Article

Development of a Potentiometric Flufenamate ISE and its Application to Pharmaceutical and Clinical Analyses

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As características, o desempenho e a aplicação de um eletrodo do tipo Pt⌠Hg⌠Hg$_2$(FF)$_2$⌠Grafite, FF=íon flufenamato, são descritas. O eletrodo responde a FF com sensibilidade de (-58,6⇓1,2) mV década^{-1} no intervalo de 1,0 ∂ 10^{-6} - 1,0 ∂ 10^{-2} mol L^{-1}, a pH 6,0 - 9,0 e com um limite de detecção de 7,1 ∂ 10^{-7} mol L^{-1}. O eletrodo é de custo relativamente baixo e facilmente construído, apresenta um rápido tempo de resposta (10-25 s) e pode ser usado por um período de 5 meses sem qualquer variação considerável nas suas características de desempenho. O eletrodo proposto mostrou boa seletividade para o íon flufenamato na presença de várias substâncias, bem como na presença de alguns carboxilatos e ânions inorgânicos. O eletrodo foi aplicado com sucesso na determinação de ácido flufenâmico em medicamentos e amostras de soro humano.

The characteristics, performance, and application of an electrode, namely, Pt⌠Hg⌠Hg$_2$(FF)$_2$⌠Graphite, where FF stands for flufenamate ion, are described. This electrode responds to FF with sensitivity of (-58.6 ⇓ 1.2) mV decade^{-1} over the range 1.0 ∂ 10^{-6} - 1.0 ∂ 10^{-2} mol L^{-1} at pH 6.0 - 9.0 and a detection limit of 7.1 ∂ 10^{-7} mol L^{-1}. The electrode is easily constructed at a relatively low cost with fast response time (within 10-25 s) and can be used for a period of 5 months without any considerable change in its performance characteristics. The proposed electrode displayed good selectivity for flufenamate in the presence of several substances as well as in the presence of some carboxylate and inorganic anions. The electrode was successfully applied to the determination of flufenamic acid in pharmaceuticals and human serum samples.

Keywords: flufenamate-sensitive electrode, potentiometry, dosage forms, blood serum

Introduction

Flufenamic acid (FFA), [N-(γ,γ,γ-trifluoro-m-tolyl) anthranilic acid] is an analgesic, anti-inflammatory and antipyretic drug. It is extensively used in the treatment of arthritis and other illnesses related to muscular-skeletal problems.[1] However the use of this drug has been implicated in some cases of nephrotoxicity.[2] Due to the importance of the assay of FFA for pharmaceutical formulations and biological fluids, several analytical methods have been developed for the quantitative determination of this drug in both pharmaceutical and biological samples.

These methods include spectrophotometry,[3-5] chromatography,[6-11] capillary electrophoresis,[12,13] atomic absorption spectrometry,[14] spectrofluorimetry,[15-17] derivative synchronous fluorescence spectrometry,[18] high-performance liquid chromatography-mass spectrometry,[19] flow injection analysis,[20] and voltammetry.[21,22] However, many of these methods are often time-consuming, technically demanding and require the use of costly, highly specialized instruments. Thus, there is an important demand for simple, low-cost, sensitive and rapid alternative methods for the determination of FFA in pharmaceuticals and biological fluids.

Potentiometric methods with ion-selective electrodes (ISE's) have proved to be effective for the analysis of pharmaceutical formulations and biological samples, 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.[23,24]

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To the best of our knowledge, there are a single report on the use of ion-selective potentiometric sensor for the determination of flufenamic acid.[25] However, this electrode has not been applied for the determination of FFA in pharmaceuticals and biological fluids, thus, precluding the assessment of its usefulness in real analysis.

Previous work from this laboratory dealt with the development of mercury(I)-carboxylate electrodes and their application to solution equilibria,[26-29] food analysis,[30] and pharmaceutical analysis[31,32] involving carboxylate bearing compounds.

