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Article

A Novel Potentiometric Naproxenate Ion Sensor Immobilized in a Graphite Matrix for Determination of Naproxen in Pharmaceuticals

Alberto O. Santini, José E. de Oliveira, Helena R. Pezza and Leonardo Pezza*

Instituto de Química, Universidade Estadual Paulista, CP 355, 14801-970 Araraquara-SP, Brazil

As características, o desempenho e a aplicação de um eletrodo do tipo Pt|Hg|$Hg_2(NAP)_2$|Graphite, onde NAP=íon naproxenato, são descritas. O eletrodo responde a NAP com sensibilidade de (58,1± 0,9) mV década^{-1} no intervalo de $5,0 \times 10^{-5}$ - $1,0 \times 10^{-2}$ mol L^{-1} , a pH 6,0-9,0 e com um limite de detecção de $3,9 \times 10^{-5}$ mol L^{-1}. O eletrodo é de baixo custo e facilmente construído, apresenta um rápido tempo de resposta (10-35 s) e pode ser usado por um período de seis meses sem qualquer divergência considerável nos potenciais. O sensor supracitado mostrou boa seletividade para naproxeno na presença de várias substâncias, tais como carboxilatos e ânions inorgânicos, sendo aplicado na análise direta de naproxeno em medicamentos (comprimidos) via método da adição de padrão. Os resultados analíticos obtidos com o eletrodo proposto estão em boa concordância com aqueles obtidos pelo procedimento preconizado na Farmacopéia Americana.

The characteristics, performance, and application of an electrode, namely, Pt|Hg|$Hg_2(NAP)_2$|Graphite, where NAP stands for naproxenate ion, are described. This electrode responds to NAP with sensivity of (58.1± 0.9) mV decade^{-1} over the range 5.0×10^{-5} - 1.0×10^{-2} mol L^{-1} at pH 6.0-9.0 and a detection limit of 3.9×10^{-5} mol L^{-1}. The electrode is easily constructed at a relatively low cost with fast response time (within 10-35 s) and can be used for a period of 6 months without any considerable divergence in potentials. The proposed sensor displayed good selectivity for naproxen in the presence of several substances, especially concerning carboxylate and inorganic anions. It was used for the direct assay of naproxen in commercial tablets 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 procedure.

Keywords: naproxenate-sensitive electrode, potentiometry, pharmaceutical formulations

Introduction

Naproxen [(+)-2-(6-metoxy-2naphthyl)propionic acid], is a non-steroidal anti-inflammatory drug that also presents analgesic and antipyretic properties often preferred to acetylsalicylic acid because of its better absortion following oral administration and fewer adverse effects.

Naproxen is extensively used in the treatment of many diseases like rheumatoid arthrits, degenerative joint disease, ankylosing spondylits, acute gout and primary dismenorrea.[1] Like other non-steroidal anti-inflammatory drugs, it inhibits the biosyntesis of prostaglandins.[1]

The United States Pharmacopeia[2] 2003 reports an high-performance liquid chromatography (HPLC) method for the determination of naproxen tablets.

Several analytical methods have been reported for the determination of naproxen in pharmaceutical preparations including UV-visible spectrophotomery,[3-5] spectrofluorimetry,[6-8] room temperature phosphorimetry,[9,10] voltametry,[11] high-performance liquid chromatography,[12-14] capillary electrophoresis,[15,16] coulometry[17] and oscilometric titration.[18]

However, most of these techniques are time-consuming, involving the use of organic solvents 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) have proved to be effective for the assay of pharmaceutical products, because these sensors offer the advantages of simple design, construction, and manipulation, reasonable selectivity, fast response time, applicability to colored and turbid solutions and possible interfacing with automated and computerized systems.

