Temperature dependence of the binding of methyl orange by crosslinked poly(4-vinylpyridine)

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Various crosslinked poly(4-vinylpyridines) (CP4VP) with different degrees of crosslinking were prepared by radical copolymerization of 4-vinylpyridine with *N,N'*-tetramethylenebisacrylamide as crosslinker. The binding abilities of these crosslinked polymers were investigated at various temperatures in a buffer solution of pH 7. The first binding constant (K_1) and thermodynamic parameters were evaluated from the equilibrium amounts of binding. The first binding constants showed bell-shaped curves when plotted against both the binding temperature and the degree of crosslinking. Lower temperature and higher degree of crosslinking at maximum binding in these bell-shaped curves were observed for this binding system when compared with those of the previously reported binding system (CP4VP/methyl orange) containing CP4VP prepared using *N,N'*-methylenebisacrylamide as crosslinker. The values of the enthalpy and entropy change increased on increasing the degree of crosslinking and decreasing the binding temperature, whereas the absolute magnitude of the free-energy change was not increased. These results could be accounted for in terms of the temperature dependence of the hole size of CP4VP in addition to the hydrophobic interactions in the binding process.

(Keywords: tetramethylenebisacrylamide; crosslinked poly(4-vinylpyridine); methyl orange; binding; hole size; hydrophobic interaction)

INTRODUCTION

When the extent of binding of small molecules by macromolecules is plotted as a function of binding temperature, a bell-shaped curve appears in some binding systems. The systems reported so far are bovine serum albumin/butyl orange[1], 2-hydroxyethyl methacrylate-*N*-vinyl-2-pyrrolidone copolymer/butyl orange[2], *N*-vinyl-2-pyrrolidone–*N*-alkyl-4-vinylpyridine copolymer/*p*-toluenesulfonate[3], crosslinked poly(vinylpyrrolidone)/pentyl orange[4], nylon-6,12/propyl orange[5], 2-dimethylaminoethyl methacrylate–*N*-vinyl-2-pyrrolidone copolymer/butyl orange[6] and crosslinked poly(4-vinylpyridine) (CP4VP)/methyl orange[7]. The main reason for the bell-shaped curves of these binding systems, except the last system[7], was reported to be a hydrophobic interaction between the polymers and the small molecules[1–6].

The results obtained from the last system, 4-vinylpyridine (4VP)–*N,N'*-methylenebisacrylamide (MBA) copolymer/methyl orange, were presented by us. In that paper[7], it was first suggested that the bell-shaped curve could be rationalized by the temperature dependence of the hole size in the crosslinked polymer, because the extent of binding was largely dependent on the binding temperature and the degree of crosslinking in the polymer. Even though such a temperature dependence was suggested, since binding systems usually do have a hydrophobic interaction, it is still not obvious whether the main reason for the bell-shaped curve in CP4VP/methyl orange is the effect of hole size of CP4VP or the hydrophobic interaction.

CP4VP used in this binding system, prepared by copolymerization of 4VP with *N,N'*-tetramethylenebisacrylamide (TMBA) as crosslinker, may have both larger crosslinked holes and larger hydrophobicity than CP4VP prepared by copolymerization of 4VP with MBA in the previous paper because of the different size of alkylene group in the two crosslinkers. Therefore, if the former polymer is used in the case of binding of methyl orange, its binding behaviour can be useful in the interpretation of the bell-shaped curve shown in the binding of methyl orange by the latter polymer.

In this study, the extents of binding of methyl orange by 4VP–TMBA copolymers have been examined. Their bell-shaped curve when plotted against the binding temperature is explained in terms of both the hole size in the crosslinked polymer and the hydrophobic interaction, in comparison with that of the binding system of methyl orange by 4VP–MBA copolymer in the previous paper[7].

EXPERIMENTAL

Materials and instruments

Commercial 4-vinylpyridine, acryloyl chloride and azobisisobutylonitrile (AIBN) were purified by the usual methods. 1,4-Diaminobutane (Aldrich Chemical Co.) was used as received. Methyl orange (Tokyo Kasei Co.) was recrystallized from a water–methanol (v/v, 1:1)

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Table 1 Extent of crosslinkage in the copolymer of 4-vinylpyridine and *N*,*N*′-tetramethylenebisacrylamide

Crosslinked poly(4-vinylpyridine)	α[a] Before crosslinking[b]	α[a] After crosslinking[c]	Conversion[d] (%)
P-I	5.0	3.1	76
P-II	10.0	6.8	80
P-III	15.0	12.1	83
P-IV	20.0	19.3	84

[a] $\alpha = ([N,N'\text{-tetramethylenebisacrylamide}]/[\text{4-vinylpyridine}]) \times 100$

[b] Ratio of the feed composition

[c] Determined from C/N of the copolymer

[d] From gravimetric measurement

mixture. All other chemicals of reagent grade were used without further purification.

