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Naproxen: Hydroxypropyl-β-Cyclodextrin:Polyvinylpyrrolidone Ternary Complex Formation

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Abstract

The aim of the present study was to investigate the effect of the presence of the water-soluble polymer polyvinylpyrrolidone (PVP) MW = 24000 g/mol, on the complexation of the phototoxic anti-inflammatory drug naproxen, in its sodium salt form, with hydroxypropil-β-cyclodextrin (HP-β-CD). The data show that the polymer interacts with the free naproxen and with the naproxen:HP-β-CD inclusion complex. The presence of different proportions of PVP, in the 0–1%(w/w) range systematically increased the K_{app} of the naproxen:HP-β-CD inclusion complex formation. The cause of this increase is that the polymer interacts with the HP-β-CD with a binding constant of $K_2 = 29000 \pm 53\ M^{-1}$; and with the naproxen:HP-β-CD inclusion complex, to give a ternary complex naproxen:HP-β-CD:PVP. The binding constant of this process was $K_3 = 5350 \pm 1\ M^{-1}$. NMR data revealed that in the ternary system, PVP is outside of the cyclodextrin, and therefore must be wholly or partially recovering the naproxen:HP-β-CD inclusion complex.

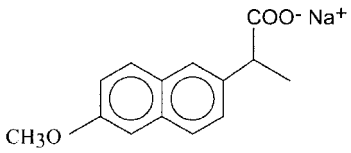
Introduction

Naproxen, (+)-6-methoxy-α-methyl-2-naphthalene-acetic sodium salt (Scheme 1), is a non steroidal anti-inflammatory typically used to treat rheumatoid and gouty arthritis. However, it can be associated with gastointestinal side-effects, drowsiness, dizziness [1] and different types of adverse cutaneous photosensitive reactions [2, 3]. These problems can be minimized through the use of suitable drug carriers. In this sense, the usual procedure is the inclusion complex formation of the drug with different cyclodextrins (CDs) [4–6].

For a variety of reasons including cost, production capability and toxicology, the amount of CDs that can be incorporated into drug formulations is limited [7]. Even under ideal conditions, CD complexation will result in a 4- to 10-fold increase in the formulation bulk. This limits the use of CD in, for example, solid oral dosage forms to potent drugs which possess good complexing properties. Likewise, the maximum CD concentration in isotonic solutions is between 20 and 25%, meaning that for some drugs a parenteral system is apparently not practical. It is, therefore, important to develop methods which can be applied in order to enhance the efficiency of drug:CD complexation [8]. It was shown that polymers interact with CDs [9], enhancing drug availability in aqueous solutions [10], solubility and dissolution rate of naproxen (in its molecular form) [11, 12].

The effect of the polymers on complexation capacity, has been little studied and the conclusions reached are different, depending on the system in question. Thus, Loftsson [7] concluded that the simple addition of the polymer without further treatment of the system has no effect on the capacity of CD to complex drugs. By contrast, Mura *et al.* [4], reported that a strong increase in the naproxen:HP-β-CD binding constant was observed when 0.1% of PVP (Scheme 2) was added.

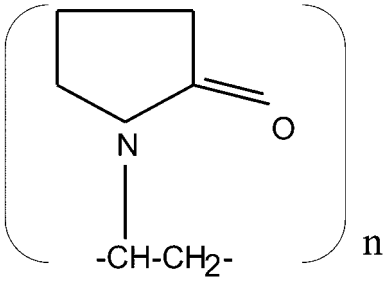
NAPROXEN



(S) 6-methoxy-α-methyl)-2-naphthaleneacetic acid, sodium salt

Scheme 1. The chemical structure of naproxen.

PVP



Scheme 2. The chemical structure of polyvinylpyrrolidone (PVP).

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Previous works carried out at our laboratory showed that the presence of PVP in naproxen:β-CD:PVP system produces a different effect on the binding constant, depending on the polymer concentration [13], whereas the presence of polyetyleneglycol (PEG) in the same system produces a decrease in the binding constant [14].

Among the different modified β-CDs, HP-β-CD and sulfoxybutylether-β-cyclodextrin are the least toxic and may be useful in the development of parenteral dosage forms of the drugs [15].

