ELSEVIER

International Journal of Pharmaceutics 153 (1997) 211–217

international journal of pharmaceutics

Interactions of naproxen with vinylpyrrolidone and β-cyclodextrin: a fluorimetric study[1]

I. Vélaz *, M. Sánchez, C. Martín, M.C. Martínez-Ohárriz, A. Zornoza

Dpto. de Química, Sección de Química-Física, Facultad de Ciencias, Universidad de Navarra, 31080 Pamplona, Spain

Received 17 February 1997; received in revised form 7 April 1997; accepted 10 April 1997

Abstract

A spectrofluorimetric method to study the interactions of naproxen with 1-vinyl-2-pyrrolidone and β-cyclodextrin in aqueous solution has been proposed. As complexation causes appreciable spectral changes, this method enables the determination of the stability constants. Complexation with β-cyclodextrin results in an enhancement of the fluorescence of naproxen whereas 1-vinyl-2-pyrrolidone involves a quenching of fluorescence. It has been supposed 1:1 complex formation. Specifically, formation constants, enthalpy and entropy values have been obtained for the aforementioned complexes at different temperatures and pH values; their associated errors are given. © 1997 Elsevier Science B.V.

Keywords: Naproxen; 1-Vinyl-2-pyrrolidone; β-Cyclodextrin; Aqueous solution; Spectrofluorimetry; Complex stability constant

1. Introduction

Naproxen (NAP), (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid is a nonsteroidal anti inflammatory drug frequently used in the treatment of rheumatic diseases and it is characterized by a low water solubility. Its binding to hydrophilic organic molecules such as polyvinylpyrrolidone (Corrigan et al., 1985; Bettinetti et al., 1988; Maruthamuthu and Subramanian, 1992; Bettinetti et al., 1992; Bettinetti and Mura, 1994) and cyclodextrins (Erden and Çelebi, 1988; Bettinetti et al., 1989; Brown et al., 1991; Otero-Espinar et al., 1992; Loftsson et al., 1993; Wang and Warner, 1993) has been widely studied by different methods. These complexes with drugs are formed in order to improve or enhance their stability, solubility, dissolution rate, bioavailability, etc.

* Corresponding author. Tel: +34 48 425600, ext. 6368; fax: +34 48 425649; e-mail: itzvelaz@mail1.cti.unav.es

[1] A part of this work was presented at the 1st World Meeting APGI/APV. Budapest, 9–11 May 1995.

PII S0378-5173(97)00107-5

Polyvinylpyrrolidone (PVP) is a synthetic polymer constituted by linear groups of 1-vinyl-2-pyrrolidone monomers (VP). PVP is frequently used in pharmaceutical formulations with anti-inflammatory nonsteroidal drugs. Bettinetti et al. (1988) have studied the complex between naproxen and the polymer and in the determination of the complex formation constants they suppose that the interaction takes place between a molecule of the drug and the PVP monomer, but they do not prove it. One of the purposes of this work is to confirm the interaction between NAP and VP.

There are no references about the NAP–VP interaction but some authors have investigated the association between NAP and the PVP polymer in solution and in the solid state (Corrigan et al., 1985; Bettinetti et al., 1988, 1992; Bettinetti and Mura, 1994). It is known that PVP is an appropriate carrier that modifies the dissolution characteristics of naproxen advantageously.

Cyclodextrins (CD) are natural cyclic oligosaccharides which form inclusion complexes with a wide range of inorganic and organic guests as long as they have a size which suitably fits within the CD cavity. Inclusion complex formation between CDs and drugs often modifies the physical and chemical properties of the guest molecules. Interaction between NAP and β-CD in solution was investigated by phase–solubility studies, and the apparent stability constant was calculated (Erden and Çelebi, 1988; Bettinetti et al., 1989; Otero-Espinar et al., 1992). The values of the stability constants obtained in these studies are not in complete accordance, probably due to the difficulty of the solution analysis.

In the present work, a general steady-state fluorescence method is described for evaluating the formation constant for NAP–VP and NAP–β-CD complexes.

