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PVC membrane sensor for diclofenac: applications in pharmaceutical analysis and drug binding studies

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A PVC membrane sensor for diclofenac based on its ion pair complex with silver is prepared. The influences of membrane composition (PVC, plasticizer, and ion pair complex), pH of test solution and presence of other anions on the performance of the electrode were investigated. The optimized membrane demonstrates Nernstian response (-58.9 ± 0.2 mV/decade) for diclofenac anions over a wide linear range from 5.2×10^{-5} to 1.1×10^{-2} M at 25 ± 1 °C. The potentiometric response is independent from pH at the range of 6.0–9.5. The advantages of the proposed sensor are: easy preparation, good selectivity and fast response time. It was successfully used for determination of diclofenac in pharmaceuticals and also in potentiometric study of interaction of diclofenac with bovine serum albumin. The results of diclofenac assay with the proposed sensor were in good agreement with the official HPLC method.

1. Introduction

Sodium diclofenac (SD), or sodium 2-[(2, 6-dichlorophenyl)amino]phenyl] acetate, is a widely used non-steroidal anti-inflammatory agent. Several methods have been reported for determination of SD in pharmaceutical preparations including ion selective electrode (Hassan et al. 1998, 2003, 2005; Pimenta et al. 2002; Santini et al. 2006; Shamsipur et al. 2005), UV-Vis spectrophotometry (Agrawal and Shivramchandra 1991; Agatonovic-Kustrin et al. 1991, 1997; Botello and Caballero 1995; Bucci et al. 1998; Cordova et al. 1998; Kamath and Shivram 1993; Kamath et al. 1994; Matin et al. 2005; Sastry et al. 1989; Sena et al. 2004), fluorimetry (Arancibia et al. 2000; Castillo and Bruzzone 2006; Damiani et al. 1999; Pimenta et al. 2002), HPLC (Klimes et al. 2001; Lee et al. 2000) and capillary electrophoresis (Aurora-Prado et al. 2002; Donato et al. 1994; Jin and Zhang 2000). In recent years, potentiometric measurements using ion selective electrodes have found widespread use in chemical, pharmaceutical, environmental and clinical analyses and study of drug interactions with other chemicals and biochemicals (Alizadeh and Mehdipour 2002; Bordbar et al. 2004a, Cosofret and Buck 1992).

Recently, cyclodextrin (Pimenta et al. 2002), hexadecylpyridinium bromide (Shamsipur et al. 2005) and immobilized ion pair complex of SD with mercury (I) in graphite matrix (Santini et al. 2006) were used to prepare SD ion selective electrodes. Cyclodextrin could form inclusion complexes with several organic molecules, hexadecylpyridinium bromide produces selectivity according to the Hofmeister pattern (i.e., selectivity based solely on the lipophilicity of the anions), also quarternary ammonium salts are usually used as additives in anion selective electrodes for increasing positive sites of membrane and the use of mercury for preparation of SD sensors is an environmental hazard and also the preparation of mercury electrodes is more difficult. In addition, mercury electrodes are not suitable for routine drug analysis, because of electrode fouling and high interference with chloride ions.

Due to the simplicity, low cost and ease of preparation of selective ionophores in development of potentiometric sensors, a PVC membrane sensor for SD anion based on diclofenac-Ag ion pair complex has been prepared in this work. The other advantages of the sensor are fast analysis and ability in large scale serial analyses which are required in pharmaceutical industry. The proposed electrode was also successfully applied to study drug interaction with bovine serum albumin (BSA) and quantification of SD in its commercially available formulations.

2. Investigations, results and discussion

2.1. Study of reaction between diclofenac and silver ion

Our preliminary experiments show that the diclofenac anion ($pH > 6$) is immediately precipitated in the presence of Ag ions. For determination of ion pair stochiometry, an appropriate amount of dry ion pair was burned in an electric furnace at about 600 °C and the residue was dissolved in 5 ml of 1.0 M nitric acid solution and diluted to 50 ml and Ag content was determined by Flame-AAS. The obtained results indicated 1 : 1 stochiometry. For further investigations, the ^{1}H NMR and FT-IR spectra were recorded and the findings confirmed the structure of ion pair.

