PCCP

Hydrogen bonding effects in the photophysics of a drug, Piroxicam, in homogeneous media and dioxane–water mixtures

Suzana M. Andrade and Sílvia M. B. Costa*

Centro de Química Estrutural, Complexo 1, Instituto Superior Técnico, 1049-001 Lisboa Codex, Portugal. Fax: (351) 1 8464455; E-mail: sbcosta@alfa.ist.utl.pt

The fluorescence emission of Piroxicam, an anti-inflammatory drug exhibiting excited-state intramolecular proton transfer (ESIPT), was studied in pure solvents and in dioxane–water mixtures. The solvent proticity was seen to play a key role in the ground-state promoting the existence of different conformers (open and closed form), and in the excited-state where it is responsible for a pronounced quenching of the tautomer emission in the aforementioned mixtures. A derived Stern–Volmer relation shows the diffusive control of the process ($k_q \approx 4.3 \times 10^9$ M^{-1} s^{-1}). The values of polarity (π^*), proticity (α) and $E_T(30)$ of dioxane–water mixtures assessed by the solvatochromism of this molecule, are in agreement with a "critical" concentration ≈ 22 M above which water presents the characteristics of the free solvent. Below that concentration, Piroxicam adopts a closed conformation through an intramolecular hydrogen bond in a six-membered ring, which is very stable and thus insensitive to microenvironmental changes.

1 Introduction

Piroxicam, 1,2-benzothiazine-3-carboxamide, 4-hydroxy-2-methyl-*N*-(2-pyridyl)-1,1-dioxide, is a molecule belonging to the wide group of non-steroidal anti-inflammatory drugs (NSAID). These drugs are used for a variety of conditions since they exhibit analgesic, antipyretic and antiplatelet properties.[1] Although they can overcome some of the negative side effects of corticosteroids, NSAID are known to induce photosensitive action.[2] Nevertheless, Piroxicam is not among the most photoactive group, covered by the 2-arylpropionic acid derivatives.[3]

This drug can exist in several prototropic species,[4] with the anionic ones dominating at neutral biological pH.[5] Different conformations have also been proposed for this molecule in the ground-state,[6] due to the ability to establish an intramolecular hydrogen bond forming a six-membered ring. This enolic species can undergo intramolecular proton transfer in the excited-state, ESIPT, converting into the keto form,[6] (Scheme 1). ESIPTs were found in a number of reactions in the recently reviewed vast literature.[7] The majority of these reactions involve the transfer of a proton (or a hydrogen atom) from an oxygen donor to an oxygen (or nitrogen) acceptor. Five-membered rings, like in 3-hydroxyflavone, and six-membered rings, like in methyl salicylate, with an intramolecular hydrogen bond between the two moieties thus facilitating proton transfer are the most commonly reported.[8,9] The dynamics of the tautomer formation encompasses a large range of time domains, ranging from the femtosecond to the microsecond, although the majority of the relevant processes occur in the sub-picosecond domain.

A well-known anti-inflammatory drug, salicylic acid, which can also undergo ESIPT,[10] presents great photochemical stability. The excited tautomer suffers a reverse isomerization on the ground-state surface, so that the entire process is cyclic, which in turn confers photochemical stability to the molecule. The same must occur with Piroxicam according to its spectroscopic behaviour where hydrogen bonds seem to play a key role. Further attempts to relate the strength of the intramolecular hydrogen bond and the photochemical stability were made.[11] Although it is clear now that ESIPT alone cannot account for all the effects of photostability, it certainly does play a crucial role, as can be envisaged from the ensemble of our data. This also, is reinforced by the fact that Piroxicam is administered to the patients in a composition that involves cyclodextrin, where it is known that the molecule adopts the closed conformation.[6]

The solvatochromism of two centrosymmetrical squaraine dyes has recently been reported in pure solvents, dioxane–water mixtures and was used to probe the polarity of AOT reverse micelles.[12] The unsubstituted squaraine dye which forms an intermolecular hydrogen bond with water exclusively in the excited state is a suitable molecule to calculate π^* (which accounts for dipole–dipole interactions between the solute and the solvent) and α (which is a measure of the solvent acidity). The hydrogen bonding observed in Piroxicam is both intramolecular and intermolecular and the prototropism exhibited contributes to more complex features.

In this paper, we show that it is also possible to apply the solvatochromic studies of Piroxicam in pure solvents and dioxane–water mixtures to explain the non-specific solute–media interactions. This drug is found to be able to report structural water changes in dioxane–water mixtures which are considered as mimetic of biological environments. Knowledge of water interactions in organic media and at organic interfaces is of crucial relevance for the understanding of a whole range of processes, including the formation and structure of microemulsions.[13,14]
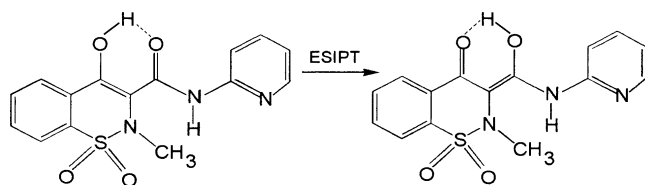
Scheme 1 Excited-state intramolecular proton transfer (ESIPT) from the enol form of Piroxicam to the keto form.