The aim of the present contribution was to develop a simple and low-cost potentiometric flufenamate ISE immobilized in a graphite matrix (Pt⌐ Hg⌐ Hg$_2$(FF)$_2$⌐ Graphite, where FF stands for flufenamate ion) and to evaluate the applicability of this sensor to the determination of FFA in dosage forms and human serum samples.

Experimental

Reagents

High purity deionized water (resistivity 18.2 MΠ 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 flufenamic acid, which was supplied by Sigma (St. Louis, MO, USA).

Sodium flufenamate stock solution was prepared in a manner similar to a previously reported procedure for preparation of sodium naproxenate.[33] This sodium flufenamate stock solution was analyzed by evaporating and drying to constant weight at 120 °C.

Standardizations of carbonate-free sodium hydroxide, nitric acid and sodium nitrate solutions were performed as described elsewhere.[26,31] Metallic mercury was purified according to a previously reported procedure.[34] Mercury (I) flufenamate was prepared by mixing, in aqueous solution, the corresponding nitrate with an excess of sodium flufenamate. 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) flufenamate indicator electrode was prepared as follows: mercury (I) flufenamate (1.6 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 (0.8 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 8.5 tons for about 5 min. The black pellet (1.6 mm thick, 12 mm o.d., and 0.7 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. Similarly to the previous mercury(I)-carboxylate electrodes reported by the authors[30-32] this electrode 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 flufenamate 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.

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.

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) = 0.500 mol L^{-1}	[NaFF](aq) = mol L^{-1}	Graphite | Hg$_2$(FF)$_2$ | Hg | Pt(+)
	[NaNO$_3$](aq) = 0.490 mol L^{-1}		[NaNO$_3$](aq) = (0.500-x)mol L^{-1}	

where FF stands for flufenamate ion and x was in the range 10^{-1}-10^{-7} 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) flufenamate electrode was assessed by measuring the emf of the aforementioned cell for 10^{-1} to 10^{-7} mol L^{-1} sodium flufenamate solutions. These solutions were freshly prepared by serial dilution of a 0.100 mol L^{-1} stock standard solution with deionized water, at constant pH (8.0 ± 0.1) and μ adjusted to 0.500 mol L^{-1} with $NaNO_3$. 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 1.
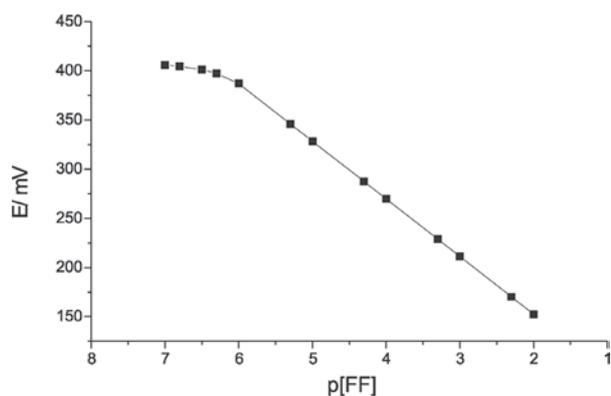
Figure 1. Calibration graph for the proposed flufenamate-sensitive electrode (pH=8.0, μ 0.500 mol L^{-1} adjusted with $NaNO_3$, T= 25 ^oC).

Determination of flufenamic acid in dosage forms

The analyzed products were purchased locally from manipulation drugstores or directly from the manufacturers and all were tested prior to the listed expiration date. Four pharmaceutical formulations containing FFA and other components were analyzed with the flufenamate-sensitive electrode.

Representative samples of finely ground tablets or opened capsules containing a quantity equivalent to about 30 mg of FFA 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 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 (with pH adjusted to 8.0 ± 0.1) for rinsing and volume completion. An aliquot of 20 mL is employed for analysis with the flufenamate-sensitive electrode.

Determination of flufenamic acid in human serum samples

Drug-free human serum used in this study was obtained from healthy volunteers. The serum was kept in a freezer until analysis.