Melting point was determined on a Büch B-510N apparatus. ^{1}H n.m.r. spectra were obtained using a Bruker WP-80-SY n.m.r. spectrometer. I.r. spectra were measured using a Bio-Rad Digilab Division FTS 20/80 spectrophotometer. Elemental analysis was performed with a Hewlett-Packard 185 instrument.

Synthesis of TMBA

A mixture of 1,4-diaminobutane (13.2 g, 0.15 mol) and acetone (500 ml) in the presence of triethylamine (41.8 ml, 0.30 mol) was placed in a 1 litre three-necked round flask fitted with a reflux condenser with $CaCl_2$ column. To the mixture was added acryloyl chloride (30.9 ml, 0.38 mol) in acetone (200 ml) over 1 h with stirring at 5°C. Then, it was stirred for an additional 10 h at 0–5°C. The precipitated triethylamine hydrochloric acid salt was immediately removed from the mixture by filtering. Evaporation of the filtered solution under reduced pressure below 25°C gave solid crude product. The resulting residue was washed with 5% $NaHCO_3$ solution and then diethyl ether. Crystallization from ethanol/water (v/v, 4:1) gave 10.4 g (yield, 35.4%) of TMBA, m.p. 127–128°C.

^{1}H n.m.r. (CD_3OD), δ ppm): 1.6 (m, 4H, $-CH_2-$), 3.3 (m, 4H, $>N-CH_2-$), 5.6–6.3 (m, 6H, $CH_2{=}CH-$).

I.r. (KBr, cm^{-1}): 3300, 1540 (N–H), 3060(=C–H), 2920 (C–H), 1655 (C=O), 1624, 980 (C=C).

Analysis: found, C = 61.5%, H = 8.5%, N = 13.3%; calculated for $C_{10}H_{16}N_2O_2$, C = 61.2%, H = 8.20%, N = 13.4%.

Preparation of CP4VP

An example (P-I) of CP4VP was prepared in the following way. An ampoule containing 4VP (23.1 g, 0.22 mol), TMBA (2.2 g, 0.01 mol) and AIBN (0.04 g, 2.3×10^{-4} mol) as initiator in methanol (200 ml) was purged with nitrogen and then sealed. The ampoule was heated at 60°C for 20 h. After copolymerization, the insoluble product was filtered, and washed repeatedly with methanol to remove all methanol-soluble materials. The crude dried product was ground to a powder and allowed to dry again at room temperature *in vacuo*. Other CP4VPs (P-II, P-III and P-IV) with different degrees of crosslinking were prepared under the same conditions as P-I, except for varying the ratio of TMBA to 4VP in the monomer feed. The conversion of copolymerization was measured by a gravimetric technique and is listed in *Table 1*. The degrees of crosslinking (α), the molar ratio of TMBA to 4VP in the copolymers, were determined from the ratio of carbon to nitrogen content, which was measured by elemental analysis, and are listed in *Table 1*.

I.r. (KBr, cm^{-1}): 3300 (N–H), 3050 (aromatic C–H), 2920 (aliphatic C–H), 1660 (C=O), 1600, 1415, 820 (pyridine).

Binding experiments

Powdered CP4VP smaller than 150 μm (100 mesh) was used in the binding experiments. The procedure for the equilibrium binding experiments was essentially the same as that described in the previous paper[7]. The test tubes that contained the solution were gently shaken in a circulating water bath at 20, 30, 35, 40 and 50°C. A 120 h period was sufficient for equilibration. After equilibrium, CP4VP powder was separated by centrifugation and the dye concentrations were determined with a Shimazu UV 2100 spectrophotometer.

RESULTS AND DISCUSSION

In order to evaluate the extent of binding of methyl orange by CP4VPs, double-reciprocal plots (Klotz plot[8], where r is the number of moles of the bound dye per 10^5 g of polymer and C is the concentration of the unbound dye) are illustrated in *Figure 1* (P-I/methyl orange) and *Figure 2* (P-III/methyl orange) as representative examples. The plots shown in *Figures 1* and *2* are essentially linear at each temperature measured. For the other systems (P-II/methyl orange and P-IV/methyl orange), linear relationships were also observed between $1/r$ and $1/C$. From the slopes of the double-reciprocal plots, the first binding constants K_1 were calculated and are listed in *Tables 2* and *3*.

