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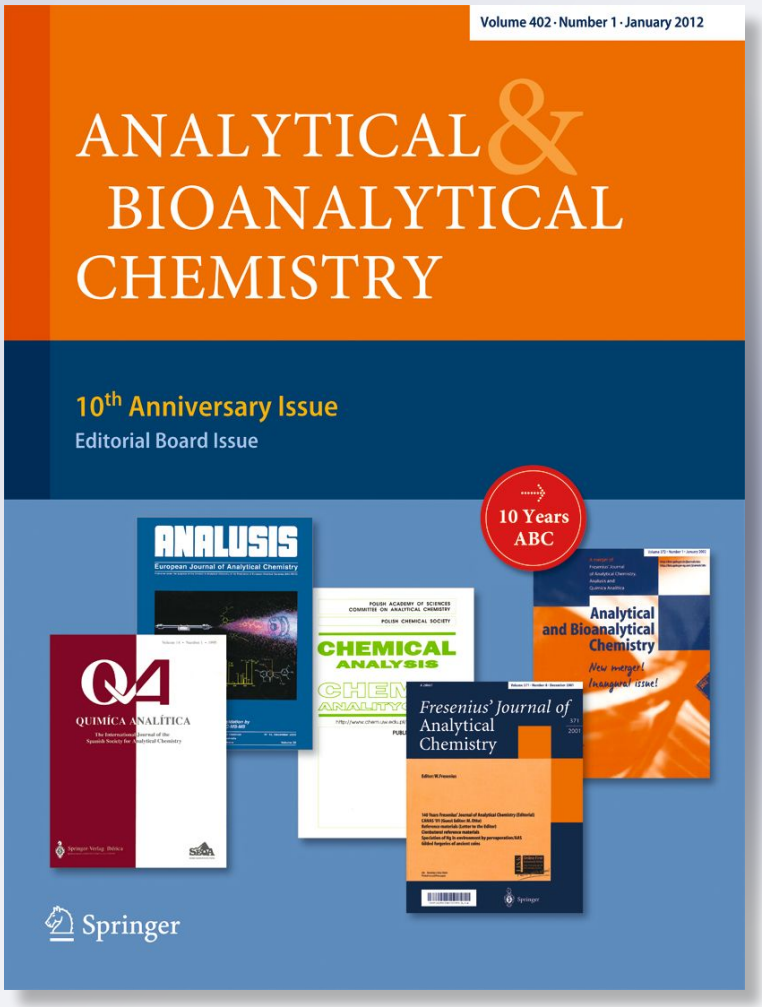

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TECHNICAL NOTE

Nylon membrane as a fluorimetric probe for the herbicide bentazone

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Abstract The fluorimetric signal produced by bentazone retained in selected solid surfaces was investigated. Among the different tested supports, only a microporous nylon membrane produced the desired signal. The quantitative study was carried out by second-order calibration using parallel factor analysis, allowing the determination in a highly interfering medium. A detection limit of 0.4 ng mL^{-1}, a prediction relative error of 8%, and a sample frequency of ten samples per hour were obtained in spiked natural waters using green analytical chemistry principles.

Keywords Optical fluorimetric probe · Nylon membrane · Second-order calibration · Bentazone

Introduction

Bentazone (BTZ) is one of the most used post-emergence herbicides in crops of rice [1], which, in turn, is one of the most consumed cereals in the world. Although BTZ levels found in environmental waters vary with cropping seasons, a health-based value of 30 ng mL^{-1} is specifically indicated for BTZ in the addendum to the guidelines for drinking water quality published in 1998 by the World Health Organization [2].

While BTZ displays a weak fluorescence in aqueous solution, more intense signals are obtained in *N*,*N*-dimethylformamide [3] and acetonitrile [4]. Very recently, our research group studied the effect of organized media in the enhancement of the fluorescence emission of BTZ in aqueous solution [5]. The current purpose was to develop an optical fluorimetric probe based on solid-surface fluorescence (SSF) of BTZ adsorbed in different plane supports.

To the best of our knowledge, a single work has reported fluorescence signals produced from BTZ sorbed on a dextran-type anion exchange gel as support [6], including the determination of the herbicide at very low concentrations [limit of detection (LOD)=0.4 ng mL^{-1}], but the procedure was somewhat laborious and time-consuming.

In the present work, different materials, solvents, and experimental conditions were analyzed in order to find the optimal conditions for developing a method for BTZ determination at trace levels. With the purpose of improving both the sensitivity and selectivity of the SSF method, a second-order chemometric calibration using parallel factor analysis (PARAFAC) [7] was applied to excitation–emission fluorescence matrices (EEFMs) of BTZ measured over the nylon membrane. Both artificial and real samples were evaluated and the results compared with those provided by a reference method.