The extraction procedure of FFA used here was similar to reported procedure by Cerretani *et al.*[7] To 5 mL of spiked human serum with different quantities of FFA, 5 mL of 1 mol L^{-1} phosphoric acid was added and mixed for 60 s. The samples were then extracted with 6 mL of dichloromethane, vortex mixed for 5 min and centrifuged at 10.000 rpm (8500 × g) for 3 min. The organic phase was then transferred to an appropriate flask and evaporated to dryness under a gentle N_2 stream, at 40 ^oC. The residue was dissolved in 10 mL of 0.500 mol L^{-1} $NaNO_3$ (pH = 8.0 ± 0.1) and an aliquot of 8 mL of reconstituent was employed for analysis with the flufenamate-sensitive electrode using the standard additions method (multiple addition method).

Results and Discussion

Electrode response

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

$$E = E^0 - S \log [FF]$$

where E^0 is the formal cell potential and S represents the Nernst coefficient (59.16 mV decade^{-1}, at 25 ^oC, for monovalent ions). Potentiometric parameters and other features associated with the mercury (I) flufenamate 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 flufenamate ion in the range of 10^{-2} to 10^{-6} mol L^{-1}. The limit of detection, as determined from the intersection of the two extrapolated segments of the calibration graph (Figure 1), was 7.1 ∂ 10^{-7} mol L^{-1}.[35] The electrode 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

For analytical applications, the response time of an electrode is an important factor. The response time of the electrode was tested by measuring the time required to achieve a steady state potential (within ± 0.2 mV min^{-1}), for 10^{-2} to 10^{-6} mol L^{-1} sodium flufenamate solutions at pH 8.0.[35,36] The electrode yielded steady potentials within 10 to

Table 1. Potentiometric response characteristics of the Mercury (I) flufenamate electrode[a]

Slope / (mV decade^{-1})[b]	Intercept, E^0 / (mV)[b]	Linear range / (mol L^{-1})	Detection limit / (mol L^{-1})
- 58.6 $\pm$ 1.2	35.4 $\pm$ 1.2	1.0×10^{-6} - 1.0×10^{-2}	7.1×10^{-7}

[a]T = 25.0 $\pm$ 0.1 °C ; pH = 8.0 $\pm$ 0.1; μ 0.500 mol L^{-1} (NaNO$_3$). [b]Average value $\pm$ SD of 35 determinations over a period of 5 months. Number of data points: 20-25. Mean linear correlation coefficient: 0.996 $\pm$ 0.004.

15 s at high concentrations ($\forall 10^{-3}$ mol L^{-1}) and about 25 s at concentrations near the detection limit. The lifetime of the electrode was found to be 5 months. During this period, the electrode did not show any significant change in working concentration range, slope and response time.

pH Effect

The influence of pH on the electrode response was tested over the pH range 4.0-10.0 for 1.00×10^{-3} and 1.00×10^{-4} mol L^{-1} flufenamate 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 flufenamate ion (pKa=4.27)[37] 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 2). 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.
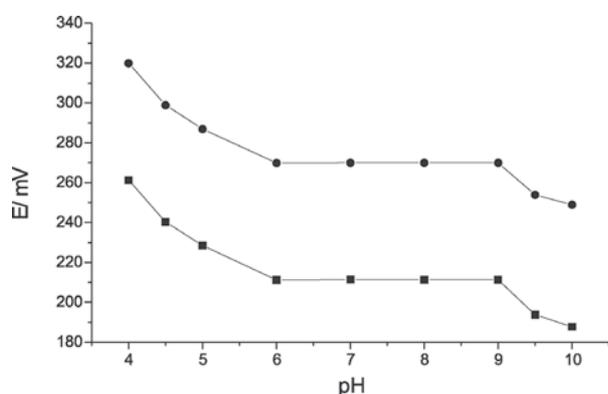
Figure 2. Effect of pH on the electrode's response at: (■) 1.0×10^{-3} mol L^{-1} FF, (●) 1.0×10^{-4} mol L^{-1} FF ; μ 0.500 mol L^{-1} adjusted with NaNO$_3$, T= 25 °C.