Table 1: Optimization of the membrane ingredients

No.	Composition (%)			Slope (mV/decade)	Linear range (M)
	PVC	Plasticizer	Ionophore		
1	28.0	67.0, DOP	5.0	-58.9 ± 0.2	$5.2\times10^{-5} - 1.1\times10^{-2}$
2	28.0	67.0, NPOE	5.0	-45.6 ± 1.5	$1.2\times10^{-4} - 1.0\times10^{-2}$
3	28.0	67.0, AP	5.0	-43.3 ± 0.8	$7.3\times10^{-5} - 2.8\times10^{-3}$
4	28.0	69.0, DBP	5.0	-32.7 ± 0.5	$1.3\times10^{-4} - 1.8\times10^{-3}$
5	28.0	69.0, BA	5.0	–	–

DOP: dioctyl phthalate
NPOE: 2-nitrophenyl octyl ether
AP: acetophenone
DBP: dibutylphthalate
BA: benzyl acetate

2.2. Optimization of membrane compositions of the electrode

It is well established that the sensitivity, linearity and selectivity of the electrode depend on the membrane composition (Umezawa et al. 1995, 1990; Bakker 1997). Although, selection of a suitable carrier is the most important parameter for obtaining a highly selective membrane towards a given analyte, but the effect of other parameters such as the membrane matrix polymer and plasticizer should not be ignored. Therefore, we investigated the influence of the plasticizer (Table 1) and amount of ionophor (Fig. 1) on the potential response of the proposed diclofenac PVC membrane sensor. As shown in Table 1, the electrode No. 1 based on silver-diclofenac ion pair complex as active material in the membrane was superior to other electrodes from both the response slope and linear concentration range viewpoints. Thus, preferably due to the Nernstian behavior, wide linear dynamic concentration range and good selectivity, the membrane composition with 5.0% ionophore, 67.0% plasticizer (DOP) and 28.0% PVC was selected as optimized membrane for further studies.

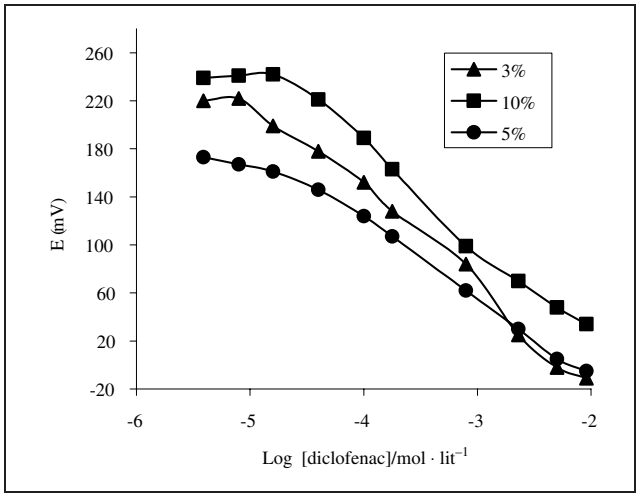

Fig. 1: Effect of ionophor amount on potential response of SD membrane sensor

2.3. EMF response characteristics of sensor

The potential responses of the optimized SD selective electrode to other common anions are shown in Fig. 2. With the exception of iodate ion, all anions tested show no considerable responses in the concentration range of 10^{-6} to 10^{-2} M. However, the potential response of the SD membrane sensor indicated a rectilinear behavior at the range of $5.2\times10^{-5} - 1.1\times10^{-2}$ M (Fig. 3). The slope of the calibration graph was -58.9 ± 0.2 mV per decade. The limit of detection of the SD ion, as determined from the intersection of the two extrapolated segments of the calibration graph, was 3.2×10^{-5} M.

