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Cucurbit[6]uril nanocavity as an enhanced spectrofluorimetric method for the determination of pyrene

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ABSTRACT

The effect of the addition of macrocyclic host cucurbit[6]uril (CB6) on the photophysical properties of polycyclic aromatic hydrocarbon pyrene (PYR) was analyzed. The fluorescence emission spectra of the aromatic compound were determined at 25.0 °C in different acidic media (HCl 18%, w/v, or HCOOH 55%, w/v) with and without CB6. A significant enhancement in the fluorescence signals in the presence of CB6 was observed. The average values of the association constant (K_A) for the 1:1 stoichiometry complex and the relative fluorescence quantum yield ratio between the complexed and free PYR ($\phi^{PYR-CB6}/\phi^{PYR}$) in acidic media were $(4.0 \pm 0.5) \times 10^2\ M^{-1}$ and (5.7 ± 0.2), respectively.

The analytical parameters improved in the presence of CB6. The relative decrease in the limit of detection was 92%. The matrix effect was evaluated in fortified samples of tap water and tea extracts. Apparent recoveries obtained by the proposed method in tap water and tea extracts were (82–103)% and (89–99)%, respectively. Selectivity studies with inorganic and organic species were performed. The method is rapid, direct, selective and simple.

1. Introduction

In the last decade, organized supramolecular systems with analytical purpose have been extensively used in several methods to improve the methods' sensitivity and selectivity [1,2]. "Supramolecular Analytical Chemistry" is the field that has studied the development of these methodologies on the basis of the intermolecular interactions between organic or inorganic structures undergoing molecular recognition and self-assembly [3]. Some of the most commonly cavitants used to obtain such organized supramolecular systems with organic compounds include cyclodextrins, calixarenes and, more recently, cucurbiturils [4].

Cyclodextrins (CDn) are cyclic oligosaccharides formed by α-D-(+)-glucopyranose units linked by α-(1,4′)-glycosidic bonds [5]. Many organic molecules have shown an important increase in their fluorescence quantum yield due to the formation of supramolecular species with CDn [6]. This advantageous effect has been implemented in the development of analytical techniques based on luminescence for the determination of different organic analytes of agrochemical [7–9] and biological [10,11] interest.

Cucurbiturils (CBn) are macrocyclic oligomers synthesized by an acid-catalyzed condensation between glycoluril and formaldehyde [12]. The skeleton of C and N atoms defines a highly rigid and symmetric cavity. The inner surface of this cavity is hydrophobic with a potential inclusion site (Fig. 1A), whereas both portals are identical and surrounded by carbonyl groups, allowing ion-dipole or hydrogen bond interactions [13,14]. These properties characterize CBn as a family of promising molecular hosts whose ability to form complex with a wide variety of species, such as cations [15], radical cations [16,17], anions [18] and neutral molecules [19], make their study particularly interesting.

As compared to CDn, relatively few studies on the fluorescence behaviour of organic substrates in the presence of CBn have been described. A significant fluorescence enhancement was reported for 2,6-anilinonaphthalene-6-sulfonate in the presence of cucurbit[6]uril (CB6) [20]. A similar behaviour was observed for the pesticide carbendazim in the presence of CB6 [21] and CB7 [22], and for 2,7-dimethyldiazapyrenium [23], acridine orange [24] and berberine hydrochloride [25] in the presence of CB7. Recently, the supramolecular interaction of a series of CBn (n = 5,6,7 and 8) and coptisine, an isoquinoline alkaloid, was studied by fluorescence enhancement [26]. By contrast, nicotine, a poorly fluorescent molecule, could be detected in cigarettes by a remarkable quenching of the fluorescence of the methylene blue–CB7 complex [27]. Similarly, the increased intensity of the 2,7-dimethylphenanthrenium-CB8 system is quenched by the

