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Interaction of 1-anilinonaphthalene-8-sulphonate with cross-linked poly(N-vinyl-2-pyrrolidone)

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Abstract. The interaction of 1-anilinonaphthalene-8-sulphonate (ANS) with cross-linked poly(N-vinyl-2-pyrrolidone) (CPVP) was studied by the adsorption technique at different temperatures and at two different pH values. Analysis by the Scatchard method and the study made in the presence of urea showed that the iceberg structure of water affects the sorption of ANS to CPVP, leading to cooperativity. The observed Giles sorption isotherms at both the pH values were of the *L*-type which indicated the interaction of ANS in flat configuration with the binding site in CPVP. The sorption of ANS to CPVP was enhanced considerably at acidic pH due to some structural factors which also resulted in multilayer sorption at this pH. Comparison of binding of ANS to CPVP and to linear poly(N-vinyl-2-pyrrolidone) demonstrated the greater contribution of hydrophobic interaction in CPVP than in the linear polymer.

Keywords. Binding; 1-anilinonaphthalene-8-sulphonate; cross-linked poly(N-vinyl-2-pyrrolidone); multilayer sorption.

1. Introduction

Poly(N-vinyl-2-pyrrolidone), a synthetic polymer is available in two different forms: (i) water-soluble linear form (abbreviated as PVP) and (ii) water-swellable cross-linked form (CPVP). Both these forms are biologically, pharmaceutically and industrially important. In our earlier study (Maruthamuthu and Subramanian 1987), we have reported the binding of the substrate, 1-anilinonaphthalene-8-sulphonate (ANS) to PVP. In the present work we report the sorption of the same substrate to CPVP.

Exceptionally high swelling pressure with water (List and Muazzam 1979), therapeutical inactivity and physiological inertness (Horn and Ditter 1982) have made CPVP a suitable tablet-disintegrator and hence it is widely used for this purpose in the pharmaceutical industry. CPVP, due to its complexing tendency with drug molecules, could affect physiological drug absorption and hence the drug activity. This complexing tendency depends on several factors such as temperature, presence of endogenous compounds in the body, variation of pH in the digestive path (in the stomach, pH = 1·4, but in the intestine, pH = 7·0) etc. Hence any binding study which takes into account some or all of these factors would be relevant and fruitful for the

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pharmaceutical application of CPVP. Keeping this in mind and taking advantage of the ability of ANS to locate hydrophobic binding sites and to indicate the microenvironmental changes of the sites (McClure and Edelman 1966), the CPVP–ANS system has been chosen for investigation. The study is made from the following points of view: (i) sorption behaviour of ANS with CPVP, (ii) interacting forces, (iii) nature of binding sites, (iv) role of the structure of water in relation to sorption, and (v) effect of pH on sorption.

2. Experimental

2.1 *Materials*

CPVP (Polyclar AT, a fine powder) and ANS were obtained from the Sigma Chemical Company, USA. Other reagents, urea, salts for buffers and HCl used were of analytical grade.

Before use, CPVP was purified by washing with repeated charges of 0·1 N NaOH and 0·1 N HCl and again with double-distilled water to remove any water-soluble UV-absorbing impurities, as checked by the UV absorption spectrum of the collections from the washings.

2.2 *Methods*

The usual adsorption technique was made use of in the present study. In a typical experiment, a known quantity (20 mg) of the purified polymer was suspended in a known volume (20 ml) of the substrate solution taken in a stoppered boiling tube and was equilibrated for about 3 h with gentle vibration in a shaker-cum-thermostatic water bath. After equilibration, the mixture was allowed to stand, thus enabling the polymer particles to settle down. It was then centrifuged in a Remi laboratory centrifuge at about 4000 rpm for 15 min. The concentration of the substrate in the centrifugate was determined spectrophotometrically in a Carl–Zeiss Specord UV VIS spectrophotometer.

