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Determination of diclofenac in pharmaceutical preparations using a potentiometric sensor immobilized in a graphite matrix

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Abstract

The characteristics, performance, and application of an electrode, namely Pt|Hg|$Hg_2(DCF)_2$|graphite, where DCF stands for diclofenac ion, are described. This electrode responds to diclofenac with sensitivity of (58.1 ± 0.8) mV/decade over the range 5.0×10^{-5} to 1.0×10^{-2} mol l^{-1} at pH 6.5–9.0 and a detection limit of 3.2×10^{-5} mol l^{-1}. The electrode is easily constructed at a relatively low cost with fast response time (within 10–30 s) and can be used for a period of 5 months without any considerable divergence in potentials. The proposed sensor displayed good selectivity for diclofenac in the presence of several substances, especially concerning carboxylate and inorganic anions. It was used to determine diclofenac in pharmaceutical preparations by means of the standard additions method. The analytical results obtained by using this electrode are in good agreement with those given by the United States Pharmacopeia procedures.

Keywords: Diclofenac-sensitive electrode; Potentiometry; Pharmaceutical preparations

1. Introduction

Diclofenac (DCF), as the sodium or potassium salt, is a benzeneacetic acid derivative, designated chemically as 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid monosodium or monopotassium salt (Fig. 1). It is a potent non-steroidal anti-inflammatory agent, extensively used for the treatment of active rheumatoid arthritis and osteoarthritis, ankylosing spondylitis, non-articular rheumatism and sport injuries [1]. Another therapeutic uses of diclofenac are as analgesic and antipyretic. This phenylacetic acid derivative acts as an inhibitor of hyaluronidase, prostaglandins synthesis and platelet aggregation [1].

The *United States Pharmacopeia 2002* [2] reports a potentiometric method using 0.1 mol l^{-1} perchloric acid for the determination of DCF tablets and an high-performance liquid chromatography (HPLC) method for simultaneous determination of DCF and its degradation product, 1-(2,6-dichlorophenyl)indolin-2-one. The potentiometric method requires about 450 mg of drug, whereas the HPLC method is sensitive, but uses an elevated volume of pure organic reagents and an expensive apparatus.

Several different methods have been reported for the determination of DCF in pharmaceutical preparations including UV–vis spectrophotometry [3–11], fluorimetry [12,13], HPLC [14,15], liquid chromatography (LC) [16], capillary electrophoresis (CE) [17,18], LC–APCI–MS [19], differential scanning calorimetry (DSC) [20] and nuclear magnetic resonance spectroscopy (NMR) [21]. However, most of these techniques are time-consuming or require expensive and sophisticated instruments and for this reason they are not suitable for routine analysis.

Potentiometric methods with ion-selective electrodes (ISE's) can provide valuable and straightforward means of assaying diclofenac in complex mixtures, as they make possible the direct determination of ions in solution with high selectivity. Most ISE's are low-cost, their use and maintenance being very simple; assay procedures involving such electrodes are generally simple and fast. These features, coupled with the reliability of the analytical information, make ISE's very attractive for the assay of pharmaceutical products.

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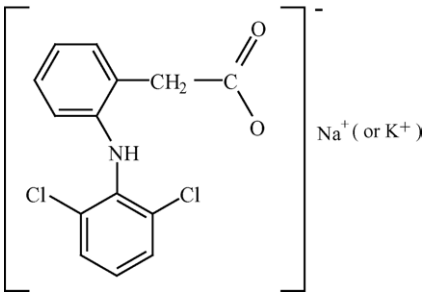

Fig. 1. Chemical structure of the sodium or potassium diclofenac.

To the best of our knowledge, there are limited reports in the scientific literature on the use of ion-selective potentiometric sensors for the determination of diclofenac in pharmaceutical preparations [22–24].