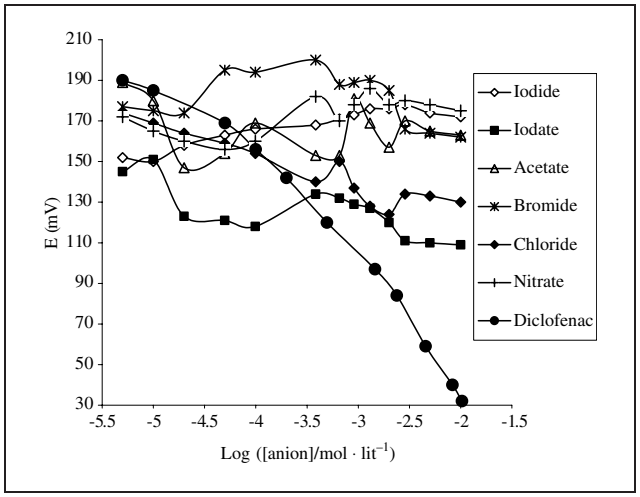

Fig. 2: Potential response of SD membrane electrode to various anions

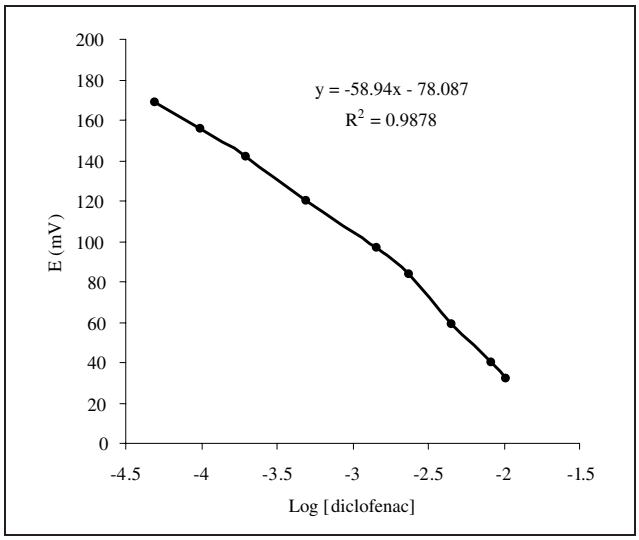

Fig. 3: Calibration curve for SD membrane sensor

Table 2: Selectivity coefficient ($K^{pot}_{A,B}$) of various interfering anions

Interferent	$K^{pot}_{A,B}$
Cl^-	3.22×10^{-4}
Br^-	7.59×10^{-4}
I^-	7.69×10^{-3}
IO_3^-	9.75×10^{-3}
NO_2^-	2.74×10^{-3}
NO_3^-	3.10×10^{-3}
Salicylate	1.91×10^{-4}
Acetate	1.75×10^{-3}
Oxalate	2.70×10^{-3}
Benzoate	1.35×10^{-3}

2.4. Selectivity of the electrode

The potentiometric selectivity coefficients are the most important characteristics of a sensor especially in direct potentiometric measurements. It is well known that the selectivity of an ion pair complex based membrane electrode depends on: a) the selectivity of the ion exchange process at the membrane-sample solution interface, b) the mobilities of the respective ions in the membrane and c) hydrophobic interactions between the primary ion and the organic membrane (Bakker 2000).

The selectivity of the proposed electrode to other anions was investigated by the matched potential method, MPM, (Gadzepko and Christian 1984). According to the MPM, the selectivity coefficient is defined as the activity ratio of the primary ion (A) and the interfering ion (B) that gives the same potential change in a reference solution. Thus, one should measure the change in potential upon changing the primary ion activity. Then, the interfering ion would be added to an identical reference solution until the same potential change is obtained. The selectivity coefficient, $K^{Pot}_{A,B}$ is determined as expression $K^{Pot}_{A,B} = \frac{\Delta A}{a_B}$ where $\Delta A = a'_A - a_A$, a_A is the initial primary ion activity and a'_A the activity of A in the presence of interfering ion, a_B. It should be added that concentration of the diclofenac anion used as a primary ion in this study was 1.0×10^{-4} M. The results show that the proposed sensor possesses good selectivity toward the diclofenac anion (Table 2).