Sorption experiments were performed at two pH values 7·1 (in KH_2PO_4–Na_2HPO_4 buffer) and 1·0 (in 0·1 N HCl) at different temperatures in the range 10–50°C. The effect of urea (2·0 mol dm^{-3}) on sorption was studied at pH 7·1. The [ANS] range in all the experiments was 2·5–150·0 μmol dm^{-3}

The experimental results were analysed using the Scatchard (1949) method and Giles sorption isotherms (Giles *et al* 1960). The Scatchard equation based on a simple site-binding model, i.e. the binding of substrates to independent indistinguishable binding sites on the polymer, is

$$r/C_F = nK - rK, \tag{1}$$

where r is the number of moles of ANS bound per basemole of CPVP, C_F the free or equilibrium concentration of ANS, n the total number of binding sites per basemole of CPVP, K the intrinsic binding constant and nK the interaction constant. The binding parameters were evaluated from the slope and intercept of the Scatchard plot. Giles sorption isotherms (plot of bound substrate concentration, C_B versus C_F) were utilised for deriving the sorption mechanism.

3. Results and discussion

Results obtained at pH 7·1 are discussed first, followed by those at pH 1·0.

3.1 *Studies at pH 7·1*

Scatchard plots and Giles isotherms for the CPVP–ANS system in the absence of urea are shown in figures 1 and 2, respectively, and those in the presence of urea are

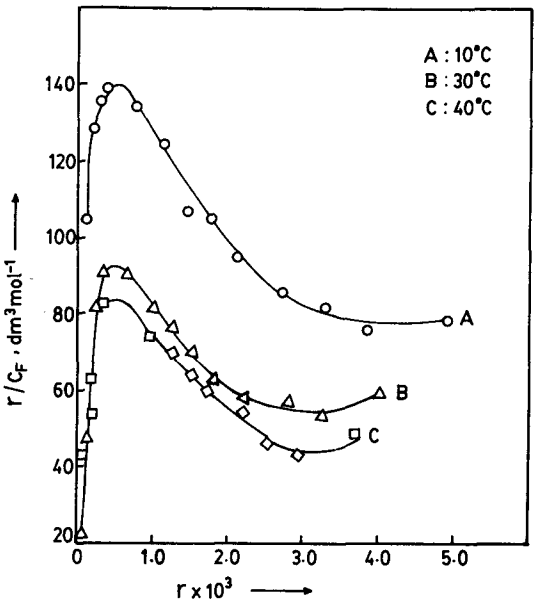


Figure 1. Scatchard plots for the CPVP–ANS system at pH 7·1. CPVP = 40 mg; V_T (total volume) = 20 ml; [ANS] = 2·5–150·0 μmol dm^{-3}.

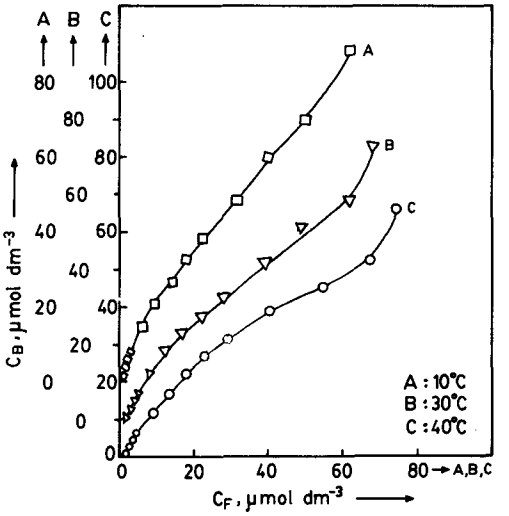


Figure 2. Giles sorption isotherms for the CPVP–ANS system at pH 7·1. Same conditions as in figure 1.

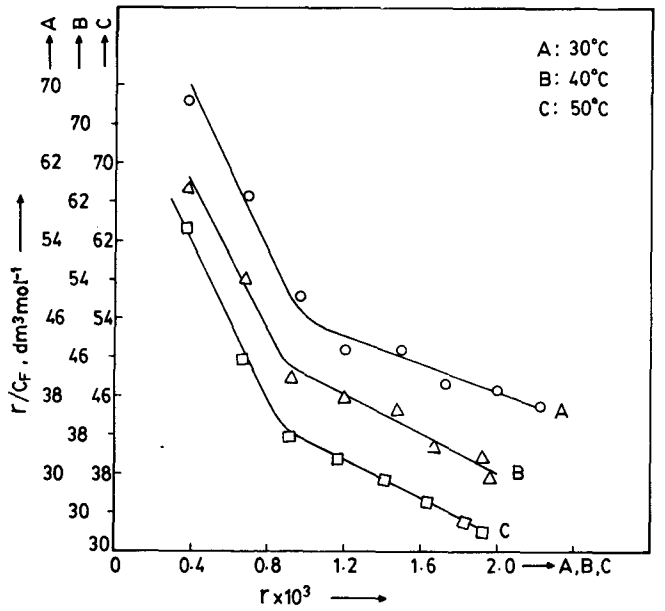


Figure 3. Scatchard plots for the CPVP–ANS system at pH 7·1 in the presence of 2·0 mol dm^{-3} urea. Other conditions are the same as in figure 1.