Another aspect to be considered in these systems is that the entire premise of using CD as a drug carrier is based on the concept that the CD will be released once the drug:CD complex comes into contact with a hydrophobic domain or with a target bio-polymer, such as protein [16, 17].

Despite the foregoing, recent work – reviewed by Stella *et al.* [17] – has shown that even in cases where the drug is strongly CD-bound or where dilution is minimal, drug release is facilitated by competitive displacement by endogenous materials, binding to plasma proteins, binding to tissue components, etc. Recent reports using model material (alcohols and bovin serum albumin (BSA)) support this hypothesis [5].

In the light of the above, we were first prompted to undertake a detailed absorption and steady-state emission study of the binding of the naproxen to HP-β-CD in the presence of increased amounts of PVP MW:24000 g/mol. In a second part of the study we explored the effect of different carrier systems on human serum albumin (HSA) binding, using the same spectroscopic techniques.

Material and methods

Materials

Naproxen, 2-(6-methoxy-α-methyl-2-naphthyl) propionic acid sodium salt was purchased from Sigma Chemical Co. HP-β-CD containing an average molar substitution of 0.8 hydroxypropyl groups per glucopyranose unit, was obtained from Sigma. The samples of PVP K25 (Fluka) had molecular weight of 24000 g/mol, stated by the manufacturers. These reagents were considered sufficiently well characterized by the manufacturer to be used without further purification. Water was treated with a Milli-Q system from Millipore.

HSA was purchased from Fluka and used without additional purification. Buffer salts (disodium hydrogen phosphate or the potassium dihydrogen phosphate) were analytical quality and were purchased from Sigma.

Methods

Three concentrated aqueous solutions were initially prepared:

1. 4.0×10^{-5}M naproxen/H_2O; prepared by weight and stirred;
2. naproxen/PVP/H_2O; prepared by weight of the required amount of PVP, using the aqueous drug solution (1), as solvent.
3. naproxen/HP-β-CD/PVP/H_2O: prepared by weight of the required amount of HP-β-CD, using the aqueous drug/polymer solution (2) as solvent.

Solutions with variable PVP concentrations were obtained by successive dilution of (2) with (1). Solutions with variable HP-β-CD concentrations, and constant PVP content, were obtained by successive dilution of (3) with (2). All measurements were carried out at 25.0 °C and at least 24 h after sample preparation to ensure that the equilibrium had been reached.

Study of the binding of the drug to HSA in the presence of different additives, was carried out as follow: 3 mL of naproxen in the absence or presence of the corresponding additives (naproxen 4×10^{-5} M , PVP 1%, and HP-β-CD 12×10^{-3} M) in phosphate buffer was added to a fluorimeter cuvette and allowed to equilibrate at 25 °C. 10 μL aliquots of HSA ($C = 8.21 \times 10^{-5}$ M) were added sequentially to the cuvette over the 0–5.47×10^{-6} M (final concentration) range. After each addition, the cuvette was stirred and the F_{355} was recorded.

Spectroscopy

UV-VIS absorption spectra were recorded on a Hitachi UV-VIS spectrophotometer, model 150-20. Fluorescence emission spectra were recorded on a Perkin-Elmer LS 50B Spectrofluorimeter. The instrumental response at each wavelength was corrected by means of a curve provided by the apparatus. Emission spectra were obtained in the λ_{em} = 325–450 nm range, with excitation at λ_{exc} = 317.0 nm. The spectral slits used were 2.5 and $<$ 2 nm (this value corresponds to the minimum possible width, which remains constant for any particular instrument). The fluorescence quantum yield, Φ, was determined using an expression described in previous works [18, 19].

Measurements of the refractive index were carried out using an Atago Abbè Refractometer, model DR-A1.

Results and discussion

Complexation of naproxen with HP-β-CD in the presence of PVP

The naproxen:HP-β-CD inclusion complex formation was initially investigated using absorption and emission spectroscopic techniques.

Fluorescence and absorption measurements were made on 4×10^{-5} M aqueous solution of the drug in form of sodium salt so, the drug will be in the anionic deprotonted form. The PVP concentration was kept constant in each study whereas the HP-β-CD concentration was changed in a range 0–12 mM.