The influence of internal solution concentration on the electrode response was also studied and it was found that the variations of concentration of the internal solution cause differences in calibration graph slope, linear range and linearity (Table 3). Due to the good linearity and Nernstian response, 1.0×10^{-3} M of diclofenac anion solution was chosen as internal solution concentration for further studies.

2.5. Effect of pH on the EMF response of the electrode

The pH dependence of the membrane electrode was tested over the pH range of 3–11 at diclofenac anion concentration of 1.0×10^{-3} M and the results are shown in Fig. 4. The electrode potential is independent of pH in the range of 6.0–9.5 and does not vary more than $\pm$2.0 mV. The effect of pH on the response characteristics in pH < 6.0 can be explained by decreasing the anionic form of diclofenac. But the interference of H^+ ions on membrane causes potential decrease and fluctuation. However, at very high alkaline solution, a cationic response is observed which is due to Donnan failure and the increased interference of OH^- ions on membrane.

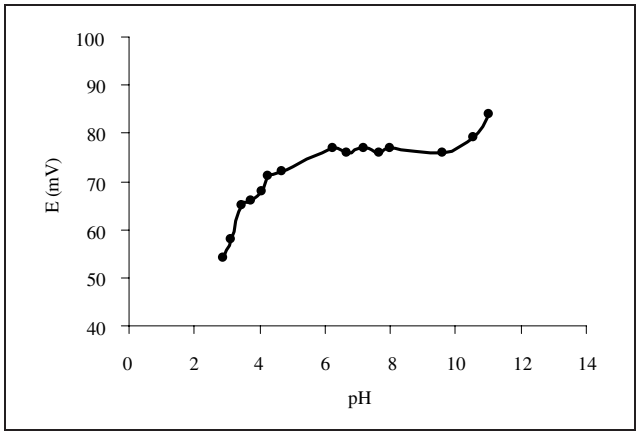

Fig. 4: Effect of the pH of test solution containing 1.0×10^{-3} M diclofenac on the EMF response of sensor

2.6. Response time and lifetime

For analytical applications, the response time of a sensor is an important factor. The response time is the average time required for the diclofenac anion selective membrane electrodes to reach a potential $\pm$1 mV of final equilibrium value after successive immersion of a series of 10 fold concentrated diclofenac anion solutions. The static response time for the proposed PVC membrane sensor was less than 15 s over all linear concentration ranges. The membrane sensor was very stable and could be used over a period of 3 months without considerable changes in response characteristics.

2.7. Determination of SD in tablets, injections and suppositories

A homogenized powder was prepared from 20 SD tablets. An appropriate amount of this powder was transferred into a 50 ml volumetric flask. Drug content of the powder was dissolved in phosphate buffer (pH 7.0) by a mechanical shaker. The solution was then made up to the mark with double distilled water. The SD anion content was then determined by titration with 0.102 M silver nitrate in the presence of the proposed sensor as indicator electrode. The resulting titration curve was shown in Fig. 5.

A 75 mg/3 ml SD ampoule was transferred into a 100 ml volumetric flask, diluted to the mark with water and then

Table 3: Optimization of the internal solution concentration

Internal standard solution concentration (M)	Linear range (M)	Slope (mV/decade)	Correlation coefficient
1.0×10^{-2} M	$2.8\times10^{-4} - 1.0\times10^{-2}$	-63.0 ± 1.1	0.9895
1.0×10^{-3} M	$5.2\times10^{-5} - 1.1\times10^{-2}$	-58.9 ± 0.2	0.9973
1.0×10^{-4} M	$7.3\times10^{-5} - 2.8\times10^{-3}$	-34.4 ± 1.8	0.9793

