

# Calorimetric determination of the enthalpy change for the binding of methyl orange to poly(vinylpyrrolidone) in aqueous solution

I. Sardharwalla\* and J. B. Lawton

Department of Biochemistry, University of Salford, Salford M5 4WT, England, UK  
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Thermodynamic parameters have been determined for the interaction of methyl orange and poly(vinylpyrrolidone) in aqueous solutions containing tris, tris and hydrochloric acid, or water only. Enthalpy changes were determined by use of a flow microcalorimeter. Free energy changes were calculated from the results of equilibrium dialysis studies. The results are interpreted in terms of the behaviour of poly(vinylpyrrolidone) in these systems. Analysis of data shows that the hydrophobic interactions in the systems are in the order: tris > water only > tris-HCl.

(Keywords: poly(vinylpyrrolidone); methyl orange; microcalorimetry; thermodynamic parameters; hydrophobic interactions; polymer configuration; dye aggregation)

## INTRODUCTION

Binding of ligands to biological polymers has been studied by a variety of techniques, for example spectrophotometry, fluorimetry, polarography and equilibrium dialysis, etc., which provide an evaluation of the binding constants. The free energy change ( $-\Delta G^\circ$ ) for many dye-anion/polycation systems has been reported, but only a few studies such as polarography<sup>1,2</sup> and equilibrium dialysis<sup>1,3,4</sup> have estimated the enthalpy ( $\Delta H^\circ$ ) and entropy ( $\Delta S^\circ$ ) changes during the interaction between the dye anion and the polycation. In the present study, for the first time, the enthalpy changes have been determined calorimetrically.

Poly(vinylpyrrolidone) (PVP) is of particular interest in biological studies in that it parallels the biopolymer serum albumin in a number of respects. Like serum albumin it displays strong binding affinity towards dissolved small molecules such as azo-dyes<sup>1,3,5,6</sup>, sodium dodecyl sulphate<sup>7</sup> and many kinds of aromatic compounds<sup>8,9</sup>, although with only one-third the affinity shown by the former<sup>5</sup>. The nature of the interactions and the thermodynamic parameters of binding of the two polymers have also been observed to be similar in many respects<sup>5,6</sup>.

Complex formation between a polymer and a small molecule is described by changes in two important parameters, enthalpy and entropy. There has been a great deal of interest in the evaluation of the contribution of hydrophobic and ionic interactions to the binding of cosolute by PVP<sup>5,10</sup>. The importance of hydrophobic interactions in the binding of anionic dyes as well as of fluorescent probes by PVP is evident in the literature. Examples include the disrupting effect of urea on the binding of methyl orange (MeO) by PVP<sup>5,10</sup>; the increasing order of binding constants and hence of free energies

of binding in the interaction of MeO and its homologues with PVP<sup>3</sup>; increase in binding constant with increase in the aromatic system<sup>8</sup>; enhancement of the fluorescence intensity on binding of 2-*p*-toluidinyl-naphthalene-6-sulphonate (TNS)<sup>11</sup> and rose-bengal<sup>12</sup> by PVP.

The objectives of the present study of the interaction of methyl orange with poly(vinylpyrrolidone) were to gain a clearer insight into the forces and the mechanisms that control the system in aqueous solutions.

## EXPERIMENTAL

### Materials

Poly(vinylpyrrolidone) (PVP; Sigma Chemical Co.) with an average molecular weight of 40 000, was purified by dialysis and the intrinsic viscosity ( $\eta$ ), at 35°C, was determined to be 22.85 ml/g<sup>13,14</sup>. Methyl orange (MeO; Eastman Kodak Co.) was purified further by recrystallization from ethanol:water mixture<sup>1</sup>. Tris-(hydroxymethyl)-methylamine (tris; BDH chemicals), and other chemicals and solvents were all of Analar Reagent Grade and were used without further purification.

### Techniques

The extent of binding of MeO by PVP was measured, at 25°C, in tris-HCl system (0.1 M; pH = 7.0), tris system (0.1 M; pH = 9.6) and glass-distilled water (pH  $\approx$  6.8) by equilibrium dialysis and calorimetric techniques. The binding was measured for solutions containing  $3.6 \times 10^{-2}$  bml<sup>-1</sup> of PVP; where bml<sup>-1</sup> = base mole of polymer per litre.

*Equilibrium dialysis.* The MeO:PVP mixtures were allowed to equilibrate in tris-HCl system, tris system and water. Blank experiments containing dye alone showed that the equilibrium had been reached within the time allowed<sup>15</sup>.