2 Experimental section

2.1 Materials

Piroxicam was kindly provided by "Laboratórios Medinfar" and used without further purification. Solvents used were all of spectroscopic grade except for n-hexanol, which was analytical grade. Water was distilled twice before use. Each solvent was checked for the absence of fluorescence at the wavelengths of experimental excitation and emission. Concentrated stock solutions of Piroxicam were prepared in spectrograde acetone. The small aliquots needed to prepare the diluted solutions were carefully bubbled by a stream of N_2 to evaporate the acetone content. The final concentration was kept below 5×10^{-6} M to guarantee that the absorbance at excitation wavelength did not exceed 0.1. All measurements were made 24 h after sample preparation in order to ensure complete dye solubilization.

2.2 Apparatus

A JASCO V560 UV/Vis spectrophotometer was employed in UV absorption measurements. Fluorescence spectra were recorded on a Perkin-Elmer LS 50B spectrofluorimeter with a sample holder thermostated at 25 °C. The instrumental response at each wavelength was carried out by means of a curve obtained using appropriate fluorescence standards (until 400 nm) including the one provided with the instrument. The fluorescence quantum yields of the aerated Piroxicam solutions were determined relative to that of quinine bisulfate in 0.5 M H_2SO_4 solution ($\phi_f \approx 0.546$ [15]) using adequate corrections for the solvent's refractive index.[16] Fluorescence lifetimes were determined in water and cyclohexane using the time correlated single photon counting method. The photon source was a Spectra-Physics set-up composed of a titanium–1 sapphire Tsunami laser pumped by an argon ion laser, with a 37 ps fwhm instrument response. Further description may be found elsewhere.[17] Data analysis was performed by a deconvolution method using a non-linear least-squares fitting programme based on the Marquardt algorithm and the goodness of the fit was evaluated by statistics parameters like reduced χ^2.

3 Results and discussion

3.1 Pure solvents

Absorption and fluorescence spectra of Piroxicam were measured in nonpolar, polar aprotic and polar protic solvents, Piroxicam being reasonably soluble in all of them, for the concentration of probe used. The data obtained are summarised in Table 1. A major bathochromic shift (≈ 30 nm) occurs with increasing solvent polarity and hydrogen bonding ability. This shift is supposed to be due to an intramolecular hydrogen bond between the –OH group of the benzothiazine ring and the carbonyl of the lateral amide group, forming a six-member ring.[6] This process is perturbed in protic solvents by the formation of an intermolecular hydrogen bond originating an open conformer or even the anionic species due to a very low pK_a (≈ 4.9). At room temperature, the fluorescence emission of Piroxicam in aprotic solvents is independent of excitation wavelength, with the band maximum around 470 nm. This extremely high Stokes shift was attributed to a rearrangement of the molecule in the excited state, originating its tautomer *via* a proton intramolecular transfer, generally denominated ESIPT.[6] In protic solvents the Piroxicam fluorescence depends on the excitation wavelength showing two bands one with a maximum around 400 nm (a so-called "normal" emission) and another around 490 nm (the tautomeric emission).

The dynamics of proton transfer can be strongly dependent on the solvent's ability to form hydrogen bonds. In fact,

Table 1 Absorption (λ_{max}^{abs}) and emission (λ_{max}^{emi}) maxima, and fluorescence quantum yield (ϕ_f) of Piroxicam in different solvents

Solvent	Label	$\phi_f \times 10^2$ ±0.05	λ_{max}^{abs}/nm ±0.5	λ_{max}^{emi}/nm ±0.5
Water	1	0.01	359.0	499.5
Methanol	2	0.01	358.2	503.0
Ethanol	3	0.02	355.0	499.0
Propanol	4	0.03	353.4	496.1
Butanol	5	0.05	350.1	493.4
Hexanol	6	0.07	334.0	491.3
Cyclohexanol	7	0.27	328.0	474.0
Ethyleneglycol	8	0.13	358.1	481.0
Glycerol	9	1.50	357.0	485.1
Dimethylsulfoxide	10	0.04	330.2	472.1
Acetonitrile	11	0.06	332.2	470.5
Acetone	12	0.07	329.0	496.0
Dichloromethane	13	0.14	325.0	472.0
Triacetine	14	0.35	324.5	470.3
Chloroform	15	0.19	325.6	472.3
Dioxane	16	0.20	329.0	480.0
Diethyl ether	17	0.05	320.5	478.2
Benzene	18	0.17	326.1	474.0
Carbon tetrachloride	19	0.18	325.0	478.1
Cyclohexane	20	0.18	325.0	470.2

proton transfer causes large electronic and structural rearrangements associated with quite large fluorescence shifts, as those that were found. In order to collect information about the properties of the electronic states involved, spectral shifts can be related to solvatochromic effects and these are usually evaluated in terms of macroscopic physical parameters of the solvent. They can be interpreted using the theoretical Ooshika, Bayliss and McRae relation (OBM).[16] This theory has been successfully applied to polar solutes as it predicts the existence of a permanent dipole for the ground-state solute.

