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Photochemically-Induced Fluorescence Properties and Determination of Flufenamic Acid, a Non-Steroidal Anti-Inflammatory Drug, in Urine and Pharmaceutical Preparation*

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The photochemically-induced fluorescence (PIF) properties of flufenamic acid (FF), a non-steroidal anti-inflammatory drug (NSAID) were investigated in acidic (pH 1.0) aqueous solutions at room temperature. An optimization procedure, including the effects of UV irradiation time, pH and solvent, was established for the determination of FF. A linear logarithmic calibration plot was obtained over a wide concentration range of four orders of magnitude. A low limit of detection of 0.14 ng/mL was found. The relative standard deviation (RSD) was 6.6% . The PIF method was applied to the quantitative analysis of FF in urine and in a pharmaceutical preparation with satisfactory recovery values.

Key Words: Photochemically-induced fluorescence; flufenamic acid; urine analysis; pharmaceutical preparation.

Introduction

Flufenamic acid (FF) (Scheme 1) belongs to a family of important nonsteroidal anti-inflammatory drugs (NSAID) with a diphenylamine structure that are widely used in the treatment of rheumatoid arthritis, other musculo-skeletal disorders and post-trauma inflammation. Several analytical methods, such as HPLC[1,2] GC-MS[3], and UV-visible spectrophotometry[4] and spectrofluorimetry[5] generally based on the formation of metal complexes, have been reported for the determination of FF and other structurally-related NSAID. Voltammetric, spectrofluorimetric and UV spectrophotometric techniques have also been proposed for FF analysis, using the cyclization reaction of FF with sulfuric acid to produce the corresponding acridone derivative[6]. The analytical usefulness of direct native fluorescence of FF is still a subject of controversy[7,8]. Recently, it has been shown that diphenylamine derivatives can undergo photocyclization and yield stable photoproducts under UV irradiation[9]. Therefore, we decided to apply the photochemically-induced fluorescence (PIF)

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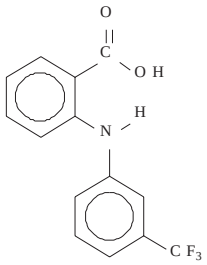
approach[10,11] to the quantitative analysis of FF. Indeed, photochemical derivatization has the advantage of being a simple, clean and efficient analytical technique, providing strongly fluorescent photoproducts from non-fluorescent or weakly fluorescent analytes[12].



Flufenamic acid $C_{14}H_{10}F_3NO_2$

In this work, we investigated the PIF properties of FF in various media. Under UV irradiation, this compound exhibited in an acidic aqueous solution, an intense fluorescence emission due to its phototransformation into stable, fluorescent photoproducts. We applied the PIF method to the determination of FF in urine and in a pharmaceutical preparation.

Experimental

Apparatus

Fluorescence and PIF spectra and intensity measurements were measured on a Kontron (Zürich, Switzerland) SFM 25 spectrofluorimeter, using a quartz cuvette (10 mm optical pathlength). An Osram (Germany) 200W high-pressure mercury arc lamp powered with a Spotlight power supply and located in a Schoeffel Instruments GmbH light-box was utilized for the photochemical studies.

Chemicals

Flufenamic acid was purchased from Sigma and used as received. Deionized water and analytical grade solvents (dioxane, ethanol, acetonitrile and dimethyl sulfoxide) were utilized for the preparation of solutions. The pH buffers were obtained from Merck. $HClO_4$ was purchased from Aldrich (spectroscopic grade). The pharmaceutical preparation Movilisin (Sanky Pharma, GmbH, Germany) was obtained in a commercial pharmacy.

Procedures

Determination of FF in standard solutions

Aliquots of FF samples, prepared in the optimal conditions and containing FF concentrations of 0.3-2,800 ng/mL, were introduced into a 1 cm quartz cuvette and then irradiated for the optimum irradiation time (defined as the irradiation time corresponding to the maximum PIF signal) at room temperature with the UV high-pressure mercury arc lamp. Then, the cuvette was transferred to the spectrofluorimeter and the fluorescence intensities were measured at the wavelengths of the excitation and emission maxima of the uncorrected PIF spectra. The procedure used for photochemical-fluorimetric measurements was the same as one previously utilized[10,11]. Afterwards, a direct calibration curve was drawn.

