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Original scientific paper

QUANTIFICATION OF DICLOFENAC IN NATURAL WATERS BY THE PHOTO-INDUCED FLUORESCENCE (PIF) METHOD

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A photo-induced fluorescence (PIF) method was developed for the quantification of diclofenac sodium, sodium 2-[2-(2,6-dichloroanilino)phenyl]acetate (NaDCF), in natural waters. Since diclofenac is not naturally fluorescent, its photoconversion under UV irradiation was carried out to produce highly fluorescent photoproduct(s) in various media (water, methanol, isopropanol, acetonitrile, ethyl acetate, dimethyl sulfoxide (DMSO), and a water-isopropanol mixture). The photoproduct responsible for the intense fluorescence of NaDCF in aqueous media was identified as chloro-carbazole by gas chromatography-mass spectrometry (GC-MS). Several PIF parameters were optimized. Using the PIF method, a highly fluorescent diclofenac photoproduct was obtained at $\lambda_{ex}/\lambda_{em}$ = 235/360 nm, with optimum fluorescence achieved after 10 min irradiation in a water-isopropanol mixture (90:10 v/v). The limit of detection (LOD) and quantification (LOQ) for diclofenac sodium in this medium were 0.11 ng ml^{-1} and 0.37 ng ml^{-1}, respectively, with low relative standard deviation (RSD) values. The PIF calibration curves demonstrated good linearity, extending over one to three orders of magnitude, with correlation coefficients (R^2) near unity, indicating good reproducibility. Analytical applications of this method to natural water samples yielded satisfactory results, with average recovery rates ranging from 84.77 to 101.72 %.

Keywords: non-steroidal anti-inflammatory drugs (NSAIDs); diclofenac; photo-induced fluorescence (PIF); environment

КВАНТИФИКАЦИЈА НА ДИКЛОФЕНАК ВО ПРИРОДНИ ВОДИ СО МЕТОД НА ФОТО-ИНДУЦИРАНА ФЛУОРЕСЦЕНЦИЈА

Развиен е метод на фото-индуцирана флуоресценција (PIF) за квантификација на натриум диклофенак, натриум 2-[2-(2,6-дихлороанилино)фенил]ацетат (NaDCF) во природни води. Бидејќи диклофенак не е природно флуоресцентен, беше извршена негова фотоконверзија со UV-зрачење за да се произведе високо флуоресцентен(ни) фотопродукт(и) во различни медиуми (вода, метанол, изопропанол, ацетонитрил, етил ацетат, диметил сулфоксид (DMSO) и смеса вода-изопропанол. Фотопродуктот одговорен за интензивната флуоресценција на NaDCF во водните медиуми беше идентификуван како хлоро-карбазол со гасна хроматографија-масена спектрометрија (GC-MS). Неколку параметри на PIF беа оптимизирани. Користејќи го методот PIF, беше добиен високофлуоресцентен фотопродукт на диклофенак на $\lambda_{ex}/\lambda_{em}$ = 235/360 nm, со оптимална флуоресценција постигната по 10 min зрачење во смеса вода-изопропанол (90:10 v/v). Границите на детекција (LOD) и квантификација (LOQ) за натриум диклофенак во овој медиум беа, соодветно, 0,11 ng ml^{-1} и 0,37 ng ml^{-1}, со ниски вредности на релативна стандардна девијација (RSD). Кривите за калибрација на PIF покажаа добра линеарност, протегајќи се од еден до три реда на големина, со коефициенти на корелација (R^2) блиску до еден, што укажува на добра

репродуктивност. Аналитичката примена на овој метод на примероци од природна вода дава задоволителни резултати, со просечен аналитички принос кој се движи од 84,77 до 101,72 %.

Клучни зборови: нестероидни антиинфламаторни лекови (NSAIDs); диклофенак; фото-индуцирана флуоресценција (PIF); животна средина

1. INTRODUCTION

Recently, human and veterinary pharmaceuticals have attracted significant interest within the scientific community due to their frequent and increasing presence in various environmental matrices (water, sediment, etc.) and their potential risks to both ecosystems and human health.[1–3] Unlike other pollutants, these drugs are synthesized to have specific biological activity beneficial to animals and humans. However, after transformation and elimination through animal or human metabolism, they may induce side effects in non-target, yet sensitive organisms.[4,5]

Among these emerging contaminants, non-steroidal anti-inflammatory drugs (NSAIDs) are particularly prominent.[6] NSAIDs are among the most commonly prescribed pharmaceuticals worldwide due to their high efficacy and low potential for abuse. More than fifty NSAIDs are used for their antipyretic, analgesic, and anti-inflammatory properties, and many of them are available without medical prescription.[7] Diclofenac, a member of this drug class, is considered as an "emerging contaminant of concern". Diclofenac and its transformation products have been detected across environmental compartments,[8,9] with nearly 75 % entering aquatic and soil environments.[10] Due to its hydrophilic potential and stability, diclofenac tends to persist in aquatic ecosystems. Recent studies have shown that diclofenac can accumulate in edible fruits and vegetables, posing direct health risks to humans.[11] Diclofenac has been observed to adversely affect various environmental species, even at concentrations less than or equal to 1 μg l^{-1},[10,12] with potential adverse effects including liver and kidney damage as well as genotoxicity.[13,14]