As shown in *Tables 2* and *3*, the range of K_1 values was 2.1×10^6 to 15.5×10^6 for the dye. These values were much higher than those, $(2 \text{ to } 11) \times 10^4$, of

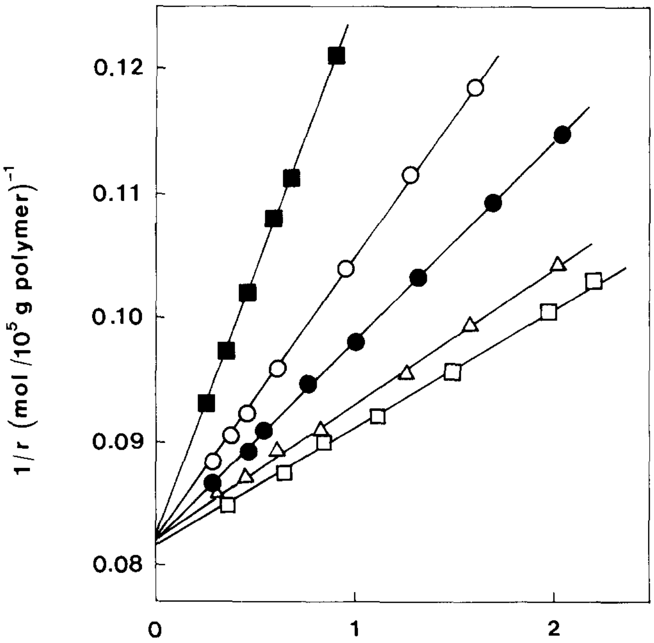

Figure 1 Relationship between $1/r$ and $1/C$ for the binding of methyl orange by P-I in 0.1 M $NaHCO_3/KH_2PO_4$ buffer, pH 7: (○) 20°C; (□) 30°C; (△) 35°C; (●) 40°C; (■) 50°C

water-soluble *N*-alkyl poly(4VP)[9,10]. The large difference in K_1 values between CP4VP and *N*-alkyl poly(4VP) can be attributed to the more compact conformation of CP4VP than that of *N*-alkyl poly(4VP), as Klotz *et al.*[11] suggested in the case of the higher K_1 values of the crosslinked polyethyleneimines/methyl orange compared with those of polyethyleneimines/methyl orange. Also, the values of K_1 of this crosslinked 4VP–TMBA copolymer/methyl orange system were almost threefold greater than those of the crosslinked 4VP–MBA copolymer/methyl orange system in the previously reported paper[7], which were known to have higher extents of binding than any other systems. The polymer of the present system has both larger crosslinked holes than and increased hydrophobicity over the one reported previously because TMBA has more methylene groups than does MBA. Therefore, the difference of K_1 values might be attributable to both the hole size and the hydrophobicity of CP4VP. In connection with these large values of K_1, the binding of methyl orange by poly(4VP) crosslinked with homologues of TMBA as crosslinkers is also being investigated.

To investigate the influence of the content of the crosslinker on the binding of methyl orange by CP4VPs, the values of K_1 at 20°C were plotted as a function of the degree of crosslinking of CP4VP in *Figure 3*. As shown in this figure, the values of K_1 in this binding system showed a bell-shaped curve against the degree of