Determination of FF in urine and pharmaceutical preparation

For these applications, the classical standard addition procedure was chosen.

In the case of FF determination in urine, a 20 μL volume of a 10^{-3} M FF aqueous solution was placed in a 10 mL flask and completed to the mark with a filtrated human urine sample. Then, 40 μL volumes of the resulting solution were put in 10 mL flasks and completed to the mark with FF aqueous standard solutions of increasing concentrations ; in these conditions, the original urine sample was diluted 250 times.

For pharmaceutical preparation, 1 mL of Movilisin® (containing 30 mg of FF) was placed in a 100 mL flask and completed to the mark with water; a 100 μL volume of this solution was introduced into a 10 mL flask and completed to the mark with water. A constant volume (100 μL) of the diluted pharmaceutical sample was spiked with FF acidic aqueous standard solutions of increasing concentrations in 10 mL flasks.

Photochemical-fluorimetric measurements were performed on these solutions.

Results and Discussion

PIF properties of FF in aqueous media

FF (10^{-5}M, 2,800 ng/mL) displayed no significant fluorescence at room temperature in an acidic aqueous medium (0.1M $HClO_4$), but upon UV irradiation of FF for 4 min in the same solution, a very strong fluorescence band appeared, with an emission maximum at 424 nm and a shoulder at 444 nm (Figure 1). The corresponding excitation spectrum showed two peaks at 256 and 390 nm. The same emission spectrum was obtained upon excitation at both wavelengths, which indicates that these excitation bands correspond to two electronic transitions of the same fluorescing molecule (Figure 1, curves a and b). This behaviour suggests that FF undergoes a photocyclization reaction, yielding strongly fluorescent photoproduct(s), in agreement with recent literature results[9].

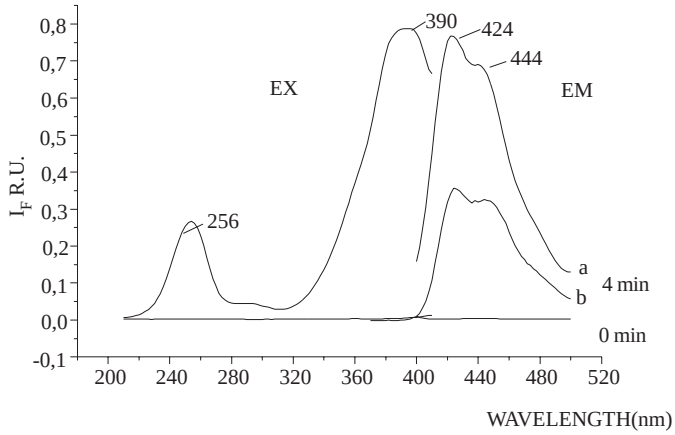


Figure 1. PIF excitation (EX) and emission (EM) spectra of flufenamic acid (10^{-5} M) in a 0.1M $HClO_4$ aqueous solution (pH = 1.0 ; t_{irr} = 4 min). Emission spectra were recorded at λ_{ex} = 390 nm (curve a) and 256 nm (curve b), respectively. Corresponding spectra are also given below for t_{irr} = 0 min.

Irradiation time effect

The curves of FF fluorescence intensity (I_F) *versus* UV irradiation time obtained in acidic aqueous medium (pH 1) at different FF concentrations exhibited an initial rapid increase in the fluorescence signal, which reached a plateau value after about 4 min for all FF concentrations (Figure 2). For irradiation times longer than about 8 min, a slight decrease in I_F was observed, probably indicating slow photolysis of the fluorescent photoproducts. An optimum irradiation time of 4 min was chosen for subsequent analytical studies.