To prevent these adverse effects, highly sensitive and selective analytical methods are needed to monitor trace levels of diclofenac in environmental matrices. Several methods have been developed for diclofenac determination in pharmaceutical formulations and natural water samples, including UV-visible spectrophotometry,[15] potentiometry,[16] capillary electrophoresis (CE),[17] immunological assays,[18] liquid chromatography (LC),[19,20] high-performance liquid chromatography (HPLC),[21,22] thin-layer chromatography (TLC),[23] gas chromatography (GC),[24] proton nuclear magnetic resonance (^{1}H NMR),[25] electrochemistry,[26] and spectrofluorimetry.[27–29] Most of these methods are chromatographic and generally require heavy and expensive equipment and, often, complex implementation procedures. In response, we developed a photo-induced fluorescence (PIF) method[30–32] that is more accessible, highly sensitive, simple, rapid, versatile, eco-friendly, and economical for diclofenac quantification. The PIF method relies on the transformation by UV irradiation of a nonfluorescent compound into fluorescent photoproduct(s) to quantify diclofenac sodium (Fig. 1) in natural waters.[30–32]

2. MATERIALS AND METHODS

2.1. *Materials*

Diclofenac sodium salt (NaDCF; CAS number: 15307-79-6; Formula: $C_{14}H_{10}Cl_2NO_2Na$; Molecular weight: 318.14 g mol^{-1}; Water solubility: 32.40 g l^{-1} at 37 °C)[33] was supplied by Sigma-Aldrich as a white powder of analytical grade with a purity > 99 %. The solvents used included ultrapure water produced by a Milli-Q system (resistivity > 18.2 MΩ cm at 25 °C), methanol (Sigma-Aldrich, Riedel-de Haen), ethanol (Honeywell), isopropanol (Riedel-de Haen), acetonitrile (Riedel-de Haen), dimethyl sulfoxide (DMSO) (Prolabo), and ethyl acetate (Sigma Aldrich).

2.2. *Instrumentation*

2.2.1. *Spectrofluorimetry*

Fluorescence and PIF measurements were performed using a Shimadzu RF-6000 spectrofluorimeter, coupled to a microcomputer equipped with the software "RF LabSolutions", which allows wavelength scanning from 200 and 900 nm. This device permitted the acquisition of both 2D and 3D fluorescence excitation and emission spectra. All fluorescence and PIF measurements were performed in parallelepipedic quartz cells (Hellma, Mullheim, Germany) with four polished faces using a 1 cm optical path.

2.2.2. *Irradiation system for the PIF method*

In the PIF method, photolysis reactions were conducted in a stationary phase using an Oriel

8000 power box equipped with a mercury lamp (HBO 200W/ 4 L1 OSRAM). Diclofenac solutions were irradiated at room temperature for a fixed time interval. A 3 ml working solution was placed in a quartz cell and positioned on an optical bench at 30 cm from the Hg lamp.

2.2.3. *Gas chromatography-mass spectrometry (GC–MS)*

To identify NaDCF photoproducts, a liquid extraction was performed be extracting 10 ml of irradiated NaDCF solution 3 times with 10 ml of ethyl acetate. The extracts were dried over magnesium sulfate, filtrated through Whatman paper, and evaporated to dryness using a magnetic stirrer heated to 80 °C. The residues were then recovered in 500 µl of the ethyl acetate, with 3.5 µl injected into a GC-MS system for analysis. GC-MS analyses were performed using a TRACE 1300 gas chromatograph (Thermo Scientific, Boston, MA, USA) coupled to an ISQ monoquadrupole mass spectrophotometer operating in electron impact mode at 70 eV. The samples were injected onto a TG-5Ms column (0.25 mm × 30 m × 0.25 mm i.d.). The temperature program for the column started at 50 °C (held for 1 min), followed by a 10 °C min^{-1} increase until reaching 330 °C, which was held for 5 min. The inlet and detector temperatures were set at 200 °C and 250 °C, respectively, with helium as the carrier gas at a flow rate of 1.2 ml min^{-1}.

2.3. *Preparation of solutions*

Diclofenac stock solutions at a concentration of 318.14 µg ml^{-1} were prepared in 10 ml flasks with the appropriate solvent for PIF studies. In most cases, the diclofenac dissolution was performed with an ultrasound apparatus. Working solutions were prepared by successive dilutions of the stock solutions to obtain the desired concentration. All solutions were stored in amber glass bottles wrapped in aluminum foil to protect them from light and were kept refrigerated until use.

2.4. *Standard addition procedure*

During 2021, natural water samples (well water and sea water) from Senegal and tap water from Marne-la-Vallée, France were collected in 0.5 l amber glass bottles. All samples were filtered through a 25 mm diameter hydrophilic PTFE membrane filter with 0.45 µm pores (Sigma-Aldrich) to remove suspended particulate matter and were stored at 4 °C.