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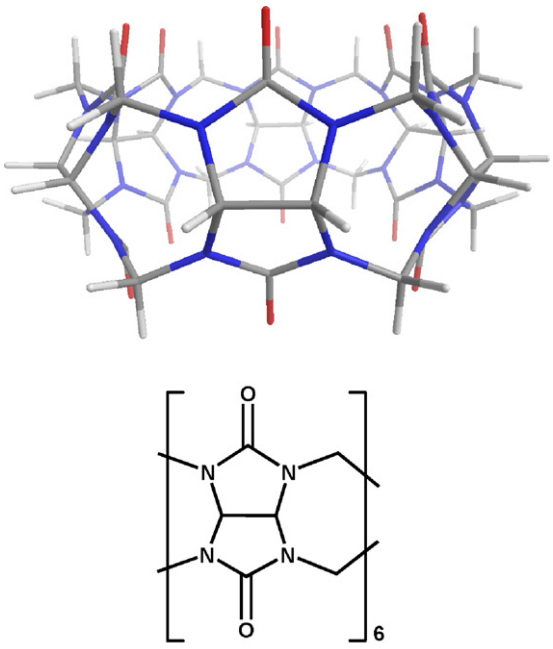
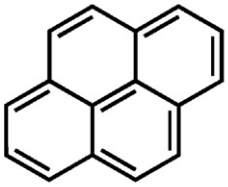


(A) cucurbit[6]uril



(B) pyrene

Fig. 1. Chemical structures: (A) cucurbit[6]uril and (B) pyrene.

addition of indole derivatives such as serotonin and tryptophan [28].

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants constituted by two or more fused aromatic rings. PAHs are generated and released during incomplete combustion or pyrolysis of organic matter in industrial processes and other human activities, or in natural processes, such as carbonization or emissions from volcanoes. These contaminants are found in the air, soil, water, or even in food contaminated by environmental PAHs or produced during processing and cooking [29].

Some studies have demonstrated that a number of PAHs are genotoxic or carcinogenic [30]. Therefore, their identification and analytical quantification are fundamental for assessing environmental pollution and for developing techniques that control their elimination [31,32].

Several analytical techniques have been described for the detection and quantification of PAHs, including fluorescence in different organized media [1] chromatographic methods, such as gas chromatography coupled with mass spectrometric detection (CG–MS) [33] or high performance liquid chromatography (HPLC) combined with chemiluminescence [34,35] or fluorescence detection [36,37] or electrochemical reduction with fluorescence detection [38]; and capillary electrophoresis (CE) with luminescence detection [39]. Furthermore, depending on the nature of the sample analyzed, separation and preconcentration steps such as liquid–liquid, solid-phase, microwave or ultrasounds extraction are in many cases required [40,41]. Several aspects of the extraction, removal and determination of PAHs have been studied by

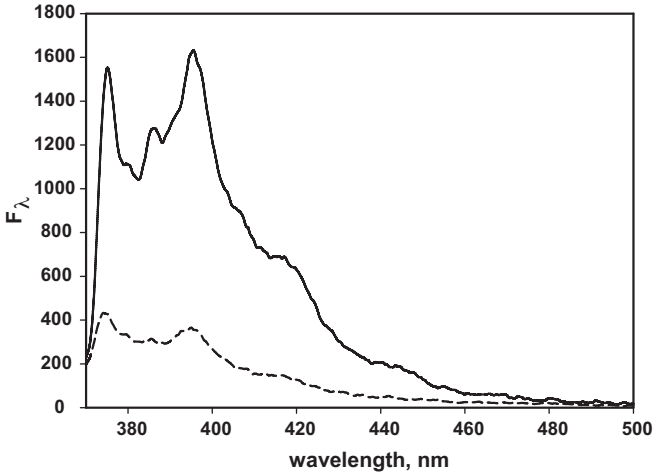


Fig. 2. Fluorescence emission spectra of PYR in the absence (- - -) and presence of 30.0 mM of CB6 (––) (0.2154 μM of PYR, HCl 18%, w/v, and CH_3OH 1%, v/v, at 25.0 °C).

the same research group in recent [42] and previous papers cited herein.

Pyrene (PYR) (Fig. 1B) is a PAH widely studied for its particularly interesting photophysical properties since its fluorescence is highly dependent on microenvironment factors [43]. Also, PYR is representative of PAHs, as stated in the Scientific Opinion of the Panel on Contaminants in the Food Chain: "*Pyrene is present in all PAH mixtures at relatively high concentrations (2-10% of the total PAHs), and in certain environments the pyrene content of the total PAHs is fairly constant*" [44]. Absorption of PAHs from the diet is determined by the size and lipophilicity of the molecule. In rats, oral absorption was reported between 42 and 99% for PYR. In mammals, in general, the oral absorption of the lower-molecular mass, i.e., 3- and 4-ring PAH (such as anthracene, phenanthrene, fluoranthene or pyrene), was higher compared with highly lipophilic higher-molecular mass PAHs [44].