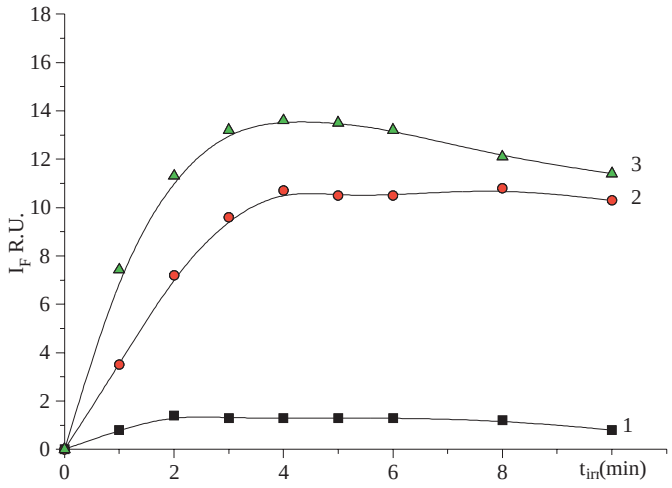


Figure 2. Influence of the UV irradiation time on the PIF intensity of flufenamic acid at concentrations of 0.3 ng/mL (curve 1) 3.0 ng/mL (curve 2) and 41 ng/mL (curve 3) in acidic (pH = 1) aqueous solutions. The curve corresponding to 41 ng/mL was obtained with a sensitivity scale divided by 10.

pH effect

We investigated the effect of pH on the FF PIF signal. The curve of I_F *versus* pH, established in the pH region of 10.0 to 1.0, indicated that I_F increased progressively for acidic pH (< 3.0) and reached a maximum at pH 1.0. This pH value was used for analytical measurements.

Solvent effect

We studied the solvent effect on the fluorescence and PIF properties of FF. In Table 1, we present the fluorescence and PIF uncorrected excitation and emission wavelengths and relative fluorescence intensities of FF in several solvents. As can be seen, FF shows a weak native fluorescence in ethanol, but yields much stronger fluorescence signals in acetonitrile, DMSO and dioxane; the fluorescence excitation and emission spectra are practically independent of the polarity of the solvent, except for dioxane for which a 20-nm blue shift of the emission maximum is observed. In contrast, the PIF properties vary considerably with the solvent: the emission maxima occur at shorter wavelengths in dioxane and DMSO than in ethanol and aqueous media. These spectral differences could be attributed to the formation of various photoproducts, according to the nature of the solvent. In all solvents with the exception of acetonitrile, the PIF signal is enhanced relative to the native fluorescence intensity. PIF signals vary considerably with the solvent according to the order: $I_F(H_2O + 0.1\ M\ HClO_4) >> I_F$ (DMSO) $> I_F$ (dioxane) $> I_F$ (EtOH) $> I_F$ (H_2O)

(Table 1). Since the PIF signal was about 12 to 590 times stronger in 0.1 M $HClO_4$ aqueous solution than in the other solvents, this medium was selected for all further analytical studies.

Table 1. Solvent effects on the fluorescence and photochemically-induced fluorescence of flufenamic acid ($1x10^{-5}$M)

Solvent	Native fluorescence			Photochemically-induced fluorescence			
	λ_{ex} (nm)	λ_{em} (nm)	I_F [a]	λ_{ex} (nm)	λ_{em} (nm)	I_F [b]	t_{irr}^{OPT} [c]
H_2O	NF [d]	NF [d]	-	258; 310; <u>390</u>	<u>430</u>	1.0	- [e]
H_2O + $HClO_4$ 0.1M	NF [d]	NF [d]	-	256; <u>390</u>	<u>424</u>; 440	589	4
EtOH	284; <u>334</u>; 368	378; <u>424</u>	1.0	256; <u>334</u>	378; <u>414</u>	2.8	- [e]
MeCN	282; <u>334</u>	<u>422</u>	54	- [f]	- [f]	- [f]	- [f]
Dioxane	282; <u>334</u>	<u>406</u>	67	285; <u>334</u>	<u>406</u>	27	5
DMSO	290; <u>338</u>	<u>426</u>	33	292; <u>338</u>	<u>392</u>	48	4

[a] Fluorescence intensity normalized relative to the corresponding signal in EtOH; [b] PIF intensity normalized relative to the corresponding signal in water; [c] Optimal irradiation time, corresponding to the photoproduct maximum fluorescence intensity; [d] Not fluorescent; [e] I_F measured at t_{irr} = 4 min. No maximum for the I_F/t curve in this solvent; [f] Decreasing I_F/ti_{rr} curve.