* e-mail: pezza@iq.unesp.br

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 naproxen in pharmaceutical formulations.[19,20]

Valsami *et al*.[19] described the construction of a NAP-selective electrode of the liquid membrane type, based on the use of a tetraheptylammonium naproxenate ion pair as the ion exchanger. This electrode responded to NAP with sensivity of (58 to 61) mV decade^{-1} over the range 1.0×10^{-1} - 1.0×10^{-4} mol L^{-1} at pH 9.0 (borate buffer). The electrode exhibited a fast response time (5 s) and had an operative life of 2 months. No interference from common ions (with the exception of chloride ion) and tablet excipients was observed. The proposed sensor was used for the determination of naproxen in pharmaceuticals.

Lenik *et al*.[20] developed an ion-selective membrane electrode based on ion-pair complex of naproxen with methyltrioctylammonium. This electrode showed Nerstian response for NAP over the concentration range of 1.0×10^{-1} – 1.0×10^{-4} mol L^{-1} at pH 5.5 – 8.5 and a detection limit of 5.0×10^{-5} mol L^{-1}. The proposed sensor exhibited a short response time (20 s) and had an operative life of 3 months. Selectivity was good over a number of organic and inorganic ions. The electrode was applied for the determination of naproxen in tablets.

Previous work from this laboratory dealt with the development of mercury(I)-carboxylate electrodes and their application to solution equilibria,[21-25] food analysis,[26] and pharmaceutical analysis[27,28] involving carboxylate bearing compounds.

In this work, the preparation of a simple and low-cost electrode, namely Pt|Hg|$Hg_2(NAP)_2$|Graphite, where NAP stands for naproxenate ion, 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 naproxen determination. Studies on the determination of naproxen in commercial tablets 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 naproxen analyses for the aforementioned samples.

Experimental

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 naproxen sodium salt, which was supplied by Sigma (St. Louis, MO, USA). Standardizations of carbonate-free sodium hydroxide, nitric acid and sodium nitrate stock solutions were performed as described elsewhere.[21,25] Metallic mercury was purified according to a previously reported procedure.[21] The sodium naproxenate stock solution was analysed by evaporating and drying to constant weight at 120 °C. Mercury(I) naproxenate was prepared by mixing, in aqueous solution, the corresponding nitrate with an excess of sodium naproxenate. 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.

Electrode preparation and conditioning

The mercury(I) naproxenate indicator electrode was prepared as follows: mercury(I) naproxenate (1.4 g) and metallic mercury (*ca*. 0.2 g) were mixed in an agate mortar and 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 tons 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., 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 Figure 1, 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 1.0×10^{-2} mol L^{-1} sodium naproxenate 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 washed with deionized water and dried with absorbent paper.

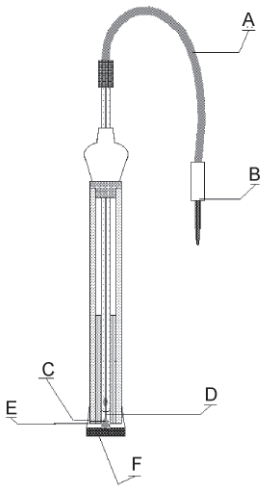

Figure 1. Mercury(I) naproxenate electrode: (A) conductor cable, (B) banana plug, (C) metallic mercury, (D) Pt wire, (E) silicone glue, (F) sensor pellet (Graphite|$Hg_2(NAP)_2$|Hg).

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(USP) employed for the assay of naproxen in tablets formulations 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-Visible 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, mod. 746 recording integrator).

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.

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}	$[NaNAP]_{(aq)}$= x mol L^{-1} $[NaNO_3]_{(aq)}$= (0.500-x) mol L^{-1}	Graphite | $Hg_2(NAP)_2$ | Hg|Pt(+)

where NAP stands for naproxenate ion and x was in the range 10^{-2} - 10^{-6} mol L^{-1}. The ionic strength of the cell compartments was kept constant at 0.500 mol L^{-1}. The outer compartment of the reference electrode was refilled periodically with fresh $NaNO_3$ solution.

The performance of the mercury(I) naproxenate electrode was assessed by measuring the emf of the aforementioned cell for 1.0×10^{-2} to 1.0×10^{-6} mol L^{-1} sodium naproxenate solutions. These solutions were freshly prepared by serial dilution of a 2.0×10^{-2} mol L^{-1} stock standard solution with deionized water, at constant pH (8.0 ± 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 Figure 2.