\* Present address: Department of Biological Sciences, City of London Polytechnic, London E1 7NT, England

**Calorimetry.** Calorimetric studies were carried out using an LKB 2107 Flow micro-calorimeter. Before starting a calorimetric experiment, microcalorimeter was allowed to equilibrate to 25°C and was calibrated<sup>15</sup>.

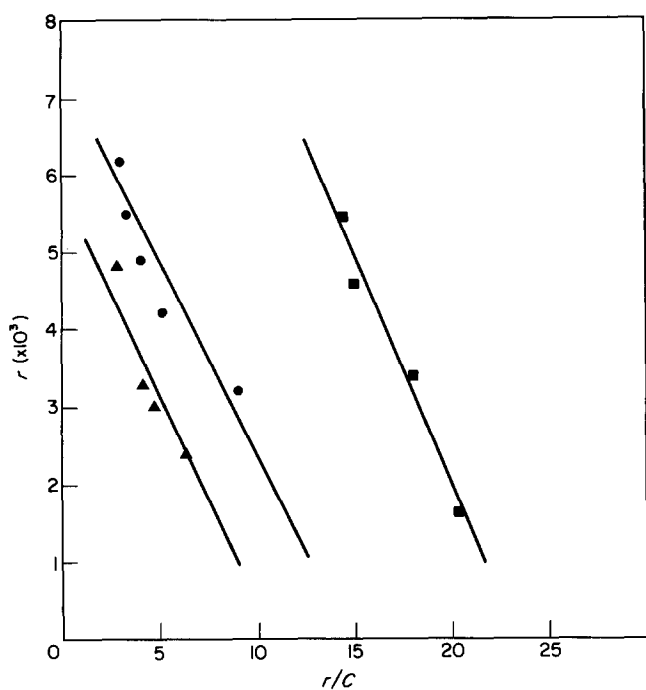
The heat measurements were carried out using a flow-mixing cell and the heat flow was recorded on a chart recorder as a deflection of the base line. The heat measurements were reproducible to within 1%. The accuracy of heat measurements was determined by diluting a standard solution of tris-HCl buffer (0.1 M; pH=7.0) with distilled water.

**RESULTS**

*Equilibrium dialysis*

The experimental results, where the extent of binding is expressed in terms of a base mole of PVP, are illustrated in Figure 1; *r* is moles of MeO bound per base mole of PVP and *C* is the concentration of free MeO at equilibrium. These values have been used to evaluate, by Langmuir isotherm as developed by Scatchard<sup>16</sup>, the equilibrium constant, *K*, and the number of moles of MeO per base mole of PVP, *n*, for each buffer system. The values of *K* and *n* have been calculated by means of the 'least squares' method. The free energy changes ( $-\Delta G^\circ$ ) of MeO:PVP binding process have been arrived at by the equation:

$$\Delta G^\circ = -RT \ln K \tag{1}$$



**Figure 1** Relationship between *r* and *r/C* for binding of methyl orange by poly(vinylpyrrolidone) at 25°C: ●, water; ■, tris-HCl buffer; ▲, tris system

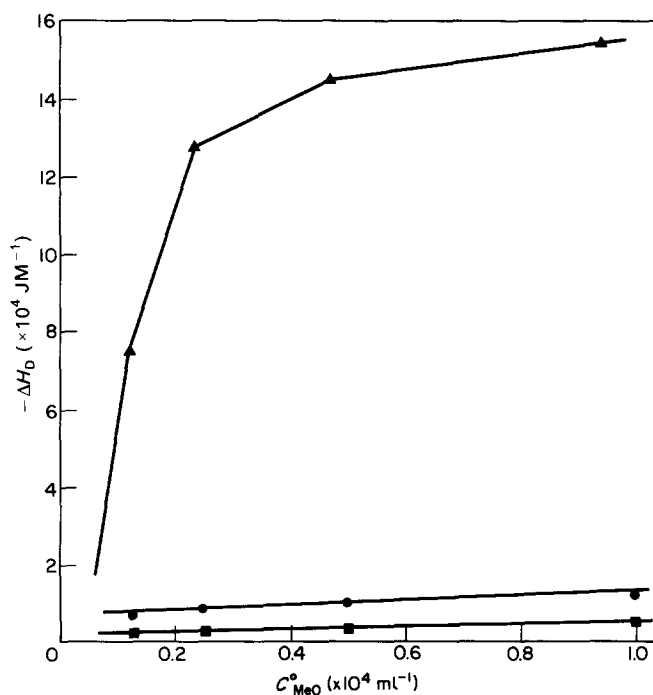
where *R* and *T* are the gas constant and the absolute temperature (K), respectively. The binding parameters and the free energy changes for each system are presented in Table 1.