An attempt to apply this theory to all types of solvents studied revealed the existence of two different trends in the absorption data, (Fig. 1a): one involving protic solvents and the other gathering the remaining aprotic ones. However, fluorescence maximum energy showed a fairly good correlation for the ensemble of tested solvents, (Fig. 1b). Therefore, there is one species which is preferentially stabilised in aprotic solvents and another one which is stabilised in the protic solvent through intermolecular hydrogen bonds. Although the two species have similar molar extinction coefficients, when excited at 350 nm their quantum yields of fluorescence are very different. In fact, the quantum yield of the tautomeric species (the one that emits further to the red) is much higher than the one from the normal emission, which is only found in a protic medium. Hence, it is understandable that the emission behaves as if only one species were present.

This behavior points to the existence of specific solute–solvent interactions. In such conditions, it is necessary to make use of empirical correlations of solvent polarity. The $E_T(30)$ polarity scale built with a betaine is undoubtedly accepted as the most popular to correlate several solvent properties. This uni-parametric scale is dependent on both the solvent dielectric properties and the hydrogen-bonding capac-
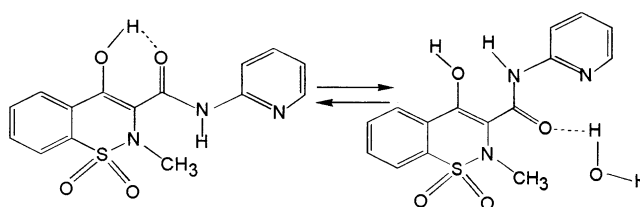
Scheme 2 Equilibrium between the closed conformation (Prx_N), in which the intramolecular H-bond prevails, and the open conformation of Piroxicam (Prx_A), in which the intermolecular H-bond prevails.

ity, but it is not affected by the solvent hydrogen bonding acceptor basicity. The Lewis acid–base specific interactions arise from the negative charge localised in the phenolic oxygen atom of the betaine.

Therefore, some important information concerning the process involved in Piroxicam solvatochromism could be inferred. As may be seen (Figs. 1c, d), there is not a linear correlation with this parameter. An inflexion point, identical for absorption and emission, around $E_T(30) = 48.5$ kcal mol^{-1}, marks the frontier between an aprotic and a protic medium. This shows that both molecules, Piroxicam and the betaine, have quite different solvatochromism, very likely due to the intramolecular hydrogen bond contribution in Piroxicam.

In view of the situation, one must look for a multi-parametric approach devised to assess separately the effects of both the polarity and hydrogen bonding capacity of the media. The Kamlet–Taft solvatochromic comparison method,[16] is one of the most studied and can be expressed by the equation

$$XYZ = XYZ_0 + s(\pi^* + \delta) + a\alpha + b\beta \tag{1}$$

where XYZ is a solute physicochemical quantity (in this case the absorption and emission energy maxima), XYZ_0 is that same quantity in an inert reference solvent, π^* is the solvent polarity/polarizability, α is the hydrogen bond donor acidity, β is the hydrogen-bond acceptor basicity and δ is a correction term for the solvent polarizability (this is different from zero only for aromatic solvents). The s, a and b coefficients are determined by energy maxima multiple linear regression analysis, leading in general to good correlations, (Figs. 1e, f).

$$\nu_{abs} = 87.97 - 8.10\alpha; \qquad r^2 = 0.961 \tag{2a}$$

$$\nu_{flu} = 61.02 - 2.14\alpha - 0.82\pi^* - 1.11\beta; \qquad r^2 = 0.958 \tag{2b}$$

The numerical values obtained showed: (i) the $\nu^0_{abs} - \nu^0_{flu}$ value around 9600 cm^{-1}, in agreement with the experimental values; (ii) an increase of charge localisation in the excited state with respect to the ground state, since both a and s are negative,[18,19] (iii) they also reflect the spectral bathochromic shifts with increasing π^* and α, as well as with increasing basicity (negative b); (iv) the solvent hydrogen bond donor ability is the parameter which further influences the spectral behaviour of this molecule ($a/s \gg 1$, while for the $E_T(30)$ molecule this ratio is 1.3[18,20]); (v) the decrease in a of the fluorescence enhances the importance of the intramolecular hydrogen bond, even though one cannot exclude the existence of a certain degree of covariance between the adjustable parameters; (vi) no significance was attributed to the δ parameter since a limited number of solvents with $\delta \neq 0$ were used and thus its statistical weight is meaningless.

In summary, the solvatochromic analysis reveals the existence of different spectroscopic species which are stabilised according to the solvent hydrogen bonding ability. The molecule seems to respond essentially to the solvent hydrogen donor capacity (Scheme 2). In protic solvents, although the dominant form of Piroxicam is the open conformer, the closed form also exists, similar to that reported for the case of salicylic acid.[21] There are, however, certain solvents which systematically fall off the correlations. They are aprotic solvents
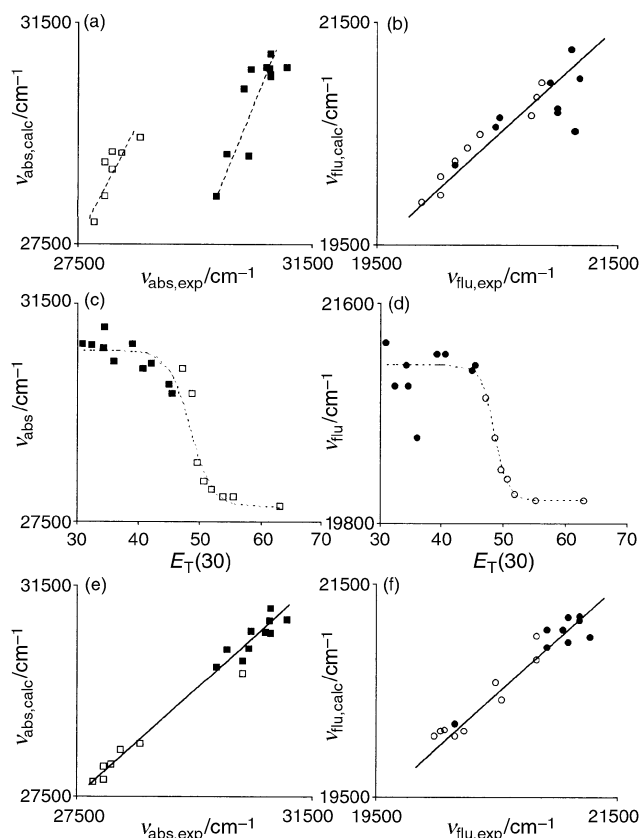