Material and methods

Reagents and solutions

Analytical reagent grade chemicals were used for the preparation of all solutions. Bentazone was obtained from Riedel-de Haën (Seelze, Germany). Nylon disks are commercially available and were used as received. Different 0.2-μm nylon membrane lots of either the same or different brands were tested, namely, Varian Inc. (Seattle, USA), Schleicher-Schuell (Dassel, Germany),

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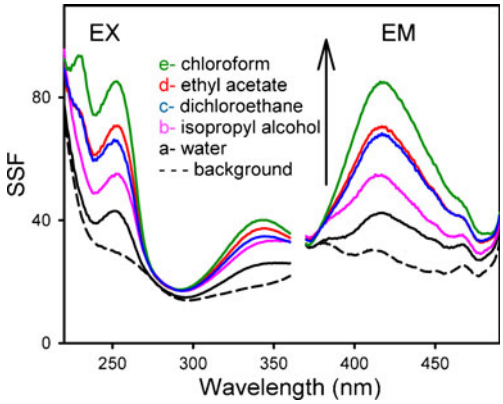

Fig. 1 Solid-surface fluorescence (*SSF*) spectra obtained after spotting in a nylon membrane 8 μL of 1,000 ng mL^{-1} BTZ dissolved in water (*a*, *black*), isopropyl alcohol (*b*, *pink*), dichloroethane (*c*, *blue*), ethyl acetate (*d*, *red*), and chloroform (*e*, *green*). The *black dotted line* corresponds to the background signal. Photomultiplier tube sensitivity=300 V; λ_{ex}=250 nm; λ_{em}=417 nm

and GE Osmonics (Trevose, USA), but no significant differences were observed either in background emissions or in the fluorescence of the retained BTZ.

Solid phase extraction (SPE) was carried out with 13-mm Empore octadecyl C18 extraction disks (Supelco, Bellefonte, PA, USA). Methanol stock solutions (approx. 1,000 μg mL^{-1}) of BTZ, fuberidazole (FBZ), 1-naphthaleneacetic acid (NAA), thiabendazole (TBZ), and carbaryl (CBL) were prepared and stored in dark flasks at 4 °C. From these solutions, working solutions were prepared by taking appropriate aliquots, evaporating the methanol by the use of dry nitrogen and diluting with the investigated solvent to the desired concentrations.

Solid-surface procedure

The nylon membrane was dissected into 8-mm diameter disks, and 8 μL of solution containing BTZ in each tested solvent was spotted on the surface. The disk was dried on a heating plate (1 min at about 100 °C) and placed in a laboratory-constructed solid substrate holder in order to collect spectra at 90°. The latter were measured in an Aminco Bowman (Rochester, NY, USA) Series 2 luminescence spectrometer equipped with a 150-W xenon lamp. The excitation and emission slit widths were 4 and 8 nm, respectively, and the photomultiplier sensitivity was fixed at 300 V. The EEFMs were recorded using excitation and emission ranges of 240–360 nm (each 4 nm) and 370–470 nm (each 1 nm), respectively.

A calibration set was prepared in triplicate with six concentrations of BTZ equally spaced in the range 0–1,000 ng mL^{-1} (18 samples). A validation set of 15 samples was prepared with concentrations different from those for calibration and following a random design. Fifteen test samples were prepared containing random concentrations of the analyte and FBZ, NAA, TBZ, and CBL in the range 5,000–20,000 ng L^{-1}. All samples were