Two interesting features of the MeO-PVP complex formation in these systems are evident from the binding data obtained. Firstly, that the amount of MeO bound by PVP decreases in the order: tris-HCl > water > tris (Figure 1) and secondly, that the equilibrium constant, *K*, decreases in the order: water > tris-HCl > tris (Table 1).

**Calorimetry**

*Enthalpy of dilution.* The heat of dilution of any solution to infinite dilution can be determined experimentally by a series of measurements followed by extrapolation. To measure heat change accompanying the actual dilution of a given solution by an infinite amount of pure solvent is, of course, not practicable. The procedure employed is to dilute a given solution in stages by using finite amounts of pure solvent. The heat changes in the various states are additive and from the result it is possible to derive the heat of dilution from a given concentration to any required concentration<sup>15</sup>.

The enthalpies of dilution,  $\Delta H_D$  (J M<sup>-1</sup> or J bm<sup>-1</sup>), of MeO and PVP in the three systems have been extrapolated by this procedure and are illustrated in Figures 2 and 3, respectively. It can be seen from these Figures that the



**Figure 2** Enthalpies of dilution of MeO, at 25°C, to a final concentration of  $0.06 \times 10^{-3}$  M: ●, water; ■, tris-HCl; ▲, tris system

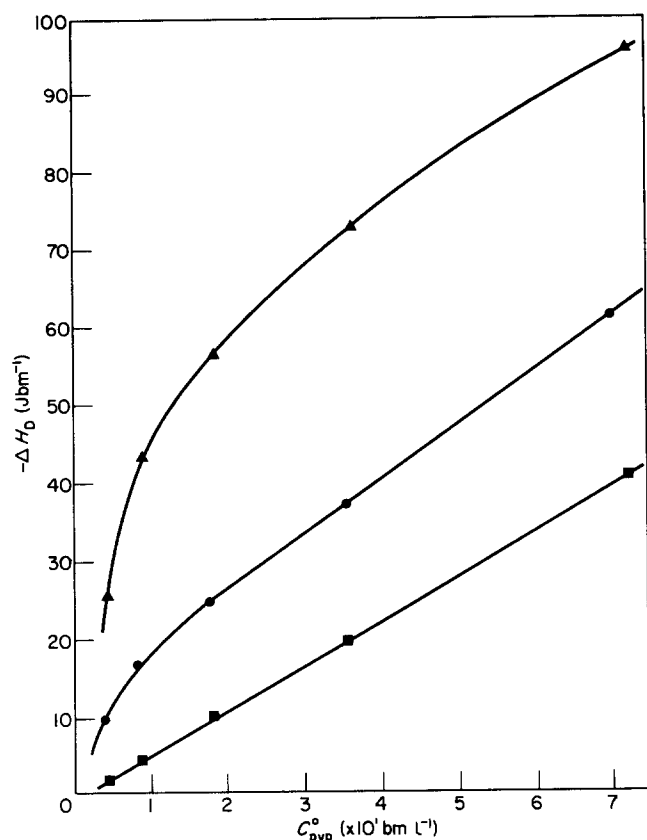
**Table 1** Binding constants and free energy changes of MeO-PVP complexes obtained by Scatchard treatment

| $C_p^\circ$<br>(x 10 <sup>2</sup> bml <sup>-1</sup> ) | Buffer system | $K^\dagger$<br>(x 10 <sup>-2</sup> mol <sup>-1</sup> l) | $\Delta G^\circ \dagger$<br>(x 10 <sup>-3</sup> JM <sup>-1</sup> ) | $n^\dagger$<br>(x 10 <sup>2</sup> ) |
|---|---------------|---|--|-------------------------------------|
| 3.6   | tris-HCl      | 16.0  | -18.3  | 1.44                                |
| 3.6   | tris          | 13.3  | -17.8  | 0.67                                |
| 3.6   | water         | 20.2  | -18.9  | 0.72                                |

\*  $C_p^\circ$  is the initial concentration of PVP  
 † measurements at 25°C

enthalpy of dilution of both MeO and PVP are exothermic over the measured concentration range and that the exothermicity of dilution for the three systems is in the order: tris > water > tris-HCl.

**Enthalpy of reaction.** The enthalpies of dilution of MeO



**Figure 3** Enthalpies of dilution of PVP, at 25°C, to a final concentration of  $0.2 \times 10^{-1}$  bm: ●, water; ■, tris-HCl; ▲, tris system

( $\Delta Q_d$ ) and PVP ( $\Delta Q_p$ ) and the enthalpies of reaction ( $\Delta Q_R$ ), all in joules/hour, for the three MeO-PVP systems are given in Tables 2 to 4. The  $\Delta Q_d$  and  $\Delta Q_p$  have been calculated by the expressions:

$$\Delta Q_d = \Delta L_d \times C_d^0 \times n_d \quad (2)$$

$$\Delta Q_p = \Delta L_p \times C_p^0 \times n_p \quad (3)$$

where  $\Delta L$  ( $J M^{-1}$  or  $J bm^{-1}$ ) is the relative molar enthalpy for the change from the initial concentration ( $C^0$ ) to the final concentration ( $C$ ), after dilution.  $C^0$  and  $n$  are the initial concentration ( $M l^{-1}$  or  $bm l^{-1}$ ) and the flow rate ( $l h^{-1}$ ) respectively, of the dye or the polymer.

