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Spectrofluorimetric study of the β-cyclodextrin–ibuprofen complex and determination of ibuprofen in pharmaceutical preparations and serum

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Abstract

The inclusion complexation of ibuprofen in β-cyclodextrin (β-CD) has been examined by means of spectrofluorimetry at both acid and alkaline pH. The results suggest that stable 1:1 complexes are formed in both media. The analysis of the pK_a values for ibuprofen in both the absence and presence of β-CD (4.12 and 4.66, respectively) suggests that in the inclusion complex the carboxylic group is located outside the cyclodextrin (CD) but interacting with it. Further structural characterization of the complex was carried out by means of AM1 semiempiral calculations. Based on the obtained results, a spectrofluorimetric method for the determination of ibuprofen in the presence of β-CD at 10 °C was developed in the range of 4.7–58 μg ml^{-1}. Better limits of detection (1.6 μg ml^{-1}) and quantification (4.7 μg ml^{-1}) were obtained in this latter case with respect to those obtained in the absence of β-CD. The method was satisfactorily applied to the quantification of ibuprofen in pharmaceutical preparations. A novel spectrofluorimetric determination of ibuprofen in the presence of β-CD was also developed for serum samples at concentration levels between 5 and 70 μg ml^{-1}. It uses second-order fluorescence excitation–emission matrices coupled to an algorithm based on self-weighted alternating trilinear decomposition (SWATLD), and avoids resorting to separative instrumental analyses.

Keywords: Ibuprofen; β-Cyclodextrin; Spectrofluorimetry; Second-order multivariate calibration

1. Introduction

Cyclodextrins (CDs) are water soluble cyclic oligosaccharides. In particular, β-CD is composed of seven D-(+) glucopyranose units. CDs have a relatively hydrophobic cavity, and are, therefore, able to form inclusion complexes in aqueous solution with a large variety of molecules of appropriate dimensions and low polarity. The incorporation of a guest compound into the CD cavity is able to change the physico-chemical properties of the former [1] and this fact can be useful in different fields such as pharmaceutical, cosmetic, and food industries [2].

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Our research group is devoted to improve the performance of analytical methods for the determination of different pharmaceuticals through the formation of guest–host complexes. Specifically, we have studied the physicochemical properties of CD complexes formed with anti-inflammatory drugs [3–7]. As part of this general program, the present report discusses the interaction between ibuprofen (Fig. 1) and β-CD. Ibuprofen (α-methyl-4-[isobutyl]phenylacetic acid) is the oldest of the newer non-steroidal anti-inflammatory drugs (NSAIDs) and, in addition, possesses analgesic and antipyretic properties [8]. Ibuprofen has been determined in biological and pharmaceutical samples by different chromatographic methods [9–13]. Recently, a spectrofluorimetric method for its quantification in pharmaceutical formulations was applied [14]. However, the technique can be further improved relying on the ability of CDs to form inclusion complexes with better fluorescent properties than those of the free compound [15–17]. This fact allowed us to obtain better figures of merit for the spectrofluorimetric determination in pharmaceuticals and to extend this method to the therapeutic monitoring of the drug in serum samples. To the extent of our literature search, this is the first time that a spectrofluorimetric method has been reported for the analysis of ibuprofen in serum. The overlapping between the fluorescence spectra for human serum and ibuprofen is significant and, in principle, it precludes the direct spectrofluorimetric determination of the studied compound. However, this problem was overcome by combining the property of β-CD of enhancing the relatively low native fluorescence of ibuprofen with a suitable chemometric analysis. Specifically, second-order data analysis was performed on excitation–emission fluorescence matrices (EEFMs) with the aid of the self-weighted alternating trilinear decomposition (SWATLD) algorithm [18]. It is important to point out that although this chemometric method seems to be of high analytical utility, reports applying this type of analyses to biological samples are rather scarce. The advantages and limitations of the method are herein discussed.

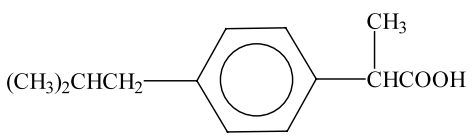

Fig. 1. Ibuprofen.

2. Experimental

2.1. Reagents and solutions

Analytical-reagent grade chemicals were used in all experiments. Ibuprofen (99.9%) was obtained from Marsing & Co (Denmark). β-CD (Aldrich, Milwaukee, WI, USA) was doubly recristalized from water. Benzene, methanol, NH_3, NaOH and NaCl were obtained from Merck (Darmstadt, Germany). Caffeine was purchased from Laboratorio Productor de Fármacos (Santa Fe, Argentina). Doubly distilled water was used in the preparations of all aqueous solutions. The analyzed pharmaceutical preparations were Druisel Ibuprofeno (Northia, Argentina), IBU–Buscapina (Boehringer Ingelheim, Argentina), and Ibupirac Fem, Ibupirac Migra and Ibupirac Flex (Monsanto Argentina). An alkaline 4.00×10^{-3} M stock solution of ibuprofen was prepared in NH_3 approximately 0.02 M. Methanolic stock solutions of both ibuprofen and caffeine were prepared at concentrations of 1500 and 5000 μg ml^{-1}, respectively.

