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Polymer–ligand interaction studies. Part I. Binding of some drugs to poly(N-vinyl-2-pyrrolidone)

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Abstract. A physico-chemical investigation on the binding of some nonsteroidal anti-inflammatory drugs, Naproxen (NP) and Ketoprofen (KP) and a drug model compound, salicylic acid (SA) to poly(N-vinyl-2-pyrrolidone) (PVP, molecular weight = 360,000), was performed at pH 7·1 by the fluorescence competition method employing 1-anilinonaphthalene-8-sulphonate (ANS) as the fluorescent probe. The binding affinities of these substrates to PVP are in the order KP < SA < NP which has been explained on the basis of their structural features and the consequent effect on the interacting forces. The π–π interaction between the carbonyl group of PVP and the π-ring system of the substrate molecule seems to be crucial in deciding the binding affinities of the substrates.

Keywords. Poly(N-vinyl-2-pyrrolidone); drug-binding; fluorescence competition method; π–π interaction.

1. Introduction

Studies on the binding of substrates to poly(N-vinyl-2-pyrrolidone) (PVP), a water-soluble synthetic polymer, are of interest (Molyneux and Vekavakayanondha 1986) from different angles because of the widespread applications of PVP in biology, pharmacy and industry (Blecher *et al* 1980). Pharmaceutically, PVP is used in different forms, for example, as an additive in biomedicine, as a blood-plasma substitute, as a tablet-adjuvant, as a detoxifying agent etc., the underlying principle – the reversible binding of substrates to PVP – being the same in all its uses. In our earlier studies (Maruthamuthu and Subramanian 1985, 1987, 1989, 1990) we investigated the interaction of some dyes and one fluorescent probe, 1-anilinonaphthalene-8-sulphonate (ANS) with PVP. In the present work, we have chosen some drugs, namely Naproxen (NP), Ketoprofen (KP) and salicylic acid (SA) and have examined their binding by the fluorescence competition method (Hsiao *et al* 1977) using ANS as the fluorescent probe. The present investigation is an attempt to obtain further insight into the general picture of the noncovalent interactions existing in polymer–substrate binding systems.

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2. Experimental

2.1 *Materials*

PVP (K90 grade; average molecular weight = 360,000), ANS and SA were obtained from Sigma, USA. NP and KP drug samples, without any additives, were kindly donated by Cipla Ltd., and the Pharmaceutical Company of India Ltd., respectively. Other reagents used were of commercially available analytical grade.

2.2 *Method*

The binding experiments were carried out at pH 7·1 in 0·05 mol dm^{-3} KH_2PO_4 – Na_2HPO_4 buffer at room temperature ($\approx$ 25°C). Fluorescence measurements were made with Aminco-Bowman spectrophotofluorometer fitted with 150 W xenon arc lamp, 1P21 (with SPF) photomultiplier tube and 1 cm pathlength four-sided quartz cuvette, under the following instrumental conditions: (1) excitation wavelength – 370 nm, (2) emission wavelength – 480 nm, (3) excitation beam band pass – 5 nm and (4) emission beam band pass – 11 nm. [PVP] was maintained at 1·2 × 10^{-2} mol dm^{-3} (in monomer terms) for routine experiments and at 0·12 mol dm^{-3} for determining the fully-bound condition of ANS. [ANS] was kept at minimum (2·0–10·0 μmol dm^{-3}) in order to avoid the self-quenching effect, and [competitor] (drug) was at 1·2 × 10^{-3} mol dm^{-3}. Besides fluorescence, the spectral technique (UV absorption spectrum of PVP recorded on a Carl-Zeiss spectrophotometer) was also made use of in analysing the binding characteristics of the system.

The fluorescence competition method (Hsiao *et al* 1977) used in the present study has the following principle. The intrinsic fluorescence of ANS, which is negligibly small, is considerably enhanced upon addition of PVP. But, when another substrate is added as competitor, the intensity is quantitatively reduced, provided the competitor binds to the same site of ANS. The concentration of bound ANS (C_B) could be determined from the relation,

$$C_B = \frac{F}{F_B} \times C_T, \tag{1}$$

by substituting the values of the fluorescence intensities of ANS in the partly bound (F) (in the presence and absence of competitor), and fully bound (F_B), conditions and C_T, the total initial [ANS]. An important condition in applying (1) is the invariance of the quantum yield of bound ANS (Steinhardt and Reynolds 1969) and this was found to be satisfied in the present system as evident from the isoemissive point (Anderson and Weber 1965) which was observed at 422 nm in between the fluoresence emission peaks of PVP (of course very weak) and ANS when their mixture was excited at 250 nm.