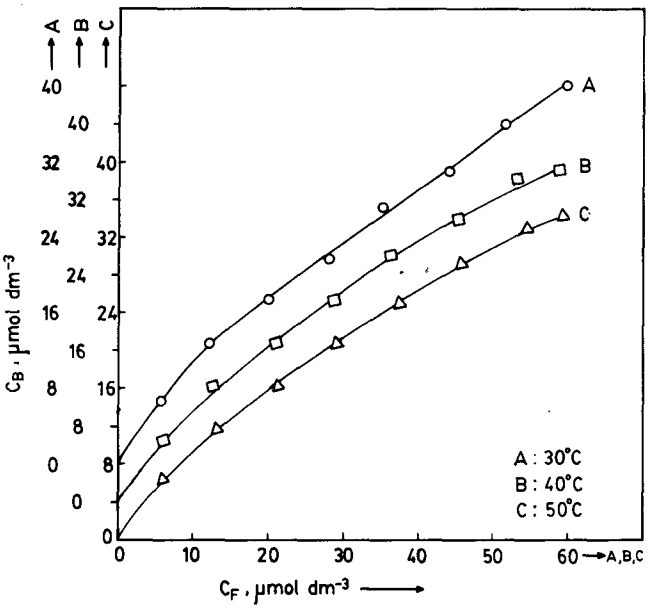


Figure 4. Giles sorption isotherms for the CPVP–ANS system at pH 7·1 in the presence of 2·0 mol dm^{-3} urea. Other conditions are the same as in figure 1.

illustrated in figures 3 and 4 respectively. As figure 1 shows, the Scatchard plots consist of initially a steep rising portion, a maximum and then a linear descending portion and finally a flat or mild rising portion. In the presence of urea, the plots have almost the same shape except the initial and final rising portions. The binding

Table 1. Binding parameters[a] for the CPVP–ANS system at pH 7·1. CPVP = 40 mg; V_T = 20 ml[b]; [ANS] = 2·5–150·0 μmol dm^{-3}.

	In the absence of urea at			In the presence of 2·0 mol dm^{-3} urea at		
Parameter	10°C	30°C	40°C	30°C	40°C	50°C
nK(dm^3 mol^{-1})	157·33	103·98	89·28	80·56	76·82	77·64
$K \times 10^{-3}$ (dm^3 mol^{-1})	30·21	21·51	16·55	31·83	34·95	39·99
$n \times 10^3$	5·21	4·83	5·39	2·53	2·19	1·94

[a] Calculated by the least squares method; [b] V_T = total volume of the substrate solution.

Table 2. Thermodynamic parameters[a] for the CPVP–ANS system at pH 7·1. Temperature = 30°C

Parameter	In the absence of urea	In the presence of 2·0 mol dm^{-3} urea
ΔH (kcal mol^{-1})	− 3·6	2·1
ΔF (kcal mol^{-1})	− 6·0	− 6·2
ΔS (eu mol^{-1})	8·00	27·48

[a] Calculated from K values.

parameters (table 1) were obtained from the slope of the descending linear portion and from the intercept on the ordinate formed by extrapolating this linear portion back to the ordinate. The thermodynamic parameters are given in table 2.

As required by (1), the Scatchard plots are not linear downward with negative slope K. This indicates that the simple site-binding model is not obeyed in the present system. Scatchard plots with a maximum have been shown (Clegg and Lindup 1984) to indicate the operation of a 'mixed' cooperativity, i.e. the ascending and descending portions of the plots represent, respectively, the positive and negative cooperativities.