2.2. Apparatus

Fluorescence measurements were done on a Aminco Bowman (Rochester, NY, USA) Series 2 luminescence spectrometer equipped with a 150 W xenon lamp and using 1.00 cm quartz cell, slit widths of 8 nm, exciting at 262 nm and obtaining the fluorescence emission at 288 nm. EEFMs were collected using 0.3 cm quartz microcell, slit widths of 8 nm, wavelength excitation range of 250–270 nm (each 1 nm) and wavelength emission range of 275–330 nm (each 1 nm). Absorbance data were obtained with a Beckman (Fullerton, CA, USA) DU 640 spectrophotometer. All spectroscopic measurements were made using a thermostatted cell holder and a thermostatic bath Lauda (Frank-

furt, Germany) RM6T. The pH of solutions were measured with a Metrohm (Herisau, Switzerland) 713 pHmeter equipped with glass and Ag/AgCl reference electrodes calibrated with standard HCl and acetate buffer at an ionic strength (μ) of 0.10 M (NaCl). Gas chromatography (GC) analyses were carried out on a Perkin Elmer (Norwalk, CT, USA) AutoSystem XL gas chromatograph equipped with a flame ionization detector. Data acquisition and data analysis were performed by use of the TURBOCHROM software. The injection, PE 17 column and detector temperatures were 250, 270 and 300 °C, respectively, and the nitrogen flow rate was 1 ml min^{-1}. A 5 μl aliquot of each sample dissolved in methanol was used for direct injection. The samples were injected in the split 1:25 mode.

2.3. Influence of the pH

The changes in the UV–visible absorption of solutions of both ibuprofen and the ibuprofen–β-CD complex as a function of pH were analyzed following a procedure previously described [19]. Briefly, to a 100 ml stirred hydrochloric acid solution of ibuprofen or ibuprofen–β-CD, NaOH solution (1–0.1 M) was added in small increments (0.05–0.2 ml). For each pH point the solution was aliquoted and the absorption spectrum was read. The initial concentration of ibuprofen in the experimental solutions was in the order of 1.40×10^{-4} M while the β-CD concentration was 5.0×10^{-3} M. The influence of pH in the fluorescence spectra of both ibuprofen and ibuprofen–β-CD systems was studied following a similar procedure to that described for the absorbance experiments. In this case, the initial concentration of ibuprofen and β-CD in the reaction vessel were 3.70×10^{-5} and 5.0×10^{-3} M, respectively. The calculation of the deprotonation constant for ibuprofen was performed by using the program EPSILON [20]. Both the spectrophotometric and spectrofluorimetric titrations were performed by duplicate under nitrogen and the ionic strength and temperature were maintained at 0.10 M (NaCl) and 25 °C, respectively.

2.4. Influence of β-CD concentration

Regarding the study of the influence of β-CD on the fluorescence spectra of ibuprofen, the concentration of the compound was held constant at 3.50×10^{-5} M while the CD concentration was varied from 0 to 5.0×10^{-3} M. These experiments were performed in both hydrochloric acid (pH 2) and ammonia (pH 10) solutions. All measurements were run at least in duplicate.

2.5. AM1 calculations

Ground-state geometry optimization of the proposed structures for the inclusion complexes was performed with the AM1 program contained in the Hyperchem (Hypercube, Gainesville, FL, USA) package, version 5.02, on a Pentium 650 microcomputer. Geometry optimization was performed until the gradient was lower than 0.1 kcal $Å^{-1}$ mol^{-1}.

2.6. Calibration curves and pharmaceutical preparations

Spectrofluorimetric calibration curves for ibuprofen both in the absence and in the presence of β-CD were constructed. Solutions for the calibration curve in the absence of β-CD were prepared by suitable dilution of a stock standard solution of ibuprofen, in order to obtain concentrations in the range 0–75 μg ml^{-1}. In the calibration solutions of ibuprofen in the presence of β-CD the concentration of the analyte was varied from 0 to 60 μg ml^{-1} while the β-CD concentration was kept constant at 5.0×10^{-3} M. Both ranges of ibuprofen concentrations were previously checked for compliance to linearity of fluorescence versus concentration.

In general, commercial pharmaceutical preparations were processed as follows: ten tablets of the corresponding preparation were weighed and the average mass of each tablet was calculated. The tablets were then triturated and mixed. A sample of 15.0 mg taken from the latter mixture was accurately weighed and treated with 6–7 ml of diluted NH_3. The mixture was sonicated for 15 min., filtered into a 10.00 ml calibrated flask and

the solution was diluted with water to the mark. For the analysis, a 60.0 μl aliquot of this solution was diluted to 2.00 ml with a β-CD solution, in such a way that the final concentration of β-CD was 5.0×10^{-3} M.

Some commercial samples required the standard addition method (see below). In these cases, the tablets were treated in the same way as described above, and appropriate aliquots of the prepared solution were distributed among six 2.00 ml volumetric flasks. Increasing volumes of ibuprofen stock solution were successively added to each flask, with the remaining reactants added in order to finally attain their optimum concentrations.