When a one-to-one complexation reaction between a substrate (S) and a ligand (L) is studied,

$$S + L \leftrightarrow SL$$

three main probable cases can be considered:

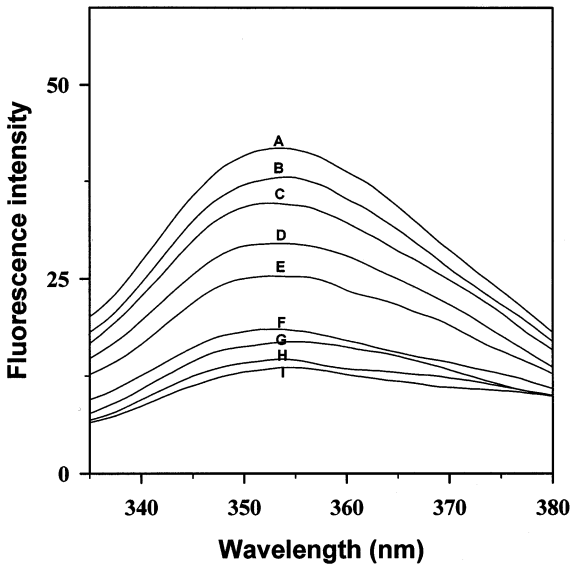

Fig. 1. Fluorescence spectra of NAP in VP aqueous solutions (pH 2). [NAP] = 2.0×10^{-6} M, [VP] (M) = 0 (A); 0.012 (B); 0.02 (C); 0.06 (D); 0.12 (E); 0.25 (F); 0.3 (G); 0.4 (H); 0.5 (I).

1.1. Case I

One of the compounds (S or L) is fluorescent but not the complex. In this case, the Stern–Volmer equation can be applied because the fluorescent substance (S, for example) is quenched by the other one (L).

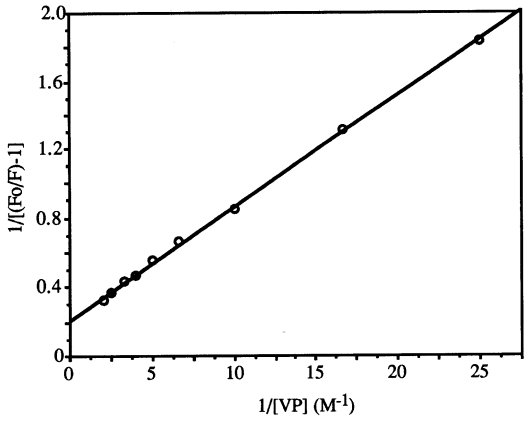

Fig. 2. Plot of the reciprocal of $(F_0/F) - 1$ against the reciprocal of [VP] obtained with NAP aqueous solutions containing VP at 25°C and pH 2.

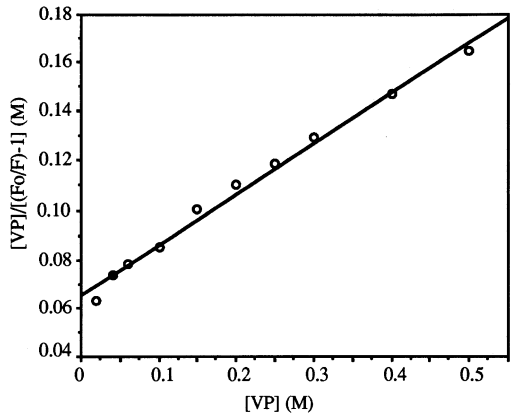

Fig. 3. Plot of $[VP]/[(F_0/F)-1]$ versus [VP] for NAP–VP system at 25°C and pH 2.

$$F_0/F = 1 + K_s[L]$$

where F_0 and F are the fluorescence intensities in absence and presence of the ligand, respectively, and [L] is the ligand concentration at equilibrium. Taking into account that, for the total ligand concentration L_t, $L_t = [L] + [LS]$, if L_t is much higher than S_t (total substrate concentration) it can be considered that $L_t = [L]$ and the resulting expression is:

$$F_0/F = 1 + K_s L_t \quad (1)$$

1.2. Case II

S and the complex are both fluorescent substances but not L, and the complex fluorescence intensity is lower than that of S. Therefore, fluorescence intensity decreases as [L] increases. When complexation takes place, the fluorescence intensity reaches a constant value which corresponds to the complex fluorescence. $S_t = [S] + [SL]$ and $L_t = [L] + [SL]$. If $L_t \gg S_t$ it can be assumed that $L_t = [L]$ and therefore the association constant is:

$$K_s = [SL]/([S]L_t) \quad (2)$$

When diluted solutions are used, $F_0 = KS_t$ and $F = K[S] + K'[SL]$, being $K = 2.3I_0\varepsilon_s\phi_s$ and $K' = 2.3I_0\varepsilon_c\phi_c$, s is referred to the substrate and c to the complex. K and K' are proportionality constants; ε is the molar absorptivity; ϕ the quantum yield and I_0 the radiation intensity. From Eq. (2), $F = K[S] + K'[S]K_sL_t$ and using the values of F_0 and F, the following equation can be inferred: where $a = K'/K = \varepsilon_c\phi_c/\varepsilon_s\phi_s$. Taking into account that $S_t = [S] + [SL]$ and combining the aforementioned equations:

$$\frac{F_0}{F} = \frac{[S] + [SL]}{[S](1 + aK_sL_t)} = \frac{1 + K_sL_t}{1 + aK_sL_t}$$

and

$$\frac{F_0}{F} - 1 = \frac{(1-a)L_tK_s}{1} + aL_tK_s \quad (3)$$

It is interesting to note that if it is a dark complex, ϕ_c and a are equal to zero. Therefore, Eq. (1) (the well-known Stern–Volmer equation) becomes a particular case of Eq. (3).

In Eq. (3), the plot of $(F_0/F) - 1$ versus L_t corresponds to a quadrangular hyperbole. In order to obtain linear plots which make the calculations easier, Eq. (3) becomes:

$$\frac{1}{(F_0/F) - 1} = \frac{a}{1-a} + \frac{1}{(1-a)K_sL_t}$$

and also:

$$\frac{L_t}{(F_0/F) - 1} = \frac{a}{1-a}L_t + \frac{1}{(1-a)K_s}$$

1.3. Case III

Substrate (S) and complex (SL) are both fluorescent, but in this case the fluorescence intensity of the complex is higher than that of S. Fluorescence increases with the ligand concentration so F_0 is lower than F. The equation obtained now is:

$$1 - \frac{F_0}{F} = \frac{(a-1)L_tK_s}{1 + aL_tK_s} \quad (4)$$

In this case, it is also possible to obtain linear plots in the same way as in Case II in order to calculate complex formation constants.

Table 1
Stability constants, (K_s/M^{-1}), for NAP–VP interaction

pH	T (°C)	8	15	20	25	35	45
2	K (M^{-1})	27.1 ± 0.8	22.8 ± 0.3	—	18.5 ± 0.1	15.3 ± 0.2	13.2 ± 0.1
7	K (M^{-1})	—	8.12 ± 0.2	7.80 ± 0.1	7.49 ± 0.1	6.92 ± 0.1	6.42 ± 0.2

2. Materials and methods

2.1. Materials

Naproxen has been kindly supplied by Syntex Latino S.A., 1-vinyl-2-pyrrolidone by Merck and β-CD by Wacker Chemie GmbH. These compounds were used as received and all the other reagents and solvents were of analytical grade. Double-distilled water was used.

All buffers were used within 1 week after their preparation. Phosphate (pH 7) and chloride (pH 2) buffers were employed. Fresh stock solutions of NAP were prepared each day.

2.2. Methods

Fluorescence studies were carried out on a Perkin-Elmer LS 50 spectrofluorimeter.

2.2.1. NAP–VP interaction in solution

Fluorescence spectra of 2.0×10^{-6} M aqueous solutions of NAP (pH 2) containing a range of concentrations of VP from 0.012 to 0.5 M were recorded. The excitation wavelength used was 330 nm and the emission one was 353 nm; the slits of the monochromators were both equal to 2.5 nm. Previously, it had been checked that VP presented neither absorption nor emission at 330 and 353 nm, respectively. A 1:1 stoichiometry for the complex has been assumed (Bettinetti et al., 1988).

Fluorescence intensities of NAP at different VP concentrations were measured at the same wavelength conditions, in 1-cm quartz cuvettes. The assays were performed in buffer solutions of pH 2 and pH 7 at different temperatures.

2.2.2. NAP–β-CD interaction in solution

The interaction of NAP with βCD was studied at different conditions of pH and temperature. NAP concentrations in each sample were always 2.0×10^{-6} M. Aqueous solutions of NAP (pH 2) containing a range of concentrations of β-CD from 0.50×10^{-4} to 2.50×10^{-4} M were used. Their fluorescence spectra were obtained at 353 nm by exciting at 271 nm.