In this paper we report on the CB6 nanosensor ability for neutral PAH from the interaction between PYR and CB6 by fluorescence spectroscopy. Accordingly, we develop an alternative analytical method based on enhanced fluorescence spectroscopy at room temperature for determining PYR in the presence of CB6. The application of the method in spiked samples of tap water and tea is demonstrated.

2. Experimental

2.1. Instruments

The UV–vis and spectrofluorimetric measurements were performed with a Shimadzu UV-2101PC or a Shimadzu MultiSpec-1501 with diode array detection (Kyoto, Japan) and a Jasco FP-777 (Tokyo, Japan), respectively. An ultrasonic bath Testlab tb02 (Bernal, Buenos Aires, Argentina) was used for solubilizing the samples. A rotavapor was used for tea extract concentration (Büchi RE 121, Switzerland). Data analysis was performed with Sigma Plot (Scientific Graph System) Version 9.00 (Jandel Scientific) and Info Stat Statistical Package, Version beta (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

2.2. Chemicals

Water was obtained from a Millipore apparatus. Cucurbit[6]uril was synthesized from glycoluril and formaldehyde according to the method described in the literature [12], implementing modifications in the separation and purification process [45]. Glycoluril

(Aldrich) and formaldehyde solution (36% in water, Fluka) were used as received. Pyrene (98% purity, Aldrich) was purified by sublimation. All compounds used in the study of interferences were commercial reagents of analytical grade. Methanol and dichloromethane were HPLC grade and hexane was PA grade (Sintorgan, Villa Martinelli, Buenos Aires, Argentina). Formic and hydrochloric acids were of analytical grade (Cicarelli, San Lorenzo, Santa Fe, Argentina). Silica gel 60 (70–230 mesh, Merck) was used for the extraction process in the tea samples.

2.3. General procedure

A concentrated solution of PYR in methanol (10 mg per 25 mL) was prepared and stored at 4 °C. The stability of this solution was periodically checked by UV–vis. The molar absorptivities ($10^2\ M^{-1}\ cm^{-1}$) in CH_3OH at 25.0 °C were (247 ± 3); (506 ± 5); (110 ± 4); (290 ± 3) and (495 ± 5) at a wavelength of 261; 271; 304; 318 and 335 nm, respectively.

The excitation wavelength used to determine the fluorescence spectra (λ_{ex} = 335 nm) was at the absorbance maximum of PYR. At this wavelength, the absorption for the highest concentration of CB6 was lower than 0.08. The photomultiplier gain was high with 3.0 nm and 1.5 nm emission and excitation bandwidths, respectively. The scanning speed was 500 nm min^{-1}. A 0.30×0.30 cm quartz cell was used. Experiments were carried out at 25.0 ± 0.1 °C, and the temperature of the cell compartment was controlled with a Haake circulator. The solutions were wrapped with aluminium foil and not degassed. A solution of 0.2154 μM of PYR at pH 7.00 (CH_3OH (1%, v/v) was used as a reference for the fluorimetric determinations. In all cases, the total area below the fluorescence spectrum (F) was measured.

2.4. Determination of the association constant

In order to obtain the value of the binding constant, solutions with different concentrations of CB6 (0–44 mM) were made up from two solutions with the same concentration of PYR (0.2154 μM), one containing the receptor for minimizing the changes in fluorescence by variations in substrate concentration. In both, acid (HCl 18%, w/v, or HCOOH 55%, w/v) and CH_3OH (1%, v/v) concentrations were kept constant. The acidic media are necessary for CB6 solubilization. The calibration curve of CB6 in the corresponding acidic media was determined in order to discount its signal.

2.5. Determination of analytical parameters

For the determination of the calibration data, solutions of PYR (0–0.70 μM) were prepared by adding the corresponding aliquot of the concentrated stock solution in the presence and absence of CB6 (30 mM) at 25.0 °C, keeping constant the percentage of acid (HCl 18%, w/v, or HCOOH 55%, w/v) and methanol (1%, v/v). To obtain the standard deviation of the blanks, replicated solutions were measured ($n \geq 25$).