With the purpose of validating the spectrofluorimetric method, ibuprofen was also quantified by GC. Different GC methods for ibuprofen determination have been proposed and validated [10,21–23]. Solutions for the GC calibration curve were prepared by suitable dilutions of methanol stock standard solutions of both ibuprofen and caffeine (internal standard), obtaining concentrations in the range 50–1000 μg ml^{-1} for ibuprofen and 500 μg ml^{-1} for caffeine. A sample of 15.0 mg of triturated and mixed tablet was treated with 1 ml of methanol, sonicated for 15 min., filtered into a 2.00 ml calibrated flask and the solution was diluted with methanol to the mark. For the analysis, a 200.0 μl aliquot of this solution was diluted to 1.00 ml with caffeine solution and methanol, in such manner that the final concentration of caffeine was 500 μg ml^{-1}.

2.7. Serum analysis

A test set of ten samples was prepared by spiking different human sera with ibuprofen, obtaining concentration levels between 5 and 70 μg ml^{-1}, which were in the order of those found in blood after administration of 400 mg doses every 6–8 h [24]. Typically, a volume of 1.50 ml of a given serum sample was placed in a capped flask and the corresponding volume of ibuprofen stock solution, 0.5 ml of 0.5 M HCl and 2.00 ml of benzene were added. The tube was then shaken for 5 min. Following a brief centrifugation (5 min at $2000 \times g$), 400 μl aliquots of the organic phase were transferred to three 1.00 ml flasks and evaporated by use of dry nitrogen. Then, 10 μl of 0.01 M NH_3, 0.5 ml of 0.01 M β-CD, and different volumes of ibuprofen stock solution were added to these flasks in order to obtain final concentrations for ibuprofen which were 0, 15 and 30 μg ml^{-1} higher than the original ones. Finally, water was added to the mark. Each serum sample and its corresponding standard additions were prepared in triplicate. The EEFMs were then read for these samples at 10 °C. The selection of the excitation and emission wavelength ranges was made upon suitable consideration of the regions with low spectral overlapping: 250–257 nm (excitation) and 275–300 nm (emission). The obtained data were subjected to second-order data analysis performed by a method employing a least-squares minimization, as implemented in SWATLD. The theory of this method was already described as a more efficient alternative of the well-known parallel factor analysis (PARAFAC) decomposition algorithm [18]. The SWATLD algorithm has the advantages of fast convergence and insensitivity to the excess factors used in calculations [18], and, according to our experience [25], offers better results than other second-order algorithms. The routine for applying SWATLD was generously supplied by N.M. Faber.

The same spiked sera used above were also analyzed by GC. For this purpose, 500 μl of each ibuprofen-containing serum was acidified with HCl and 200 μl of benzene was added. After centrifuging, 150 μl of the organic phase were evaporated and then 30 μl of 500 μg ml^{-1} caffeine in methanol (internal standard) were added. Each sample was prepared in triplicate.

3. Results and discussion

3.1. Acid–base behavior

Preliminary studies showed that β-CD significantly modifies the fluorescence spectra of ibuprofen, suggesting an interaction between both compounds and thus a concomitant complex formation. Since the fluorescence signals for ibuprofen change in going from acid to basic media, studies of the influence of pH were carried

out before calculating the magnitude of its interaction with β-CD. The carboxylic group of ibuprofen may or may not be included into the CD and conclusions about the spatial distribution of this group could be obtained by comparison of the pK_a values calculated in the presence and in the absence of β-CD [5]. The acid–base behavior of ibuprofen in the ground-state was investigated by spectrophotometric titrations. Under absorptimetric conditions, the compound was found to be soluble in the analyzed range of pH (2–12). Fig. 2A shows the experimental profile of ibuprofen as a function of pH at the absorption maximum. From the changes in the absorbance with pH a value of pK_a of 4.12 ± 0.08 for the carboxylic group was obtained. The acidity constant calculated from the spectrophotometric profile of ibuprofen in the presence of β-CD (Fig. 2B) was $pK_a' = 4.66 \pm 0.05$ and is slightly larger than that obtained in its absence, suggesting certain interaction between the carboxylic proton and an external hydroxyl group of the CD.

Fig. 2C and D show the fluorescence intensity of ibuprofen and ibuprofen–β-CD as a function of pH. These fluorescence profiles resemble those shown in Fig. 2A and B, and thus the acidity