Fig. 1 Maximum absorption (■, □) and emission (○, ●) wave-numbers of Piroxicam in protic (open symbols) and non-protic (closed symbols) solvents, calculated using OBM theory, (a) and (b); against solvent $E_T(30)$ values, (c) and (d); and calculated using eqns. (2) (e) and (f).

like acetonitrile and chloroform. In this case perhaps there is not only one dominating species but a mixture of states occurs, making possible the presence of both open and closed conformers. In fact, these solvents are known to establish hydrogen bonds.[22]

Molecules such as Piroxicam, exhibiting ESIPT usually display fluorescence lifetimes of the order of picoseconds (or even femtoseconds). In fact, we have tried the measurement of fluorescence lifetimes of Piroxicam in several solvents in an apparatus with a 60 ps resolution. However, the signal-to-noise ratio was such that a clear trustworthy analysis is not feasible. The data obtained in water (protic) and cyclohexane is presented in Table 2 together with some literature values. Good agreement between cyclohexane and the reported values of toluene (non-protic solvents) is found. In water, the decay is very complex and a third, much longer component was detected, in contrast with the reported data.[6] In view of the results obtained they could not be used in a quantitative way. In microemulsions where the quantum yields of fluorescence are higher, a complete study was carried out[23,24] but, the determination of both radiative and non-radiative rate constants was not possible, since the decays were multi-exponential and an average lifetime value would not have any physical meaning in this system. Therefore, a partial

Table 2 Fluorescence decay times (τ_i) and preexponential factors (a_i) for Piroxicam decays in solvents, at 23 °C, ($\lambda_{exc} = 296$ nm; $\lambda_{emi} = 460$ nm)

Solvent	τ_1/ns	τ_2/ns	τ_3/ns	a_1 (%)	a_2 (%)	a_3 (%)	χ^2
Water (pH = 7)	0.017	1.80	0.15	97.8	1.3	0.9	1.59
(pH = 4)[6]	<0.020	3.74	—	98.0	2.0	—	—
Cyclohexane	0.058	—	0.18	95.0	—	5.0	1.60
Toluene[6]	0.060	—	0.20	92.0	—	8.0	—

study of Piroxicam photophysics was attempted using only fluorescence quantum yields assuming the approximation $\phi_f^{-1} - 1 \propto k_{nr}$ to be valid. Significant differences among the solvents studied were detected (Table 1), showing a decrease of ϕ_f for protic solvents. Nevertheless, solvents like glycerol, ethyleneglycol or cyclohexanol show relatively high values. An attempt to correlate the quantum yields with the solvent macroscopic viscosities was made. All the protic solvents fall in the same line, (Fig. 2a), which can be expressed by

$$\phi_f = \frac{c}{\eta^{\gamma}} \qquad (3)$$

where $c = -8.6 \pm 0.3$ and $\gamma = 0.62 \pm 0.05$. This power dependence on viscosity with $0 < \gamma < 1$ has been reported for different types of molecules[25] under various conditions of solvent, temperature and pressure. Several distinct interpretations have been given.[25,26] A value of γ close to unity corresponds to the Smoluchowski limit and leads to simple hydrodynamics in the Stokes–Einstein relation, while a very low γ generally means an almost total absence of viscosity dependence. Our results do not let us decide whether the process has a barrier or not. In the excited state Piroxicam can undergo intramolecular proton transfer, which is normally a very fast process. The absence of an energy barrier would lead to proton or hydrogen atom transfer rates in the sub-picosecond region; H-tunnelling through a small barrier would also lead to the same kind of picture.[27] A competitive process for tautomeric emission in a protic environment would involve breaking the hydrogen intramolecular bond. This rupture requires a relatively low energy ($10–12$ kJ mol^{-1} [28]) and it is usually an adiabatic process. Therefore, if the barrier exists it is not likely to be high.