Electrode selectivity

The most important characteristic of any ion-selective electrode 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) flufenamate electrode ($K_{FF,M}$) were determined, for a number of anions

(M), by the matched potential method (MPM).[38-40] 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.[41] Mainly for these reasons, it has increased in popularity in the last few years.[42]

The MPM-selectivity coefficients ($K_{FF,M}$) were determined under the following conditions: Initial reference solution (pH=8.0) contains 0.500 mol L^{-1} NaNO$_3$ as a supporting electrolyte and 1.0×10^{-5} mol L^{-1} of the primary ion (flufenamate). The selectivity coefficients were calculated from the concentration of the interfering ion (M), which induced the same amount of the potential change ($\pm$emf = 15.0 mV) as that induced by increasing the concentration of primary ion. The resulting values of $K_{FF,M}$ are presented in Table 2.

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

Anion	$K_{FF,M}$
Formate	1.3×10^{-4}
Acetate	1.8×10^{-3}
Propionate	2.1×10^{-3}
Citrate	2.9×10^{-3}
Lactate	3.2×10^{-3}
Tartrate	2.7×10^{-3}
Benzoate	3.3×10^{-3}
Salicylate	3.1×10^{-3}
Phthalate	3.5×10^{-3}
Oxalate	2.2×10^{-3}
Chloride	2.3×10^{-1}
Sulphate	4.3×10^{-4}
Borate	5.2×10^{-4}
Perchlorate	7.4×10^{-6}
Nitrate	8.2×10^{-6}

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

The results comprised in the aforementioned Table 2 show that the selectivity of the mercury(I) flufenamate

electrode towards all tested organic acid anions is good. No interference was noted for most of the common excipients used in commercial formulations (tablets and capsules) such glucose, lactose, starch, sodium glycolate, magnesium stearate, hydroxypropylmethylcellulose, microcrystalline cellulose, croscarmellose sodium, titanium dioxide, silicon dioxide, povidone, polyethyleneglycol, hypromellose, methylcellulose, vanillin.

Sulphate and borate have a low selectivity coefficient (Table 2); Nitrate and perchlorate have a very low selectivity coefficient and they can therefore be used as background electrolytes or ionic strength adjusters for flufenamate solutions before performing potentiometric measurements.

Some interference by chloride ion might be expected as shown in Table 2. In the dosage forms analyzed in this work (tablets and capsules), chloride ion is seldom found and hence the proposed sensor can be used for direct determination of FFA in these samples without previous extraction procedures. Concerning the human serum samples analyzed by the potentiometric electrode, it should be noted that analytical procedure adopted in this work is based on dichloromethane extraction of FFA from acidified biological matrices followed by its reversion to the aqueous phase (0.500 mol L^{-1} NaNO$_{3(aq)}$; pH = 8.0 ± 0.1) as flufenamate ion. The chloride content found in the last mentioned aqueous phase (which originates from the analyses of human blood serum) was always < 1μg L^{-1}, as analyzed by the mercury thiocyanate method.[43] Therefore, the working procedure removes chloride interference.

Analytical application

In order to test the analytical applicability of the proposed electrode, it has been applied for the determination of FFA in dosage forms and human serum samples using a standard additions method (multiple addition method).[44,45]

Table 3 shows statistical analysis of the results obtained by using the presently proposed flufenamate-sensitive electrode and the official method of B.P (non-aqueous alkalimetry)[46] for FFA determination in certain dosage forms. In all cases, the calculated F and t values did not exceed the theoretical values, indicating that there is no significant difference between the performance of the two methods as regard accuracy (*t*-test) and precision (*F*-test).

Flufenamic acid is readily absorbed after oral ingestion and a peak plasma (or serum)concentration occurs about 2 h.[47] FFA is given orally in doses of 200-1000 mg daily; this leads to a final blood concentration of *ca.* 4-20 μg mL^{-1}.[47] In humans, FFA is mainly metabolized into acyl glucuronides.[48-50] Taking into account the role of the flufenamate ion in the electrode response, along with the very low concentration of glucuronides in serum,[49,50] no interference from these metabolites is expected in the determination of FFA in human blood serum by the proposed electrode.