3.2 *Positive cooperativity due to iceberg structure of water*

The initial rising portion of the plots (figure 1), representing positive cooperativity, appears to be due to the effect of the iceberg structure of water, surrounding the binding sites, on the sorption of ANS. The presence of this structure of water in the polymer matrix is definitely possible because (i) CPVP contains both hydrophilic and hydrophobic groups in its structure, (ii) CPVP has very great affinity for water as evident from its high swelling pressure, and (iii) a particular volume of the CPVP sample is doubled due to swelling with water as we have observed. The initial rising portion is seen at very low [ANS], as there are only a few ANS molecules in solution under this condition. Hence it is difficult for them to break the water structure, reach the binding sites and get sorbed. But once a few ANS molecules are sorbed, further sorbing molecules find it easier because the water structure has already been broken to a considerable extent. This leads to the positive cooperativity in the initial stage.

This explanation is supported by the observation of Scatchard plots in the presence of urea (figure 3). Urea, being a water-structure breaking agent (Nemethy 1967), disrupts the iceberg structure leading to loss of cooperativity and hence the absence of the initial rising portion in the Scatchard plots.

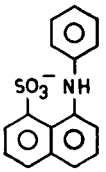
The linear descending portion of the Scatchard plots both in the presence and absence of urea may be due to the apparent anticooperativity (negative cooperativity) caused by the statistical effects (Cantor and Schimmel 1980).

3.3 *Second sorption layer of ANS at pH 7·1*

The flat or mild rising portion appearing in the final part of the Scatchard plots (figure 1) could be considered to denote the formation of a second sorption layer of ANS molecules on the already bound ANS in the first layer. This inference could also be obtained from the shape of the corresponding Giles isotherms (figure 2) which contain, especially at higher temperature, a second rise after the inflection point. This inference is further supported by the shape of the Scatchard plots (figure 3) and Giles isotherms (figure 4) observed in the presence of urea, which contain no mild rising portion or second rise respectively. Since ANS is a hydrophobic probe, the formation of a second ANS layer would mainly involve hydrophobic bonding which is inhibited in the presence of urea and hence the observed shape of the Scatchard plots and the Giles isotherms.

3.4 *Sorption mechanism at pH 7·1*

We have already observed and reported (Maruthamuthu and Subramanian 1987) that aromatic compounds containing negatively charged groups in their structures do not interact with CPVP at neutral pH. Later it was found that CPVP contains some negative centres which experience electrostatic repulsion with the negatively charged groups of the substrate molecules and hence the latter are not bound. On the other hand, ANS, an anionic substrate, is sorbed by CPVP under the same condition, i.e., at



ANS

pH 7·1. This is possible only when the sulphonato group is away from the binding sites, probably exposed to the solvent medium. A previous study (McClure and Edelman 1966) on the binding of 2-*p*-toluidinonaphthalene-6-sulphonate (a probe structurally analogous to ANS) to bovine serum albumin has also reported that the sulphonato group does not play a significant role in binding at neutral pH.

The CPVP–ANS system exhibits an *L*-type Giles isotherm (figure 2) which implies that the ANS molecules are sorbed flat with two-point attachment to the sites. The bifunctional groups which effect this two-point attachment with CPVP may be the

naphthalene and aniline rings. These two rings lie in the same plane during sorption, thus facilitating effective interaction between ANS and CPVP. This should be the actual binding situation because ANS fluoresces only when the two rings are in the same plane, and this happens during binding to polymers. The formation of a second sorption layer also (as already discussed) is possible only when ANS interacts in this flat configuration. This binding situation is further supported by the fact that both energetic and hydrophobic forces (negative ΔH and positive ΔS; table 2) operate in the CPVP–ANS system. Observation of negative enthalpy is possible when there is an effective interaction between ANS and CPVP, as could occur easily in the above binding situation.

3.5 *Studies at pH 1·0*

Figures 5 and 6 show the Scatchard plots and Giles sorption isotherms respectively for the CPVP–ANS system at pH 1·0. The shapes of these Scatchard plots and Giles isotherms are somewhat different from those observed at pH 7·1. The Scatchard plots at pH 1·0 have an initial steep rising portion with a maximum and then an almost horizontal plateau instead of the descending linear portion of the Scatchard plots at pH 7·1 (figure 1). Similarly the Giles isotherms at pH 1·0 (figure 6) do not have any well-defined inflection as the one possessed by the isotherms at pH 7·1 (figure 2) but rise steadily. The binding parameters at pH 1·0 were determined by extrapolating the horizontal plateau of the Scatchard plots to the ordinate and then evaluating the slope and intercept values. These values are given in table 3 along with the thermodynamic parameter values.