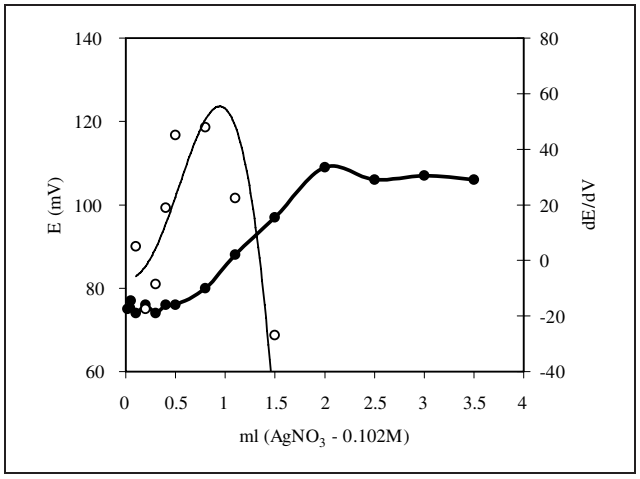

Fig. 5: Potentiometric titration curve (●) and its corresponding derivative curve (○) for diclofenac solution (145 mg of diclofenac tablet sample in 100 ml distilled water) with 0.102 M $AgNO_3$ standard solution using the proposed sensor as an indicator electrode

the potentiometric procedure for determination of SD was followed.

Five 50 mg SD suppositories were grinded and accurately weighed amount of the mixture was dissolved in an appropriate volume of hexane. All excipients were dissolved and SD was precipitated, then the drug was separated by filtration. The solid phase on the filter was dissolved in water, the solution transferred quantitatively into a 50 ml volumetric flask, diluted to the mark with water and then the potentiometric procedure was followed.

Obtained results of potentiometric determinations compared with the official HPLC method of British Pharmacopoeia (stationary phase: ODS column with 25 cm length, 4.6 mm I.D and 5 μm particle size, mobile phase: a mixture of 34%, 2.6 g/L phosphate buffer, pH = 2.5 and 66% methanol, UV detector 254 nm) (British Pharmacopoeia 1993). The results indicated a satisfactory agreement between diclofenac anion contents determined by the proposed sensor and the official HPLC method (Table 4).

The analytical characteristics of the proposed sensor against other published methods for SD analysis were listed in Table 5. The proposed sensor is superior to most of other methods in simple fabrication, low cost, wide linear range and no need for sample preparation steps (separation, extraction and derivatization).

2.8. Study of diclofenac binding to bovine serum albumin

Serum albumin is a principle protein component of plasma and is remarkable for its power to bind a wide variety of molecules. Equilibrium dialysis techniques were used to investigate the interactions of the serum albumin and a number of drugs. In the present work, the results obtained from potentiometric study of interaction of diclofenac with bovine serum albumin (BSA) using SD selective membrane sensor were reported.

The electrode's responses in the presence and absence of BSA at 25 °C (pH = 7.0) are shown in Fig. 6. Deviation from Nernst equation in the presence of BSA is due to diclofenac-BSA interaction. From this deviation, the amount of diclofenac bound to BSA ($[diclofenac]_b$) could be calculated. The average number of diclofenac molecules bound per BSA molecule, ν, was calculated as follows:

$$\nu = \frac{[\text{diclofenac}]_{\text{total}} - [\text{diclofenac}]_{\text{free}}}{[\text{BSA}]_{\text{total}}} \qquad (1)$$

Analysis of the binding isotherms (plotting of ν *versus* the logarithm of free concentration of diclofenac) using the binding capacity concept (Wyman et al. 1988; Bordbar et al. 1996, 2004b, 2004c) indicated that there are two binding sets for diclofenac interaction with BSA (Fig. 7). The corresponding Hill equation parameters (Hill 1910) of diclofenac binding to BSA using binding capacity concept were presented in Table 6.