Experiments were performed to determine the feasibility of using the flufenamate-sensitive electrode for determination of FFA in human serum samples. Serum samples previously spiked with 4, 8, 12, 16 and 20 μg mL^{-1} of FFA were treated and analyzed as described under "*Determination of Flufenamic Acid in Human Serum Samples*". The concentrations of FFA were selected from pharmacokinetic data of this compound.[51] The recovery values found by application of the proposed potentiometric sensor and the comparative HPLC method[52] are given in Table 4. Satisfactory recovery values were

Table 3. Determination of flufenamic acid in commercial formulations

Product (laboratory)[a]	Label to content[b]	Electrode Found[c] / (mg unit^{-1})	Method RSD[e] / (%) (n = 6)	B.P. method[46] Found[c] / (mg unit^{-1})	RSD[e] / (%) (n = 6)
Capsules					
A (Alexandria)	100	98.1 ± 1.6 t[d] =1.17, F[d] = 2.42	1.6	97.8 ± 1.8	1.8
B (Parke Davis)	100	99.1 ± 1.8 t[d] = 1.13, F[d]= 2.74	1.8	99.4 ± 2.1	1.9
Tablets					
C (Manipulation drugstore)	50	50.7 ± 1.2 t[d] = 1.18, F[d]= 2.88	1.9	51.2 ± 1.1	2.1
D (Manipulation drugstore)	75	74.7 ± 1.5 t[d] = 1.16, F[d]= 2.35	2.1	74.1 ± 1.3	1.7

[a]These contain some or all of the following substances/materials: glucose, lactose, starch, sodium glycolate, magnesium stearate, hydroxypropylmethylcellulose, microcrystalline cellulose, croscarmellose sodium, titanium dioxide, silicon dioxide, povidone, polyethyleneglycol, hypromellose, methylcellulose, vanillin. [b]Declared concentration of flufenamic acid in mg unit^{-1}. [c]Values found are the average of six independent analyses (n = 6) ± the corresponding standard deviation (SD). Expressed as flufenamic acid. [d]Values of t and F at 95% confidence level. Theoretical values: *t* = 2.23, *F*= 5.05. [e]Relative standard deviation (RSD).

Table 4. Determination of flufenamic acid added to human serum samples

FFA added to blood serum[a] / (πg mL^{-1})	Electrode Found[b] / (πg mL^{-1})	Method Recovery / (%)	Comparative Found[b] / (πg mL^{-1})	Method [52] Recovery / (%)
4	3.9 ± 0.1	97.5	3.9 ± 0.1	97.5
8	7.9 ± 0.2	98.7	8.1 ± 0.2	101.2
12	12.2 ± 0.4	101,7	12.1 ± 0.3	100.8
16	16.2 ± 0.4	101.2	16.1 ± 0.3	100.6
20	20.3± 0.5	101.5	20.2 ± 0.4	101.0

[a]These concentrations for FFA were selected from pharmacokinetic data.[51] [b]Values found are the average of six determinations (n=6) ± the corresponding standard deviation (SD). Expressed as flufenamic acid.

obtained indicating that the endogenous compounds of serum samples do not interfere in the detection of FFA using the proposed method. Therefore, the proposed electrode can be used for the determination of FFA in dosage forms and human serum samples.

Conclusions

The results of this study show that the potentiometric method based on flufenamate ion-selective electrode immobilized in a graphite matrix may provide an attractive alternative for the determination of flufenamic acid.

The proposed potentiometric electrode is easy to prepare, exhibits long lifetime, show high sensitivity and wide dynamic range. Good selectivity, very low detection limit, rapid response and low cost make this electrode suitable for analysis of FFA in dosage forms and human serum samples.

Acknowledgments

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