3.6 *Enhanced sorption at pH 1·0*

At this acidic pH, both the sulphonato and –NH– groups of ANS are protonated and therefore are present as $-SO_3H$ and $-\overset{+}{N}H_2-$, respectively. Further, the negative

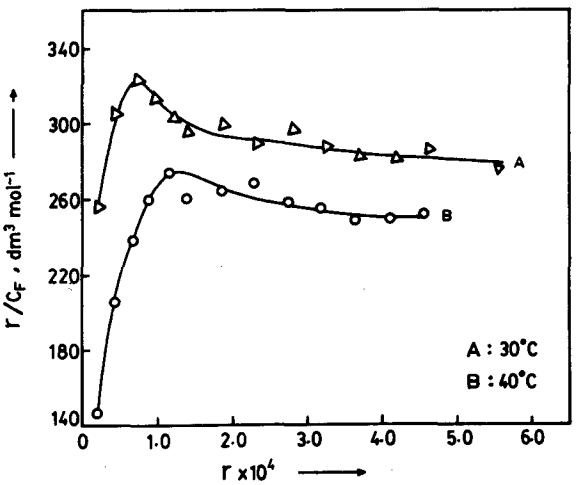


Figure 5. Scatchard plots for the CPVP–ANS system at pH 1·0. CPVP = 40 mg; V_T = 20 ml; [ANS] = 5·0–120·0 μmol dm^{-3}.

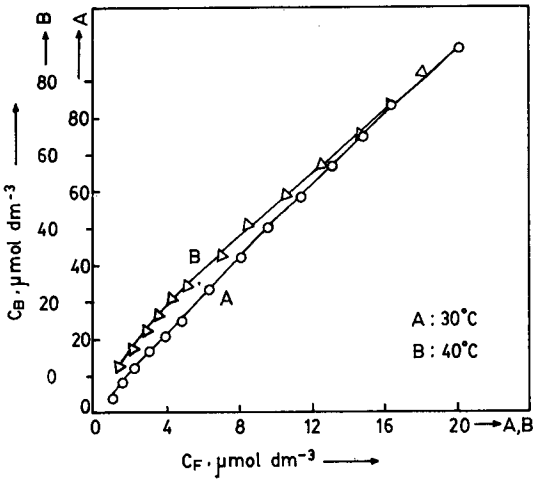


Figure 6. Giles sorption isotherms for the CPVP–ANS system at pH 1·0. Same conditions as in figure 5.

Table 3. Binding and thermodynamic parameters for the CPVP–ANS system at pH 1·0.
CPVP = 40 mg; V_T = 20 ml;
[ANS] = 5·0–120·0 µmol dm^{-3}

Parameter	30°C	40°C
nK (dm^3 mol^{-1})	303·13	278·36
$K \times 10^{-3}$ (dm^3 mol^{-1})	4·80	6·81
$n \times 10^3$	63·15	40·85
ΔH (kcal mol^{-1})	6·4	
ΔF (kcal mol^{-1})	− 5·1	
ΔS (eu mol^{-1})	37·95	

centres in CPVP, which are mainly the $-COO^-$ groups, are not present at pH 1·0. In addition to these, there is a possibility of the $-SO_3H$ group interacting with CPVP through hydrogen-bonding, since the latter contains a carbonyl group in the pyrrolidone ring, which is a good hydrogen acceptor. However the involvement of the $-\overset{+}{N}H_2-$ group in hydrogen bonding with CPVP seems to be negligible, as evident from the previous study (Horn and Ditter 1982), the reason being the poor strength of such bonding. All the above structural features are expected to enhance the sorption of ANS to CPVP at pH 1·0 relative to that at pH 7·1. In fact the observed experimental results are consistent with this expectation. Figure 7 which depicts the binding isotherms at both the pH values clearly illustrates the enhanced sorption of ANS at pH 1·0. The Scatchard plots evidently show the increased sorption at this pH. This enhanced sorption is reflected in the interaction constants also, for example, the interaction constant (nk) values at pH 1·0 (table 3) are higher than those at pH 7·1 (table 1).