The study of selectivity was performed by adding an aliquot of the interfering compound to a solution of PYR. The maximum allowed concentration of the interference caused a relative error not higher than 5% in the PYR signal. When the variation of the analytical signal was higher than the accepted value, the concentration of the foreign species was gradually decreased until interference ceased.

2.6. Tap water recoveries

Solutions of PYR (0.060–0.701 μM) containing 10% (v/v) of tap water and CB6 (30 mM) were prepared by adding the corresponding aliquot of the stock solutions of the substrate and CB6, keeping constant the percentage of acid (HCl 18%, w/v, or HCOOH 55%, w/v) and CH_3OH (1%, v/v). PYR was not detected in the blank solutions. The method of standard addition (MOSA) was applied for the apparent recoveries of triplicate analysis.

2.7. Extraction and clean-up procedure of tea sample

The method described by Lin et al. was followed for the extraction and cleanup of the tea sample [46]. The tea infusion was prepared by adding 20 g of the tea sample in 500 mL of boiling water. A conical flask was capped and placed in a water bath at 90 °C for 30 min. Solid tea residues were filtered and the water phase was kept and cooled at room temperature. The tea liquor was extracted by ultrasonication at 30 °C for 30 min with 50 mL of dichloromethane. The mixture was decanted for 10 min and the organic phase was collected. This procedure was repeated three times and finally all extracts were combined. The remaining water was eliminated by the addition of anhydrous Na_2SO_4. The sample was washed with 10 mL of hexane:dichloromethane (1:1, v/v) and then concentrated by solvent evaporation. The extract was dissolved in 2.5 mL of hexane and transferred into a minicolumn (internal diameter = 8 mm) prepared with silica gel and conditioned with 10 mL of hexane:dichloromethane 1:1 (v/v). The extract was eluted with 14 mL of this solvent mixture and then concentrated. The extract was retaken with 2 mL of methanol and filtered with a nylon membrane filter (0.22 μm).

Finally, apparent recovery experiments by MOSA were performed with solutions of PYR (0.173–0.692 μM) containing 10% (v/v) of the tea extract. The concentrations of CB6 (30 mM), the percentage of acid (HCl 18%, w/v, or HCOOH 55%, w/v) and CH_3OH (1%, v/v) were kept constant.

3. Results and discussion

3.1. Effect of the addition of CB6 on the fluorescence of PYR

The fluorescence emission spectra of PYR showed a significant enhancement in the presence of CB6 (Fig. 2). There was no significant shift in the maxima emission wavelengths. The characteristic broad band centred on 473 nm corresponding to the PYR excimer formation was not observed in the fluorescence emission spectra [47–50] as shown in Fig. 2 with CB6 (full line) or without the receptor (broken line).

The addition of glycoluril (weight equivalent to the quantity used for CB6) did not show any changes in the fluorescence signals. This evidence suggests that there is some specific interaction between the substrate and CB6.

On the basis of literature data [51], changes in signal intensity or shifts in the maxima of the spectrum could indicate complex formation. As seen in the case reported here, both changes are not necessarily observed together in other CBn complexes [22,28] and examples from supramolecular literature [6,9]. According to Eq. (1), the spectral change could be attributed to the complex reaction between the fluorophore and the host:

$$\mathrm{PYR} + \mathrm{CB6} \overset{K_A}{\rightleftarrows} \mathrm{PYR\text{–}CB6} \quad (1)$$

The change of the observed fluorescence as a function of CB6 concentration is provided by Eq. (2), where F and F_0 represent the total fluorescence in the presence and absence of CB6, respectively; K_A is the association constant for the 1:1 complex and $\phi^{PYR\text{–}CB6}/\phi^{PYR}$ is the fluorescence quantum yield ratio between the complexed and