Fluorescence intensities of NAP at different β-CD concentrations ranged from 0.4×10^{-4} up to 2.2×10^{-4} M were measured at excitation and emission wavelengths of 330 and 353 nm, respectively. The assays were carried out at pH 2, 3, 4, 5 and 6 and at different temperatures. The pH value was adjusted with H_2SO_4.

It was supposed a 1:1 stoichiometry for the complex (Bettinetti et al., 1989). This assumption is in accordance with the results reported by other authors. Brown et al. (1991) have studied the inclusion complexation of NAP with four different cyclodextrins and they have observed that the most adequate geometrical fitting occurs between naproxen and β-cyclodextrin. Besides, the examination of the Corey–Pauling–Koltun space-filling molecular models confirms that the internal diameter of the β-cyclodextrin cavity is just great enough to allow the naphthalene moiety to reside inside (Brown et al., 1991). Recent computer-aided molecular modelling studies of the NAP–β-CD complex (Otero-Espinar et al., 1992) and negative ion fast atom bombardment (FAB) measurements (Wang and Warner, 1993) confirm the existence of a 1:1 inclusion complex of naproxen and β-CD.

All the experiments were repeated at least three times. Each value is the average of five measures.

3. Results and discussion

3.1. NAP–VP interaction in solution

Fluorescence spectra of naproxen in aqueous solutions (pH 2, 25°C) at different concentrations of VP are shown in Fig. 1. It can be observed that the fluorescence intensity is decreased on addition of VP. Such changes in NAP spectrum can be attributed to the existence of an interaction between the drug and the monomer. At higher concentrations of VP fluorescence remains constant, indicating that a fluorescent complex is formed. Therefore, it is not possible to apply the Stern–Volmer equation to calculate the complex stability constant. The equation shown in Case II has been used. In addition, no broad excimer-like emission has been observed, indicating that the fluorescence does not occur from naproxen aggregates (Nakajima, 1983). It can be also seen that there is no variation in the spectral fluorescence maximum, indicating that exciplex formation does not occur (Froehlich and Wehry, 1976; Jiang and Wang, 1994)

The plot of the reciprocal of $(F_0/F)-1$ against the reciprocal of [VP] is linear (Fig. 2). The plot of $[VP]/[(F_0/F)-1]$ versus [VP] is also linear (Fig. 3). K_s can be calculated from the intercept and the slope of the straight lines. The constants obtained at different pH values and temperatures are shown in Table 1. It can be seen that the stability constants decrease as temperature increases, indicating the presence of static quenching and complex formation in the ground state. In addition, the highest complex stability occurs at low temperatures. Moreover, it is observed that the interaction is greater at pH 2, when NAP is in its acid form. It shows that an acid-base interaction takes place between NAP and VP.

Table 2
Thermodynamic parameters for the NAP–VP complex in aqueous solution

pH	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})
2	-7.25 ± 0.16	-14.50 ± 0.44	-24.29 ± 0.06
7	-4.97 ± 0.02	-6.05 ± 0.10	-3.49 ± 0.01

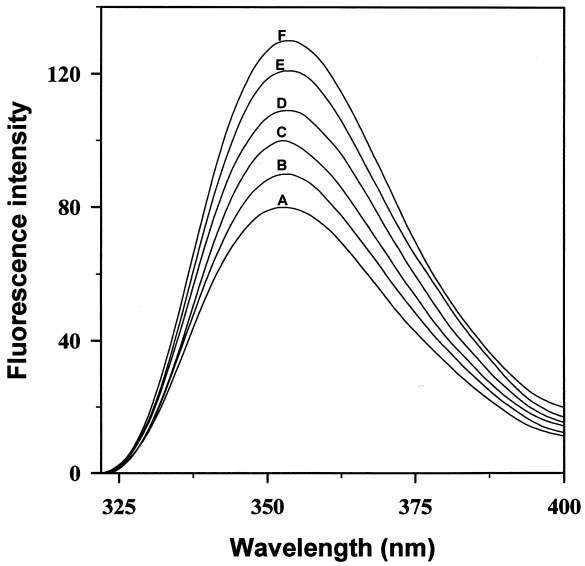

Fig. 4. Fluorescence spectra of NAP in β-CD aqueous solutions (pH 2). $[NAP] = 2.0 \times 10^{-6}$ M, $[\beta\text{-CD}] \times 10^4$ (M) = 0 (A); 0.5 (B); 1.0 (C); 1.5 (D); 2.0 (E); 2.5 (F).