Table 4: Results of determination of SD in pharmaceutical formulations

Sample	Labeled	Official method	RSD (%)	Proposed method	RSD (%)
Tablet	25 mg	25.53 ± 0.52	2.03	25.26 ± 0.65	2.57
Ampoule	75 mg/3 ml	75.40 ± 0.18	0.24	74.83 ± 1.57	2.09
Suppository	50 mg	50.54 ± 1.17	2.31	51.02 ± 0.92	1.81

Table 5: Comparison of analytical characteristics of some methods used for SD determination

SD determination method	Working range ($\mu g \cdot ml^{-1}$)	Recovery	Pretreatment step	Reference
HPLC	0.05–10.0	90.2	Separation	(Castillo and Bruzzone 2006)
CE	5.0–45.0	NR	None	(Jin and Zhang 2000)
Spectrophotometry	5.0–40.0	NR	Extraction	(Agarwal and Shivramchandra 1991)
	0.8–6.4	NR	Extraction	(Matin et al. 2005)
	3.0–14.8	95.6	None	(Sena et al. 2004)
	5.0–50.0	98.3–100.6	None	(Kamath et al. 1994)
	1.0–30.0	99.4–99.9	None	(Botello and Caballero 1995)
Fluorimetry	0.3–29.0	NR	Filtration	(Castillo and Bruzzone 2006)
	0.12–0.6	NR	None	(Castillo and Bruzzone 2006)
Potentiometry	1.10–3180.0	99.0	None	(Hassan et al. 2003)
	2.93–2930.0	101.0–103.0	None	(Shamsipour et al. 2005)
	1.0–3180.0	99.7	None	(Hassan et al. 2005)
	15.0–3100.0	99.8–102.0	None	This work

NR: Not reported

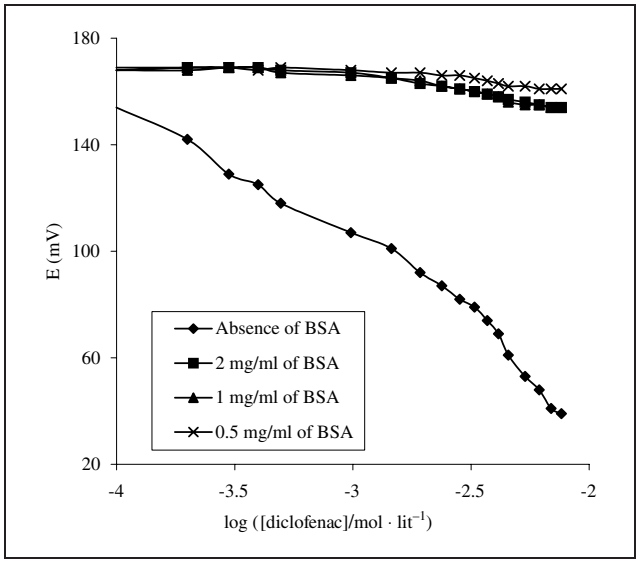

Fig. 6: EMF response of diclofenac electrode at various concentrations of BSA

The process showed positive cooperativity in both binding sets ($n_{Hi} > 1$) for all studied conditions. It can be concluded that by increasing BSA concentration, the second binding set affinity decreased, that it is due to protein-protein interactions at high concentrations of the protein.

In conclusion, our study has shown that the potentiometric assay of diclofenac anion in pharmaceutical preparations using the membrane electrode is reliable and accurate. The wide dynamic concentration range, low detection limit, fast response time, good selectivity make the membrane sensor suitable for measuring the concentration of diclofenac anion in various samples, without the need for pretreatment steps and without significant interaction from other ionic species present in the samples. The prepared sensor was also employed to investigate protein binding of diclofenac.

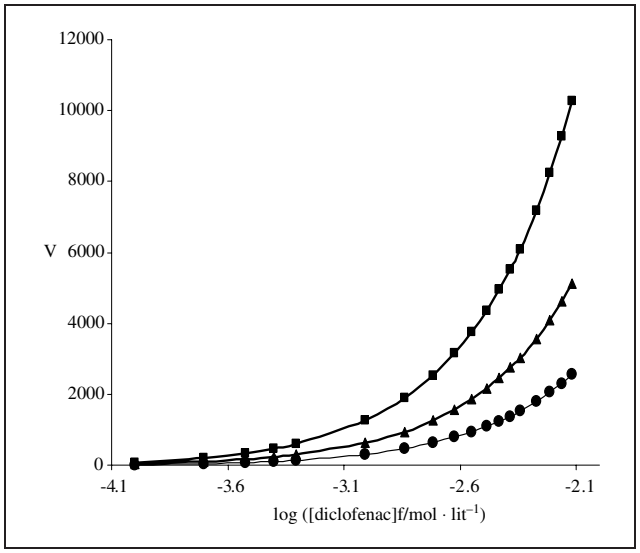

Fig. 7: Binding plots for interaction of diclofenac with BSA at 25 °C at various BSA concentration: 2 mg/ml (●), 1 mg/ml (▲) and 0.5 mg/ml (■)