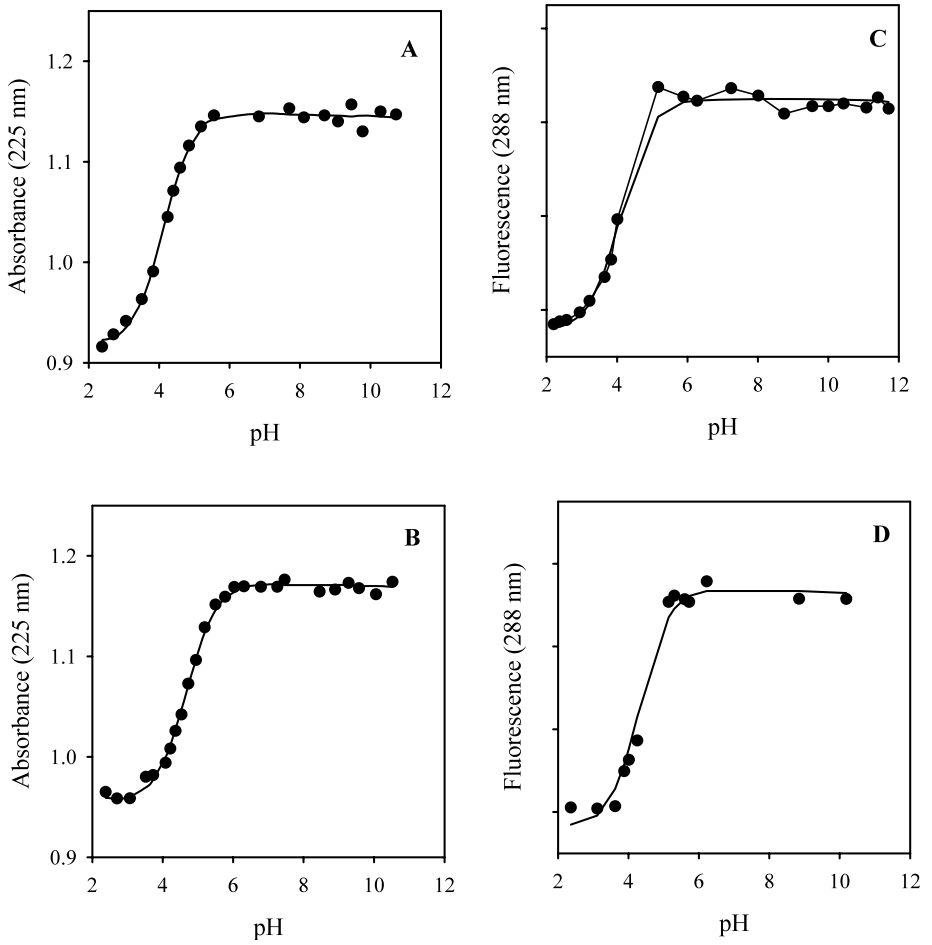

Fig. 2. Experimental absorbance vs. pH in (A) ibuprofen and (B) ibuprofen–β-CD solutions ($C_{ibuprofen} = 1.40 \times 10^{-4}$ M, $C_{\beta\text{-CD}} = 5.0 \times 10^{-3}$ M), and experimental fluorescence values vs. pH in (C) ibuprofen and (D) ibuprofen–β-CD solutions ($C_{ibuprofen} = 3.70 \times 10^{-5}$ M, $C_{\beta\text{-CD}} = 5.0 \times 10^{-3}$ M). The solid line is the non-linear fit of the data. $\mu = 0.10$ M (NaCl), $t = 25$ °C.

constant values calculated (4.10 ± 0.08 and 4.42 ± 0.05, respectively) are essentially the same as those obtained from the spectrophotometric titrations. These facts suggest that the reaction rates in the excited state are significantly smaller than the rates of fluorescence emission of the excited species. Therefore, the process probed by the fluorescence-pH study does also correspond to the deprotonation in the ground-state [26].

3.2. Inclusion complexes of β-CD with ibuprofen

The fluorescence emission of ibuprofen is significantly increased with increasing concentrations of β-CD (Fig. 3). These changes occur at both acid and alkaline pH, and allow to determine the inclusion constant values for the complexes formed by protonated ibuprofen and its anionic form, respectively. Further, with the purpose of evaluating the temperature influence on the complex formation, the experiments were conducted at 10 and 25 °C. Fig. 4 displays plots of fluorescence intensities against the analytical concentration of β-CD for different working conditions. From these profiles, the association constants were determined by applying non-linear regression analysis [5]: $1.8(1) \times 10^4$ and $1.39(7) \times 10^4$ M^{-1} for the protonated form at 10 and 25 °C, respectively, and $8.8(5) \times 10^3$ and $9.2(6) \times 10^3$ M^{-1} for the deprotonated form at 10 and 25 °C, respectively. The good refinement obtained in all systems confirms the 1:1 stoichiometry for the studied complexes. The calculated constant values indicate a strong interaction between the studied compound and β-CD, notably when ibuprofen is in its protonated form and at low temperature. However, from an analytical point of view, the best working conditions should lead to maximum analyte sensitivity, i.e. to maximum fluorescence intensity. These conditions are reached at alkaline pH, where the inclusion-driven fluorescence enhancement adds to the intrinsically higher fluorescence of the anionic form with respect to acid ibuprofen (see Fig. 3).