The  $\Delta Q_R$  has thus been arrived at by the expression:

$$\Delta Q_R = (\Delta Q_R^{obs}) + (\Delta Q_d) + (\Delta Q_p) \quad (4)$$

where  $\Delta Q_R^{obs}$  ( $J h^{-1}$ ) is the observed heat of reaction.

Three points may be concluded from the data presented in Tables 2 to 4. Firstly, that the  $\Delta Q_R$  for all three systems has an exothermic value. Secondly, the exothermic nature of  $\Delta Q_R$  is a function of the initial concentration of MeO and decreases with a decrease in concentration, and finally, that the exothermic  $\Delta Q_R$  for the three systems is in the order: tris-HCl > water > tris.

**Evaluation of the thermodynamics of binding.** The thermodynamic parameters for the binding of MeO by PVP are presented in Tables 2 to 5.

The enthalpy of binding,  $\Delta H_B$  ( $J M^{-1}$ ), for MeO-PVP complex formation, has been calculated by the relationship:

$$\Delta H_B = \Delta Q_R / \sigma \quad (5)$$

where  $\sigma$  is the amount of dye bound in moles/hour. The value of  $\sigma$  has been evaluated from the equilibrium dialysis data by the expression:

**Table 2** Thermodynamic parameters for the binding of methyl orange by poly(vinylpyrrolidone) in water (pH  $\approx$  6.8) at 25°C

| $C_p^0$<br>( $\times 10^2$ bm<br>$l^{-1}$ ) | $C_d^0$<br>( $\times 10^4$<br>$M l^{-1}$ ) | $\Delta Q_p$<br>( $\times 10^2$<br>$J h^{-1}$ ) | $\Delta Q_d$<br>( $\times 10^2$<br>$J h^{-1}$ ) | $\Delta Q_R$<br>( $\times 10^2$<br>$J h^{-1}$ ) | $\sigma$<br>( $\times 10^6$<br>$M h^{-1}$ ) | $\Delta H_B^\dagger$<br>( $\times 10^{-3}$<br>$J M^{-1}$ ) | $\Delta G^\circ$<br>( $\times 10^{-3}$<br>$J M^{-1}$ ) | $\Delta S^\circ$<br>( $J \cdot deg^{-1}$<br>$M^{-1}$ ) |
|---|--|---|---|---|---|--|--|--|
| 3.59  | 27.2                                       | -0.60   | -14.9   | -1.90   | 2.67  | -7.12  | -18.9  | 39.5   |
| 3.60  | 22.1                                       | -0.60   | -18.4   | -1.89   | 2.66  | -7.11  | -18.9  | 39.6   |
| 3.61  | 17.0                                       | -0.61   | -18.6   | -1.83   | 2.56  | -7.15  | -18.9  | 39.4   |
| 3.62  | 12.0                                       | -0.60   | -12.8   | -1.62   | 2.29  | -7.08  | -18.9  | 39.7   |
| 3.58  | 7.1  | -0.60   | -4.3  | -1.32   | 1.84  | -7.17  | -18.9  | 39.4   |

\*  $C_p$  and  $C_d$  are the concentrations of PVP and MeO, respectively, in the flow-mixing cell of the microcalorimeter

†  $\Delta H_B \equiv \Delta H^\circ$

**Table 3** Thermodynamic parameters for the binding methyl orange by poly(vinylpyrrolidone) in tris-HCl system (0.1 M; pH = 7.0) at 25°C