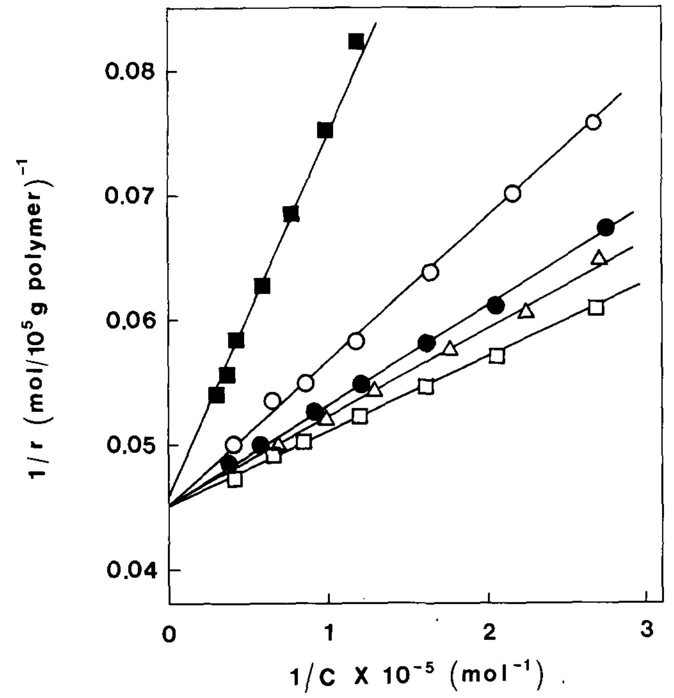

Figure 2 Relationship between $1/r$ and $1/C$ for the binding of methyl orange by P-III in 0.1 M $NaHCO_3/KH_2PO_4$ buffer, pH 7: (○) 20°C; (□) 30°C; (△) 35°C; (●) 40°C; (■) 50°C

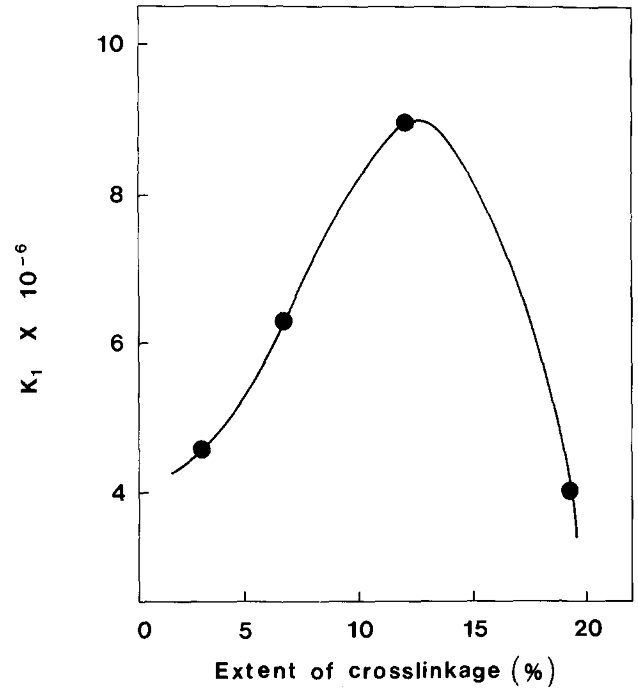

Figure 3 Relationship between the first binding constant K_1 and the extent of crosslinkage for the binding of methyl orange by CP4VPs at 20°C

Table 2 Thermodynamic parameters for the binding of methyl orange by P-I

Temperature (°C)	$K_1 \times 10^{-6\,a}$	ΔG (cal mol^{-1})	ΔH (cal mol^{-1})	ΔS (cal mol^{-1} K^{-1})
20	4.57	−8930	14050	78.4
30	9.57	−9740	−1000	28.8
35	8.04	−9730	−3500	20.2
40	6.10	−9710	−6700	9.6
50	2.08	−9340	−11300	−6.1

[a] Measurements in 0.1 M $NaHCO_3/KH_2PO_4$ buffer, pH 7. Calculated from r values computed for 10^5 g of polymer

Table 3 First binding constants and thermodynamic parameters for the binding of methyl orange by crosslinked poly(4-vinylpyridines)

	$K_1 \times 10^{-6\,a}$							
Polymer	20°C	30°C	35°C	40°C	50°C	ΔG^b (cal mol^{-1})	ΔH^b (cal mol^{-1})	ΔS^b (cal mol^{-1} K^{-1})
P-I	4.57	9.57	8.04	6.10	2.08	−9680	−2000	25.4
P-II	6.28	10.36	9.82	7.14	2.28	−9740	−1000	28.8
P-III	8.97	15.51	14.60	12.19	2.96	−9970	0	32.9
P-IV	3.99	12.34	13.02	9.98	3.25	−9380	610	51.1

[a] Measurements in 0.1 M $NaHCO_3/KH_2PO_4$ buffer, pH 7. Calculated from r values computed for 10^5 g of polymer
[b] Measurements at 30°C