With the purpose of characterizing the inclusion binary complexes, semiempirical MO calculations using the AM1 program were performed. This analysis provides some information about the possible orientation of the ibuprofen molecule inside the β-CD cavity. Isolated β-CD and ibuprofen in both protonation forms were first optimized and then the corresponding inclusion complexes were refined. These calculations were performed by placing the complexes in a box

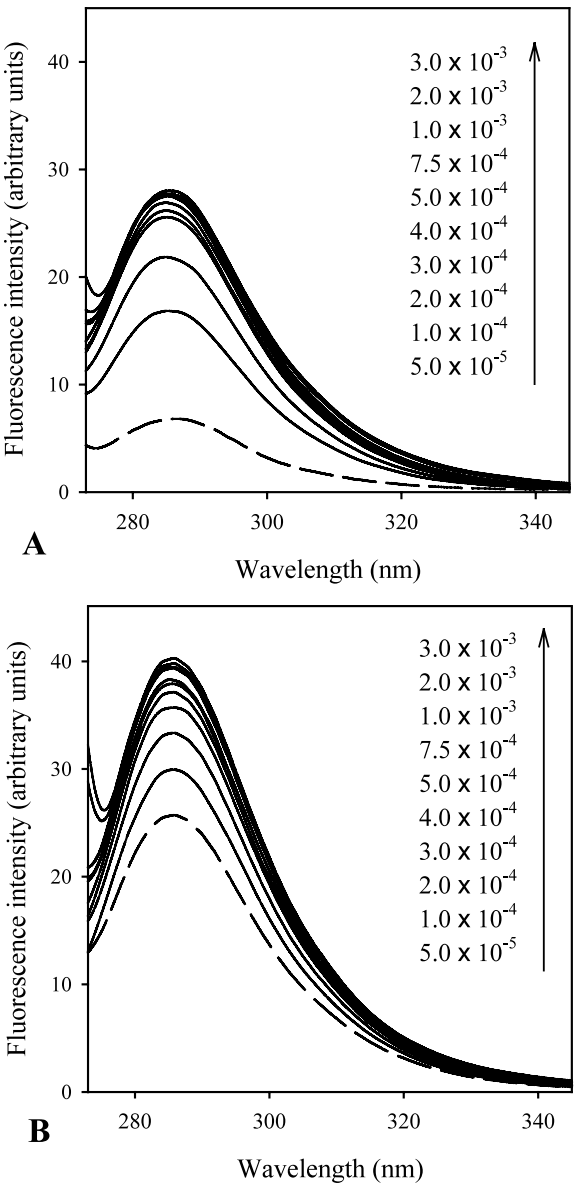

Fig. 3. Fluorescence spectra of ibuprofen (-----) and ibuprofen with different concentrations (M) of β-CD, as indicated, (A) pH 2, $t = 10$ °C, (B) pH 10, $t = 10$ °C; $C_{\text{ibuprofen}} = 3.5 \times 10^{-5}$ M.

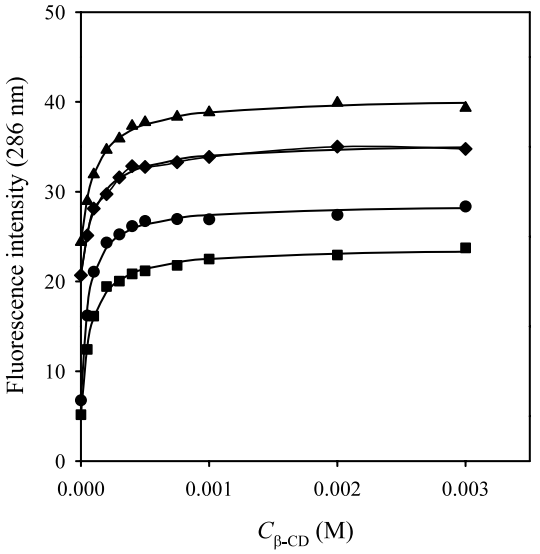

Fig. 4. Influence of β-CD concentration on the fluorescence signal of ibuprofen in an acid medium at (■) 25 °C and (●) 10 °C, and in an alkaline medium at (◆) 25 °C and (▲) 10 °C. The solid line is the non-linear fit of the data assuming the formation of an 1:1 ibuprofen–β-CD complex.

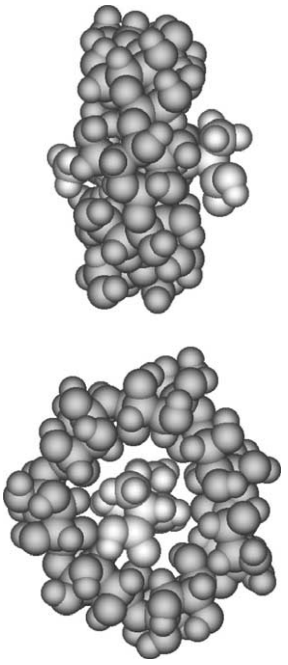

Fig. 5. Two views of the model for the complex formed between ibuprofen (protonated form) and β-CD.

containing 830 water molecules. Geometry optimization shows that ibuprofen prefers one of the two possible axial types of inclusion: the form with the carboxylic group towards the wider CD end is more stable by 51 kcal mol^{-1}. In this most stable arrangement, the aromatic ring of ibuprofen appears to be completely included inside the CD cavity, with the carboxylic group near the CD external hydroxyl groups, in accordance with the acidity results of ibuprofen in the presence and absence of β-CD (see above). Hydrogen bonds between ibuprofen and CD hydroxyl groups were not detected. This fact suggests that only hydrophobic interactions are responsible for the complex formation. Fig. 5 shows the structure corresponding to the complex formed with protonated ibuprofen.