Analytical figures of merit

The analytical figures of merit were obtained for the PIF determination of FF in optimal conditions ($\lambda_{ex}/\lambda_{em}$ = 390 nm/424 nm; 0.1 M $HClO_4$ aqueous solution; t_{irr} = 4 min). A linear logarithmic plot of the FF photoproduct fluorescence intensity *versus* initial FF concentration was established over a linear dynamic range (LDR) of about four orders of magnitude in concentration (0.3 – 2,800 ng/mL). The corresponding regression equation was: $\log I_F = 1.04 \log C + 0.37$. The correlation coefficient (r = 0.999) was very close to unity, indicating a satisfactory precision for the analytical curve. The small relative standard deviation (RSD = 6.6%) demonstrated the good reproducibility of the PIF measurements.

In addition, the IUPAC limit of detection (LOD) was very low with a value of 0.14 ng/mL, and the absolute LOD was 70 pg of FF (corresponding to 1 picomol of compound). This LOD value is much lower than those obtained in the literature by HPLC (absolute LOD: 500 pg)[1], by fluorescence of Al(III)-FF complexes (LOD : 2 ng/mL)[5] or by FF direct fluorescence in micellar media (LOD not reported, but LDR range: 460-6,750 ng/mL)[6]. When compared to other literature analytical techniques, our photochemical-fluorimetric method also yields the largest LDR reported so far in the case of FF, which constitutes a distinct advantage for analytical, biomedical and pharmaceutical applications.

Analytical applications

We applied our PIF method to the determination of FF in human urine samples and in a pharmaceutical preparation (Movilisin) using the standard addition procedure.

A good linearity and a good precision were obtained for the standard addition plots (r=0.995). The slopes were very close to that measured for the calibration curve: for instance, slopes of 3.30 and 3.56 mL/ng were found for the calibration and standard addition linear plots, respectively, in the case of urine samples. These results indicate the absence of significant interference from the matrix effects in the urine samples as well as in the pharmaceutical preparation. Satisfactory recoveries were found at various FF concentrations,

ranging from about 103 to 110% for the urine samples and from about 100 to 108% for the pharmaceutical preparation (Table 2).

Table 2. PIF determination of flufenamic acid in urine and in a pharmaceutical preparation (Movilisin)

Type of sample	Flufenamic acid [a] (ng/ml) Added	Found[b]	Recovery (%)[c]
Urine	3.66	3.97	108.5
	5.06	5.21	102.9
	10.69	12.09	113.1
	16.31	17.95	110.0
	24.75	26.70	107.9
			Mean: 106.5
Pharmaceutical Preparation (Movilisin)	24.60	26.69	108.5
	26.00	27.05	104.0
	31.63	34.06	107.7
	37.25	39.30	105.5
	40.06	40.84	101.9
	45.70	45.80	100.2
			Mean: 104.6

[a] Including a spiked constant concentration of 2.25 ng/mL of FF for urine samples or an added constant amount of Movilisin corresponding to 23.20 ng/mL of FF.

[b] Number of replicate measurements = 3 for each FF concentration

[c] The relative standard deviation (RSD) values were 2.7% and 2.8% , for urine samples and pharmaceutical preparations, respectively.

Conclusion

We have shown the analytical value of using PIF detection for the easy determination of flufenamic acid, which is naturally non-fluorescent or weakly fluorescent in most media. The PIF method is simple, rapid and very sensitive, allowing detection of picomol (corresponding to about 70 pg) amounts of FF in acidic aqueous solution. It is possible to assay FF in urine and pharmaceutical formulation, with satisfactory recovery values. Work is now in progress to apply our method to other non-steroidal anti-inflammatory drugs.

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