For the standard addition procedure applied to the PIF method, filtered water samples were enriched with NaDCF standard solutions in 10 ml vials. Six 1 ml samples of these fortified solutions were transferred into 5 ml vials. The first 5 ml vial served as the blank, while the remaining vials contained increasing concentrations of NaDCF ranging from 0.03 to 0.64 µg ml^{-1}. Each vial was then adjusted to the mark with the appropriate solvent. The PIF signal was subsequently measured at the maximum emission wavelength after irradiation for the optimal duration.

3. RESULTS AND DISCUSSION

3.1. *NaDCF conventional fluorescence study*

Diclofenac exhibited very low natural fluorescence intensity. Furthermore, our study revealed significant instability in this natural emission across all solvents examined. We concluded that the NaDCF molecule is likely photolabile,[34] as the intensity of the spectrofluorimeter lamp was probably sufficient to photodegrade the molecule. This result aligns with the work of Li et al.,[27] who utilized fluorescent kinetics combined with fourth-order calibration to determine NaDCF levels in environmental water. They observed that NaDCF fluorescence was unstable and varied significantly with UV irradiation time. Consequently, we decided to use the PIF method to quantify this drug.

3.2. *NaDCF PIF study*

3.2.1. *NaDCF photochemical reactivity and PIF spectral properties*

Recently, the photolysis of diclofenac was investigated, with several studies proposing pathways for its photochemical degradation when exposed to different light sources.[34–37] Some authors used UV wavelengths greater than 315 nm for the photodegradation of diclofenac under natural or simulated sunlight,[35,36] while others employed the photo-Fenton reaction to identify the main intermediates and degradation pathways.[37]

The two main degradation photoproducts of diclofenac were identified as carbazole-1-acetic acid and its 8-chloro derivative.[35,38] It was also found that small amounts (1 %) of isopropanol or other organic solvents acted as proton sources for the photoreduction of 8-chlorocarbazole-1-acetic acid.[34,36–38] Furthermore, the presence of two chlorine atoms in the aromatic nucleus of NaDCF was expected to favor singlet-triplet intersystem cross-

ing by increasing spin-orbital coupling, which diminished the fluorescence quantum yield. This observation is consistent with the low fluorescence intensity of NaDCF.

We subjected the NaDCF solutions prepared in different media to UV irradiation to examine the formation of several photoproducts. We obtained 3D PIF spectra of NaDCF in ultra-pure water (Fig. 1 – A) and in a water-isopropanol mixture (90:10 v/v) (Fig. 1 – B) after irradiation for 8 and 10 min, respectively.

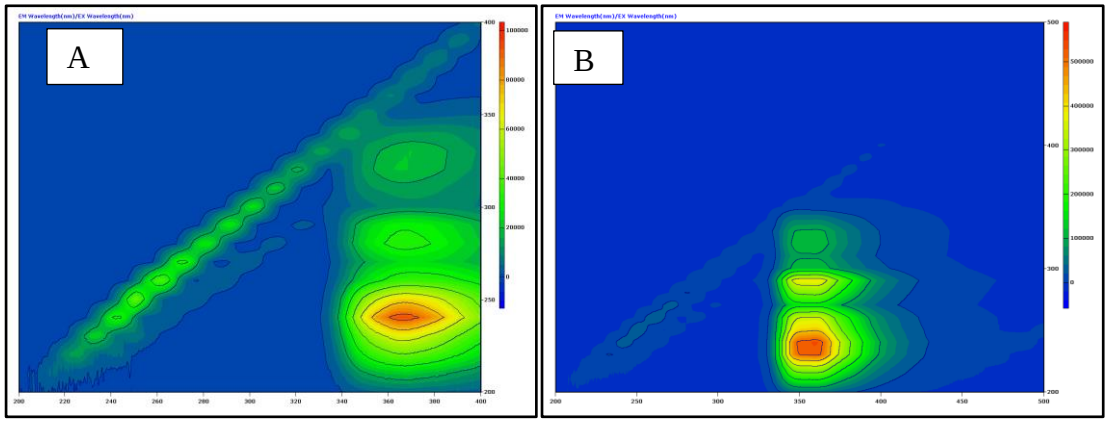

Fig. 1. 3D excitation and emission PIF spectra of diclofenac sodium (0.95 µg ml^{-1}) after 8 min and 10 min UV irradiation times, respectively, in ultra-pure water [A] and in water-isopropanol mixture (90 :10 v/v) [B]

The different colored spots indicated the presence of several fluorescent NaDCF photoproducts. In both solvents, two to three spots of varying intensity were observed for the same emission wavelength, corresponding to three excitation wavelengths. This result was confirmed by the 2D excitation and emission PIF spectra of diclofenac, obtained after UV irradiation (Fig. 2), which revealed three excitation bands at 239 nm, 287 nm, and 329 nm in ultra-pure water, and at 235 nm, 289 nm, and 323 nm in the 90:10 v/v water-isopropanol mixture. In contrast, the emission spectra were characterized by a single, broad band located between approximately 348 nm and 395 nm, depending on the solvent (Fig. 2 and Table 1).