Thermodynamic parameters, $\Delta H°$ and $\Delta S°$, were calculated from the temperature dependence of K_s by considering the equation $\ln K_s = (-\Delta H°/R)(1/T) + (\Delta S°/R)$ and $\Delta G°$ was obtained from $\Delta G° = \Delta H° - T\Delta S°$ (Table 2). The binding of NAP to VP is an exothermic process. The small enthalpy values are indicative of a weak interaction, mainly at pH 7. The unfavourable entropy

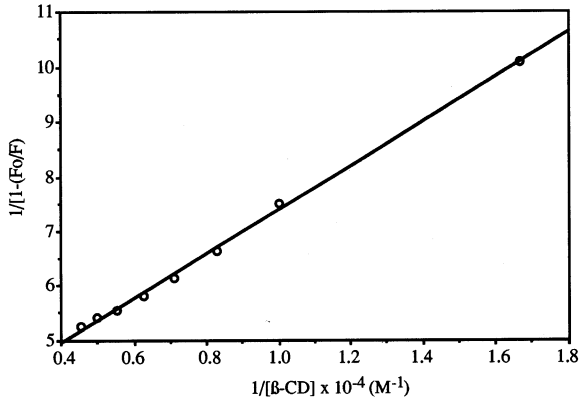

Fig. 5. Plot of the reciprocal of $(F_0/F)-1$ against the reciprocal of $[\beta\text{-CD}]$ obtained with NAP aqueous solutions containing β-CD at 25°C and pH 2.

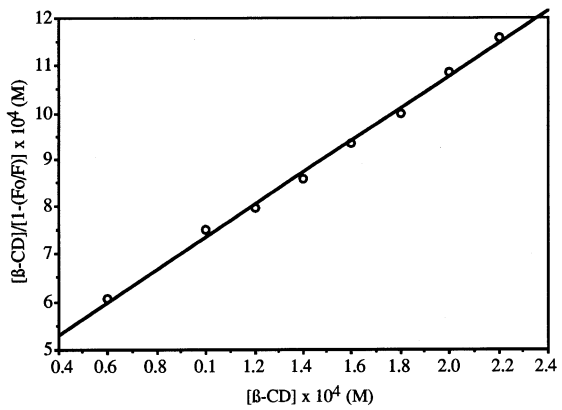

Fig. 6. Plot of $[\beta\text{-CD}]/[(F_0/F)-1]$ versus $[\beta\text{-CD}]$ for NAP–βCD system at 25°C and pH 2.

values reasonably suggest the development of a more ordered system due to complexation.

3.2. NAP–β-CD interaction in solution

The effects of β-CD concentration on the emission spectrum of NAP (pH 2, 25°C) are shown in Fig. 4. The results show that as β-CD concentration increases, there is a pronounced increase in the peak emission intensity. The inclusion of NAP in the CD cavity results in the formation of an inclusion complex which produces a significant enhancement of fluorescence.

The equation shown in Case III has been used to calculate the stability constants of the inclusion complex at different pH and temperature values. The plot of the reciprocal of $[1-(F_0/F)]$ against the reciprocal of $[\beta\text{-CD}]$ is linear (Fig. 5). The plot of $[\beta\text{-CD}]/[1-(F_0/F)]$ versus $[\beta\text{-CD}]$ is also linear (Fig. 6). K_s can be calculated from the intercept and the slope of the straight lines. The obtained constants at different pH values and temperatures are shown in Table 3. Thermodynamic parameters, $\Delta H°$, $\Delta S°$ and $\Delta G°$, were obtained as described above (Table 4). In all cases, the standard errors are given.

The relatively high negative enthalpy and negative entropy values indicate that hydrogen bonds and Van der Waals' forces together with hydrophobic interactions are the driving forces of complexation.

It is worthy of note the high pH dependence of the stability constant, because the neutral form of NAP has more affinity for the β-CD cavity than the ionized form.

It is interesting to indicate that, in both cases, according to the linear fit of the plots, it is confirmed that the ratio of the two complexes is 1:1 and that the theoretical equations and the assumptions done are valid.