In this work, the preparation of a simple and low-cost electrode, namely Pt|Hg|$Hg_2(DCF)_2$|graphite, is described. The investigation of the experimental variables that contribute to the electrode response led to the development of a simple, selective and reliable method for diclofenac determination. Studies on the determination of diclofenac in pharmaceutical formulations, particularly tablets dosage formulations and injectable ampoules were carried out to illustrate the feasibility of the proposed method. Furthermore, as both the electrode and the standard potentiometric equipment are low-cost, the developed procedure also allows small laboratories with limited resources to run diclofenac analyses for the aforementioned samples.

2. Experimental

2.1. Reagents

High-purity deionized water (resistivity 18.2 MΩ cm) obtained by using a Milli-Q Plus system (Millipore Corp., Bedford, MA, USA) was used throughout. All reagents employed were of analytical grade and obtained from E. Merck (Darmstadt, Germany) except diclofenac sodium, which was supplied by Fluka (St. Louis, MO, USA). Standardizations of carbonate-free sodium hydroxide, nitric acid and sodium nitrate solutions were performed as described elsewhere [25,26]. Metallic mercury was purified according to a previously reported procedure [25]. The sodium diclofenac stock solution was analysed by evaporating and drying to constant weight at 120 °C. Mercury(I) diclofenac was prepared by mixing, in aqueous solution, the corresponding nitrate with an excess of sodium diclofenac. The resulting precipitate was filtered through a sintered glass funnel, washed with deionized water until nitrate-free, and then dried in a desiccator, over calcium chloride under reduced pressure, at room temperature, to constant mass. A white powder was obtained as the final product.

2.1.1. Pharmaceutical preparations

The following commercial dosage forms were analysed with the diclofenac-sensitive electrode: Voltaren® tablets (Novartis), labeled to contain 50 mg of diclofenac(sodium salt) per tablet. Medicamento Genérico tablets (EMS), labeled to contain 50 mg of diclofenac(sodium salt) per tablet. Medicamento Genérico tablets (Medley), labeled to contain 50 mg of diclofenac(sodium salt) per tablet. Voltaren® injectable ampoules (Novartis), labeled to contain 75 mg of diclofenac(sodium salt) per ampoule. Artren® injectable ampoules (Merck), labeled to contain 75 mg of diclofenac(sodium salt) per ampoule. Setacen® injectable ampoules (Itafarma), labeled to contain 75 mg of diclofenac(sodium salt) per ampoule. Medicamento Genérico injectable ampoules (Medley), labeled to contain 75 mg of diclofenac(sodium salt) per ampoule. Medicamento Genérico injectable ampoules (EMS), labeled to contain 75 mg of diclofenac(sodium salt) per ampoule.

2.2. Electrode preparation and conditioning

The mercury(I) diclofenac indicator electrode was prepared as follows: mercury(I) diclofenac (1.4 g) and metallic mercury (ca. 0.2 g) were mixed in an agate mortar and

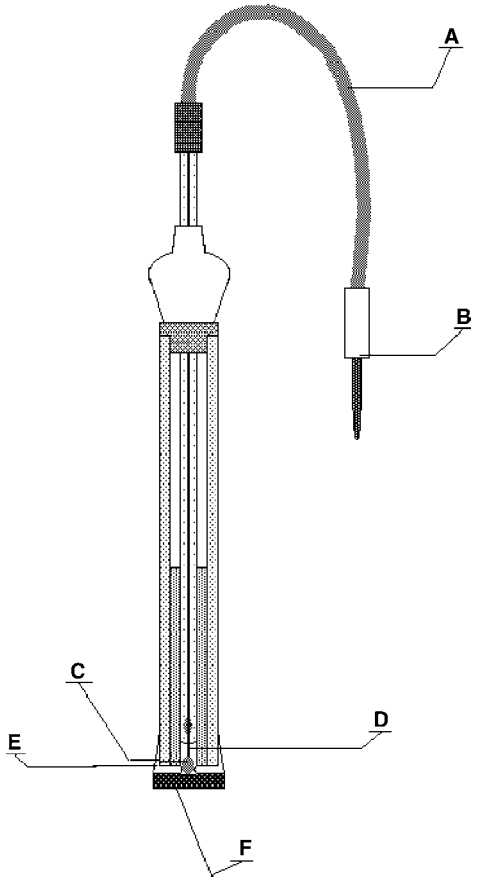

Fig. 2. Mercury(I) diclofenac electrode: (A) conductor cable, (B) banana plug, (C) metallic mercury, (D) Pt wire, (E) silicone glue and (F) sensor pellet (graphite|$Hg_2(DCF)_2$|Hg).