3.3. Analytical parameters and application

The univariate spectrofluorometric determination of ibuprofen in the presence of β-CD involves the construction of the corresponding calibration curve. In order to attain a high and pH independent fluorescence emission, an alkaline medium (pH 10) was selected according to the above commented results. Together with the calibration curves in the presence of β-CD at two working temperatures, additional ones without the addition of β-CD were built with the purpose of comparing the results. The equation for the calibration graph is in all cases: $F = a + bC$, where F is the fluorescence intensity (in arbitrary units) and C is the concentration of ibuprofen. The results obtained are displayed in Table 1, which indicate that in the absence of β-CD, calibration parameters do not significantly improve in going from 25 to 10 °C. However, the addition of β-CD to the calibration solutions at alkaline pH produces better results than those obtained in the absence of CD. Specifically, the analytical sensitivity increases by about 100% in the presence of β-CD (when the experiment is carried out at 10 °C) with respect to the value obtained in its absence. This fact demonstrates how the inclusion complex magnifies the sensitivity of the method.

Although it is recommended that ibuprofen should not be administered with other anti-in-

Table 1
Calibration results of ibuprofen[a]

	LR[b] (μg ml^{-1})	R[c]	a[d]	b[e]	γ[f] (μg^{-1} ml)	LOD[g] (μg ml^{-1})	LOQ[h] (μg ml^{-1})	R.S.D.[i] (%)
Without β-CD								
10 °C	9.3–72	0.998	0.4 (0.1)	0.266 (0.003)	0.7	3.2	9.3	2.4
25 °C	9.3–72	0.998	0.4 (0.1)	0.229 (0.003)	0.7	3.2	9.3	2.4
With β-CD								
10 °C	4.7–58	0.999	0.5 (0.1)	0.469 (0.003)	1.5	1.6	4.7	1.2
25 °C	5.4–58	0.999	0.5 (0.1)	0.411 (0.003)	1.3	1.8	5.4	1.5

[a] The number of data for each calibration curve corresponds to eight different concentration levels, with three replicates for each level ($n = 24$).
[b] Linear range.
[c] Correlation coefficient.
[d] Intercept (S.D. within parentheses).
[e] Slope (S.D. within parentheses). This value is the calibration sensitivity according to IUPAC [33].
[f] Analytical sensitivity: $\gamma = b/S_S$, where S_S is the S.D. of the regression residuals [34].
[g] Limit of detection: calculated according to Clayton [30] using 0.05 as assurance probabilities.
[h] Limit of quantification.
[i] Relative standard deviation. In all cases three replicates were measured ($C_{ibuprofen} = 34.3$ μg ml^{-1}).

flammatory agents [8], it is generally present in commercial formulations with other active principles, and some of them may prevent its direct spectrofluorimetric determination. Unlike ibuprofen, caffeine, ergotamine and chlorzoxazone do not fluoresce under the conditions herein studied, but the fluorescence emission of ibuprofen decreases due to the high absorbance of these compounds across its emission maximum (288 nm). The inner filter produced by these compounds could be overcome by using the standard addition method (see Section 2). In these cases, a least-squares fit to a straight line of the obtained fluorescence intensity values versus ibuprofen concentration was performed, and its concentration in the unknowns was calculated as the quotient a/b (a, intercept and b, slope of the fitted line). The values obtained from the different systems are shown in Table 2 and indicate that the applied method yields satisfactory results in all

Table 2
Determination of ibuprofen in pharmaceutical preparations (tablets)

Proprietary name	Composition of tablets	Found (mg per tablet)[a]	
		Spectrofluorimetry[b]	GC
Druisel Ibuprofeno (Northia)	Ibuprofen, 400 mg	404 (5) [101%]	408 (9) [102%]
IBU–Buscapina (Boehringer Ingelheim)	Ibuprofen, 400 mg; Hyoscine *N*-butyl bromide, 20 mg	416 (5) [104%]	392 (9) [98%]
Ibupirac Fem (Monsanto)	Ibuprofen, 400 mg; Homatropine methyl bromide, 4 mg	410 (5) [103%]	397 (11) [99%]
Ibupirac Migra[c] (Monsanto)	Ibuprofen, 400 mg; Caffeine, 100 mg; Ergotamine tartrate, 1 mg	402 (13) [101%]	408 (12) [102%]
Ibupirac Flex[c] (Monsanto)	Ibuprofen, 400 mg; Chlorzoxazone, 250 mg	418 (10) [105%]	396 (12) [99%]

[a] S.D. in parentheses. Average of three determinations. The recoveries (in square brackets) are based on the amount reported by the laboratory.
[b] The method was developed in the presence of β-CD and at 10 °C.
[c] Results of standard addition using six concentrations levels. The confidence limits (at 95% level) were calculated according to Ref. [29].

studied cases. The absence of interference from the excipients was confirmed by the very good recoveries, all in the range recommended by Pharmacopoeias for this type of analyses [27,28].