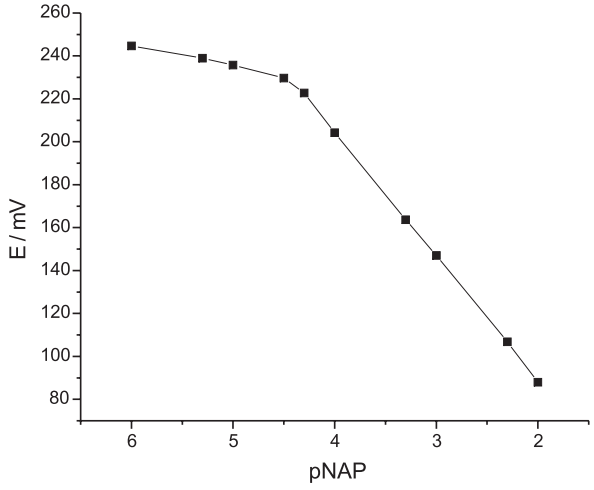

Figure 2. Calibration graph for the proposed naproxenate –sensitive electrode (pH 8.0, μ =0.500 mol L^{-1} adjusted with $NaNO_3$, T= 25 °C).

Determination of naproxenate ion in commercial tablets

The analysed products were purchased locally or directly from the manufacturers and all were tested prior to the listed expiration date. Six pharmaceutical formulations containing the active compound as naproxen or sodium naproxen and other components were analysed with the naproxenate-sensitive electrode.

Twelve 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 25 mg of naproxen or sodium naproxenate was accurately weighed and placed in a glass vessel; 70 mL of water was added and magnetically stirred for 10 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 8.0 ± 0.1 with 1.0×10^{-2} mol L^{-1} NaOH or 1.0×10^{-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 = 8.0 ± 0.1) for rinsing and volume completion. An aliquot of 25 mL is employed for analysis with the naproxenate-sensitive electrode.

Results and Discussion

Effect of the ionic strength

The choice of a suitable ionic strength value at which the potentiometric sensor exhibits the best response is also of prime importance in quantitative analysis.[21,28] The potential values of the mercury(I) naproxenate electrode at different ionic strengths (0.500 – 3.00 mol L^{-1}, $NaNO_3$) have been determined at 25 °C, pH 8.0 and naproxenate concentrations between 5.0×10^{-5} to 1.0×10^{-2} mol L^{-1}. It was found that the electrode followed a near-Nernstian behaviour for μ comprised between 0.500 and 3.00 mol L^{-1}. Therefore, for practical purposes the ionic strength was kept constant at 0.500 mol L^{-1} (adjusted with $NaNO_3$) during the potentiometric measurements.

Electrode response

Experiments carried out as described in the subsection "Potentiometric Cell" led to the following linear relationship between the measured emf (E, in mV) and naproxenate ion concentration;

$$E = E^0 + S\ p[NAP]$$

where E^0 is the formal cell potential and S represents the Nernst coefficient (59.16 mV $decade^{-1}$, at 25 °C, for monovalent ions). Potentiometric parameters and other features associated with the mercury(I) naproxenate 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 naproxenate ion in the range of 1.0×10^{-2} to 5.0×10^{-5} mol L^{-1}. The limit of detection,[29] as determined from the intersection of the two extrapolated segments of the calibration graph (Figure 2), was 3.9×10^{-5} mol L^{-1} . 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.

Response time and lifetime of the electrode

The response time of the electrode[29] was tested by measuring the time required to achieve a steady state potential (within ± 0.3 mV min^{-1}), for 1.0×10^{-2} to 5.0×10^{-5} mol L^{-1} sodium naproxenate solutions at pH 8.0. The electrode yielded steady potentials within 10 to 15 s at high concentrations ($\geq 1.0 \times 10^{-3}$ mol L^{-1}) and about 35 s at concentrations near the detection limit. Detectable loss of performance characteristics has not been found after using the electrode up to 6 months.

pH effect

The influence of pH on the electrode response was tested over the pH range 4.0-10.0 for 1.00×10^{-2}, 1.00×10^{-3} and 1.00×10^{-4} mol L^{-1} naproxenate ion concentrations. The resulting solutions' pH(s) were adjusted with diluted HNO_3 or NaOH solutions.