As described in previous papers the absorption spectrum of the aqueous solution of the drug presents two systems of bands centered at 260, 270 nm for the first one and around 317, 330 nm for the second one. Whereas the emission spectrum presents a non-structured band centered around 355 nm [19–22].

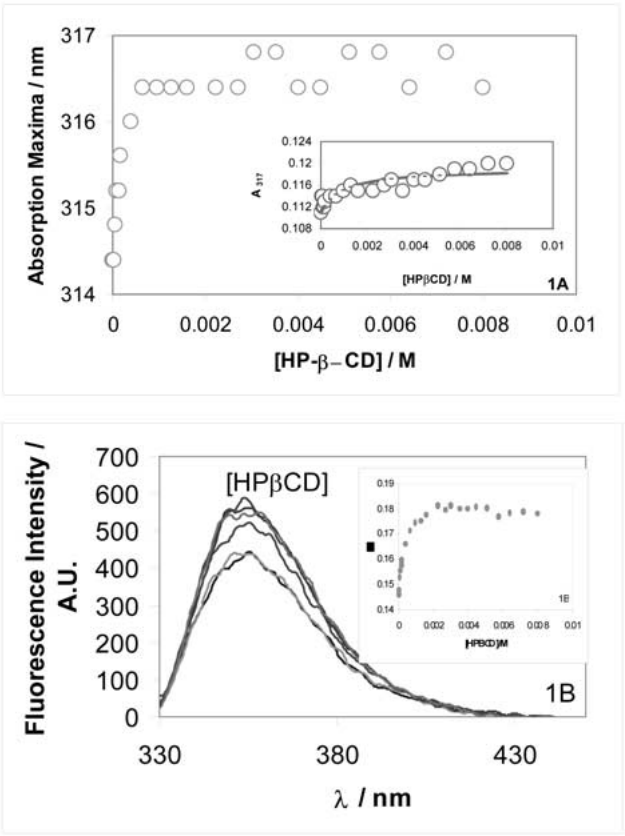

Figure 1. Effect of HP-β-CD addition on the naproxen aqueous solutions in presence of PVP A: on the absorption maxima position, inset: on the absorbance; B: on the emission spectra.

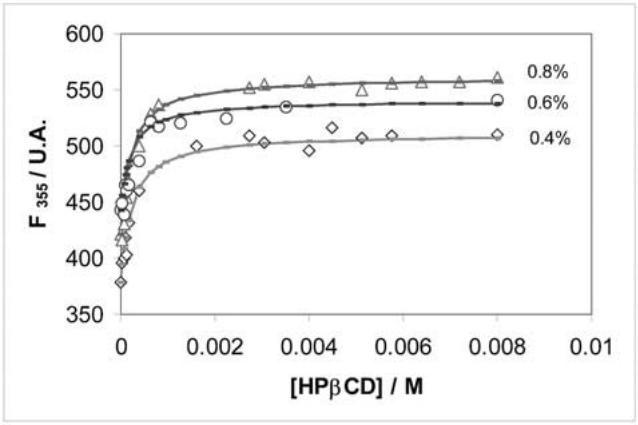

Figure 2. Fitting of the fluorescence data of the binding of the naproxen to the HP-β-CD to Equation (2).

Addition of HP-β-CD to naproxen aqueous solution containing a fixed PVP concentration, resulted in appreciable spectral changes.

It was observed that in the whole range of PVP studied, the absorption wavelength was red-shifted when increasing amounts of HP-β-CD were added (Figure 1A). As can be observed, this property reaches a plateau value, indicating that most drug molecules are included in a different microenvironment. A similar bathochromic effect has been detected in weakly polar solvents [19] and when the drug is included in the hydrophobic region of *N*-acetyl-*N*,*N*,*N*-trimethyl-ammoniumbromide (CTAB) micelles [22] and β-CD [13]. These changes clearly indicate that naproxen is changing from the polar water solution to a more hydrophobic medium, in good agreement with the inclusion of the drug within the apolar cavity of the CD. In addition a hyperchromic effect is also produced on the absorption (Figure 1A inset) and emission spectra (Figure 1B).