| $C_p^0$<br>( $\times 10^2$ bm<br>$l^{-1}$ ) | $C_d^0$<br>( $\times 10^4$<br>$M l^{-1}$ ) | $\Delta Q_p$<br>( $\times 10^2$<br>$J h^{-1}$ ) | $\Delta Q_d$<br>( $\times 10^2$<br>$J h^{-1}$ ) | $\Delta Q_R$<br>( $\times 10^2$<br>$J h^{-1}$ ) | $\sigma$<br>( $\times 10^6$<br>$M h^{-1}$ ) | $\Delta H_B^\dagger$<br>( $\times 10^{-3}$<br>$J M^{-1}$ ) | $\Delta G^\circ$<br>( $\times 10^{-3}$<br>$J M^{-1}$ ) | $\Delta S^\circ$<br>( $J \cdot deg^{-1}$<br>$M^{-1}$ ) |
|---|--|---|---|---|---|--|--|--|
| 3.55  | 5.11                                       | -0.23   | -0.97   | -2.80   | 3.23  | -8.67  | -18.3  | 32.3   |
| 3.56  | 4.07                                       | -0.23   | -1.06   | -2.29   | 2.66  | -8.61  | -18.3  | 32.5   |
| 3.54  | 3.32                                       | -0.23   | -0.97   | -1.84   | 2.16  | -8.52  | -18.3  | 32.8   |
| 3.54  | 2.56                                       | -0.23   | -0.87   | -1.51   | 1.77  | -8.53  | -18.3  | 32.8   |
| 3.57  | 1.78                                       | -0.23   | -0.55   | -1.15   | 1.34  | -8.58  | -18.3  | 32.6   |
| 3.57  | 1.27                                       | -0.23   | -0.36   | -0.90   | 1.05  | -8.58  | -18.3  | 32.6   |
| 3.55  | 0.51                                       | -0.23   | -0.07   | -0.62   | 0.72  | -8.57  | -18.3  | 32.6   |

\*  $C_p$  and  $C_d$  are the concentrations of PVP and MeO, respectively, in the flow-mixing cell of the microcalorimeter

†  $\Delta H_B \equiv \Delta H^\circ$

**Table 4** Thermodynamic parameters for the binding of methyl orange by poly(vinylpyrrolidone) in tris system (0.1 M; pH = 9.6) at 25° C

| $C_p^*$<br>( $\times 10^2$ bm<br>l <sup>-1</sup> ) | $C_d^*$<br>( $\times 10^4$<br>Ml <sup>-1</sup> ) | $\Delta Q_p$<br>( $\times 10^2$<br>Jh <sup>-1</sup> ) | $\Delta Q_d$<br>( $\times 10^2$<br>Jh <sup>-1</sup> ) | $\Delta Q_R$<br>( $\times 10^2$<br>Jh <sup>-1</sup> ) | $\sigma$<br>( $\times 10^6$<br>Mh <sup>-1</sup> ) | $\Delta H_B^\dagger$<br>( $\times 10^{-3}$<br>JM <sup>-1</sup> ) | $\Delta G^\circ$<br>( $\times 10^{-3}$<br>JM <sup>-1</sup> ) | $\Delta S^\circ$<br>(J. deg <sup>-1</sup><br>.M <sup>-1</sup> ) |
|--|--|---|---|---|---|--|--|---|
| 3.61   | 9.35   | -1.69   | -12.0   | -0.59   | 1.47  | -4.01  | -17.8  | 46.3  |
| 3.63   | 7.48   | -1.72   | -9.7  | -0.57   | 1.41  | -4.04  | -17.8  | 46.2  |
| 3.66   | 4.68   | -1.73   | -14.0   | -0.51   | 1.27  | -4.02  | -17.8  | 46.3  |
| 3.58   | 2.34   | -1.70   | -20.9   | -0.46   | 1.14  | -4.04  | -17.8  | 46.2  |
| 3.61   | 0.94   | -1.72   | -13.3   | -0.43   | 1.07  | -4.02  | -17.8  | 46.3  |

\* $C_p$  and  $C_d$  are the concentrations of PVP and MeO, respectively, in the flow-mixing cell of the microcalorimeter

†  $\Delta H_B \equiv \Delta H^\circ$

**Table 5** Thermodynamic parameters for the binding of methyl orange by poly(vinylpyrrolidone) in the three systems, at 25° C

| Buffer system | $K$<br>( $\times 10^{-2}$ mol <sup>-1</sup> l) | $\Delta G^\circ$<br>( $\times 10^{-3}$ JM <sup>-1</sup> ) | $\Delta H_B^*$<br>( $\times 10^{-3}$ JM <sup>-1</sup> ) | $\Delta S^\circ$<br>(J.deg <sup>-1</sup> .M <sup>-1</sup> ) |
|---------------|--|---|---|---|
| Water         | 20.1   | -18.9   | -7.2  | 39.4  |
| tris-HCl      | 16.0   | -18.3   | -8.7  | 32.3  |
| tris          | 13.3   | -17.8   | -4.0  | 46.3  |

\* $\Delta H_B \equiv \Delta H^\circ$

$$\sigma = C^b \times n_T \quad (6)$$

where  $C^b$  is the bound dye in moles/litre and  $n_T$  is the total flow rate in litres/hour, in the microcalorimeter.