In order to test the accuracy of the proposed method concerning the content of ibuprofen in the studied tablets, the samples were also assayed by a CG method and the paired t test was applied [29]. Using the data shown in Table 2, the experimental t value obtained was 1.61, whereas the critical $t_{\alpha,\nu}$ ($\alpha = 0.05$, $\nu = n-1 = 4$) was 2.78. Therefore, since the experimental t value is lower than the critical one, both methods give results, which are not statistically different.

3.4. Human serum

As was previously mentioned, the spectral overlapping of a normal serum and ibuprofen is very significant and hinders its direct fluorimetric determination in this type of matrix. Even after extraction with organic solvents, serum components, which are present in the organic phase, can interfere with the ibuprofen quantification. Fig. 6 shows the excitation–emission matrices of ibuprofen and a serum sample free of ibuprofen, both of them subjected to the pre-treatment indicated in the Section 2. As can be appreciated, the degree of overlapping of both spectra avoids the use of an univariate method. This problem can be overcome by the use of EEFMs, which provide trilinear second-order data sets. They allow spectra and relative concentrations of individual mixture components to be extracted directly, in the presence of any number of uncalibrated constituents (itself known as the 'second-order advantage'). Fig. 7 shows examples of EEFMs for a typical serum containing ibuprofen, before and after standard additions. Table 3 shows the nominal and predicted analyte concentrations obtained by applying this method. The corresponding statistical values were: root mean square errors of prediction (RMSEP) = 1 μg ml^{-1}, relative error of prediction (REP) = 3% and square correlation coefficient (R^2) = 0.998, calculated as:

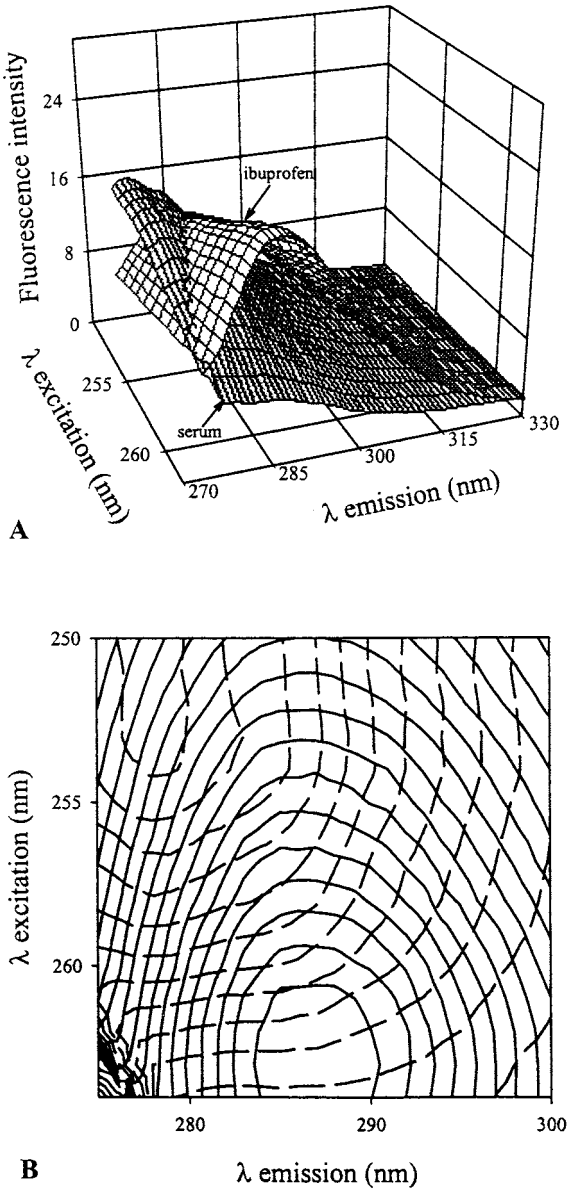

Fig. 6. (A) Three-dimensional plots of the EEFMs for the extracts of a serum sample and ibuprofen 25 μg ml^{-1}. Final $C_{\beta\text{-CD}} = 5.0 \times 10^{-3}$ M, pH 10. (B) Corresponding two-dimensional projections: dotted line, serum sample and solid line, ibuprofen.

$$\mathrm{RMSEP} = \left[\frac{1}{I-1}\sum_{1}^{I}(c_{\mathrm{act}} - c_{\mathrm{pred}})^2\right]^{1/2} \quad (1)$$

where I is the number of prediction samples, c_{act}

and c_{pred} are the actual and predicted concentrations, respectively,

$$\mathrm{REP} = \frac{100}{\bar{c}} \times \mathrm{RMSEP}, \quad (2)$$

where $\bar{c}$ is the average component concentration, and

$$R^2 = 1 - \frac{\sum_{1}^{I}(c_{act} - c_{pred})^2}{\sum_{1}^{I}(c_{act} - \bar{c})^2} \quad (3)$$

The limit of detection for a second-order analysis of ibuprofen in serum was empirically calculated from the product between the average of the standard deviations (S.D.) corresponding to diluted solutions and the coefficient used by Clayton [30] in univariate calibration. The value obtained for serum (3 μg ml^{-1}) corresponds to a concentration of 1 μg ml^{-1} in the reading cell, after the sample treatment indicated in the Section 2. An inspection of Table 3 indicates that the recoveries and the statistical parameters are reasonably good. The rather high value of the relative standard deviation (R.S.D.) corresponding to the serum sample with the lowest ibuprofen concentration (5 μg ml^{-1}) can be ascribed to this value being in the vicinity of the lowest predictable concentration.