It is well known that the enhancement of the luminescent processes of luminophores partially or wholly encapsulated by the CD cavity is a result of better protection from quenching and other processes that occur in the bulk solvent. But taking into account that the presence of the HP-β-CD also results in an increase of the absorbance at the excitation wavelength, the hyperchromic effect on the emission of fluorescence would be due exclusively to this fact. For this reason the quantum yield of the naproxen at different HP-β-CD concentration was determined. As can be observed, Figure 1B inset, a clear increase in the quantum yield of the drug is produced by the increase in the CD concentration, what means without doubt that the CDs provide a protective effect to the drug.

Thus, the spectroscopic data show that naproxen:HP-β-CD inclusion complex is formed at all the PVP percentages studied.

When the fluorescence intensity or absorbance values are plotted as a function of [HP-β-CD] a typical binding isotherm [23] is observed (Figure 1A inset and Figure 2).

Formation of a 1:1 naproxen:HP-β-CD complex is assumed, based on previous literatura date [13, 20, 21, 24, 25].

$$\text{Naproxen} + \text{HP-}\beta\text{-CD} \Leftrightarrow \text{Naproxen:HP-}\beta\text{-CD}. \quad (1)$$

The treatment for extracting the value of K from the binding isotherm has been described previously [13, 20, 21]. Essentially the binding isotherm data are fit by the model (Equations (2) and (3)), for the emission and adsorption data, respectively

$$F = (F_D + F_B K[CD])/(1 + K[CD]) \quad (2)$$

and

$$A = (A_D + A_B K[CD])/(1 + K[CD]). \quad (3)$$

The values of the binding constant, K, of the inclusion complex formed between naproxen and HP-β-CD in the presence of PVP at different concentrations (between 0–1% (w/w)) were determined using the fluorescence intensity and absorption data at 355 nm and 317 nm respectively. A good fit of the data obtained by both techniques is obtained (Figure 1A inset and Figure 2) at all PVP studied; what confirms that the stoichiometry of the complex is 1:1 as it was observed in absence of any additive [21] and in presence of PEG [14] or β-CD with PVP [13]. The data obtained from the absorption data present more error, due to the small change produced in the absorbance. Therefore only the binding constant obtained from fitting of the fluorescence data are going to be considered.

Table 1. Binding constant of complexation of aqueous naproxen Na with HP-β-CD in presence of PVP

% PVP (w/w)	$K_{app} \times 10^{-3}$ (M^{-1})[a]
0	1.06 ± 0.053
0.01	1.52 ± 0.048
0.05	2.67 ± 0.020
0.1	3.40 ± 0.084
0.2	4.09 ± 0.112
0.3	4.42 ± 0.081
0.4	4.61 ± 0.091
0.5	4.74 ± 0.186
0.6	4.83 ± 0.113
0.7	4.89 ± 0.111
0.8	4.98 ± 0.092
0.9	4.98 ± 0.095
1	5.02 ± 0.096

[a]Ref. [21].

Table 2. Spectroscopic parameters of the Naproxen free and complexed with HP-β-CD in presence of increased amounts of PVP

	Naproxen		Naproxen:HP-β-CD	
% PVP (W/W)	Absorption maximum position nm	Φ	Absorption maximum position nm	Φ
0.01	318.0	0.226	319.2	0.286
0.05	317.6	0.212	318.8	0.276
0.1	318.0	0.343	318.6	0.366
0.2	316.4	0.206	318.0	0.246
0.3	316.4	0.182	318.4	0.230
0.4	316.0	0.2175	317.6	0.260
0.5	316.0	0.561	317.6	0.630
0.6	315.6	0.153	317.0	0.187
0.7	315.6	0.158	315.1	0.181
0.8	316.0	0.150	317.1	0.138
0.9	315.2	0.146	316.4	0.185
1.0	314.4	0.146	316.6	0.179

The association constants obtained for the formation of the naproxen:HP-β-CD inclusion complex at different PVP percentages are presented in Table 1.

The binding constant values show that the presence of PVP, produces a systematic increase in the affinity of the HP-β-CD to the naproxen, in good agreement with the data existing in the literature [4, 26]. This is an important result because the effect of several additives studied such as surfactants [27], alcohols [28] even polymers (PEG [14] or PVP [13]) produce a decrease in the binding constant. Furthermore, in general enhancements of the complexation ability and increased drug availability in CD solutions are usually obtained exclusively by heating aqueous solutions containing polymers, CD and drug in autoclave or in a sonicator but not by simple addition of the polymer. On this basis it is possible to speculate that if the system follows these procedures a more strong increase in the complexation ability must be obtained.