The free energy change,  $-\Delta G^\circ$  (JM<sup>-1</sup>), has been evaluated from the equilibrium dialysis data as described above (equation (1)).

The entropy change of binding,  $\Delta S^\circ$ , for MeO-PVP complex formation, has thus been arrived at by the relationship:

$$\Delta S^\circ = (\Delta H_B - \Delta G^\circ)/T \quad (7)$$

where  $\Delta H_B \equiv \Delta H^\circ$  and  $T$  is the absolute temperature (K).

It can be seen from Tables 2 to 5, that the  $\Delta G^\circ$  is exergonic for the three systems encountered here and increases in the order: water > tris-HCl > tris. These Tables also illustrate that the binding is an exothermic process and that the value of  $-\Delta H_B$  is independent of the concentration of MeO. The exothermic nature of the enthalpy change is apparent from these data to be decreasing in the order: tris-HCl > water > tris.

The positive entropy changes ( $\Delta S^\circ$ ) of binding for the three systems fall in the order: tris > water > tris-HCl. The entropy change is clearly independent of the concentration of MeO.

## DISCUSSION

### Enthalpies of dilution of methyl orange

Figure 2 illustrates the enthalpies of dilution ( $-\Delta H_D$ ) of MeO in water and in the presence of two buffer systems and shows the exothermicity of the dilution process to be in the order: tris  $\gg$  water > tris-HCl.

Since MeO does not exhibit spectral anomalies at the pH range of 5 to 10<sup>1</sup>, these differences in  $-\Delta H_D$  are not likely to be a consequence of this factor. This is also evident from the enthalpy of dilution of MeO in sodium acetate buffer (0.1 M; pH = 4.34) being not very much lower than in tris-system (0.1 M; pH = 9.6)<sup>15</sup>. These differences in  $-\Delta H_D$ , however, may be explained in terms of the extent of aggregation of MeO in these systems. From the nature of the values of  $-\Delta H_D$ , it is evident that

the nature of the forces responsible for the aggregation are changing with the buffer system.

The enthalpy of dilution, for dyes, can be expressed in terms of at least four major factors. These being hydrolysis, electrostatic interactions, London-van der Waals forces and non-electrostatic interactions. The enthalpy of dilution thus may be expressed by the relationship:

$$\Delta H_D = \Delta H_H + \Delta H_e + \Delta H_o + \Delta H_L \quad (8)$$

where  $\Delta H_H$  is the enthalpy of hydrolysis,  $\Delta H_e$  denotes the enthalpy change of electrostatic interactions,  $\Delta H_o$  that of non-electrostatic interactions (hydrophobic interactions, for example) excluding the hydrolysis ( $\Delta H_H$ ), and  $\Delta H_L$  that of London-van der Waals interactions.

Since the electrostatic interactions between aggregated dye ions is an endothermic process<sup>17</sup>, disaggregation upon dilution must result in the release of energy, that is  $\Delta H_e$  must be exothermic in nature. The London-van der Waals interactions for the aggregation of dye ions is exothermic in nature<sup>17</sup>, the disaggregation upon dilution, therefore, would be expected to be endothermic in nature. Non-electrostatic interactions (particularly hydrophobic) for the aggregation is endothermic, the disaggregation upon dilution would therefore be exothermic in nature. Finally, hydrolysis is an exothermic process, thus  $\Delta H_H$  must be exothermic in nature. Equation (8) thus may be written as follows:

$$\Delta H_D = (-\Delta H_H) + (-\Delta H_e) + (-\Delta H_o) + (\Delta H_L) \quad (9)$$

On the assumption that the contribution of  $\Delta H_H$  to the  $\Delta H_D$  for all three systems is of the same order of magnitude, it is possible to attribute the extent to which the other three enthalpy changes may contribute towards the overall enthalpy change observed.

In water, that is in the absence of cosolutes, MeO does not aggregate to a great extent even at very high concentrations<sup>1,18</sup>. The very small  $-\Delta H_D$  value observed is a result of the contribution of each of the three enthalpies ( $\Delta H_e$ ,  $\Delta H_L$  and  $\Delta H_o$ ) to the disaggregating system. In the presence of a cationic cosolute, tris-H<sup>+</sup>Cl<sup>-</sup>,

however, due to the screening of the electrostatic interactions between dye anions by the counterions, the major contribution to the overall  $-\Delta H_D$  comes from  $\Delta H_L$  and  $\Delta H_0$ . In the presence of tris, although  $\Delta H_L$  contributes to a degree, the overall large exothermic value of the dilution process probably results from the contributions from  $\Delta H_0$  and  $\Delta H_e$ .