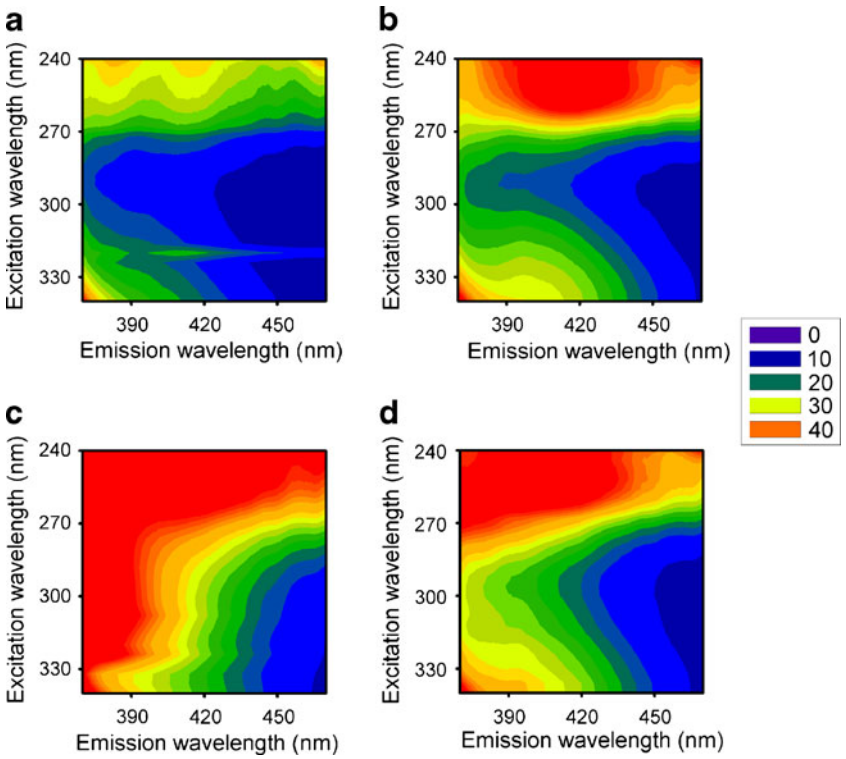

Fig. 2 Contour plots of the EEFMs obtained over the nylon surface after spotting 8 μL of chloroform (blank solution) (**a**), a validation sample containing 500 ng mL^{-1} BTZ (**b**), a test sample containing 540 ng mL^{-1} BTZ and 20,000 ng mL^{-1} FBZ, 14,000 ng mL^{-1} TBZ, 10,000 ng mL^{-1} CBL, and 9,000 ng mL^{-1} NAA (**c**), and a chloroformic extracted of a stream sample containing 321 ng mL^{-1} BTZ (16 ng mL^{-1} BTZ in the original water matrix) (**d**). The *color bar on the right* indicates the vertical scale (in arbitrary fluorescence units)

Table 1 Statistical results for the determination of BTZ by second-order calibration in samples without interferences (validation), with FBZ, TBZ, NAA, and CBL as interferences (test), and in spiked natural waters

	Validation samples[a]	Test samples[a]	Real water samples[b]
RMSEP (ng mL^{-1})	40	46	1.8
REP (%)	8	10	8
LOD (ng mL^{-1})	4	5	0.4
LOD (ng/spot)	0.03	0.04	0.003
LOQ (ng mL^{-1})	12	15	1.2
LOQ (ng/spot)	0.10	0.12	0.01

RMSEP root-mean-square error of prediction, *REP* relative error of prediction, *LOD* limit of detection, *LOQ* limit of quantitation (calculated as LOD×(10/3.3))

[a] Fifteen samples

[b] The results refer to the original water sample before SPE. Four different natural waters evaluated at three BTZ levels each

subjected to the above spotting procedure, and the obtained EEFMs were analyzed with PARAFAC.

Real samples

Stream, canal, and underground water samples were collected near zones with profuse farming activity. Since the samples did not contain BTZ at the level detected by the proposed method, a recovery study was performed. Different volumes of the methanol BTZ solution were evaporated and reconstituted with 10 mL of the studied natural water. A few microliters of diluted HCl were added before completing to the mark (pH ~2.5). The samples were filtered through filter paper and then SPE through a C18 disk was applied. The retained BTZ was eluted with 500 μL of chloroform, and this final solution was employed for spotting 8 μL on the nylon membrane. High-performance liquid chromatography (HPLC) was used as a reference method, as suggested in the literature [8].

Algorithm and software

The PARAFAC theory is well known [9]. PARAFAC codes written in MATLAB 7.0 are available on the Internet [10] and were implemented using the MVC2 graphical interface, which is also available on the Internet [11].

Results and discussion

Fluorimetric properties of BTZ in nylon

From exploratory experiments using filter paper, cellulose acetate, cellulose nitrate, octadecyl C18, silica gel, and nylon membranes as supports, it was concluded that fluorescence was only detected in the latter one. The retention of BTZ on nylon may be due to the interaction of the keto-enol oxygen and/or the sulfonyl group of BTZ with the amide nylon groups. A new disk is used in each measurement, and thus the sensing capability of the probe remains unaltered, with a good reproducibility of the obtained spectra.

The fluorescence intensity produced by a given BTZ concentration spotted on the nylon surface varies according to the solvent. Among 24 non-polar, polar aprotic, and polar protic solvents investigated, chloroform produced the best fluorescence signal. Figure 1 shows the fluorescence spectra obtained when solutions of BTZ prepared in selected solvents are separately spotted on nylon membranes.