the material was crushed until a homogeneous solid was obtained. Pure powdered graphite (0.7 g) was then added and the crushing process was continued until perfect homogenization was attained. Part of the resulting solid was transferred to a press mold, being compressed at 8 t for about 5 min. The black pellet (1.5 mm thick, 12 mm o.d. and 0.6 g mass) was fixed at one end of a glass tube (12 mm o.d. and 80 mm long) with a silicone–rubber glue ("Rhodiastic", Rhône-Poulenc, France) and allowed to dry for 48 h. Sufficient metallic mercury (ca. 0.6 g) was then introduced into the tube to produce a small pool on the inner pellet surface; electric contact was established through a platinum wire plunged into the mercury pool and a subsequent conductor cable. The resulting electrode is diagrammed in Fig. 2, showing that it is sealed. This feature, coupled with the small amount of metallic mercury placed inside the electrode (ca. 0.6 g), stresses that the considered sensor does not offer significant risk to the operator's health and can thus be recognized as safe.

When not in use, the electrode's pellet was kept immersed in a small volume of 0.010 mol l^{-1} sodium diclofenac solution whose ionic strength (μ) was adjusted to 0.500 mol l^{-1} with a sodium nitrate solution. Before carrying out each experiment, the external surface of the aforementioned pellet was polished with an alumina paper (polishing strip 30144-001, Orion Instruments Inc., Cambridge, MA, USA), washed with deionized water and dried with absorbent paper.

2.3. Instruments

The electromotive force (emf) values were read to the nearest 0.1 mV with a Metrohm model 692 pH|ion meter (Metrohm Ltd., Herisau, Switzerland).

The reference electrode was a Metrohm Ag|AgCl double junction, model 6.0726.100. The pH of aqueous solutions was adjusted and monitored with the aid of a Metrohm pH electrode, model 6.0234.100. A thermostated titration cell (25.0 ± 0.1 °C) was employed.

The standard procedure of the United States Pharmacopeia employed for the assay of diclofenac in injectable ampoules utilizes an HPLC method [2]. Chromatographic analysis were carried out on a Shimadzu model SPD-10A liquid chromatograph (Shimadzu Seisakusho, Kyoto, Japan), equipped with a LC-10 AS pump (Shimadzu), variable UV–vis detector (model SR-10A, Shimadzu) set at 254 nm, gradient control (Waters, model 680; Waters Chromatography Div., Milford, MA, USA) and a "Rheodyne" 20 μl injector (Rheodyne Inc., Berkeley, CA, USA). A stainless steel "Microsorb LC-18" analytical column (250 mm × 4.6 mm i.d., Varian, Walnut Creek, CA, USA) with 5 μm particle size packing material was used. Before injection the samples were filtered through a Millex unit (Millex-HV, 0.45 μm, Millipore). Chromatograms were recorded and the areas were measured with an integrator (Waters, model 746 recording integrator).

The standard procedure of the United States Pharmacopeia (USP) employed for the assay of diclofenac in tablets is based on a potentiometric titration using 0.1 mol l^{-1} perchloric acid in glacial acetic acid media [2]. All potentiometric measurements were carried out using a Metrohm model 716 DMS potentiometric titrator (Metrohm Ltd., Herisau, Switzerland) and a combined glass electrode Metrohm, model 6.0234.100.

Volume measurements (±0.001 ml) were performed with a Metrohm model 665 automatic burette.

All experiments were performed in a thermostated room, maintained at 25 ± 1 °C.