The binding data were analysed by the Klotz method (Klotz *et al* 1946) making use of the equation

$$\frac{1}{r} = \frac{1}{nKC_F} + \frac{1}{n} \tag{2}$$

where r is the ratio, C_B/[PVP], n the total number of binding sites per mole, K the

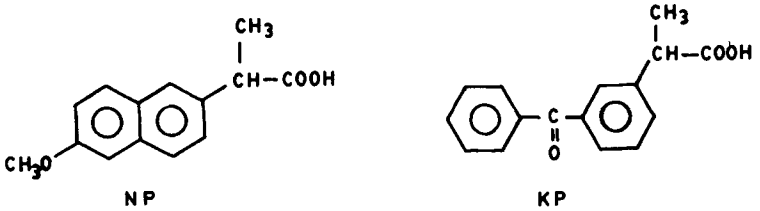
intrinsic binding constant and C_F the free or unbound [ANS]. From the slope of the Klotz plot [(1/slope) = nK], the binding constants for the PVP–ANS system in the absence ($nK = K_1'$) and in the presence ($nK = K_1''$) of the competitor were evaluated and using them, the binding constant of the competitor, K_c, was calculated using the equation,

$$K_c = \frac{1}{C_c}\frac{K_1'}{K_1''} - 1. \tag{3}$$

Here C_c is the free or unbound [competitor] which could not be experimentally determined. However, since [competitor] $\gg$ [ANS], the $[\text{competitor}]_{\text{bound}}$ would be very much less and hence the approximation $C_c \simeq [\text{competitor}]_{\text{initial}}$ becomes valid and is applied in (3) to evaluate K_c.

3. Results and discussion

Naproxen [NP, *d*-2-(6-methoxy-2-naphthyl)propionic acid] and Ketoprofen [KP, 2-(3-benzoylphenyl)propionic acid] are structurally related (scheme 1) nonsteroidal anti-inflammatory drugs. They also produce analgesic and antipyretic effects in



Scheme 1. Structure of Naproxen and Ketoprofen.

physiological systems. Salicylic acid (SA, 2-hydroxybenzoic acid) is a drug model compound and is also the starting compound for the synthesis of many other drugs, e.g. aspirin (acetylsalicyclic acid). All these compounds, NP, KP and SA, have certain common structural characteristics, i.e., the π-electron ring system and the carboxyl group. Hence, it is of interest to study the binding characteristics of these substrates with PVP.

3.1 *Fluorescence of ANS and its reduction due to competition*

The fluorescence competition method is relatively easy and less time-consuming. Further, the condition that only the bound fluorescent probe should emit fluorescence at the wavelength (480 nm) at which the fluorescence is measured but not the polymer or the competitor, was found to be obeyed in the present system. Also the intrinsic fluorescence of PVP and the free or bound drug at their respective emission wavelengths (345 and 360 nm) are negligibly small and are by no means interfering. Hence, this method was chosen for the present study. Figure 1 shows the representative fluorescence emission spectra of PVP-bound ANS in the absence (curve 1) and in the presence of the competitor, NP (curves 2–4), at different concentrations. The emission maximum of all the curves lies at about 480 nm in accordance with the literature

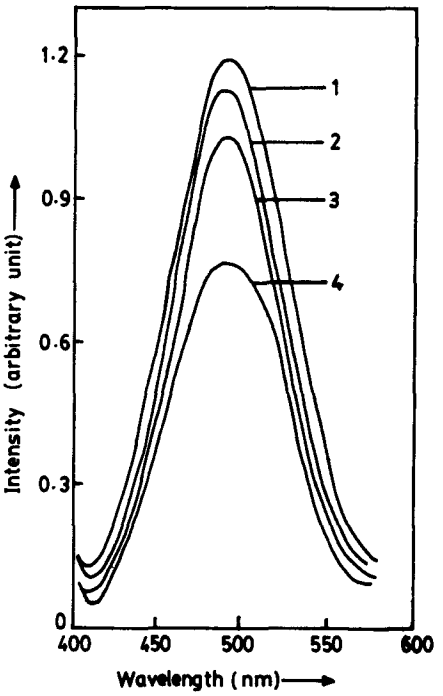


Figure 1. Fluorescence emission spectra of PVP–ANS complex in the presence and in the absence of NP. Excitation wavelength = 370 nm; pH = 7·1; [ANS] = 2·0 μmol dm^{-3}; [PVP] = 1·2 × 10^{-2} mol dm^{-3}; [NP] (in mmol dm^{-3}) = (1) 0, (2) 0·2, (3) 0·4 and (4) 1·2.