T a b l e 1

Spectral properties and parameters of NaDCF PIF analytical curves in organic solvents and aqueous media

Solvent	$\lambda_{ex}/\lambda_{em}$ [a] (nm)	t_{irr}^{opt} [b] (min)	LOD [c] (ng.ml^{-1})	LOQ [d] (ng.ml^{-1})	R^2 [e]	LDR [f] (ng.ml^{-1})	% RSD [g] $n = 3$	I_f [h]
Water	239/365	8	0.25	0.85	0.996	1.59–954	0.78	1
Methanol	233/348	40	0.21	0.72	0.995	1.59–954	0.31	2.51
Isopropanol	235/348	35	0.19	0.63	0,996	1.59–954	0.32	2
Acetonitrile	232/348	20	0.34	1.13	0.996	1.59–954	0.67	1.07
DMSO	292/352	23	2.17	7.24	0.992	31.8–954	2.80	0.35
Ethyl acetate	288/348	27	0.77	2.57	0.998	31.8–954	0.50	0.34
Water-isopropanol 90 : 10 v/v	235/360	10	0.11	0.37	0.997	1.59–1272	1.06	5.01

[a] $\lambda_{ex}/\lambda_{em}$: maximum excitation wavelength/ maximum emission wavelength; [b] t_{ir}^{opt} : optimal irradiation time; [c] LOD : limit of detection defined as the concentration of analyte giving a signal-to-noise ratio (S/N) of 3 (IUPAC criterion); [d] LOQ : Limit of quantification defined as the concentration of analyte giving a S/N ratio of 10 (IUPAC criterion); [e] R^2 : Correlation coefficient; [f] LDR : linear dynamic range; [g] RSD : Relative standard deviation; [h] I_f : Relative PIF intensity, normalized relative to the NaDCF PIF intensity in water.

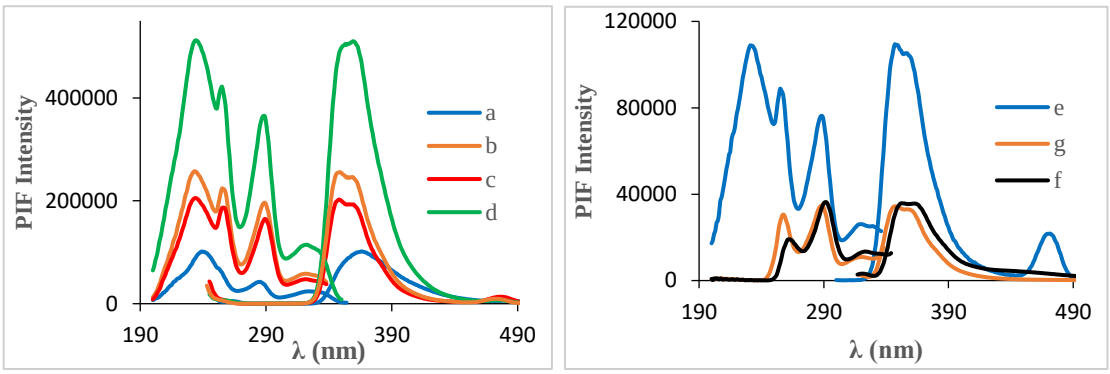

Fig. 2. NaDCF (3×10^{-6} M) excitation and emission PIF spectra at optimum irradiation times in: a) water; b) methanol; c) isopropanol; d) 90:10 v/v water-isopropanol mixture; e) acetonitrile; f) DMSO; and g) ethyl acetate

3.2.2. *Optimization of analytical conditions*

Several physicochemical factors, including UV irradiation time, pH, and solvent, influenced the fluorescence emission. Therefore, an analytical study was required to optimize these different parameters and obtain the best analytical performance.

3.2.2.1. *Effect of the UV irradiation time*

The effect of UV irradiation time (t_{irr}) on NaDCF PIF intensity was investigated in various solvents, including:

ultra-pure water (λ_{ex} = 239 nm, λ_{em} = 365 nm),
methanol (λ_{ex} = 233 nm, λ_{em} = 348 nm),
isopropanol (λ_{ex} = 233 nm, λ_{em} = 348 nm),
a 90:10 v/v water-isopropanol mixture – (λ_{ex} = 235 nm, λ_{em} = 360 nm),
acetonitrile (λ_{ex} = 232 nm, λ_{em} = 348 nm),
DMSO (λ_{ex} = 292 nm, λ_{em} = 352 nm), and
ethyl acetate (λ_{ex}=288nm, λ_{em}=348nm) (Fig. 3).

The optimum (maximum) t_{irr} value varied significantly depending on the medium, ranging from approximately 8 to 40 min. The lowest t_{irr} value (8 min) was observed in water, while the highest t_{irr} value (40 min) was obtained in isopropanol. For the optimal medium, the 90:10 v/v water-isopropanol mixture, the optimum t_{irr} value was relatively short at 10 min.