For pH values below 6.0, significant fractions of naproxenate ion (pKa=4.48)[30] changes to the corresponding protonated form which is not detected by the electrode. For pH > 9.0, the hydroxide ion interferes with the electrode's response (Figure 3). The emf values are independent of pH in the range 6.0-9.0; this can be taken as the working pH range of the electrode.

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) naproxenate electrode ($K_{NAP,M}$) were determined, for a

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

Slope (mV $decade^{-1}$)[b]	Intercept, E^0 (mV)[b]	Linear range (mol L^{-1})	Detection limit (mol L^{-1})
58.1 ± 0.9	-27.7 ± 1.6	$5.0 \times 10^{-5} – 1.0 \times 10^{-2}$	3.9×10^{-5}

[a]T = 25.0 ± 0.1 °C; pH 8.0 ± 0.1; μ = 0.500 mol L^{-1} ($NaNO_3$). [b]Average value ± SD of 25 determinations over a period of 6 months. Number of data points: 22-25. Mean linear correlation coefficient: 0.998 ± 0.004.

number of anions (M), by the matched potential method (MPM).[31-33] 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.[34] Mainly for these reasons, it has increased in popularity in the last few years.[35]

The MPM-selectivity coefficients ($K_{NAP,M}$) were determined under the following conditions: Initial reference solution (pH 8.1) contains 0.500 mol L^{-1} $NaNO_3$ as a supporting electrolyte and 5.0×10^{-5} mol L^{-1} of the primary ion (naproxenate). 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 $K_{NAP,M}$ are presented in Table 2.

The results comprised in the aforementioned Table 2 show that the selectivity of the mercury(I) naproxenate electrode towards all tested organic acid anions is good. No interference was noted for most of the common excipients used in tablet formulations such as glucose, lactose, talc, starch, magnesium stearate, cellulose, microcrystaline cellulose, hydroxypropyl-methylcellulose, titanium dioxide, silica, polyethyleneglycol, polyvinylpirrolidone, povidone, mannitol and sorbitol.

Sulphate and borate have a very 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 naproxenate solutions before performing potentiometric measurements.

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

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

Anion	$K_{DCF,M}$
Formate	2.3×10^{-4}
Acetate	1.9×10^{-3}
Propionate	1.4×10^{-3}
Citrate	3.8×10^{-3}
Lactate	3.1×10^{-3}
Tartrate	2.0×10^{-3}
Benzoate	5.3×10^{-3}
Salicylate	6.7×10^{-3}
Phthalate	4.8×10^{-3}
Oxalate	3.5×10^{-3}
Chloride	2.1
Sulphate	3.9×10^{-5}
Borate	4.3×10^{-6}
Perchlorate	no interference
Nitrate	no interference

[a]Selectivity coefficients were determined by matched potential method. See subsection "Electrode Selectivity" for details.

Analytical application

A standard addition method (multiple addition method)[36-38] was employed for potentiometric naproxen estimation in tablets formulations by using the presently proposed naproxenate – sensitive electrode.