2.4. Potentiometric cell

The following cell was used:

(-)Ag | AgCl	$[NaCl]_{(aq)}$ = 0,010 mol l^{-1} $[NaNO_3]_{(aq)}$ = 0.490 mol l^{-1}	$[NaNO_3]_{(aq)}$ = 0.500 mol l^{-1}	$[NaDCF]_{(aq)}$=x mol l^{-1} $[NaNO_3]_{(aq)}$= (0.500-x)mol l^{-1}	Graphite | $Hg_2(DCF)_2$ Hg | Pt(+)

where DCF stands for diclofenac ion and x is in the range 10^{-2} to 10^{-6} mol l^{-1}. The ionic strength of the cell compartments was kept constant at 0.500 mol l^{-1}. No flow of chloride ions from the reference electrode into the test solution could be detected during the measurements.

The performance of the mercury(I) diclofenac electrode was assessed by measuring the emf of the aforementioned cell for 10^{-2} to 10^{-6} mol l^{-1} sodium diclofenac solutions. These solutions were freshly prepared by serial dilution of a 2.6×10^{-2} mol l^{-1} stock standard solution with deionized water, at constant pH (7.0 ± 0.1). The emf readings were obtained for solutions under stirring and recorded when they became stable. A typical calibration plot of the electrode is shown in Fig. 3.

2.5. Determination of diclofenac ion in pharmaceutical formulations

The analysed products were purchased locally or directly from the manufacturers and all were tested prior to the listed expiration date. Eight pharmaceutical formulations containing diclofenac as the sodium salt and other components were analysed with the diclofenac-sensitive electrode.

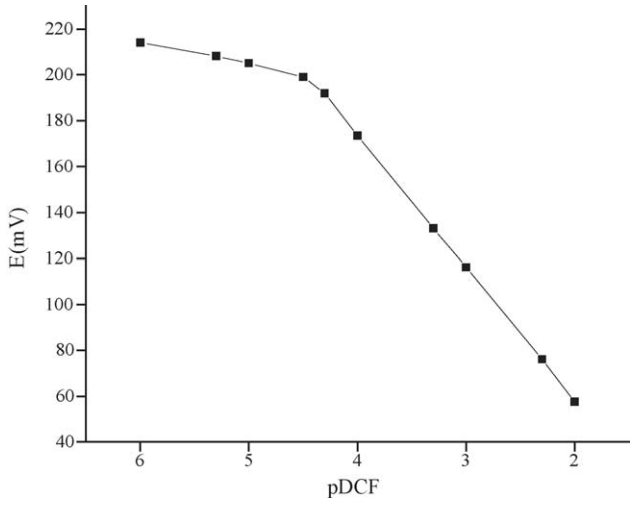

Fig. 3. Calibration curve for the diclofenac-sensitive electrode.

2.5.1. Liquid samples

Liquid samples were appropriately diluted with deionized water to obtain a diclofenac concentration level within the linear range of the electrode's calibration curve. The ionic strength was adjusted to 0.500 mol l^{-1} with $NaNO_3$ and the pH to 7.0 ± 0.1 with 10^{-2} mol l^{-1} HNO_3 or 10^{-2} mol l^{-1} NaOH. For the samples containing sulphite, oxygen was bubbled into the sample for 15 min (rate: 200 cm^3 min^{-1}). Finally, each sample was analysed with the diclofenac-sensitive electrode.

2.5.2. Solid samples

Fifteen tablets of each sample were weighed to calculate the average tablet weight. They were finely powdered and homogenized. A quantity of the resulting powder equivalent to about 30 mg of diclofenac was accurately weighed and placed in a glass vessel; 70 ml of water was added and magnetically stirred for 15 min. The resulting mixture was filtered and its ionic strength was adjusted to 0.500 mol l^{-1} with $NaNO_3$ and the pH to 7.0 ± 0.1 with 10^{-2} mol l^{-1} NaOH or 10^{-2} mol l^{-1} HNO_3 before volume completion. The resulting solution was quantitatively transferred to a 100 ml volumetric flask using deionized water (pH 7.0 ± 0.1) for rinsing and volume completion. An aliquot of 25 ml is employed for analysis with the diclofenac-sensitive electrode.