Some information about the origin of this increase may be obtained considering the spectroscopic parameters of the drug free in water, and when complexed with the CD in the absence and presence of different amounts of PVP. The position of absorption maxima and quantum yield of the drug at [HP-β-CD] = 0 and the asymptotic values of these parameters, taken from the curves λ and F_{355} vs [HP-β-CD] at different PVP concentrations, are included in Table 2. The first two columns correspond to the spectroscopic data of the free drug ([HP-β-CD] = 0) and the other two to the complexed drug (asymptotic part of the curves). As can be observed, the addition of PVP to an aqueous solution of free naproxen produced a blue shift in the position of the absorption maxima, indicating that its microenvironment was more polar than in water. Also, the presence of the polymer caused a quenching of the fluorescence of the drug. All the data are in good agreement with the naproxen:PVP interaction via ion–dipole through the carboxilate group described in previous works [13].

The same trend in the spectroscopic parameters was observed when the PVP was added to an aqueous solution of the naproxen complexed with HP-β-CD. As can be seen (columns 3 and 4 of Table 2), the addition of PVP leads to a shift in the absorption maxima to a lower wavelength, accompanied by a simultaneous decrease in the quantum yield of the drug. Such changes show that in these systems the drug also interacts with the PVP. It is also possible to note that at all PVP concentrations studied, the absorption maximum appeared at lower wavelengths when the HP-β-CD was present; this means that the drug was in a more hydrophobic environment than in its absence, and hence inside the CD cavity, as described previously. Moreover, the quantum yield of the drug in the systems containing HP-β-CD were higher than in its absence, showing that in these systems the drug was partially protected by its inclusion in the CD, to the effect of the PVP. Accordingly, all the foregoing data suggest that in the presence of PVP a ternary complex – naproxen:HP-β-CD:PVP – was formed in which the drug was included within the CD cavity and was interacting with the PVP; most probably *via* ion–dipole interaction through the carboxilate group, as described for the naproxen:β-CD:PVP system [15]. According to the NMR data, no change is observed in the position of the hydrogen atoms of the naphthalene ring nor on the methoxy group of the drug. This shows that the polymer in the ternary complex was not included within the HP-β-CD cavity. Therefore, the naproxen:HP-β-CD must be recovering total or partially by the PVP, as previously described for the ternary drug:β-CD:PVP complex [13].

In these conditions the binding constant values presented in Table 1, correspond to an apparent binding constant, K_{app} that is related with the equilibrium constants of the processes involved; so the K_{app} values estimated at different [PVP] can be used to obtain the equilibrium constants of these processes.

In our case, two additional equilibria must also be considered:

$$\text{PVP} + \text{HP-}\beta\text{-CD} \Leftrightarrow \text{HP-}\beta\text{-CD:PVP} \quad K_2 \qquad (4)$$

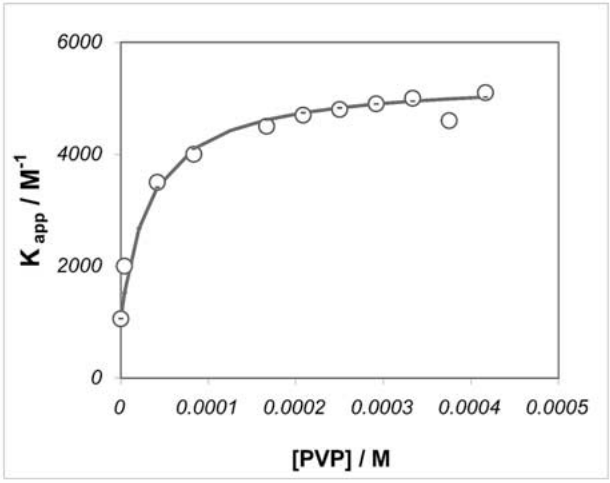

Figure 3. Fitting of Kapp, Table 1, to Equation (6).

$$\text{PVP} + \text{Drug:HP-}\beta\text{-CD} \Leftrightarrow \text{Drug:}\beta\text{-CD:PVP} \quad K_3. \qquad (5)$$