value (Kono *et al* 1970) and this does not undergo any change due to competition from the drug. Moreover, there is a gradual reduction in fluorescence intensity, as the concentration of drug is increased. The fact that the reduction in fluorescence intensity of bound ANS on adding the drug is only due to competition and not due to any intermolecular quenching was confirmed by the independent dialysis technique and also by studying the fluorescence of ANS in methanolic solution in the presence and absence of drug. It was found in the latter study that ANS (1·0 × 10^{-5} mol dm^{-3}) exhibits a strong emission at 470 nm and that this emission is not influenced by the increasing addition of drug (0·25 – 4·0 × 10^{-3} mol dm^{-3}). Further, the absorbing regions of all the three drugs do not overlap with the emission region of ANS, thus eliminating the possibility of intermolecular quenching. All the above observations therefore lead to the conclusion that the added drug competes with ANS for the binding sites in PVP and also that it causes no change in the conformational state of the polymer.

To prove that there is quantitative reduction of fluorescence intensity of the bound ANS at its various concentrations in the presence of the competitor, figure 2 is depicted, again with NP as the example for competitor. It shows the variation of fluorescence intensity with [ANS] in the fully bound (no NP) and partly bound (with/without NP) conditions. The linearity of the plots and their passage through the origin clearly indicate that there is no self-quenching effect in the present system. Further the reduction in fluorescence intensity at a particular [ANS] in presence of the competitor is a measure of the competing ability of the competitor at that particular concentration.

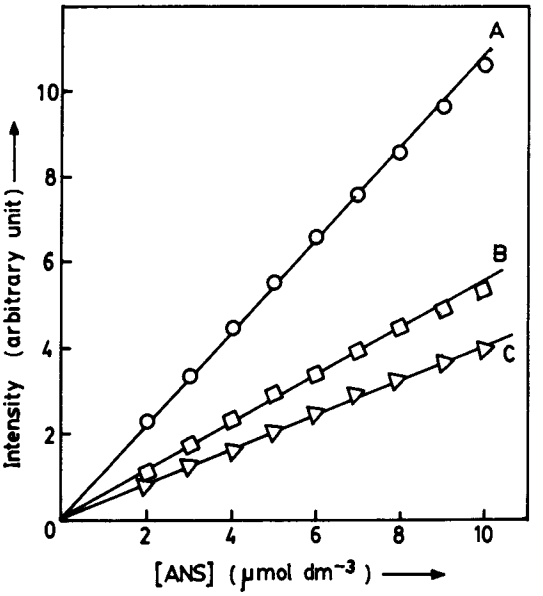


Figure 2. Variation of fluorescence intensity of PVP–ANS complex at 480 nm with [ANS]. Excitation wavelength = 370 nm; pH = 7·1; [ANS] = 2·0–10·0 μmol dm^{-3}; (A) fully bound, [PVP] = 1·2 × 10^{-1} mol dm^{-3}; (B) partly bound, [PVP] = 1·2 × 10^{-2} mol dm^{-3}; (C) partly bound, [PVP] = 1·2 × 10^{-2} mol dm^{-3}; and [NP] = 1·2 × 10^{-3} mol dm^{-3}.

3.2 *Klotz method of analysis*

Figure 3 shows the Klotz plots for the PVP–ANS and PVP–ANS/competitor systems and table 1 presents the binding parameters. All the plots are linear and have a common intercept whereby they indicate that the simple site-binding model (Klotz *et al* 1946) (binding of ligands to independent indistinguishable binding sites) is obeyed in the present system and that both the ANS and competitor have common binding sites in PVP. The very high value of $1/n$, i.e., 400 (number of monomer units in one binding site) is primarily due to the large uncertainty associated with the determination of the intercept in Klotz plots (Klotz 1950) in spite of the determination of slope being accurate. This uncertainty is an intrinsic drawback of the double-reciprocal plot to which the Klotz plot also belongs. The binding constant of ANS (K_1' = 100·03 dm^3 mol^{-1}) determined in the present study closely agrees with the value (91·24 dm^3 mol^{-1}) determined from the dialysis method of our previous work (Maruthamuthu and Subramanian 1987). This agreement shows the validity of the fluorescence method.