3. Results and discussion

3.1. Electrode response

Experiments carried out as described in Section 2.4 led to the following linear relationship between the measured emf (E, in mV) and diclofenac ion concentration:

$$E = E^0 + Sp[\mathrm{DCF}]$$

Table 1
Potentiometric response characteristics of the mercury(I) diclofenac electrode[a]

Slope (mV/decade)[b]	58.1 ± 0.8
Intercept, E^0 (mV)[b]	-58.8 ± 1.2
Linear range (mol l^{-1})	5×10^{-5} to 10^{-2}
Detection limit (mol l^{-1})	3.2×10^{-5}

[a] $T = 25.0 \pm 0.1$ °C; pH 7.0 ± 0.1; $\mu = 0.500$ mol l^{-1} ($NaNO_3$).
[b] Average value ± S.D. of 30 determinations over a period of 5 months. Number of data points: 20–25. Mean linear correlation coefficient: 0.994 ± 0.002.

where E^0 is the formal cell potential and S represents the Nernst coefficient (59.16 mV/decade, at 25 °C, for monovalent ions). Potentiometric parameters and other features associated with the mercury(I) diclofenac electrode are given in Table 1. The above calibration equation and the slope value (Table 1) show that the electrode provides a near-Nernstian response to the diclofenac ion in the range of 10^{-2} to 5×10^{-5} mol l^{-1} (Fig. 3). The sensor response displayed good stability and repeatability over the tests; the last mentioned feature is illustrated by the standard deviation values shown in Table 1.

3.2. Response time and lifetime of the electrode

The response time of the electrode was tested by measuring the time required to achieve a steady state potential (within ±0.5 mV/min), for 10^{-2} to 5×10^{-5} mol l^{-1} sodium diclofenac solutions at pH 7.0 [27]. The electrode yielded steady potentials within 10–15 s at high concentrations ($\geq 10^{-3}$ mol l^{-1}) and about 30 s at concentrations near the detection limit. Detectable loss of performance characteristics has not been found after using the electrode up to 5 months.

3.3. pH effect

The influence of pH on the electrode response was tested over the pH range 2.0–10.0 for 1.00×10^{-1}, 1.00×10^{-2} and 1.00×10^{-3} mol l^{-1} diclofenac ion concentrations. The resulting solutions' pH(s) were adjusted with diluted HNO_3 or NaOH solutions.

For pH values below 7.0, progressive formation and precipitation of the free diclofenic acid, protonation of the secondary amino group of the diclofenac to form cationic species and interference by $[H^+]$ cause potential fluctuation.

For pH > 9.0, the hydroxide ion interferes with the electrode's response. The emf values are independent of pH in the range 7.0–9.0; this can be taken as the working pH range of the electrode.

3.4. Electrode selectivity

The most important characteristic of any ion-sensitive sensor is its response to the primary ion in the presence of

other ions present in solution, which is expressed in terms of the potentiometric selectivity coefficient. The potentiometric selectivity coefficients for the mercury(I) diclofenac electrode ($K_{DCF,M}$) were determined, for a number of anions (M), by the matched potential method (MPM) [28–30]. In this method, the selectivity coefficient is defined by the ratio of the activity of the primary ion relative to an interfering ion, when they generate identical potentials in the same reference solution. In the MPM method, both monovalent and divalent ions are treated in the same manner and the valence of the ions does not influence the selectivity coefficient. Furthermore, the MPM can be used with no regard to the electrode slopes being Nernstian or even linear [31]. Mainly for these reasons, it has increased in popularity in the last few years [32].

The MPM-selectivity coefficients ($K_{DCF,M}$) were determined under the following conditions: Initial reference solution (pH 7.1) contains 0.5 M $NaNO_3$ as a supporting electrolyte and 1.0×10^{-5} M of the primary ion (diclofenac). The selectivity coefficients were calculated from the concentration of the interfering ion (M), which induced the same amount of the potential change (Δemf = 20.0 mV) as that induced by increasing the concentration of primary ion. The resulting values of $K_{DCF,M}$ are presented in Table 2.