Therefore if we consider the three possible equilibria involved, Equations (1), (4) and (5), each described by its own equilibrium constant: K_1, K_2 and K_3, the relation between K_{app} and these three equilibrium constant is given by [24, 29, 30].

$$K_{app} = (K_1 + K_2K_3[\text{PVP}])/(1 + K_2[\text{PVP}]). \qquad (6)$$

As can be seen, Figure 3, a good fitting of the data to Equation (6) was obtained, clearly pointing to the formation of the ternary complex in addition to the PVP:HP-β-CD interaction. The equilibrium constants obtained from the fit were K_1 = 1060 ± 4 M^{-1}, K_2 = 29000 ± 53 M^{-1} and K_3 = 5350 ± 1 M^{-1}. In the absence of added PVP the K_{app} value corresponded to that of K_1. The K_1 value obtained in this study corresponds closely to the value of 1065 ± 53 [21] reported previously. The binding constant value of the formation of the ternary complex is in good agreement with the strong increase in the apparent binding constant of formation of the naproxen:HP-β-CD complex observed.

Binding of naproxen to HSA in the different systems

As mentioned above, the use of CDs as drug carrier is based on the concept that the drug will release once the complex comes in contact with a hydrophobic domain. But drug release is often facilitated by competitive displacement by endogenous materials. On the basis of the above observations, we continued the search, studying the binding to the HSA of the naproxen – both free and complexed with HP-β-CD in the presence and abscence of PVP. Experiments on the binding of naproxen, naproxen:PVP, naproxen: HP-β-CD, and naproxen:HP-β-CD:PVP in phosphate buffer pH = 7.0, to HSA at 25 °C were carried out. The samples were excited at 320 nm (in the systems without HP-β-CD) and 340 nm (in the systems with HP-β-CD) to avoid light absorption by the protein. Under these conditions, the protein alone exhibits no fluorescence.

Table 3. Binding constant of naproxen Na in phosphate buffer with HSA in presence and absence of HP-β-CD or/and PVP. Fluorescence maxima position of the drug in these systems with and without HSA

System	$K \times 10^{-3}/M^{-1}$	$\lambda_{fluorescence}$/nm without HSA	$\lambda_{fluorescence}$/nm with HSA
Naproxen	141.0 ± 8	354.2	354.0
Naproxen:PVP	149.0 ± 8	349.0	353.1
Naproxen:HP-β-CD	175.0 ± 9	349.0	350.7
Naproxen:HP-β-CD:PVP	183.0 ± 6	350.2	349.7

The addition of HSA to the buffered drug solution produced a variation in the intensity of the naproxen fluorescence spectrum (Figure 4A). The addition of the HSA produced a quenching of fluorescence of the drug, indicating an interaction between the fluorophore and the protein. Based on the recording F355 data, we calculate K_{app} for the binding of naproxen to HSA, assuming a 1:1 binding model (Figure 5). The value obtained (Table 3) is in good agreement with that publishing for the drug with BSA [5].

The same changes in the fluorescence spectrum were observed when HSA was added to naproxen:PVP (Figure 4B), naproxen:HP-β-CD (Figure 4C) and naproxen:HP-β-CD:PVP (Figure 4D) systems, buffered to pH = 7.0. HP-β-CD was present in sufficient amounts to ensure that the drug is in its inclusion complex form, and the PVP was in 1% w/w. As in the absence of any additive, the trend of fluorescence intensity at 355 nm – wavelength corresponding to the maximum of emission of the drug – with the HSA concentration fitted the Equation [2] well, Figure 5. The values of the binding constant of the drug to the protein in the different systems are shown in Table 3.

As can be seen, the presence of the inclusion complex increased the binding to the HSA; further, this increase was more pronounced when the ternary complex was formed. It is clear that if the HSA disrupts the complex between HP-β-CD and naproxen, the stronger the binding between them, the more difficult it would be for the protein to break it. In our case, the result was clearly the opposite, suggesting that the complex was not disrupted by the HSA. However, our results also show that binding with the protein did occur. The picture suggests that it is the complex that binds to the protein and not the free drug. The spectroscopic parameters point in the same direction. If we consider the position of the emission maximum centered around 350 nm, of the four systems in the presence and absence of HSA (Table 3) it is possible to observe that:

– No change in the maximum position occurs when the drug is free or bound to the HSA.
– In the system naproxen:PVP, the binding to HSA produces a red shift in the emission maximum of the drug as a result of the binding to the protein; but the maximum position of the drug-HSA bound does not differ from that seen in the absence of PVP.