For the complete set of solvents tested, eqn. (3) is unable to report all the changes in ϕ_f, as far as aprotic solvents are concerned. Solvent polarity must also play an important role in the case of Piroxicam. Molecules like salicylic acid or methyl salicylate also showed a lack of correlation between k_{nr} and viscosity for non-alcoholic solvents, while a moderate dependence on relative permittivity was detected.[9] Similar situations have already been reported for molecules in reactions involving dipoles or ions.[29,30] To account for this "polarity" effect, the empirical parameter $E_T(30)$ was used, giving the best results (Fig. 2b). Since $\phi_f^{-1} - 1 \propto k_{nr}$, the following empirical equation, eqn. (4), and a multiple regression analysis was applied:

$$\ln(\phi_f^{-1} - 1) = A + B \ln \eta + C/E_T(30) \qquad (4)$$

where $A = 11.5$; $B = -0.60$; $C = -178$ and $r^2 = 0.97$. This last correlation allows the apolar solvents to be in the same line as the protic ones. Once again, solvents like acetonitrile, and acetone failed to correlate. These solvents have a rather important basicity, which influences Piroxicam emission, eqn. (2b). However, $E_T(30)$ does not account for it.[31]

3.2 Mixtures of dioxane–water

The major contribution of the solvent hydrogen intermolecular bonding in the tautomeric emission of Piroxicam led us to explore the dioxane–water mixtures since several physicochemical properties of these mixtures are now well known and they are frequently used as mimetic media to study probes in membranes and interfaces.[32,33] These two solvents are miscible in all proportions thus allowing one to perform measurements in a broad range of solvent polarity. Furthermore we have also studied photophysical properties of Piroxicam in microemulsions.[5,23,24] The spectroscopic behaviour of Piroxicam was studied in a wide range of water concentrations, the probe being soluble in all of them. For lower water concentrations, the spectra resemble those in aprotic solvents, with a strong absorption band with a maximum around 329 nm. As the water concentration is increased this band is red shifted and a second one appears at higher energies similar to what happens in protic solvents. The main aspect in these mixtures is the existence of an isosbestic point ($\lambda = 290$ nm), (Fig. 3), anticipating an equilibrium in the ground-state controlled by the presence of water. This equilibrium can be expressed as:

$$Prx_N + nH_2O \underset{}{\overset{K_b}{\rightleftharpoons}} Prx_A\text{–}nH_2O$$
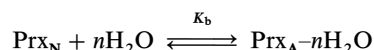
where Prx_N is the neutral form of Piroxicam (with an intramolecular hydrogen bond) and $Prx_A\text{–}nH_2O$ is the molecule where intermolecular hydrogen bonding with water competes to disrupt the intramolecular hydrogen bond; n is the number of water molecules necessary to lead the "intra" species to the
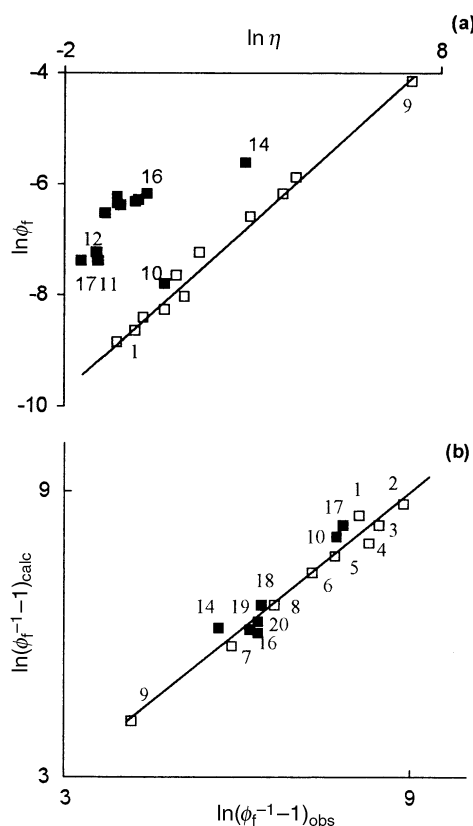

Fig. 2 Fluorescence quantum yield of Piroxicam in protic ($\square$) and non-protic solvents ($\blacksquare$), $\lambda_{exc} = 350$ nm and $T = 25\,°C$, (a) fit using eqn. (3); (b) multiple regression analysis with eqn. (4).
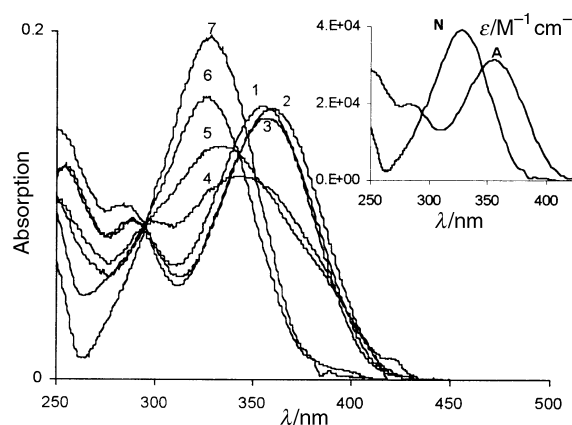

Fig. 3 Absorption spectra of Piroxicam in dioxane–water mixtures at different compositions (1–7): [water] = 55.6 M; 38.9 M; 27.8 M; 16.7 M; 11.1 M; 5.6 M; 0 M. Insert: absorption spectra of Prx_N and Prx_A (see text) calculated by global analysis using eqn. (6).