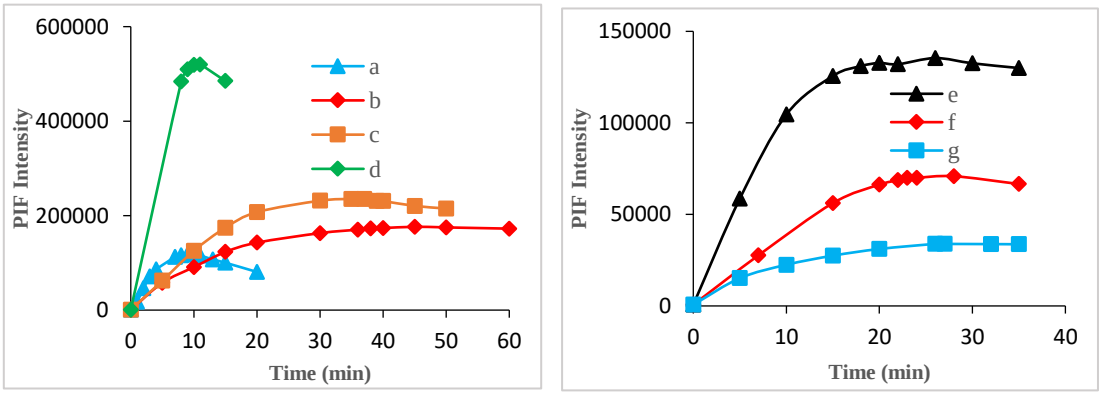

Fig. 3. Effect of UV irradiation time on the PIF intensity of 3×10^{-6} M NaDCF in: a) water, λ_{ex} = 239 nm, λ_{em} = 365 nm; b) methanol, λ_{ex} = 233 nm, λ_{em} = 348 nm; c) isopropanol, λ_{ex} = 233 nm, λ_{em} = 348 nm; d) water-isopropanol 90:10 v/v mixture, λ_{ex} = 235 nm, λ_{em} = 360 nm; e) acetonitrile, λ_{ex} = 232 nm, λ_{em} = 348 nm ; f) DMSO, λ_{ex} =292 nm, λ_{em} =352 nm; g) ethyl acetate, λ_{ex} = 288 nm, λ_{em} = 348 nm

3.2.2.2. *Effect of pH on the NaDCF photo-induced fluorescence intensity*

Figure 4 shows that the photoproduct fluorescence intensity (I_F) increased rapidly with pH, reaching a plateau between pH 5 and pH 7, followed by a second maximum at pH 8. Subsequently, I_F quickly decreased until pH 14. The presence of two maxima at pH 5 and pH 8 may be attributed to the existence of two protonation sites in the molecular structure of

NaDCF. The very low PIF intensity values observed in the pH range of 1 – 3 and 11 – 14 are probably due to fluorescence inhibition at these pH levels. The optimum pH for the best working conditions was found in the range of pH 5 – 8.

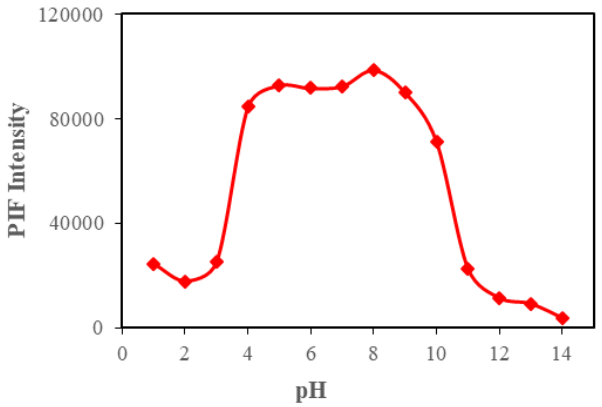

Fig. 4. Effect of pH on the NaDCF PIF intensity (0.95 μg ml^{-1}) in water (t_{irr}^{opt} = 8 min)

3.2.2.3. *Effect of the isopropanol percentage on the NaDCF PIF intensity*

Diclofenac (pK_a = 4)[39] has a Lewis acid-base character due to the presence of the NH group, which can function as an electron donor or proton acceptor, in addition to the carboxyl group. Previous studies have shown that water-organic solvent mixtures, particularly water-alcohol mixtures, yield larger PIF signals for several organic compounds.[30–32] Therefore, we investigated the effect of isopropanol concentration on the NaDCF PIF intensity and spectra in isopropanol-water mixtures.

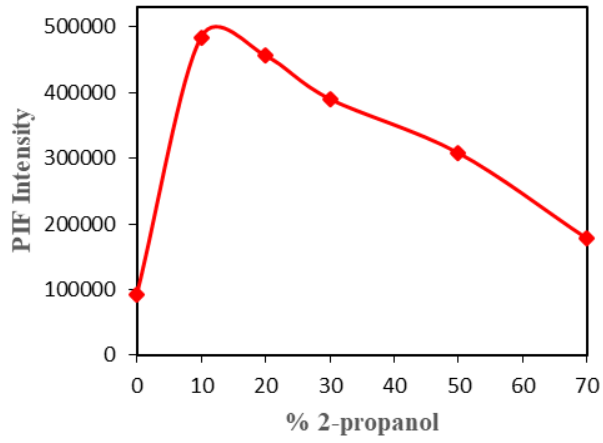

Fig. 5. Effect of the isopropanol % on the NaDCF (0.95 μg ml^{-1}) PIF intensity in water-2-propanol solutions

Our findings revealed that the addition of isopropanol significantly modified both the PIF signal and the characteristics of the PIF spectra (Fig. 5). The PIF intensity increased rapidly with the isopropanol percentage up to a 10% isopropanol-water mixture, after which the PIF signal gradually decreased. The NaDCF PIF emission spectra in the 10% isopropanol-water were characterized by the presence of two shoulders located at approximately 348 and 360 nm, while the PIF emission spectra in pure water consisted of only one maximum at about 360 nm.