3.3 *Structure-affinity relationship of the competitors*

A comparison of the binding constant values of the competitors shows the order

$$KP < SA < NP.$$

Since binding constant is a measure of binding affinity, it is evident from K_c values (table 1) that KP and SA have comparable binding affinities and NP has a higher

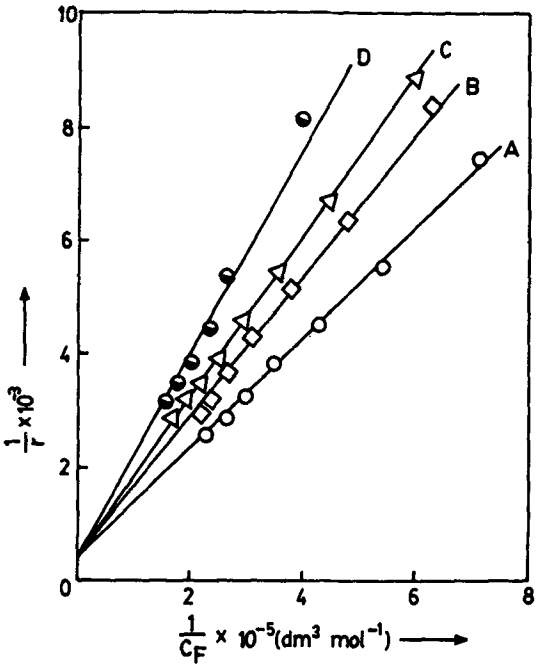


Figure 3. Klotz plots for the PVP–ANS/competitor system. pH = 7·1; [PVP] = 1·2 × 10^{-2} mol dm^{-3}; [ANS] = 2·0–10·0 μmol dm^{-3}; [competitor] = 1·2 × 10^{-3} mol dm^{-3}; (A) PVP–ANS; (B) PVP–ANS/KP; (C) PVP–ANS/SA; and (D) PVP–ANS/NP.

Table 1. Binding parameters* for the PVP–ANS/competitor system.

Temperature ≃ 25°C; [PVP] = 1·2 × 10^{-2} mol dm^{-3}; [ANS] = 2·0–10·0 μmol dm^{-3}; [competitor] = 1·2 × 10^{-3} mol dm^{-3}; pH = 7·1.

System	K_1'(dm^3 mol^{-1})	K_1''(dm^3 mol^{-1})	K_c(dm^3 mol^{-1})	1/*n*
PVP–ANS	100·03			
PVP–ANS/NP		46·06	976·44	400
PVP–ANS/KP		75·21	275·01	
PVP–ANS/SA		72·58	315·17	

*Calculated by the least-squares method

affinity (3- or 4-fold) to PVP than KP or SA. This trend in affinity is explicable on the basis of the structure of these drugs (scheme 1).

All the substrates have, in common, one anionic carboxylate group (since experimental pH = 7·1) and a nonpolar π-electron ring system. NP has the fused naphthyl ring which is large in dimension compared to the benzene ring, and also a polar methoxyl group. KP also has two benzene rings as in NP but they are not fused and are separated from each other by the intervening polar electron-withdrawing (– *M* effect) carbonyl group. This carbonyl moiety by virtue of its nature, could inhibit the effective interaction of the nonpolar benzene rings with PVP. Although NP also possesses a polar group (– OMe), it may not offer hindrance, as this electron-releasing (+ *M* effect) group is present at the extreme end of the molecule. SA, in contrast to

the other two substrates, has only one phenyl ring but it has one hydroxyl group which may form a hydrogen bond with the carbonyl group of PVP, despite its involvement in intramolecular hydrogen bonding. This intramolecular hydrogen bonding may not be operative to a considerable extent in presence of PVP, because when the phenyl ring of SA interacts with PVP, there would be, naturally, a competition between the >C=O group of PVP and the $-COO^-$ group of SA to form a hydrogen bond with the hydroxyl group.