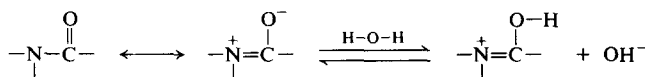
*Enthalpies of dilution of poly(vinylpyrrolidone)*

It is evident from Figure 3 that the enthalpy of dilution of PVP is exothermic for the three systems and increases in the order: tris > water > tris-HCl. A possible explanation for this difference in the observed  $-\Delta H_D$  may lie in the behaviour of this polymer, in solution, upon varying the pH ionic strength and the buffer system.

PVP has been suggested to exist in a loose configuration in water<sup>13</sup>. This is evident by its high intrinsic viscosity (20 ml/g) in contrast to a much lower value for serum albumin (3.8 ml/g)<sup>5,13</sup>, a polymer with a highly compact locally concentrated conformation. This difference in the conformation of these polymers is also evident from the observation that MeO bound to serum albumin shows an absorption maximum near 435 nm, but the complex with PVP has a maximum near 470 nm. The free dye in bulk water has a peak near 465 nm. Thus dye bound to PVP has a much more waterlike environment than that bound to serum albumin<sup>5,10</sup>.

PVP has been shown to shrink in size in the presence of non-ionic cosolutes<sup>8</sup>. This shrinkage is suggested to be solely and directly due to the binding of the cosolute to the PVP molecule. These bound cosolutes produce labile 'crosslinks' through the formation of hydrogen bonds between the substituent hydroxyl groups and another section of the same polymer molecule. The binding of tris, a non-ionic cosolute, by PVP seems feasible and would involve the interactions between the tris molecule and the non-polar sections of the polymer molecule. The binding process would therefore involve forces which are non-electrostatic in nature. Shrinkage of the PVP molecule in the presence of tris may occur via hydrogen bonding between the three hydroxyl groups on tris molecule and a section of the same PVP chain.

Presence of small amounts of salts have been shown to enhance the solubility of proteins in solution by a phenomena known as salting-in<sup>19</sup>. In order to parallel the biopolymer serum albumin<sup>3,5,6</sup>, PVP must be sensitive to such factors. Thus the addition of tris-H<sup>+</sup>Cl<sup>-</sup>, a neutral salt, may enhance the solubility of PVP in solution resulting in a conformation which is much more extended than in water. Presence of 'cationic' loci on PVP molecule, in aqueous solution, have been suggested and represented by Takagishi *et al.*<sup>3</sup> by the following scheme:



These cationic loci may be involved in the greater solubility of PVP in tris-H<sup>+</sup>Cl<sup>-</sup> buffer system.

From the discussion so far it may be inferred that the compactness of a PVP molecule increases in the order: tris >> water > tris-HCl, and interestingly is the same as the values of exothermic enthalpies of dilution in these systems.

Several enthalpic features may contribute to the overall

observed enthalpy of dilution,  $\Delta H_D$ , of a polymer and may be represented by the following expression:

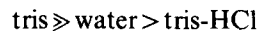
$$\Delta H_D = \Delta H_e + \Delta H_H + \Delta H_0 \tag{10}$$

where  $\Delta H_e$  is the enthalpy of dilution of electrostatic interactions,  $\Delta H_H$  that of hydrolysis and  $\Delta H_0$  is the enthalpy of dilution of non-electrostatic interactions (such as the hydrophobic ones).

Although the presence of 'cationic' loci on PVP molecules has been suggested<sup>3</sup>, the  $\Delta H_e$  and  $\Delta H_H$  for this polymer may be assumed to be negligibly small and of the same order of magnitude for all three systems. This makes  $\Delta H_0$  to be the sole contributor to the observed differences in the enthalpy of dilution of PVP in these systems.

The basis for hydrophobic interactions is that the  $\Delta H^\circ \geq 0$  and  $\Delta S^\circ > 0$ . The positive nature of entropy and less exothermic nature of enthalpy, of a hydrophobic system, is a feature obtained by the highly ordered water structure formed in the vicinity of the non-polar loci of the polymer. The process of dilution would thus proceed by a gradual change of the ordered water structure to a less ordered one resulting, therefore, in a decrease in the positive nature of the entropy change. Consequently the less negative initial enthalpy change for a system will increase in its exothermic nature approaching a constant value as dilution proceeds to infinity. This feature of  $-\Delta H_D$  for PVP is evident from Figure 3.

This argument is consistent with the suggestions above that PVP exists in a much more folded conformation, or in other words in a much more hydrophobic environment, in tris than either water or tris-HCl. The higher exothermic value of  $\Delta H_D$  thus corresponding to the enthalpy of dilution of a more hydrophobic system. The conformation of PVP in these systems in terms of hydrophobicity may therefore be represented as follows:



Three conclusions may be drawn from the above considerations. Firstly that tris binds to the PVP molecule, secondly that PVP exists in a much more folded conformation in tris than either water or tris-HCl, and thirdly that the exothermic nature of enthalpy of dilution is greater for a PVP-system which is more hydrophobic in nature.