3.2.3. *Analytical performances of the PIF method*

After optimizing the physicochemical factors affecting the PIF intensity and evaluating the spectral characteristics of NaDCF, we determined the analytical performances of the PIF method. A summary of analytical parameters is detailed in Table 2.

All NaDCF calibration curves were linear, covering approximately one to two orders of magnitude. The correlation coefficient (R^2) values were very close to unity, indicating good linearity of the calibration curves (Table 2). The limit of detection (LOD) values were calculated for the NaDCF concentration corresponding to a signal-to-noise ratio (S/N) of 3, while the limit of quantification (LOQ) values were determined for an S/N ratio of 10, following IUPAC criteria. The LOD and LOQ values varied significantly with the solvent, with the lowest values obtained in the water-isopropanol 90:10 v/v mixture (0.11 and 0.37 ng ml^{-1}, respectively). The relatively low RSD values (0.31–2.80 %) indicated satisfactory reproducibility of our measurements.

Furthermore, we compared our analytical performance with that of other techniques reported in the literature. For instance, Kermia et al.[40] examined the fate and removal of diclofenac and other pharmaceuticals in waste water treatment plants using solid phase extraction–gas chromatography–mass spectrometry (SPE-GC-MS) and found a very low LOD of 0.033 ng ml^{-1}. In another study, Nakhaei et al.[40] utilized a solvent–assisted dispersive solid phase extraction-high-performance liquid chromatography (SADSPE-HPLC) method and determined an LOD value of 0.47 ng ml^{-1} for NaDCF in human serum and pharmaceutical tablets. Recently, Safwat et al.[29] quantified NaDCF in tap water using derivative synchronous and micellar-enhanced spectrofluorimetric methods, reporting LOD and LOQ values of 0.47 and 1.7 ng ml^{-1}, respectively. These results, which are very close to ours, indicate a good sensitivity of our PIF method.

3.2.4. *Analytical application of the PIF method*

To verify the usefulness of our PIF method, we tested it on three samples of natural water: tap water collected in Marne-la-Vallée (France), sea water, and well water from Dakar (Senegal). To ensure a matrix free of organic contaminants, all samples were filtered through a 25 mm diameter PTFE filter membrane with 0.45 µm pores. The applications were conducted using the standard addition method, where water samples were initially fortified with 0.1 µg ml^{-1} of diclofenac.

Linear standard addition curves were obtained, with slope values closely matching those measured for the calibration curves across the three types of natural water (Fig. 6). This similarity indicates that there were no significant matrix effects in these water samples.

The mean recovery rates ranged from 84.77 % to 101.72 % (Table 2). The reproducibility of the measurements was satisfactory, with RSD values between 0.31 % and 1.42 %. Recovery rates were much lower in sea water, likely due to its nature as a surface water body influenced by significant wastewater discharge, which may have introduced additional substances that can affect fluorescence measurements.

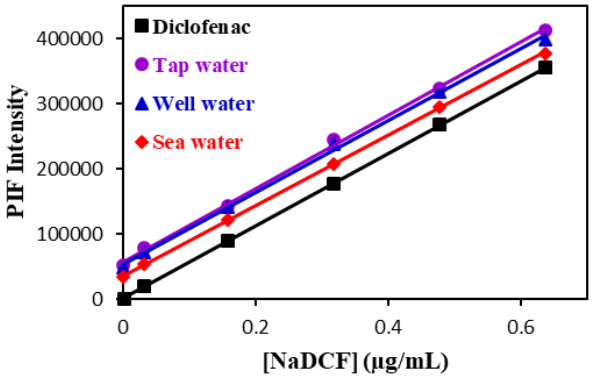

Fig. 6. Calibration curves and standard addition curves of NaDCF in fortified samples of tap, well, and sea waters

Our results were consistent with those reported in the literature. For instance, Kermia et al. and Nakhaei et al.[40,41] found recovery rates of 101 % and 96.7 – 97.9 % for diclofenac in surface waters (Valley of El-Harrach) and in pharmaceutical formulations, respectively. Additionally, Safwat et al.[29] reported recovery rates of 96.6 % using derivative synchronous and micellar-enhanced spectrofluorimetric methods.