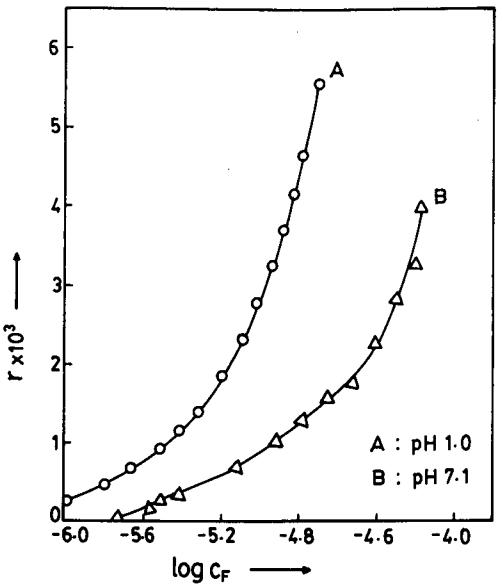


Figure 7. Binding isotherms for the CPVP–ANS system at pH 1·0 and pH 7·1. Temperature = 30°C; CPVP = 40 mg; V_T = 20 ml; [ANS] = 2·5–140·0 μmol dm^{-3}.

3.7 *Multilayer sorption at pH 1·0*

The horizontal plateau of the Scatchard plots and the steady rise without inflection of the Giles isotherms at pH 1·0 obviously demonstrate the ANS sorption being several layers deep. The enhanced sorption of ANS at pH 1·0 as compared to that at pH 7·1 also supports this inference. This multilayer sorption is definitely possible, as evident from the structural considerations. The following three are the main structural factors which can easily explain the multilayer sorption at pH 1·0.

(i) The aniline and naphthalene rings in ANS during interaction with the binding site in CPVP are planar.
(ii) These two rings in the first layer can, therefore, provide a non-polar surface for the ANS in the second layer, just as in CPVP.
(iii) The bifunctional hydrogen-bonding nature of the $-SO_3H$ group in ANS, i.e., the sulphonyl oxygen (=S=O) can interact with a hydrogen donor while the sulphonyl hydroxyl (–S–OH) can function as the hydrogen donor in hydrogen-bond formation.

These factors facilitate multilayer sorption, maintaining the original affinity of the binding sites of CPVP in the subsequent layers also. This type of multilayer sorption facilitated by the structural factors of the substrate molecule has already been reported in the study of the adsorption of *p*-nitrophenol on chromatographic alumina from benzene (Chipalkatti *et al* 1954).

In the formation of this type of multilayer, hydrophobic interaction plays a significant role. Our observation indeed proves the same. The sorption of ANS by CPVP at pH 1·0 is exclusively an entropy-driven process ($\Delta S = 37{\cdot}95$ eu mol^{-1}; table 3) but at pH 7·1, it is enthalpy- as well as entropy-controlled (table 2).

3.8 *Comparison of binding of ANS to PVP and CPVP at pH 7·1*

If nK value is considered as the basis for comparison, then it is inferred that k90PVP (molecular weight = 360,000) and CPVP are comparable. The nK value for the k90PVP–ANS system at 25°C is 91·24 $dm^3\ mol^{-1}$ (Maruthamuthu and Subramanian 1987) but that for CPVP–ANS system at 30°C is 103·98 $dm^3\ mol^{-1}$ (table 1). On comparison of thermodynamic parameters, we notice that ΔH is more or less the same but ΔS is higher for the CPVP–ANS system (ΔS^0 for the k90PVP–ANS system = 4·58 eu mol^{-1}; ΔS at 30°C for the CPVP–ANS system = 8·00 eu mol^{-1}). This comparison leads to the interpretation that the hydrophobic interaction operates to a greater extent in CPVP than in k90PVP. Such a conclusion has already been arrived at in the interaction of methyl orange homologues to CPVP (Takagishi *et al* 1980). They have shown that CPVP contains hydrophobic domains which enhance the hydrophobic contribution to the binding process.

4. Conclusions

The following conclusions can be made from the present study.

(i) Water-structure plays a significant role, as it leads to cooperativity in the sorption process.
(ii) Of the two pH's 7·1 and 1·0, the participation of the sulphonato group in binding becomes evident at pH 1·0 as revealed by the enhanced sorption at this pH.
(iii) Multilayers are formed at both the pH's but their formation is very much facilitated at pH 1·0.
(iv) Hydrophobic interaction operates to a greater extent in CPVP than in PVP.

Acknowledgement

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