The results in Table 2 show that the selectivity of the mercury(I) diclofenac electrode towards all tested organic acid anions is good. No interference was noted for most of the common components found along diclofenac in pharmaceutical formulations such as glucose, lactose, talc, starch, magnesium stearate, cellulose, microcrystalline cellulose, croscarmellose sodium, titanium dioxide, silica, polyethyleneglycol, polyvinylpirrolidone, polysorbate 80, sodium saccharin, aspartame, mannitol, benzilic alcohol, methyl- and *n*-propyl-*p*-hydroxybenzoate. The mentioned esters of *p*-hydroxybenzoic acid are also extensively used as preservatives in pharmaceutical formulations. Sulphate has a low selectivity coefficient (Table 2); no interference at all is caused by nitrate or perchlorate and they can therefore be used as background electrolytes or ionic strength adjusters for diclofenac solutions before performing potentiometric measurements.

Table 2
Selectivity coefficients ($K_{DCF,M}$) for various anions[a]

Anion	$K_{DCF,M}$
Formate	1.8×10^{-4}
Acetate	1.2×10^{-3}
Propionate	1.6×10^{-3}
Citrate	4.2×10^{-3}
Lactate	3.6×10^{-3}
Tartrate	2.8×10^{-3}
Benzoate	8.3×10^{-3}
Salicylate	9.7×10^{-3}
Phthalate	7.8×10^{-3}
Oxalate	8.8×10^{-3}
Chloride	2.3
Sulphate	1.4×10^{-4}
Perchlorate	No interference
Nitrate	No interference

[a] Selectivity coefficients were determined by matched potential method. See Section 3.4 for details.

Chloride ion interferes as shown in Table 2. However, the influence due to this ion can be eliminated by a preliminary *n*-octanol extraction procedure. In the samples analysed in this work (tablets and injectable ampoules), chloride ion is seldom found and hence the proposed electrode can generally be used for direct determination of diclofenac in these pharmaceutical formulations without previous extraction procedures.

Sulphite converts mercury(I) to elemental mercury at the electrode's surface and seriously affects its response. Previous oxidation of this species, as described in the analytical procedure (Section 2.5.1) completely eliminates its interfer-

Table 3
Diclofenac determination in pharmaceutical preparations

Samples[a]	Nominal content	Electrode method		USP [2]	
		Found[b] (mg diclofenac unit^{-1})	R.S.D.[d] (%) (n = 6)	Found[b] (mg diclofenac unit^{-1})	R.S.D.[d] (%) (n = 6)
Tablets					
1	50 mg/tablet	50.5 ± 0.6, t^c = 1.25, F^c = 2.71	1.2	49.2 ± 0.9	1.9
2	50 mg/tablet	49.6 ± 0.5, t^c = 1.08, F^c = 2.41	1.0	50.8 ± 1.0	2.5
3	50 mg/tablet	49.3 ± 0.8, t^c = 1.27, F^c = 2.78	1.6	51.1 ± 1.2	3.0
Ampoules					
4	75 mg/ampoule	74.7 ± 0.9, t^c = 1.05, F^c = 2.43	1.2	74.1 ± 1.1	2.0
5	75 mg/ampoule	74.8 ± 0.8, t^c = 1.38, F^c = 2.50	1.1	75.9 ± 0.8	2.5
6	75 mg/ampoule	75.8 ± 0.7, t^c = 1.37, F^c = 2.85	0.9	76.5 ± 1.2	
7	75 mg/ampoule	74.5 ± 0.8, t^c = 1.23, F^c = 2.79	1.1	76.3 ± 1.0	2.1
8	75 mg/ampoule	75.6 ± 0.6, t^c = 1.19, F^c = 2.47	0.8	74.6 ± 0.7	3.0

