×10^{-3}
Ca^{2+}	7.9×10^{-4}
Mg^{2+}	6.0×10^{-4}
Na^+	5.0×10^{-2}
K^+	1.0×10^{-3}
Glycine	2.5×10^{-3}
L-Histidine	5.0×10^{-3}
Glucose	1.47×10^{-3}
Benzyl alcohol	1.2×10^{-4}

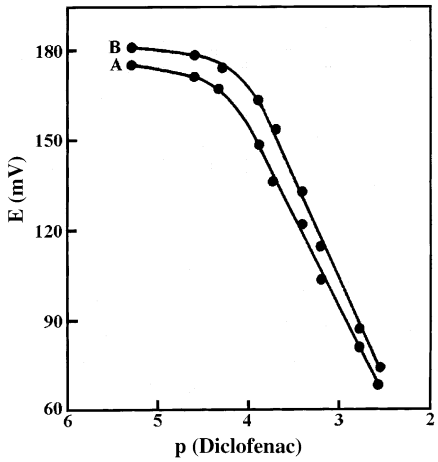

Fig. 4. Calibration curve in the absence (1) and in the presence (2) of 0.01 M β-cyclodextrin.

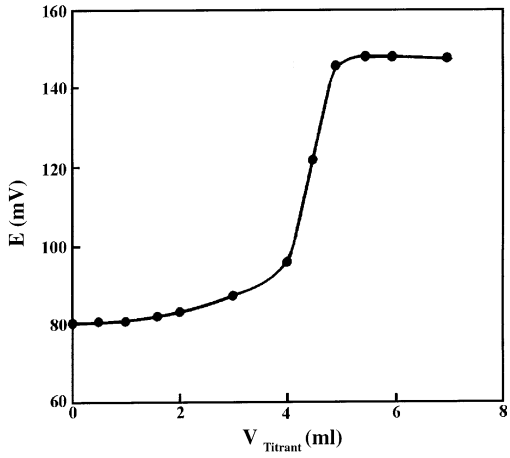

Fig. 5. Potentiometric titration curve of 25.0 ml diclofenac sodium (0.001 M) with HDPB (0.005 M), using the proposed diclofenac sensor as an indicator electrode.

surable effect on the potential response of the proposed sensor.

3.5. Applications

It is known that cyclodextrins (CDs) have the property of forming inclusion complexes with guest molecules with suitable characteristics of polarity and dimension [19]. This ability has been widely used in studies of general inclusion phenomena and enzyme–substrate interactions, applied to food and pharmaceutical studies.

In this work, the proposed electrode was applied to evaluate the equilibrium constant of the β-cyclodextrin-diclofenac inclusion complex:

$$\text{Diclofenac} + \beta - \text{cyclodextrin} \overset{K_s}{\rightleftharpoons} \text{Inclusion compound} \quad (1)$$

Fig. 4 shows the emf response of the diclofenac-selective electrode in the absence and presence of β-cyclodextrin. It should be noted that, after each titration of the cyclodextrin solution, an experiment was performed in the absence of cyclodextrin to confirm the reproducible response of the proposed ion-selective electrode. The data were analyzed by first assuming that equilibrium (1) between diclofenace and the cyclodextrin involves a 1:1 complexation. In this case, the equilibrium constant, K_s, for each solution can be evaluated from the emf data using the classical Scatchard equation in the following form [20,21]:

$$\frac{\upsilon}{m_1} = K - K\upsilon \quad (2)$$

where υ is the concentration of diclofenace complexed with β-cyclodextrin over the total concentration of β-cyclodextrin and m_1 is the drug monomer concentration. A plot of υ/m_1 versus υ for the data involving diclofenace binding to β-cyclodextrin was found to be quite linear, The linearity of this plot confirms the 1:1 stoichiometry of the complex. The binding constant obtained for the diclofenac complex with β-cyclodextrin, obtained from Eq. (2), was found to be $3100\ \text{mol}^{-1}\ \text{dm}^3$, as it compared with the result determined spectroflourimetrically as $3300\ \text{mol}^{-1}\ \text{dm}^3$ [1].

The proposed sensor was successfully used as an indicator electrode in the potentiometric titration of 25.0 ml of 0.001 diclofenac with 0.005 M HDPB. Fig. 5 shows the resulting titration curve. As is obvious, the concentration of diclofenac can be accurately determined by the proposed electrode.

In order to investigate the applicability of the new sensor to the determination of the drug in the biological fluids, it was applied to the recovery of diclofenac from urine and blood serum samples. A 2.5 ml portion of 0.001 M diclofenac solution was transferred into a 10 ml volumetric flask. After addition of a 2.5 ml portion of urine or serum samples, the solution was diluted to the mark with water. The diclofenac content of the solution was then determined by the proposed electrode, using the calibration method. The recovery from three replicate measurements was found to be 103 and 101%, respectively.

The new sensor was also used for assay of diclofenac content in tablets and ampoules. The results for the determination of diclofenac amount in some pharmaceutical samples are shown in Table 3. As it is seen, the results are in satisfactory agreement with the labeled amounts.

Table 3
Potentiometric determination of diclofenac in some pharmaceutical formulations

Application sample	Labeled amount (mg/tab. or amp.)	Found (mg/tab. or amp.)
Diclofenac Tablet, Sobhan Company, Iran	25.0	25.0 ± 0.5
Diclofenac Ampoule, Chemidarou Company, Iran	75.0	73.0 ± 2.0
Voltaren Ampoule	75.0	74.0 ± 1.0

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