The spectrofluorimetric determination of ibuprofen by second-order analysis was compared with the results provided by a GC method (see Table 3), by applying a least squares regression analysis of predicted concentrations for the samples shown in Table 3 [31,32]. As both methods should provide the same concentration values for the same samples, values for the intercept and slope of 0 and 1 must be, respectively, obtained. The calculations show that the joint confidence region indeed contains the theoretical (0, 1) point, and, therefore, the conclusion is that the accuracies of the proposed and the reference method are not significantly different.

With the purpose of evaluating the proposed method in real samples, two different sera of patients administered with a single oral dose of 400 mg of ibuprofen were investigated. The values obtained (average of three replicates) by the spectrofluorimetric second-order method [5.0 (0.5) and 22.0 (0.6) μg ml^{-1}, S.D. in parenthesis] are statistically comparable to those, respectively,

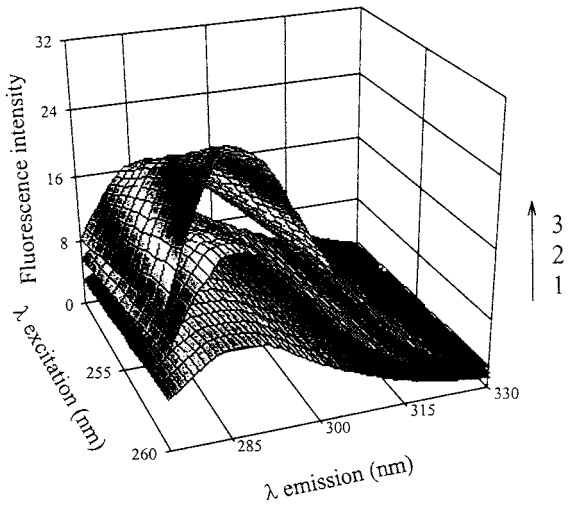

Fig. 7. Three-dimensional plots of the EEFMs for a typical validation serum sample containing (1) 49.7 μg ml^{-1} ibuprofen, and after the standard addition of (2) 15 μg ml^{-1} of ibuprofen and (3) 30 μg ml^{-1} of ibuprofen. $C_{\beta\text{-CD}} = 5.0 \times 10^{-3}$ M, pH 10.

Table 3
Determination of ibuprofen in human serum

Nominal value (μg ml^{-1})	Found (μg ml^{-1})[a]	
	Spectrofluorimetry[b]	GC
5.0	5 (1) [100%]	5.3 (0.1) [106%]
10.0	10.9 (0.8) [109%]	10 (1) [100%]
15.2	15.0 (0.6) [99%]	15.6 (0.2) [103%]
20.0	19.5 (0.7) [98%]	19.0 (0.5) [95%]
29.4	31 (3) [105%]	29 (3) [99%]
35.1	37 (3) [105%]	34 (1) [97%]
40.0	40 (3) [100%]	41 (3) [103%]
49.7	51 (2) [103%]	50 (2) [101%]
59.2	58 (2) [102%]	60 (1) [101%]
70.5	71 (1) [101%]	72 (3) [102%]

[a] S.D. in parentheses. Average of three determinations. The recoveries are indicated in square brackets.

[b] Found from second-order data using the SWATLD algorithm. Two fluorescent species were considered to be present in all cases.

obtained with the GC reference one [4.8 (0.1) and 22 (1) μg ml^{-1}]. These results suggest that the proposed method does not suffer the interference of ibuprofen metabolites.

4. Conclusions

On the basis of spectrofluorimetric analysis, the complex formation between ibuprofen and β-CD was studied. Equilibrium constant values and 1:1 stoichiometries for the inclusion complexes were evaluated at both acid and alkaline pH. The rather large constant values found suggest a significant interaction between β-CD and ibuprofen, especially with the protonated form of the latter. By comparing the acidity constants for ibuprofen in both the presence and absence of β-CD, it was inferred that in the inclusion complex the carboxylic group interacts with a secondary hydroxyl group of the CD. Further structural information was obtained from semiempirical MO studies, which suggested that the aromatic ring resides within the β-CD cavity.

A spectrofluorimetric method for the determination of ibuprofen in pharmaceutical preparations was developed and the results showed an improvement in performance with respect to that carried out in the absence of β-CD.

Second-order multivariate calibration of fluorescence data in the presence of β-CD was satisfactorily applied to the determination of ibuprofen in serum. Since the interferences of the complex matrix studied are completely eliminated and the method is simple to implement, rapid and inexpensive, the strategy of employing this chemometric tool leads to extremely useful results.

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