Table 6: The Hill parameters for interaction of diclofenac with BSA at 25 °C (pH = 7)

[BSA] (mg/ml)	ln K_{H1} (M^{-1})	ln K_{H2} (M^{-1})	n_{H1}	n_{H2}
0.50	9.55	8.46	4.16	6.10
1.00	9.69	5.75	4.21	4.57
2.00	9.42	3.78	4.11	3.52

3. Experimental

3.1. Apparatus

All potentiometric and pH measurements were made at 25 ± 1 °C using a digital 744 pH meter (Metrohm, Switzerland). Ag/AgCl reference electrodes were purchased from Azar Electrode Co. (Urmia, Iran). The following assembly was used for EMF measurements: Ag−AgCl, 3 M KCl | internal solution (1.0×10^{-3} M diclofenac) | PVC membrane | test solution | Ag−AgCl, 3 M KCl. A high performance liquid chromatograph (HP 1100, Agilent technologies, USA) controlled by ChemStation software, equipped with diode array detector was used for measurement of SD using official method. Flame atomic absorption spectrophotometer (AA-670, Shimadzu, Japan) was used for determination of silver content of the ion pair. NMR spectra were recorded in dimethylsulphoxide on a Varian Unity INOVA NMR Spectrometer (400 MHz for ^{1}H) using the residual solvent peaks as internal standard. IR spectra were recorded using Nicolette Nexus 670 FT-IR spectrometer.

3.2. Reagents

Reagent grade 2-nitrophenyl octyl ether (NPOE), dibutylphethalate (DBP), dioctyl phthalate (DOP), acetophenone (AP), benzyl acetate (BA) and high relative molecular weight PVC were purchased from Aldrich and used as received. Tetrahydrofuran (THF), chloroform and salts (all from Merck) were of the highest purity available and used without further purification. Standard solutions and buffers were prepared using double distilled water. Pure SD and its tablets and suppositories were gifts from Sobhan Pharmaceutical Co. (Rasht, Iran). SD ampoules (Voltarene®) were purchased from a pharmacy store.

3.3. Preparation of ion pairs

A 25 ml of aliquot of 1.0×10^{-2} M aqueous solution of SD was slowly mixed with 25 ml of 1.0×10^{-2} M silver nitrate solution with continuous stirring until precipitation was completed. The resulting ion pair precipitates was filtered, washed with double distilled water and dried at 40 °C.

3.4. Electrode preparation

For each electrode the membrane composition was optimized using an orthogonal experimental design with the electrode linear response range, slope and selectivity for SD anion as the object function for optimization. The optimum composition obtained for diclofenac selective solvent polymeric membrane was 5.0% (w/w) ionophore, 67.0% (w/w) DOP, and 28.0% (w/w) PVC. The master membrane was constructed by dissolving 42.0 mg of powdered PVC, 100.5 mg of plasticizer DOP, 7.5 mg of ion pair complex in 5 ml of THF. The resulting mixture was transferred into a glass dish of 2 cm diameter. THF was evaporated slowly until an oily concentrated mixture was obtained. A Pyrex tube (3–5 mm O.D.) was dipped into the mixture for 10 s so that a membrane of about 0.5 mm thickness was formed. The tube was then pulled out from the mixture and kept at room temperature for about 4 h. A 1.0×10^{-2} M of SD solution (pH = 7.0) was used as internal reference solution. The electrode was finally conditioned by soaking in 1.0×10^{-2} M SD solution (pH = 7.0) for 24 h. A 1.0×10^{-2} M of SD solution (ph = 7.0) was used as internal reference solution.

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