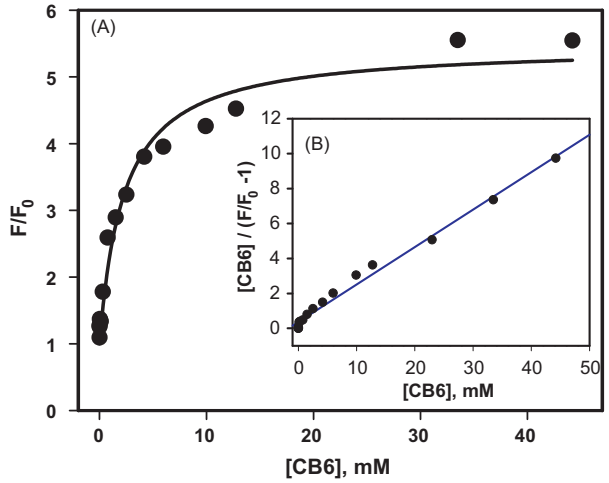


Fig. 3. Relative fluorescence of PYR as a function of CB6 concentration (0.2154 μM of PYR, HCl 18%, w/v, and CH_3OH 1%, v/v, at 25.0 °C). (A) Non-linear plot (the solid line shows the best fit of the data to Eq. (2)). (B) Plot according to a linearized equation in Ref. [51].

free analyte.

$$\frac{F}{F_0} = \frac{1 + (\phi^{PYR-CB6}/\phi^{PYR})K_A[CB6]}{1 + K_A[CB6]} \quad (2)$$

The values of K_A and $\phi^{PYR-CB6}/\phi^{PYR}$ determined in HCl 18% (w/v) from the non-linear fitting of the data to Eq. (2) were $(4.4 \pm 0.2) \times 10^2\ M^{-1}$ and (5.5 ± 0.2), respectively (Fig. 3A). In HCOOH 55% (w/v), these values were the same within the experimental error ($3.6 \pm 0.5 \times 10^2\ M^{-1}$ and 5.9 ± 0.1, respectively). These results indicate that the identity of the acidic species or the value of the acidic pH does not influence the results. In both cases, the guest-host complex stoichiometry was confirmed to be 1:1 from a linear double-reciprocal plot (Fig. 3B is the corresponding plot in HCl 18%, w/v) [51].

The preceding interpretations of the observed changes in PYR fluorescence produced by CB6 were based mainly from the evidence of complex formation [51], hydrophobic interactions and literature data [52], but they provide limited structural information on the geometry and mode of complexation (inclusion or exclusion). Direct evidence in solution can be obtained from NMR studies, which can show some specific interactions between specific parts of the guest and host, and thus direct evidence of particular mode of interaction (inclusion or exclusion) [53,54]. In the case studied here, no more insight was provided by NMR studies. The insolubility of the minimal concentration of PYR required for a NMR experiment, in the aqueous acidic media necessary for CB6 solubilization, makes it impossible.

Although the structural parameters for CB6 (portal diameter: 3.9 Å; cavity diameter: 5.8 Å; cavity volume: 164 $Å^3$; outer diameter: 14.4 Å; and height: 9.1 Å) [55] could indicate no interaction with benzene ring which has van der Waals dimensions (6 Å diameter × 4 Å thick) larger than the estimated internal cavity of CB6, spectroscopic evidences (Uv–vis, NMR) suggested complex formation [52]. The phenomena must be associated with encapsulation of the aryl ring into the cavity of CB6, which provides a nonpolar environment like of a hydrocarbon solvent. As is reported in the literature [52], the cavity of CB6 can accommodate the benzene ring of arylamines with different affinity depending of the substituents of the arene. These antecedents suggest the possible partial inclusion of PYR in the CB6 cavity.

The $\phi^{PYR-CB6}/\phi^{PYR}$ values are higher than five indicating that the receptor provides a more protected environment for the excited state of PYR. This interesting property allows us to propose an enhanced fluorimetric analytical method for the determination of the analyte.

Table 1
Analytical parameters[a].

Solvent % (w/v)	Species	s_B ($\times 10^{-2}$)	m ($\times 10^6$ M^{-1})	L_D (ng mL^{-1})	L_Q (ng mL^{-1})
HCl 18	PYR	3.18	2.92 ± 0.05	7.3 ± 0.1	22.0 ± 0.4
	PYR–CB6[b]	1.06	12.4 ± 0.1	0.570 ± 0.006	1.73 ± 0.01
HCOOH 55	PYR	2.69	2.2 ± 0.1	8.1 ± 0.4	25 ± 1
	PYR–CB6[b]	1.32	13.7 ± 0.3	0.64 ± 0.01	1.95 ± 0.04

[a] CH_3OH 1% (v/v), at 25.0 °C. Analytical parameters (s_B, m, L_D and L_Q) as defined in the text.
[b] 30 mM of CB6.