Second-order calibration

The coupling of SSF with second-order calibration is proposed using an algorithm achieving the second-order advantage, which is the ability of resolving analyte

Table 2 Recovery study of BTZ for spiked natural water samples

Nominal	Underground water[a]		Stream water[b]		Canal water[c]		Canal water[d]	
	HPLC	PARAFAC	HPLC	PARAFAC	HPLC	PARAFAC	HPLC	PARAFAC
4.3	4.1 (0.5)	4 (1)	3.9 (0.4)	4.3 (0.2)	3.8 (0.1)	4.1 (0.3)	3.3 (0.1)	5.1 (0.2)
16.0	15.1 (0.3)	17 (1)	15.2 (0.3)	17 (1)	15.6 (0.1)	16 (1)	15.1 (0.1)	15.0 (0.4)
48.2	48.9 (0.1)	48 (2)	46.9 (0.7)	49 (1)	49.1 (0.7)	50 (4)	48.1 (0.2)	50 (1)

Values in nanograms per milliliter. Mean of duplicates. Standard deviation between parentheses

[a] From Bombal City (Santa Fe, Argentina)

[b] From Ludueña Stream (Santa Fe, Argentina)

[c] From Ibarlucea Canal (Santa Fe, Argentina)

[d] From Salvat Canal (Santa Fe, Argentina)

concentrations and spectra in the presence of unexpected constituents [12]. EEFMs were first recorded for calibration and validation samples and then for more complex synthetic test samples containing other agrochemicals as interferents.

Figure 2a, b shows the contour plots of EEFMs for a blank (BTZ-free) and a typical validation sample, respectively. PARAFAC was initialized with the best-fitting loadings after some trial runs [9]. The number of components was selected considering the core consistency [13] and fitting residuals [7]. This is a critical aspect for obtaining reliable predictions, but the aforementioned methods provide reliable estimations of the number of components. The latter was estimated as 2 for the three-way arrays composed by calibration and validation data, corresponding to the analyte and to the background signals.

The PARAFAC predictions for the 15 validation samples are in good agreement with the nominal values. Both the test of the elliptical joint confidence region (EJCR) [14] and the statistical indicators shown in Table 1 support the latter conclusion. The achieved LOD (4 ng mL^{-1}) was calculated according to [15].

The selectivity of the proposed method was evaluated quantifying BTZ in test samples containing high concentrations of additional agrochemicals, which fluoresce in nylon membranes and overlap their spectra with BTZ (Fig. 2c). The potential effect of cations and anions as interferences was not investigated because BTZ is extracted in its neutral form in a non-polar solvent. The good recoveries obtained when the method was applied to natural waters where ions are usually present at very high levels (see below) support this conclusion.

The number of components required for modeling the three-way arrays composed by the calibration and test sample data was also 2, although in principle the test samples may contain several responsive components. It seems that PARAFAC is not able to discern between the profiles of the background and the foreign compounds, a phenomenon already observed in other systems [5, 9]. Nevertheless, good predictions were obtained (Table 1), demonstrating the usefulness of the method. The LOD in the presence of interferents (5 ng mL^{-1}) is similar to that for the validation samples, where only BTZ was present.

Natural waters

BTZ must be previously extracted in chloroform to carry out the determination, and this was exploited in order to concentrate the analyte, attaining a low LOD. Thus, a volume of water (e.g., 10 mL) was extracted with a C18 membrane, eluting the retained BTZ with 500 μL of chloroform. In this way, both the preconcentration and the conditioning are simultaneously achieved.

Figure 2D shows the contour plots of the EEFM of a natural stream sample added with BTZ. The total fluorescent signal (background plus analyte) is different from that obtained in either validation or test samples (Fig. 2b, c). It is clear that chloroform does also extract additional compounds retained in the C18 surface, which are adsorbed in the nylon membrane along with BTZ. The obtained results in water samples (Table 2) were compared with those provided by an HPLC method by applying the EJCR analysis [14], showing similar prediction ability.

It is apparent that the applied treatment to real samples yields a favorable LOD (Table 1), significantly lower than those obtained for both validation and test samples, which were unextracted and directly prepared in chloroform. Finally, since the experimental time elapsed between consecutive measurements (including EEFM recording) was approximately 6 min, a suitable sample frequency of ten samples per hour is achieved.

Conclusion

Nylon membrane showed to be an appropriate surface as a fluorescent probe of the herbicide bentazone. The obtained signal was very sensitive to the solvent used for spotting the analyte over the membrane, with chloroform being the most suitable one for quantitative purposes. A simple method for the determination of bentazone was developed, coupling solid-surface fluorescence to second-order multivariate calibration. The proposed approach allowed the successful determination of the herbicide in natural waters.

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