T a b l e 2

Analytical applications of the PIF method for NaDCF determination in fortified natural water samples and assessment of recovery rates by the standard addition method

Sample [a,b]	Added (µg. ml^{-1})	Found (µg. ml^{-1})	Recovery (%) [c]	Mean recovery (%)	RSD [d] (%)
Tap water	0.13	0.14	107.69	101.72	1.42
	0.26	0.25	96,15		
	0.42	0.44	104.76		
	0.58	0.58	100		
	0.74	0.74	100		
Well water	0.13	0.13	100	98.072	0.31
	0.26	0.25	96.15		
	0.42	0.42	100		
	0.58	0.57	98.27		
	0.74	0.71	95.94		
Sea water	0.13	0.09	69.23	84.77	0.86
	0.26	0.22	84.61		
	0.42	0.37	88.09		
	0.58	0.53	91.38		
	0.74	0.67	90.54		

[a] Samples initially spiked with 0.1 ng ml^{-1} of NaDCF; [b] PIF measurements in water-isopropanol 90 :10 v/v; Optimum irradiation time = 10 min; [c] Triplicate measurements for each concentration; [d] RSD = relative standard deviation.

Statistical analyses (Table 3) show that the slopes of the various calibration and standard addition lines are very similar, with relative errors below 5 %. Furthermore, variance analysis for each sample at 5 % confidence level (α = 0.05) was conducted (Table 3). In all cases, the *p*-values were significantly greater than the confidence level α, ranging from 0.509 to 0.733. Additionally, the calculated Fischer values (F = 0.125 – 0.476) were well below the critical value of F (5.318). Therefore, the analytical application of the PIF method using the standard addition method can be validated for these samples.

T a b l e 3

Statistical evaluation of standard addition curves

Samples	**Slope**	**Relative error on slope**	**ANOVA**						
Пure water	567389		**Source**	**Sum of squares**	***df***	***Mean square***	***F***	**p-value**	***F* crit**
Tap water	569027	0.29 %	Between groups	8580991825	1	8580991825	0,4764872	0,5095444	5,3176551
			Within groups	1,44071E+11	8	18008860051			
			Total	1,52652E+11	9				
Well water	556363	1.94 %	Between groups	6717308018	1	6717308018	0,3815373	0,5539534	5,3176551
			Within groups	1,40847E+11	8	17605902140			
			Total	1,47565E+11	9				
Sea water	548837	3.27 %	Between groups	2167470006	1	2167470006	0,1246878	0,7331286	5,3176551
			Within groups	1,39065E+11	8	17383176831			
			Total	1,41233E+11	9				

df: degree of freedom; *F*: calculated Fischer value; *F* crit: critical value of *F*

3.2.5. *Interference study of foreign chemical species*

To evaluate the selectivity of the PIF method, which is a non-separative analytical method, we investigated the influence of foreign chemical species on NaDCF PIF measurements. The interfering chemical species included four NSAIDs commonly used in Senegal, namely paracetamol, ibuprofen, mefenamic acid, and piroxicam, as well as several inorganic ions (K^+, NO_3^-, Na^+, HCO_3^-, Mg^{2+}, Cl^-, and SO_4^{2-}) that may also be present in Senegal's natural waters. Therefore, we examined the effects of varying concentrations of these interfering chemical species on the NaDCF PIF signal within the appropriate ranges (Table 4).

The tolerance limit for foreign interfering chemical species was defined as the maximum concentration of these compounds at which the relative error of the PIF signal did not exceed ± 5 %. For each concentration of interfering species, the variation in the PIF signal was calculated using the following expression:

$$\Delta F = \frac{F0-F}{F0} \times 100 \qquad (1)$$

where F_0 represents the NaDCF PIF intensity without interfering species, and F represents the NaDCF PIF intensity in the presence of interfering species.

We maintained a constant NaDCF concentration of 0.318 μg ml^{-1} and evaluated the interference effects of increasing concentrations of the selected foreign species on the emission spectra and PIF intensity. The addition of foreign species did not significantly change the shape of the NaDCF PIF emission spectra, nor was there any spectral shift in the maximum emission wavelength. An increase in the PIF signal was observed following addition of all other drugs and inorganic ions. However, in the case of ibuprofen, a decrease in the PIF signal was noted, accompanied by the appearance of a new emission band at approximately 285 nm.

The tolerance limits for foreign species were estimated and summarized in Table 4. Among NSAIDs drugs, the highest interference effect on NaDCF PIF signals was observed with paracetamol (tolerance limit = 0.04 μg ml^{-1}), while the lowest interference effect was found with ibuprofen, which had a tolerance limit of 0.41 μg ml^{-1}. The inorganic ions studied also yielded notable interference effects on the PIF signals of NaDCF, with tolerance limits ranging from 0.17 to 1.02 μg ml^{-1}.