*Enthalpies of binding of methyl orange by poly(vinylpyrrolidone)*

The binding of dyes and other cosolutes onto PVP has been investigated quite extensively in the past<sup>1,3-10</sup>, and the involvement of hydrophobic interactions, in addition to energetic ones, have been suggested to play a major role in the process of binding.

From the equilibrium data, presented in Table 1 and illustrated in Figure 1, two features of the MeO-PVP complex formation in these systems are evident. Firstly, that much more MeO is bound in tris-HCl than either water or tris and secondly, that the strength of binding is greater in the absence of cosolutes.

In its natural configuration, that is in water, a PVP molecule can accommodate only a certain number of dye anions. Upon addition of tris-H<sup>+</sup>Cl<sup>-</sup>, however, more dye anions can bind to the PVP molecule as a consequence of two factors. Firstly, the screening of the electrostatic

interactions between dye anions by the large excess of counterions promotes dye aggregation and secondly, as described above, PVP exists in a much more extended conformation in this buffer, rather like polycation, which facilitates the interaction between the aggregated dye molecules and the binding sites on the PVP chain. The occupation of most of its binding sites and its folded state configuration in tris-system is evident by the lower amount of dye bound by PVP<sup>21</sup>.

The thermodynamic parameters for the MeO-PVP complex formation in the three systems may be summarized as follows:

|                         |                  |
|-------------------------|------------------|
| water > tris-HCl > tris | $\Delta G^\circ$ |
| tris-HCl > water > tris | $\Delta H_B$     |
| tris > water > tris-HCl | $\Delta S^\circ$ |

The binding process, as illustrated by Tables 2-5, for all three systems is accompanied by an exothermic enthalpy change and an entropy gain which is consistent with the literature<sup>3,20</sup>. From the value of these parameters it is evident that the nature of the forces responsible for the binding of MeO by PVP are changing considerably with the buffer system. Although the MeO-PVP complex formation is to a large extent an entropy-driven process, the trend in the values of  $\Delta S^\circ$  and  $\Delta H_B$ , in going from tris-HCl to water to tris, suggests that the process is gradually changing from an enthalpy directed one to a much more entropy directed one.

In going from tris-HCl system to water system an increase in the binding constant, that is an increase in the free energy change, and a decrease in  $\Delta H_B$  are evident from Tables 2 and 3. Comparing the effects of the positive entropy and the negative enthalpy changes on the binding of MeO by PVP in water, it is likely that the former predominates over the latter in the enhancement of the binding. The opposite, however, would be true in the case of MeO-PVP complex formation in tris-HCl buffer. That is the less positive entropy change and more negative enthalpy change as compared to water would suggest that the latter predominates over the former in this buffer, as would be expected for a system where electrostatic interactions are of importance<sup>20</sup>. Thus the interpretation of these results in terms of the hydrophobic nature of the binding system shows that the contribution of hydrophobic interactions to the total binding forces is greater in water than in tris-HCl. The contribution of energetic forces being considerably greater in tris-HCl buffer because of the more extended very open conformation of this polymer in this buffer<sup>20</sup>.

In going from water to tris, a decrease in the free energy change and a decrease in the absolute magnitude of  $\Delta H_B$  is evident from Tables 2 and 4. The increase in the entropy term in the presence of tris appears to partially compensate for the decrease in  $\Delta H_B$  and the net effect is a slight decrease in  $\Delta G^\circ$ . These results are consistent with our suggestion above that tris binds to the PVP molecule thus resulting in a much more compact conformation. This conformation provides clusters of non-polar parts which can offer a very favourable environment for the formation

of hydrophobic bonds between the dye and the polymer. Accordingly, the hydrophobic forces would be principal contributors to the MeO-PVP complex formation. The large positive entropy of binding, coupled with the small enthalpy of binding, supports the hypothesis that hydrophobic forces play a major role in the MeO-PVP complex formation in tris. The decrease in the amount of MeO bound in tris provides further support to the suggestions above that tris binds to PVP and competes with the dye anions for the binding sites<sup>21</sup>.

## CONCLUSIONS

The above discussion can be summarized to give a fairly clear and consistent pattern. The main conclusion is that the forces involved in stabilizing the MeO:PVP complex are both energetic and hydrophobic in nature. In addition the results obtained in this paper and discussed above suggest that the hydrophobic natures of the binding processes for the three systems differ in magnitude and increase in the order: tris > water > tris-HCl.

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