Table 4
Thermodynamic parameters for the NAP–β-CD complex in aqueous solution

pH	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})
2	-21.28 ± 0.26	-33.95 ± 0.66	-41.82 ± 0.06
3	-20.95 ± 0.19	-31.04 ± 0.36	-33.31 ± 0.04
4	-20.16 ± 0.20	-29.79 ± 0.18	-31.77 ± 0.02
5	-18.41 ± 0.26	-28.06 ± 5.79	-31.85 ± 0.58
6	-17.95 ± 0.17	-25.76 ± 1.61	-25.77 ± 0.16

Table 3
Stability constants, ($K_s \times 10^{-3}$/M^{-1}), for the NAP–β-CD inclusion complex

T (°C)	pH				
	2	3	4	5	6
15	9.54 ± 0.09	7.70 ± 0.08	5.52 ± 0.27	3.01 ± 0.03	2.08 ± 0.07
25	5.73 ± 0.09	5.09 ± 0.63	3.68 ± 0.58	1.55 ± 0.04	1.49 ± 0.07
35	3.76 ± 0.29	3.35 ± 0.26	2.47 ± 0.05	1.14 ± 0.10	1.11 ± 0.00
45	2.49 ± 0.26	2.28 ± 0.12	1.71 ± 0.00	0.99 ± 0.09	0.74 ± 0.02

References

Bettinetti, G.P., Mura, P., 1994. Dissolution properties of naproxen in combinations with polyvinylpyrrolidone. Drug. Dev. Ind. Pharm. 20, 1353–1366.

Bettinetti, G.P., Mura, P., Giordano, F., Setti, M., 1992. Thermal behaviour and physicochemical properties of naproxen in mixtures with polyvinylpyrrolidone. Thermochim. Acta 199, 165–171.

Bettinetti, G.P., Mura, P., Liguori, A., Bramanti, G., 1988. Solubilization and interaction of naproxen with polyvinylpyrrolidone in aqueous solution and in the solid state. Il Farmaco 43, 331–343.

Bettinetti, G.P., Mura, P., Liguori, A., Bramanti, G., 1989. Solubilization and interaction of naproxen with cyclodextrins in aqueous solution and in the solid state. Il Farmaco 44, 195–213.

Brown, S.E., Coates, J.H., Easton, C.J., Lincoln, S.F., Luo, Y., Stephens, A.K.W., 1991. Cyclodextrin inclusion complexes of two non-steroidal antiinflammatory drugs and of an analgesic drug. Aust. J. Chem. 44, 855–862.

Corrigan, O.I., Holohan, E.M., Reilly, M.R., 1985. Physicochemical properties of indomethacin and related compounds co-spray dried with polyvinylpyrrolidone. Drug. Dev. Ind. Pharm. 11, 677–695.

Erden, N., Çelebi, N., 1988. A study of the inclusion complex of naproxen with β-cyclodextrin. Int. J. Pharm. 48, 83–89.

Froehlich, P., Wehry, E.L., The study of excited state complexes ('exciplexes') by fluorescence spectroscopy. In: Wehry, E.L. (Ed.), Modern Fluorescence Spectroscopy 2, Plenum Press, New York, 1976, pp. 346–373.

Jiang, Y.-B., Wang, X.-J., 1994. Stoichiometric-dependent intramolecular charge transfer fluorescence of *p*-dimethylaminochalcone in β-cyclodextrin host-guest systems. J. Photochem. Photobiol. A Chem. 81, 205–209.

Loftsson, T., Ólafsdóttir, B.J., Fríóriksdóttir, H., Jónsdóttir, S., 1993. Cyclodextrin complexation of NSAIDs: physicochemical characteristics. Eur. J. Pharm. Sci. 1, 95–101.

Maruthamuthu, M., Subramanian, E., 1992. Polymer-ligand interaction studies. Part I. Binding of some drugs to poly(*N*-vinyl-2-pyrrolidone). Proc. Indian Acad. Sci. Chem. Sci. 104, 417–424.

Nakajima, A., 1983. A study of the system of pyrene and β-cyclodextrin in aqueous solution utilizing the intensity enhancement phenomenon. Spectrochim. Acta 39A, 913–915.

Otero-Espinar, F.J., Anguiano-Igea, S., García-González, N., Vila-Jato, J.L., Blanco-Méndez, J., 1992. Interaction of naproxen with β-cyclodextrin in solution and in the solid state. Int. J. Pharm. 79, 149–157.

Wang, J., Warner, I.M., 1993. Studies of the naproxen–β-cyclodextrin inclusion complex. Microchem. J. 48, 229–239.