T a b l e 4

Interference study of foreign chemical species with NaDCF

Foreign species	Tested concentration range of foreign species (μg/ml)	Tolerance limit of foreign species (μg/ml) [b]
NSAIDs [a]		
Paracetamol	0.03–0.30	0.04
Ibuprofen	0.04–2.06	0.41
Mefenamic acid	0.05–0.48	0.09
Piroxicam	0.06–0.66	0.13
Inorganic ions [a]		
K^+ ; NO_3^-	0.07–40.44	0.17
Na^+ ; HCO_3^-	0.06–0.33	0.17
Mg^{2+} ; $2Cl^-$	0.14–1.22	1.02
$2Na^+$; SO_4^{2-}	0.10–0.99	0.85

[a] Fixed concentration of diclofenac sodium = 0.318 μg ml^{-1}; [b] See text for definition

3.2.6. *Identification of NaDCF photoproducts by GC-MS and phototransformation pathway*

The identification of the photoproduct(s) responsible for the intense fluorescence observed after optimal NaDCF irradiation time in water was conducted using GC-MS. Identification was based on the analysis of the mass spectra (*m*/*z*) of the various fragments present in the analyte (Table 5). The initial study was performed on an unirradiated NaDCF sample, followed by the analysis of the irradiated NaDCF sample in aqueous media after 8 minutes of irradiation. We were able to identify six photoproducts (Table 5) from the MS software library, designated as A1, A2, A3, B1, B2, and B3 (Fig.7).

T a b l e 5

GC-MS detection of photodegradation products formed during photolysis of diclofenac sodium (NaDCF)

Product	**(*m*/*z*)**	**t_r (min)**
DCF	295	32,00
A1	251	22,11
A2	281	37,62
A3	203	20,19
B1	265	26,23
B2	231	25,40
B3	201	23,12

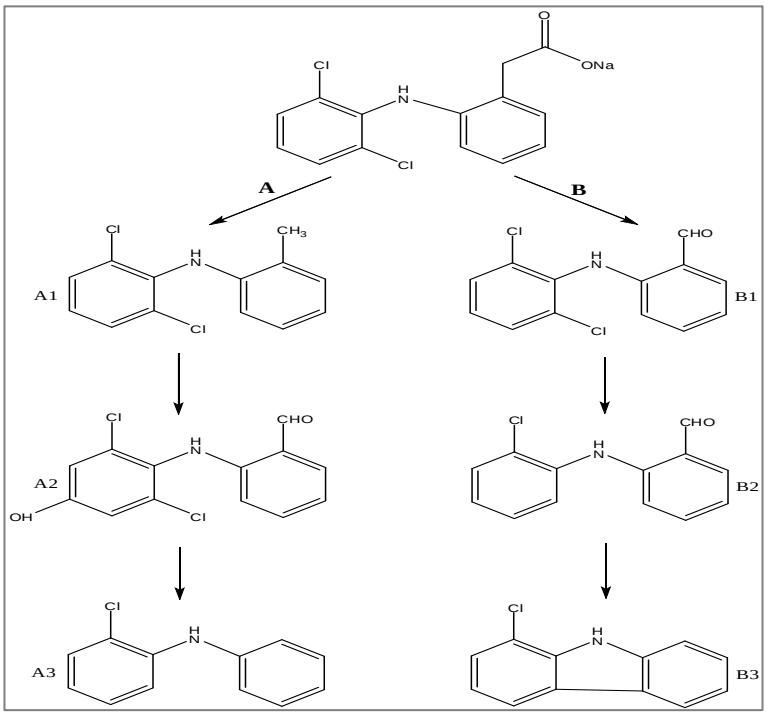

Fig. 7. Proposed mechanism of photoproduct formation of NaDCF ($c = 10^{-5}$ M) irradiated for 8 min (optimum irradiation time) in aqueous medium

Among the detected compounds, B3 (chloro-carbazole) may be responsible for the intense fluorescence observed after NaDCF irradiation. In comparison with the literature, the absorption spectrum of carbazole (2×10^{-5} M) in ethanol, as shown in the work of Encinas et al.,[34] has a shape that is virtually identical to that recorded in our previous study[42] for NaDCF in a 90:10 v/v water-isopropanol mixture after optimal irradiation time.

On the basis of the photoproducts identified in our study, two main pathways for diclofenac photolysis have been proposed: pathway A and pathway B (Fig. 7). The results obtained for the identification of photoproducts in this work align with findings in the literature. Notably, Agüera et al.[43] identified similar photoproducts of diclofenac in water under direct solar irradiation.

4. CONCLUSION

In this work, we developed a simple, inexpensive, sensitive, and precise PIF method for determining the nonsteroidal anti-inflammatory drug (NSAID) diclofenac sodium in water samples collected in Senegal (Dakar) and in France (Marne-la-Vallée). We demonstrated the analytical utility of the PIF method, which performed optimally in a 90:10 v/v water/isopropanol mixture. The analytical parameters confirmed good sensitivity and satisfactory precision of the PIF method for the diclofenac determination. Additionally, we demonstrated the applicability of the PIF method for quantifying NSAID residues in natural water samples, achieving good recovery rates. The PIF method was also satisfactorily validated using UV-visible absorption spectrophotometry as a reference method. Finally, our study highlights the significant analytical potential of the PIF method for the quantitative analysis of NSAID drugs in environmental water samples. Six photoproducts were identified by GC-MS, with chloro-carbozole being a likely source of the intense fluorescence observed.

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