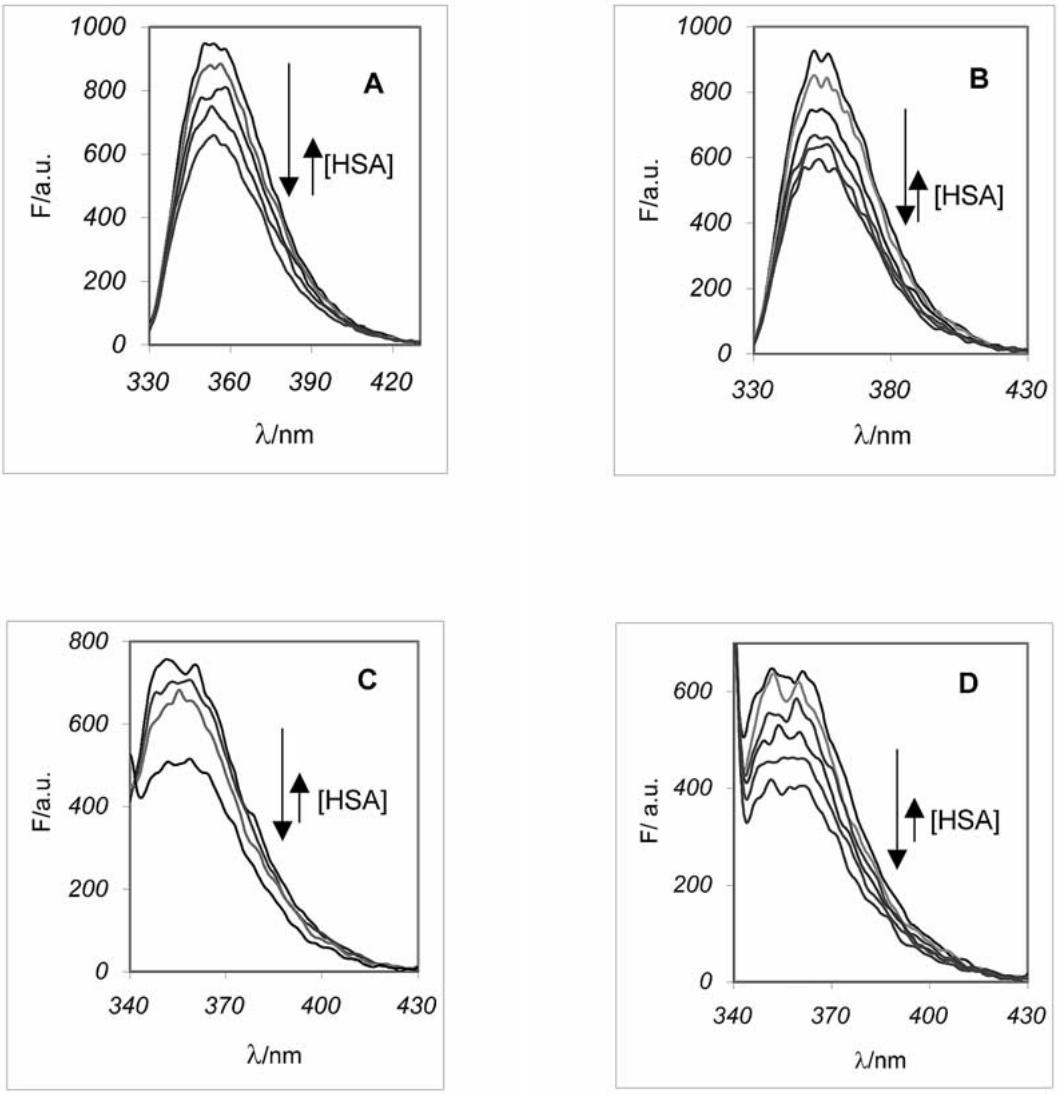

Figure 4. Fluorescence spectra of naproxen (A), naproxen:PVP (B), naproxen:HP-β-CD binary complex (C), naproxen:HP-β-CD:PVP ternary complex (D) in presence of increased amounts of HSA.