"inter" one; K_b is an equilibrium constant defined by the equation

$$K_b = \frac{[\text{Prx}_A - n\text{H}_2\text{O}]}{[\text{Prx}_N][\text{H}_2\text{O}]^n} \quad (5)$$

Considering that the absorption species detected follows a Lambert–Beer law and using a mass balance, a final expression can be derived which allows one to calculate K_b, n and the spectral band shapes of the two species involved in the equilibrium according to the expression

$$A^\lambda = [\text{Prx}]_0 \frac{\varepsilon_N^\lambda + \varepsilon_A^\lambda K_b[\text{H}_2\text{O}]^n}{1 + K_b[\text{H}_2\text{O}]^n} \quad (6)$$

where ε_N^λ and ε_A^λ represent the molar extinction coefficients of both species. The insert of Fig. 3 gives the absorption spectra of these two species following this formalism. A value of $K_b = 0.003 \pm 0.002$ M^{-1} is obtained ($n = 2.5 \pm 0.2$), which means that, e.g., for the mixture 22 M in water, 87% of Piroxicam is hydrogen-bonded. The emission spectra ($\lambda_{\text{exc}} = 350$ nm) once again show the tautomer fluorescence. Its intensity is tremendously affected by water concentration.

Absorption and emission maxima may be used to gain an insight into solvent characteristics sensed by the probe. Using eqn. (2a), the Kamlet–Taft parameter α can be directly evaluated. The results show that the ability of dioxane–water mixtures to donate hydrogen bonds (α) is very poor for lower water concentrations.[34] But, for concentrations above 25 M it increases rapidly up to a value (1.06) which is near the one reported for pure water (1.17[18,20]). Recent work indicates that α values generally have an uncertainty of ± 0.10 per unit,[35] which makes this a fair result.

Since β values for water and dioxane are quite similar (0.38 for water[36] and 0.37 for dioxane[18]), and assuming that β will not change much, one can obtain a π^* for the different solution compositions. The π^* calculated in this way predict a value of 0.59 for pure dioxane (0.55 in the literature[18]) and 1.02 for water (1.09 in the literature[18]) which are in agreement with those previously reported. π^* variation is much less significant than α, for these mixtures, probably reflecting the formation of (dioxane)$_m$(water)$_n$ complexes as evidenced by an infrared study of the vibrational modes of water in dioxane.[37] Microwave dielectric measurements[38] confirm the existence of such complexes and of a breaking point in the analysed properties of the mixture for a molar water fraction of 0.83 (≈ 25 M), above which the properties are similar to pure water with a tetrahedral structure.[39,40]

For a better comparison with results from the literature for these mixtures, one should convert them into $E_T(30)$ values. This can be achieved using the following equation:[41]

$$E_T(30) = 31.2 + 11.5\pi^* + 15.2\alpha \ (\text{kcal mol}^{-1}) \quad (7)$$

These can be compared with those found for pure solvents (Fig. 4a) and with those determined by Langhals[42] (Fig. 4b) using the following empirical relation:

$$E_T(30) = E_D \ln\left(\frac{C_p}{C^*} + 1\right) + E_T^0(30) \quad (8)$$

where C_p is the molar concentration of water, $E_T^0(30)$ is the value in dioxane and E_D and C^* are adjustable empirical parameters. From the former, one can envisage the probe sensing the possibility of establishing hydrogen bonds with the solvent "later" than the solvent is able to do it. The inflection point of the sigmoidal trend corresponds to a water concentration close to 25 M. This is also the concentration above which α approaches its value in pure water. The latter comparison reinforces this view. Above a water concentration which Langhals denominated as a "critical concentration", $C_k \approx 22$ M, there is a reasonable agreement between the two sets of results, while for lower concentrations Piroxicam reports a solvent less available to interact through hydrogen
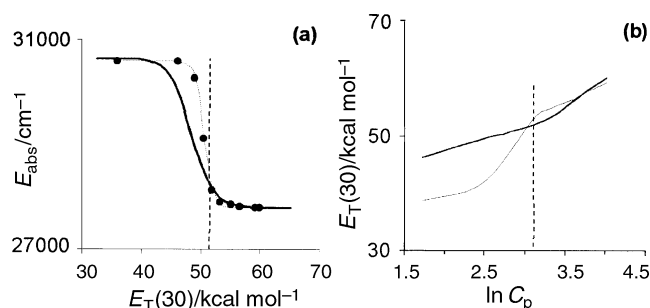

Fig. 4 (a) Absorption maxima variation with the solvent parameter $E_T(30)$ in pure solvents (bold line) and dioxane–water mixtures (dotted line); (b) comparison of Langhals $E_T(30)$ values (bold line) and those predicted by the solvatochromism of Piroxicam (thin line) for the dioxane–water mixtures.

bonding. The equilibrium between the "inter" and the "intra" conformers of Piroxicam is able to detect this transition and to report correctly the solvent characteristics above 22 M water concentration. But, below this value, it must be more favourable for water, from an energetic viewpoint, to interact with dioxane rather than to break the intramolecular hydrogen bond of Piroxicam. This would also explain the difference detected by comparison with betaine, where the intramolecular hydrogen bond is absent.