The results, along with those obtained by applying the official method of USP[2] to the same samples, are given in Table 3. For all samples assayed, the results obtained by official method and electrode method were compared by applying the *F*-test 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

Table 3. Naproxen determination in tablets formulations

Samples	Label to content	Electrode	Method	USP[2]	
		Found (mg $unit^{-1}$)	RSD^f (%) (n = 6)	Found (mg $unit^{-1}$)	RSD^f (%) (n = 6)
Tablets					
1	275[a]	277.9 ± 4.6[c] t^e =1.25, F^e = 2.71	1.6	273.8 ± 4.9[c]	1.8
2	550[a]	545.2 ± 9.8[c] t^e = 1.08, F^e= 2.41	1.8	556.6 ± 8.9[c]	1.6
3	500[b]	496.3 ± 7.4[d] t^e = 1.27, F^e= 2.78	1.5	497.1 ± 8.2[d]	1.6
4	250[b]	246.7 ± 3.9[d] t^e = 1.05, F^e= 2.43	1.6	247.8 ± 4.3[d]	1.7
5	500[a]	507.2 ± 7.1[c] t^e = 1.38, F^e= 2.50	1.4	505.9 ± 8.8[c]	1.7
6	250[b]	252.6 ± 3.7[d] t^e = 1.37, F^e= 2.85	1.5	249.1 ± 5.2[d]	2.1

[a]Declared concentration of naproxen (sodium salt) in mg $unit^{-1}$. [b]Declared concentration of naproxen in mg $unit^{-1}$. [c]Values found are the average of six independent analyses (n = 6) ± the corresponding Standard Deviation (SD), expressed as sodium naproxenate. [d]Values found are expressed as naproxen. [e]Values of *t* and *F* at 95% confidence level; theoretical values : *t* = 2.23 , *F*= 5.05. [f]Relative Standard Deviation (RSD).

and accuracy in the determination of naproxen in pharmaceuticals.

In order to investigate the presence of matrix effects on the proposed method, a recovery study was carried out. In this study 100, 200 and 300 mg L^{-1} of naproxenate reference solutions were added in three representative pharmaceuticals (samples 1, 3, 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 SDs were within 0.9-1.5.

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 potenciometric measurements.

The time required for performing analyses by the electrode method was 20-30 minutes *per* sample.

Table 4. Recovery data for sodium naproxenate spiked in pharmaceutical formulations

Formulation	Concentration added (mg L^{-1})	Concentration found (mg L^{-1})	Recovery[a] (% ±SD)
	100	97.9	97.9 ± 1.3
1 (tablet)	200	196.8	98.4 ± 0.9
	300	297.9	99.3 ± 1.0
	100	98.1	98.1 ± 1.1
3 (tablet)	200	201.8	100.9 ± 1.0
	300	302.1	100.7 ± 0.9
	100	98.3	98.3 ± 1.5
6 (tablet)	200	198.8	99.4 ± 1.3
	300	303.6	101.2 ± 1.1

[a]Average of six determinations ± standard deviation (SD).

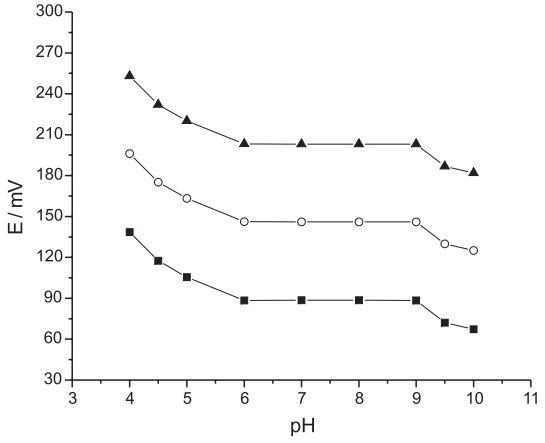

Figure 3. Effect of pH on the electrode's response at : (■) 1.0×10^{-2} mol L^{-1} NAP, (○)1.0×10^{-3} mol L^{-1} NAP, (▲)1.0×10^{-4} mol L^{-1} NAP ; μ=0.500 mol L^{-1} adjusted with $NaNO_3$, T= 25 °C.

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 naproxen determination in real samples, particularly for some commercial naproxen tablets was demonstrated suggesting its use as a reliable and advantageous alternative to the USP method[2] as well as to most other previously reported methods in the routine control of naproxen concentration in these samples. The electrode developed in this laboratory is superior (especially concerning lifetime and simplicity) as compared with naproxenate ion selective electrodes described in the literature.[19,29]

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