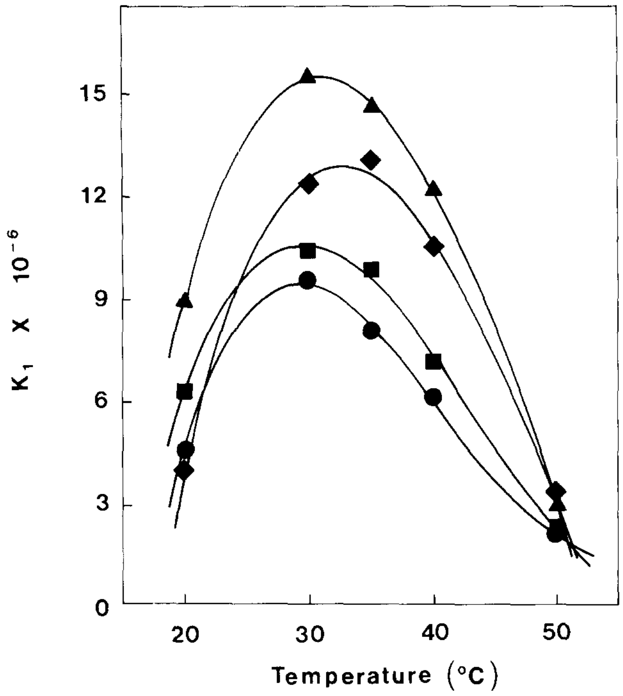

Figure 4 Relationship between K_1 and temperature for the binding of methyl orange by CP4VPs: (●) P-I; (■) P-II; (▲) P-III; (◆) P-IV

system showed a bell-shaped curve against the degree of crosslinking. This result was first found in the methyl orange/CP4VP system among small molecule/crosslinked macromolecule systems. In *Figure 3*, over the range $\alpha = 3.1$ (P-I) to $\alpha = 19.3$ (P-IV), the extents of binding of CP4VPs for methyl orange increased in the following order: P-IV < P-I < P-II < P-III. The order of P-I < P-II < P-III can be accounted for in terms of more extensive hydrophobic domains in the crosslinked polymer, which enhances hydrophobic interactions in the binding process. However, the order of P-IV < P-III cannot be explained by the hydrophobicity of CP4VP. This phenomenon can be interpreted in terms of the hole size of CP4VP containing different degrees of crosslinking as follows. The hole sizes of P-I and P-II are larger than those of P-III because the degrees of crosslinking of P-I and P-II are smaller than that of P-III as shown in *Table 1*. On the other hand, the hole size of P-IV is smaller than that of P-III because of their crosslinkage. Accordingly, the hole size of P-III ($\alpha = 12.1$), which has a moderate degree of crosslinking, might match with the hole size of methyl orange more nicely than that of the other three polymers. Also, the degree of crosslinking ($\alpha = 9.8$) of maximum binding of the previous polymer[7] was lower than that of this polymer. This result seems to indicate that the hole sizes of this polymer are larger than those of the previous polymer at the same degree of crosslinking.

In *Figure 4*, the first binding constants in *Table 3* are plotted against the binding temperature to illustrate the effect of temperature on the binding of methyl orange by CP4VPs. The respective extents of binding of methyl orange by P-I, P-II and P-III, which have lower degree of crosslinking than P-IV, increased with increasing temperature from 20 to 30°C (endothermic process), and then decreased from 30 to 50°C (exothermic process). And the extent of binding of methyl orange by P-IV increased with increasing temperature up to 35°C and then decreased above 35°C. Thus, not only does the plot of K_1 against temperature show a bell-shaped curve, but also the temperature of maximum K_1 was observed in the range of 30–35°C. Among the binding of small molecules by crosslinked macromolecules, this phenomenon was first observed in the case of binding of methyl orange by CP4VP. This result can be explained by the change of the hole size in CP4VP with temperature variation as follows[7]. When the temperature increases from 20 to 30°C, the hole size of P-I, P-II and P-III becomes large enough to hold methyl orange due to swelling. Accordingly, the first binding constants at 30°C were larger than those at 20°C and reached maximum values at 30°C except for P-IV. The hole size of P-IV at 30°C is still too small for the methyl orange to squeeze into the polymer. Increasing the temperature to 35°C, the hole size of P-IV becomes larger for the same reason, and might nicely match with the size of methyl orange. Therefore, the values of K_1 of P-IV/methyl orange at 35°C were larger than that at 30°C. However, when the temperature is increased to 50°C, the hole sizes of P-I, P-II, P-III and P-IV become too big for the polymer to hold methyl orange, and the values of K_1 of all four systems become small and approximately the same. The value of K_1 of the P-IV/methyl orange system at the lowest temperature (20°C) was smallest compared with the other three systems. This result indicates that the hole size of P-IV at 20°C was smallest due to the lowest binding temperature and the highest degree of crosslinking. Therefore, the hole size of P-IV may be too small for the methyl orange to bind to it. This concept of hole size could be responsible for the fact that the value of K_1 of the P-III/methyl orange system is larger than the other three systems.