[a] These contain many or all of the following substances/materials: glucose, lactose, talc, starch, magnesium stearate, cellulose, microcrystaline cellulose, croscarmellose sodium, titanium dioxide, silica, polyethyleneglycol, polyvinylpirrolidone, polysorbate 80, sodium saccharin, sodium sulphite, aspartame, mannitol, benzilic alcohol, sodium hydroxide, potassium hydroxide, methyl- and *n*-propyl-*p*-hydroxybenzoate. Ampoules contain bi-distilled water.
[b] Values found are the average of six independent analyses (n = 6) ± the corresponding standard deviation (S.D.).
[c] Values of t and F at 95% confidence level. Theoretical values: t = 2.23, F = 5.05.
[d] Relative standard deviation (R.S.D.).

ence. Moreover, the oxidation product, i.e., sulphate, has a low selectivity coefficient (Table 2).

3.5. Analytical application

A standard additions method [33,34] was employed for potentiometric diclofenac estimation in some pharmaceutical preparations by using the presently proposed diclofenac-sensitive electrode.

The results, along with those obtained by applying the official methods of USP [2] to the same samples, are given in Table 3. For all samples assayed, the results obtained by official methods and electrode method were compared by applying the *F*- and *t*-test at 95% confidence level. In all cases, the calculated *F*- and *t*-values did not exceed the theoretical values, indicating that there is no significant difference between either methods in concerning precision and accuracy in the determination of diclofenac in pharmaceuticals.

In order to investigate the presence of matrix effects on the proposed method, a recovery study was carried out. In this study, 50, 100 and 200 mg l^{-1} of diclofenac reference solutions were added in four representative pharmaceuticals (samples 1, 3, 5, 6) from those listed in Table 3. The results presented in Table 4 show that the recoveries were found to be close to 100%; the S.D.s were within 0.8–1.4.

The statistical parameters and the recovery data reveal good accuracy and precision of the proposed method and the absence of significant matrix effects on the potentiometric measurements.

The time required for performing analyses by the electrode method was 20–30 min per sample.

Concerning analyses' costs the NMR, LC–APCI–MS, HPLC, LC, CE, DSC and fluorimetric techniques are more expensive than the electrode method, if expenses regarding reagents, solvents and initial investment on good quality standard equipment associated with each of the named techniques are considered.

Table 4
Recovery data for diclofenac spiked in pharmaceutical formulations

Formulation	Concentration added (mg l^{-1})	Concentration found (mg l^{-1})	Recovery[a] (%, ±S.D.)
1 (tablet)	50	48.4	96.8 ± 1.2
	100	98.3	98.3 ± 0.8
	200	198.6	99.3 ± 0.9
3 (tablet)	50	49.2	98.5 ± 1.2
	100	100.4	100.4 ± 1.1
	200	200.4	100.2 ± 0.9
5 (ampoule)	50	49.1	98.2 ± 1.4
	100	99.5	99.5 ± 1.2
	200	202.2	101.1 ± 0.8
6 (ampoule)	50	50.2	100.4 ± 1.2
	100	99.6	99.6 ± 1.1
	200	201.7	100.9 ± 0.9

[a] Average of five determinations ± standard deviation (S.D.).

4. Conclusions

The proposed electrode exhibits long lifetime, good stability, sensitivity, precision, accuracy and selectivity. It is low-cost, easy to prepare and to use. Its usefulness for diclofenac determination in real samples, particularly for some commercial pharmaceutical preparations was demonstrated suggesting its use as a reliable and advantageous alternative to the USP methods [2] as well as to most other previously reported methods in the routine control of diclofenac concentration in these samples. The electrode developed in this laboratory is superior (especially concerning lifetime and simplicity) as compared with diclofenac ion-selective electrodes described in the literature [22–24].