From the above structural consideration of the substrate molecules, one would first expect that there should be a nonpolar or hydrophobic interaction between the π-ring system of the substrate and the nonpolar groups of PVP (polymer backbone and a part of the pyrrolidone ring) and a polar interaction between the $-COO^-$ group of the substrate and the positive end of the dipole of the pyrrolidone ring of PVP. This second type of interaction, however, seems to be impractical because the $-COO^-$ group in NP and KP is out of the plane of the π-ring system and, in the case of SA, it experiences competition from the adjoining ortho-hydroxyl group. Another type of interaction due to the dipole-induced dipole forces between the carbonyl group of PVP which is a permanent dipole (Molyneux 1975) ($\mu = 4{\cdot}07$ D) and the easily polarisable π-ring system of the substrate molecules, is also possible and this is denoted as the π–π interaction. This π–π interaction is dependent upon the π-electron density of the substrate molecule which, in turn, is dependent upon the electron-withdrawing or -releasing nature of the substituents. In the case of NP and SA, as we have seen earlier, there is a likelihood of increase in electron density due to the presence of –OMe and –OH groups, respectively, which are electron releasing by the $+M$ effect, although electron-attracting by the $-I$ effect; but in KP a reverse situation exists due to the presence of the electron-withdrawing ($-M$ effect) carbonyl group. Consequently the π–π interaction would be stronger with NP and SA but weaker with KP. This must be, probably, the reason for the observed order of binding constants mentioned earlier.

Spectra of PVP (figure 4) recorded in the presence of the drug by maintaining the

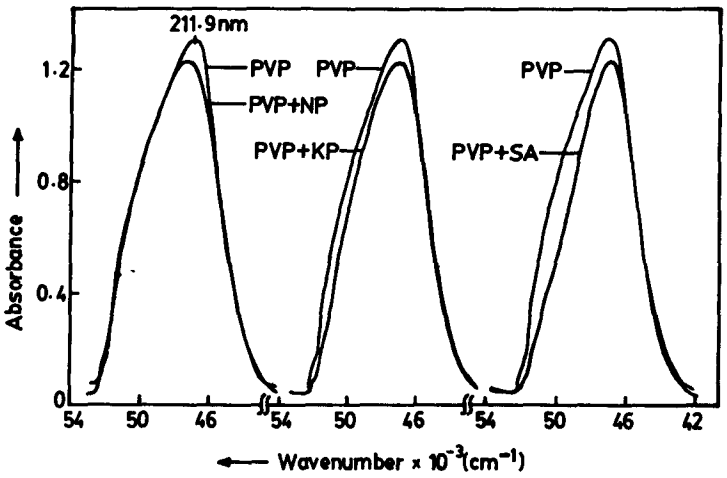


Figure 4. Spectrum of PVP with/without drug. [PVP] = $2{\cdot}5 \times 10^{-4}$ mol dm^{-3}; [drug] = $2{\cdot}5$ μmol dm^{-3}. In recording the spectrum of PVP–drug sample, the same [drug] was kept as reference.

same [drug] in both the reference and sample columns of the spectrophotometer, provide good evidence for the preceding interpretation. PVP shows a maximum at 211·9 nm ($47{\cdot}18 \times 10^3\ cm^{-1}$) which gets slightly blueshifted, unchanged and redshifted in the presence of NP, KP and SA respectively. This change in λ_{max} may not be well-pronounced but the respective ascending portion of the spectral curves documents this change clearly. The redshift and the associated large gap between the ascending portion of the curves of PVP and PVP–SA samples indicate the predominance of the polar type of interaction in the PVP–SA binding system relative to others. This is possible because SA is capable of exhibiting both hydrogen bonding and π–π interaction. KP, having an intervening carbonyl group, has a polar binding environment though not to the same extent as does SA because the carbonyl group is unable to exhibit a polar interaction like the hydroxyl group. The interaction of KP with PVP has, therefore, both polar and nonpolar characteristics. NP, on the other hand, having no such polar group as carbonyl or hydroxyl, facilitates the creation of a nonpolar binding environment which is reflected in the spectrum as the blueshift. This nonpolar environment is a favourable situation for the enhancement of π–π interaction, as the latter has the tendency to decrease if the binding system happens to interact with polar molecules such as water at its microenvironmental level. Hence the interaction of NP with PVP is maximum and is also of relatively a nonpolar type.

The emerging conclusion from the present study is that the π–π interaction is of prime importance in binding, as its contribution to interacting forces is greater, especially in the case of substrates having large π-ring systems.

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