In addition, as shown in *Figure 4*, the temperature of maximum binding of methyl orange by CP4VPs was in the range of 30–35°C. On the other hand, the temperature of maximum binding of methyl orange by the crosslinked polymer in the previous paper[7] was in the range of 40–50°C. That is, the temperature of maximum binding was shifted to a lower temperature in going from 4VP–MBA copolymer/methyl orange system to 4VP–TMBA copolymer/methyl orange system. If the main reason for the bell-shaped curve is a hydrophobic interaction, the temperature of maximum binding of this binding system should be higher than that of the previous binding system because the hydrophobic interaction is an endothermic process[12]. This result indicates that the temperature dependence of the hole size for the bell-shaped curve that appears in our binding system was more operative than the hydrophobic interaction.

For purposes of discussing the thermodynamic behaviour involved in this binding system, the thermodynamic parameters were evaluated from the first binding constants and the binding temperatures (i.e. $\ln K_1$ vs. $1/T$ plot[13]). The resultant thermodynamic parameters for the binding of methyl orange with P-I in *Table 2* and also of methyl orange with each of four different CP4VPs in *Table 3* are listed.

The peculiar temperature dependence observed for CP4VP/methyl orange was deduced to be a result of the balance of the negative enthalpy changes due to electrostatic interactions (ΔH_e) and the positive enthalpy change contributed from hydrophobic interactions (ΔH_h) involved in the binding[1]. As shown in *Figure 4* and

Table 2, when these two enthalpy terms, ΔH_e and ΔH_h, of opposite sign, balance each other at about 28°C, the overall binding enthalpy ΔH becomes athermal. If the latter term exceeds the former one in magnitude, an endothermic enthalpy change appears. The binding process exhibits exothermic reaction in the reverse case. When the hydrophobicity of both or either of the binding entities, the small molecule and the polymer, becomes sufficiently large and the temperature of the environment is low enough, the energy requirement for the hydrophobic interactions should be larger and the binding process tends more to endothermicity. As shown in *Table 2*, the enthalpy change varied markedly with the binding temperature and becomes more endothermic with decreasing temperature. The entropy change tends to increase gradually as the temperature decreases. Also, the absolute value of ΔG increased with increasing temperature from 20 to 28°C. Accordingly, the increasing value of absolute ΔG from 20 to 28°C was dominated by the ΔH_h term rather than the ΔH_e term, which is known to be a characteristic of the hydrophobic interaction. However, the absolute value of ΔG decreased with increasing temperature from 28 to 50°C. The decreasing value of absolute ΔG from 28 to 50°C was dominated by the ΔH_e term rather than the ΔH_h term, and thus the contribution of the hydrophobic interactions in the binding appears to be weakened with increasing temperature from 28 to 50°C. The ΔS data can be explained in a similar manner as ΔH: the contribution of the entropy term to the free-energy change tends to increase with decreasing binding temperature. Therefore, it seems likely that the bell-shaped curve appears in binding systems in which the contribution of hydrophobic interactions is large.

As shown in *Table 3*, the values of ΔH and ΔS associated with binding of methyl orange by CP4VPs increased with increasing degree of crosslinking. This indicates that the contribution of hydrophobic interaction increases from P-I to P-IV in the binding process, since the hydrophobic interaction contains both endothermic enthalpy change and increasing entropy change[12,13]. However, the absolute values of ΔG and first binding constants were not increased in that order. That is, the value of ΔG increased from P-I to P-III and then decreased from P-III to P-IV. The maximum value of ΔG appeared at P-III among the four CP4VPs. The magnitude of ΔG or K_1 leads to the relations of the nature of energetic and hydrophobic interactions involved in the binding process[2]. Increasing absolute magnitude of ΔG from P-I to P-III can be explained on the basis of the hydrophobic interaction between the hydrophobic moieties of the polymer and the small molecule as previously mentioned. On the other hand, decreasing absolute magnitude of ΔG from P-III to P-IV might be explainable in terms of the energetic interaction between the sulfonate group of the small molecule and the nitrogen atoms on the polymer.

CONCLUSION

It was observed that the first binding constant of this binding system of methyl orange by CP4VPs showed bell-shaped curves against both the binding temperature and the degree of crosslinking. This fact can be explained in terms of the temperature dependence of the hole size of CP4VP together with the hydrophobic interaction involved in the binding process.

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