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References

[1] H.E. Paulus, D.E. Furst, S.H. Drogmoole, Drugs for Rheumatic Disease, Churchil Livingstone, New York, 1987, pp. 413–414.
[2] United States Pharmacopeia National Formulary, USP 25, NF 20, Rockville, 2002, p. 554.
[3] J.C. Botello, G.P. Caballero, Talanta 42 (1995) 105.
[4] M.S. Bhatia, S.R. Dhaneshwar, East. Pharm. 38 (1995) 133.
[5] Y.K. Agrawal, K. Shivramchandra, J. Pharm. Biomed. Anal. 9 (1991) 97.
[6] S. AgatonovicKustrin, L. Zivanovic, D. Radulovic, M. Vasiljevic, Analyst 116 (1991) 753.
[7] C.S.P. Sastry, A.S.R.P. Tipirneni, M.V. Suryanaryana, Analyst 114 (1989) 513.
[8] B.V. Kamath, K. Shivram, Anal. Lett. 26 (1993) 903.
[9] S. AgatonovicKustrin, L. Zivanovic, M. Zecevic, D. Radulovic, J. Pharm. Biomed. Anal. 16 (1997) 146.
[10] R. Bucci, A.D. Magri, A.L. Magri, Fresenius J. Anal. Chem. 362 (1998) 577.
[11] M.L.F. Cordova, P.O. Barrales, A.M. Diaz, Anal. Chim. Acta 369 (1998) 262.
[12] P.C. Damiani, M. Bearzotti, M.A. Cabezón, A.C. Olivieri, J. Pharm. Biomed. Anal. 20 (1999) 587.
[13] J.A. Arancibia, M.A. Boldrini, G.M. Escandar, Talanta 52 (2000) 261.
[14] R.T. Sane, R.S. Samant, V.G. Nayak, Drug Dev. Ind. Pharm. 13 (1987) 1307.
[15] J. Klimes, J. Sochor, P. Dolezal, J. Körner, Int. J. Pharm. 217 (2001) 153.
[16] N. Beaulieu, E.G. Loveringe, J. Lefrancois, H. Ong, J. Assoc. Off. Anal. Chem. 73 (1990) 698.
[17] M.S. Aurora-Prado, M. Steppe, M.F.M. Tavares, E.R.M. Kedor-Hackmann, M.I.R.M. Santoro, J. Assoc. Off. Anal. Chem. 85 (2002) 333.
[18] W.R. Jin, J. Zhang, J. Chromatogr. A 868 (2000) 101.
[19] M.E. Abdel-Hamid, L. Novotony, H. Hanza, J. Pharm. Biomed. Anal. 24 (2001) 587.
[20] R. Bucci, A.D. Magri, A.L. Magri, J. Therm. Anal. Calorim. 61 (2000) 369.

[21] S.A. Abdel Fattah, S.Z. El-Khateeb, S.A. Abdel Razeg, M.S. Tawakkol, Spectrosc. Lett. 21 (1988) 533.
[22] S.S.M. Hassan, R.M. Abdel-Aziz, M.S. Abdel-Samad, Analyst 119 (1994) 1993.
[23] S.S. Hassan, W.H. Mahamoud, M.A. Elmosallamy, M.H. Almarzooqui, Pharmazie 58 (2003) 29.
[24] M. Shamsipur, F. Jalali, S. Ershad, J. Pharm. Biomed. Anal. 37 (2005) 943.
[25] L. Pezza, M. Molina, M. Moraes, C.B. Melios, J.O. Tognolli, Talanta 43 (1996) 1689.
[26] A.C.V. Cavalheiro, M. Moraes, L. Pezza, Ecl. Quím. 25 (2000) 123.
[27] R.P. Buck, E. Lindner, Pure Appl. Chem. 66 (1994) 2527.
[28] V.P.Y. Gadzekpo, G.D. Christian, Anal. Chim. Acta 164 (1984) 279.
[29] G.D. Christian, Analyst 119 (1994) 2309.
[30] Y. Umezawa, K. Umezawa, H. Sato, Pure Appl. Chem. 67 (1995) 507.
[31] E. Bakker, P. Bühlmann, E. Pretsch, Chem. Rev. 97 (1997) 3083.
[32] M. Mazloum, M.K. Amini, I.M. Baltork, Sens. Actuators B63 (2000) 80.
[33] G.D. Christian, J.E. O'Reilly, Instrumental Analysis, Allyn & Bacon, New York, 1986, p. 42.
[34] O.M. Bader, J. Chem. Educ. 57 (1980) 703.