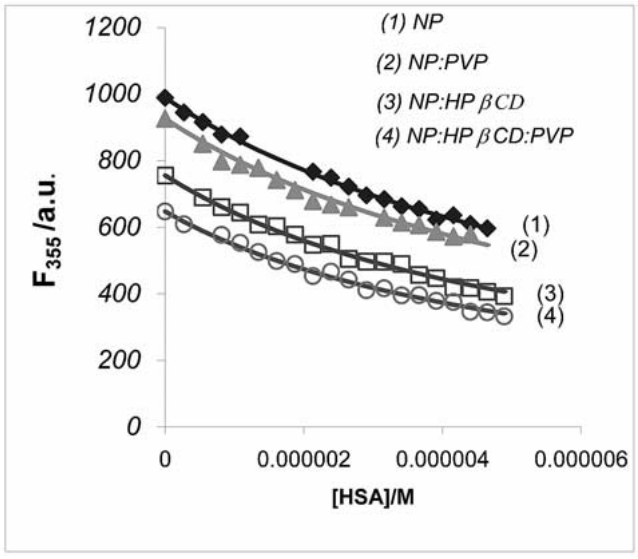

Figure 5. Fitting of the fluorescence of the binding to the HSA to Equation (2).

– In systems containing HP-β-CD (both in the absence and presence of PVP), the binding to the HSA does not change the maximum position, but the wavelength is 2 or 3 nm lower than the corresponding to when no cyclodextrin is present.

These data suggest that the drug bound to the HSA is in a different environment when the cyclodexrin is present, both in the absence and presence of PVP, whereas the same environment is detected by the drug when binding to the protein occurs in the presence or absence of PVP. All the above data thus suggest that the binary – naproxen:HP-β-CD – and ternary – naproxen:HP-β-CD:PVP – complexes bind to the HSA, while the binary one naproxen:PVP does not.

The use of CDs to suppress the photosensitizing side effect of NSAIDs is based on the drug remaining CD complexed even in the presence of bio-membranes, tissue and proteins. Clearly given the high concentrations of lipid-like domains and bio-polymers in the body, the association of

drug and CD is likely to be undetermined. Even so, there is a clear evidence that CDs do suppress the photosensitizing power of some NSAIDs [28–32]. Some authors [2, 27] have suggested that the observed reduction in bio-damage may be due in part to CD complexation of toxic photoproducts, and in part to the possibility of CD trapping of radical species formed during drug photolysis. Nevertheless, the products of drug photolysis are structurally similar to the drugs themselves, and therefore conditions that inhibit CD binding of tolmentin or naproxen should also inhibit binding of their photoproducts. Our results show that the presence of additives can modify in the interaction between species in an important way, and support some evidence about the persistence of the drug inside the CD in presence of the HSA. These observations show that the results obtained in model systems are initially a good approximation of the behaviour of the different carrier systems in the body.

Conclusions

PVP interacts with free naproxen and with the naproxen:HP-β-CD inclusion complex. The experimental data show that a naproxen:HP-β-CD inclusion complex is formed at all the PVP percentages studied with a 1:1 stoichiometry. The presence of PVP elicits a strong increase in the affinity of the HP-β-CD for the naproxen.

The increase in the apparent binding constant is due to the simultaneous formation of the binary naproxen:β-CD and ternary naproxen:HP-β-CD:PVP complexes, in addition to the PVP:HP-β-CD interaction. The equilibrium constants obtained for these processes are $K_1 = 1060 \pm 4\ M^{-1}$, $K_2 = 2.9 \times 10^4 \pm 53\ M^{-1}$ and $K_3 = 5350 \pm 1\ M^{-1}$.

The presence of the binary or ternary HP-β-CD complexes, naproxen:HP-β-CD and naproxen:HP-β-CD:PVP respectively, produces an increase in the binding constant of the drug to the HSA, whereas no change is observed when the naproxen:PVP complex is formed. The data suggest that in the presence of PVP only the drug is bound to the protein, whereas in the presence of the CD it is the inclusion complex that is bound to the HSA; this situation seems to occur both in the presence of the PVP and in its absence.

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