The fluorescence quantum yield of tautomeric Piroxicam ($\lambda_{\text{exc}} = 350$ nm) is strongly affected by water concentration, as was expected by what was seen in pure protic solvents. This fluorescence quenching sensed by Piroxicam, was also reported for other organic molecules like squaraines[14] and 9-anthroates.[43] The establishment of intermolecular bonds promotes the nonradiative processes via additional vibration modes with the consequent increase in internal conversion.[7] The ratio of the fluorescence quantum yield of Piroxicam in the absence ϕ_f^0, and in the presence ϕ_f, of water, are plotted against water concentration, (Fig. 5). As it is seen this Stern–Volmer plot is non-linear. Usually, this is related to the existence of static quenching. In the present case this static contribution is bound to be associated with a ground-state complex, as it was detected and accounted for by the absorption. A way of confirming the value calculated for the complex equilibrium constant K_b, would be through a plot of $(\phi_f^0/\phi_f)/(\tau^0/\tau)$ vs. [H$_2$O], which contains data not available to us.

The Stern–Volmer constant $K_{\text{SV}} = k_q\tau_O$, extracted from the fitting of all data points, is 0.56 M^{-1}. In order to have an estimate of the magnitude of the quenching rate constant, k_q, and since we do not know the fluorescence lifetime of the molecule in dioxane, we used a value close to that reported for Piroxicam in toluene (Table 2), $\tau \approx 100$ ps. In such conditions, $k_q \approx 4.3 \times 10^9$ M^{-1} s^{-1}. This value is only slightly inferior to the diffusion-controlled rate constant, k_{diff}, in the dioxane–water mixtures, which range from 5.9×10^9 to 10×10^9 M^{-1} s^{-1}, (insert of Fig. 5). This range was calculated through the use of the Smoluchowski relation, $k_{\text{diff}} = 4\pi D'R'N_a$. D' is the mutual diffusion coefficient, $D' = D_{\text{H}_2\text{O}} + D_{\text{Piroxicam}}$, $D_{\text{Piroxicam}}$ can be obtained through the Stokes–Einstein equation, where the viscosity of the mixtures[44] as well as the values of $D_{\text{H}_2\text{O}}$[45] were taken from the literature. A closer look at Fig. 5 shows the existence of two regions. One for [H$_2$O] > 25 M, where a linear trend with the total water concentration confirms that almost all the water is available to interact with Piroxicam. Secondly, for [H$_2$O] < 22 M, a clear departure from linearity is seen, revealing that not all the water is being effectively used to interact with the probe. Curiously the transition region found corresponds to a water concentration of 22–25 M, which as we saw before shows significant changes in water aggregation and coincides with the minimum detected in K_{diff}.
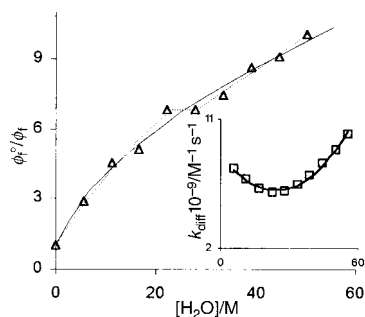
Fig. 5 Stern–Volmer plot for the quantum yield ratio against water concentration. Insert: variation of diffusional rate constant, k_{diff}, with water concentration.

4 Conclusions

The role of the solvent on the spectroscopic behaviour of the drug Piroxicam was the aim of the present study. This molecule exhibits a very high Stokes shift due to a tautomeric emission arising from an intramolecular proton transfer in the excited state. In protic solvents a second species is present, probably as an open conformer. The existence of specific interactions explains the failure of the theoretical relation of Ooshika, Bayliss and McRae and the success of the multiparametric Kamlet–Taft equation to explain the data. An empirical equation involving both viscosity and polarity ($E_T(30)$) could describe the solvent influence on ϕ_f, pointing to a process with a low barrier.

The absorption spectra of Piroxicam in dioxane–water mixtures show the existence of a ground-state complex between the probe and water. In the excited state water contributes to the quenching of the tautomer emission with a diffusion-controlled rate constant process. The π^* and α parameters of these mixtures were calculated from spectral shifts showing good agreement with reported results for a water concentration above 22 M. The data obtained confirm the picture of a critical water concentration, as proposed by Langhals,[42] above which properties similar to bulk water are sensed by the probe. These are important features to bear in mind when working with this drug in systems as reversed micelles, which are considered as mimetic of biological environments.