3.2. Analytical parameters

The analytical parameters for PYR were calculated according to current IUPAC definitions (Table 1) [56]. The precision of the method was determined by analyzing 10 replicate samples of 0.2154 μM of PYR with a relative error not higher than 5%.

The effect of the variation of the percentage of the acid on the fluorescence signals of PYR in the presence of CB6 was evaluated applying a one-way analysis of the variance (one-way ANOVA) [57] between randomized experiments with four levels of formic acid (50% w/v, 55% w/v, 65% w/v and 75% w/v) or with three levels of hydrochloric acid (16% w/v, 18% w/v and 20% w/v). According to ANOVA analysis, spectra show no significant differences at the percentages of acid studied.

The calibration graphs of relative fluorescence areas as a function of PYR concentrations ($F_R = F_B + m$[PYR]), where F_B is the average relative fluorescence areas of the blanks, were linear at the range studied (at least 7 different levels by triplicate), with good correlation coefficient (at least 0.997). All plots corresponding to the residuals in regression [58] obtained from $(y - y_{estimated})$ values (representing the differences between the experimental y-values and the fitted y-value) versus the $y_{estimated}$ show a normal distribution with variance independent of $y_{estimated}$.

The assessment of the fitted regression model was checked by F-test of linear model, which evaluates the null hypothesis H_0: x and y are not linearly related, according to ANOVA principles [58]. In all cases, the value of the ratio obtained between the mean square regression and the mean square for residuals exceeds the critical value of F for the corresponding degree of freedom (1 and at least 8) in each case at 95% confidence level. Therefore the fitted linear model appears to be statistically valid.

The values of the sensitivity (m, slope of the calibration graph) in the presence of CB6 were four-fold higher than in its absence (Table 1). The limits of detection, L_D, calculated as 3.29 s_B/m (where s_B is the standard deviation of the blank), were better for the PYR–CB6 complex (0.6 ng mL^{-1} average value). The improvement in L_D, defined as the percentage of the relative decrease obtained for the better L_D with respect to the higher value $[(L_D{}^{PYR} - L_D{}^{PYR-CB6})/L_D{}^{PYR} \times 100\%]$, was 92% in both acidic media.

In order to verify the selectivity of the method proposed, the influence of foreign species [59,60] on the analytical signal of PYR in the presence of the receptor was established. The interferences analyzed were: inorganic cations and anions with different charge and naphthalene, phenanthrene and anthracene. In all cases the maximum accepted concentration of the interference caused a relative error not higher than 5% in the analytical signal of PYR (Table 2). The absorption of organic interferences as naphthalene and phenanthrene at the λ_{ex} (335 nm) was negligible, thus fluorescence from these compounds was not possible. Therefore, the analyte could be determined in the presence of a higher concentration of these

Table 2
Selectivity study[a].

Foreign species	Tolerated interference/analyte ratio (w/w)[b]
Na^+, K^+, NH_4^+, Al^{3+}, Cl^-, I^-, SO_4^{2-}, PO_4^{3-}, CO_3^{2-}, NO_3^-	50,000[c]
F^-	5,000
Ca^{2+}	10,000
Naphthalene	1,000[c]
Phenanthrene	1,000[c]
Anthracene	0.7

[a] 1.886×10^{-7} M of PYR, 30 mM of CB6, HCl 18% (w/v), CH_3OH 1% (v/v) at 25.0 °C.
[b] Maximum concentration of the interference causing a relative error not higher than 5% in the analytical signal.
[c] Maximum ratio tested.

organic and inorganic interfering compounds (interference/analyte 1000–50,000, w/w). Other PAHs, like anthracene that absorbs in the same region as PYR, could interfere at lower concentration than other organic foreign species, depending on their emission region and the affinity for CB6. As shown in Table 2 the allowed ratio anthracene/PYR was 0.7 (w/w). The overlapping of the emission bands for both compounds requires a mathematical treatment (derivative or synchronic spectrofluorimetry) for interference/analyte ratio higher than this.

The L_D value obtained for the determination of PYR with CB6 as nanosensor is lower than or similar to those obtained by other methods based on the luminescence of static systems or less easily implemented. For example, by spectrofluorimetry in aqueous micellar media, using the anionic surfactant sodium dodecylsulfate (SDS) in tap and river water samples, the L_D determined is 2 ng mL^{-1} [61]. In addition, by heavy atom-induced room-temperature phosphorescence (HAI-RTP), using thallium (I) nitrate ($TlNO_3$) as the heavy atom perturber, the L_D value informed is 0.31 ng mL^{-1} [62]. In both cases L_D were calculated as $3s_B/m$, thus these values are 2.2 and 0.34 ng mL^{-1} according to the current IUPAC recommendation and the values calculated in this work.

The L_D values reported by HPLC methods (0.1–0.5 ng mL^{-1}) [36,46,63,64] are lower to or similar than those obtained by spectrofluorimetric method; however, these techniques are time-consuming, require expensive supplies and large amounts of organic solvents.

The enhanced fluorescence of PYR observed from the interaction with CB6 contrasts with the behaviour shown in other supramolecular systems as the quenching of PYR fluorescence by calix [4] arene and calix [4] resorcinarenes [65]. It also contrasts with the slightly variation of integrated fluorescence intensity of PYR in the presence of CD [66], in both cases with no analytical applications.

3.3. Applicability

The applicability of the proposed method to real samples was demonstrated from apparent recovery experiments in spiked tap water and tea samples. One of the ways to estimate the accuracy of an analytical method is by performing recovery essays. According to IUPAC specifications [67], the recoveries are classified, depending on the moment in which the sample is fortified, in apparent calibration recovery (R^C) or recovery factor (R^*). In the former, the fortification is carried out before the measurement procedure; then related to systematic errors due to the matrix effect [68]. In the recovery or recovery factor, the samples are fortified at the beginning of the extraction process and related to the overall systematic error of the whole analytical procedure [68].

Apparent recovery assays were carried out by MOSA in fortified samples of tap water (Table 3) and tea extracts (Table 4). The R^C values were (82–103)% with R.S.D. = 7% in tap water and (89–99)% with R.S.D. = 3% in tea extracts.

Table 3
Apparent recoveries of PYR in tap water samples[a].

Amount added ($\times 10^{-6}$ M)	Amount found ($\times 10^{-6}$ M)	R^C (%)[b]
0.060[c]	0.053	88 (5)
0.200[c]	0.175	88 (7)
0.351[c]	0.327	93 (9)
0.701[c]	0.621	89 (8)
0.060[d]	0.060	100 (8)
0.092[d]	0.095	103 (10)
0.200[d]	0.164	82 (4)
0.222[d]	0.225	101 (5)
0.701[d]	0.603	86 (10)

[a] 30 mM of CB6, CH_3OH 1% (v/v), at 25.0 °C.
[b] Apparent calibration recovery. Relative standard deviations of triplicate determination are in parentheses.
[c] HCl 18% (w/v).
[d] HCOOH 55% (w/v).

Table 4
Apparent recoveries of PYR in tea samples[a].

Amount added ($\times 10^{-6}$ M)	Amount found ($\times 10^{-6}$ M)	R^C (%)[b]
0.173[c]	0.162	94 (3)
0.692[c]	0.615	89 (2)
0.173[d]	0.157	91 (4)
0.346[d]	0.338	98 (3)
0.692[d]	0.683	99 (1)

[a] 30 mM of CB6, CH_3OH 1% (v/v) at 25.0 °C.
[b] Apparent calibration recovery were corrected by matrix effect. Relative standard deviations of duplicate determination are in parentheses.
[c] HCl 18% (w/v).
[d] HCOOH 55% (w/v).

4. Conclusions

The microenvironment created by CB6 as nanosensor allows PYR to modify its physical and chemical properties. These results indicate that the use of CB6 improves the sensitivity and selectivity for the determination of PYR by spectrofluorimetry. The method is simple and only requires the addition of an aliquot of the analyte to a receptor solution and the measurement of the fluorescence at room temperature.

The analytical methodology described here could be extended to the study of other PAHs. In addition, the enhanced fluorescence of PYR produced in the presence of CB6 could be applied in HPLC with fluorescence detection analysis as a post-column derivatization method. This modification could increase the selectivity and sensitivity of the chromatographic method.

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