References

1 R. L. Miller, P. A. Insel and K. L. Melmon, in *Clinical Pharmacology*, Macmillan Publishing Co., New York, 2nd edn., 1978.
2 (a) G. Serrano, J. Bonillo, A. Aliaga, E. Gargallo and C. Pelufo, *J. Am. Acad. Dermatol.*, 1984, **11**, 113; (b) I. E. Kochevar, *Photochem. Photobiol.*, 1987, **45**, 891.
3 B. L. Diffey, T. J. Daymond and H. Fairgreaves, *Br. J. Rheumatol.*, 1983, **22**, 239.
4 R.-S. Tsai, P.-A. Carrupt, N. El Tayar, Y. Giroud, P. Andrade and B. Testa, *Helv. Chim. Acta*, 1993, **76**, 842.
5 S. M. Andrade and S. M. B. Costa, *Prog. Colloid Polym. Sci.*, 1996, **100**, 195.
6 Y. H. Kim, D. W. Cho, S. G. Kang, M. Yoon and D. Kim, *J. Luminesc.*, 1994, **59**, 209.
7 S. J. Formosinho and L. G. Amaut, *J. Photochem. Photobiol. A: Chem.*, 1993, **75**, 21.
8 A. J. G. Strandjord and P. F. Barbara, *Chem. Phys. Lett.*, 1983, **98**, 21.
9 S. Nagaoka, N. Hirota, M. Sumitani, K. Yoshihara, E. Lipczynska-Kochany and H. Iwamura, *J. Am. Chem. Soc.*, 1984, **106**, 6913.
10 H. C. Joshi, H. B. Tripathi, T. C. Pant and D. D. Pant, *Chem. Phys. Lett.*, 1990, **173**, 83.
11 M. Miranda, F. Vargas and G. Serrano, *J. Photochem. Photobiol. B: Biol.*, 1991, **8**, 199.
12 C. A. T. Laia and S. M. B. Costa, *Phys. Chem. Chem. Phys.*, 1999, **1**, 4409.
13 L. P. Novaki and O. A. El Seoud, *Ber. Bunsen-Ges. Phys. Chem.*, 1997, **101**, 105.
14 C. A. T. Laia and S. M. B. Costa, *Chem. Phys. Lett.*, 1998, **285**, 385.
15 S. R. Meech and D. Phillips, *J. Photochem.*, 1983, **23**, 193.
16 N. Mataga and T. Kubota, *Molecular Interactions and Electronic Spectra*, Marcel Dekker Inc., New York, 1970.
17 H. Laguitton-Pasquier, R. Pansu, J.-P. Chauvet, A. Collet, J. Faure and R. Lapouyade, *Chem. Phys.*, 1996, **212**, 437.
18 M. J. Kamlet, J.-L. Abboud, M. H. Abraham and R. W. Taft, *J. Org. Chem.*, 1983, **48**, 2877.
19 M. M. Velazquez, M. Valero, L. J. Rodriguez, S. M. B. Costa and M. A. Santos, *J. Photochem. Photobiol. B: Biol.*, 1995, **29**, 23.
20 J. H. Park, A. J. Dallas, P. Chau and P. W. Carr, *J. Phys. Org. Chem.*, 1994, **7**, 757.
21 S. Nagaoka, N. Hirota, M. Sumitani and K. Yoshihara, *J. Am. Chem. Soc.*, 1983, **105**, 4220.
22 C. Chudoba, E. T. J. Nibbering and T. Elsaesser, *Phys. Rev. Lett.*, 1998, **81**, 3010.
23 S. M. Andrade, S. M. B. Costa and R. Pansu, *Photochem. Photobiol.*, submitted for publication.
24 S. M. Andrade, S. M. B. Costa and R. Pansu, *J. Colloid Interface Sci.*, submitted for publication.
25 S. P. Velsko, D. H. Waldeck and G. R. Fleming, *J. Chem. Phys.*, 1983, **78**, 249.
26 H. Forster and G. Hoffman, *Z. Phys. Chem. Neue Folge*, 1971, **75**, 63.
27 A. J. G. Strandjord and P. F. Barbara, *J. Phys. Chem.*, 1985, **89**, 2355.
28 A. J. Benigno, E. Ahmed and M. Berg, *J. Chem. Phys.*, 1996, **104**, 7382.
29 T. O. Harju, J. E. I. Korppi-Tommola, A. H. Husier and C. A. G. Varma, *J. Phys. Chem.*, 1996, **100**, 3592.
30 J. Hicks, M. Vandersall, Z. Babarogic and K. B. Eisenthal, *Chem. Phys. Lett.*, 1985, **116**, 18.
31 G. M. Anstead, K. E. Carlson, P. R. Kym, K.-J. Hwang and J. A. Katzenellenbogen, *Photochem. Photobiol.*, 1993, **58**, 785.
32 M. Belletête, M. Lachapelle and G. Durocher, *J. Phys. Chem.*, 1990, **94**, 5337.
33 E. C. C. Melo, S. M. B. Costa, A. L. Maçanita and H. Santos, *J. Colloid Interface Sci.*, 1991, **141**, 439.
34 E. Casassas, G. Fonrodona and A. de Juan *J. Solution Chem.*, 1992, **21**, 147.
35 W. J. Cheong and P. W. Carr, *Anal. Chem.*, 1988, **60**, 820.
36 C. Laurence, M. Berthelot, M. Helbert and K. Sraïdi, *J. Phys. Chem.*, 1989, **93**, 3799.
37 E. Zoidis, J. Yarwood, T. Tassaing, Y. Danten and M. Besnard, *J. Mol. Liq.*, 1995, **64**, 197.
38 S. Mashimo, N. Miura, T. Umehara, S. Yagihara and K. Higasi, *J. Chem. Phys.*, 1992, **96**, 6358.
39 Y. Tominaga and S. M. Takeuchi, *J. Chem. Phys.*, 1996, **104**, 7377.
40 N. Agmon, *J. Phys. Chem.*, 1996, **100**, 1072.
41 Y. Marcus, *J. Solution Chem.*, 1991, **20**, 929.
42 H. Langhals, *Angew. Chem. Int. Ed. Engl.*, 1982, **21**, 724.
43 A. L. Maçanita, F. P. Costa, S. M. B. Costa, E. C. C. Melo and H. Santos, *J. Phys. Chem.*, 1989, **93**, 336.
44 K. Ibuki and M. Nakahara, *J. Chem. Phys.*, 1986, **84**, 6979.
45 G. Atkinson, S. Rajagopalan and B. L. Atkinson, *J. Chem. Phys.*, 1980, **72**, 3511.

Paper 9/06213K