Interaction and structure of PVA–Cu(II) complex: 2. Effect of microstructure of PVA chain on binding affinity of p-aminoazobenzene

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The effect of the content and distribution of the acetyl groups of partially acetylated poly(vinyl alcohol) (PVA) on the binding affinity of p-aminoazobenzene (AAB) to PVA–Cu(II) complex has been investigated. The binding affinity of the dye increased with increasing content of acetyl groups on the PVA chain. The enhancement of the binding affinity was larger for the PVA with a block-type distribution of the acetyl groups than for the PVA with a random distribution of the acetyl groups. Although the dye has a binding affinity to the partially acetylated PVA even in the uncomplexed state, this enhancement was far larger than the simple sum of the contributions from the Cu(II) complex of perfectly saponified PVA and from the uncomplexed acetylated PVA. The effects of pH and temperature on the binding affinity have been also investigated in connection with the hydrophobic character of the polymer chain.

(Keywords: poly(vinyl alcohol)–Cu(II) complex; cupric hydroxide cluster; dye binding; acetylated poly(vinyl alcohol); sequential distribution)

INTRODUCTION

In the previous paper[1] (part 1 of this series), we have reported that a hydrophobic dye, p-aminoazobenzene (AAB), shows a binding affinity to poly(vinyl alcohol) PVA–Cu(II) complex. We proposed a binding mechanism based on a 'Cu cluster model'. In this model, PVA plays a role in the protection of the ultrafine copper hydroxide cluster. We found that the binding affinity of the dye is determined only by the concentration of Cu, being almost independent of the concentration of PVA.

In order to gain further insight into the mechanism of the binding of AAB to the complex, the effect of the content and distribution of the acetyl groups of partially acetylated PVA was investigated in this paper. It is well known[2,3] that the physicochemical properties of partially acetylated PVA are strongly dependent upon the method of preparation. Partially acetylated PVA can be regarded as a copolymer composed of vinyl alcohol (VA) and vinyl acetate (VAc). The main cause of the diversity of the properties of VA–VAc copolymers is believed to be the difference in their sequential distribution. N.m.r. studies have revealed that the sequential distribution of VA and VAc units of the copolymer prepared by reacetylation of PVA is essentially random, while that prepared by the saponification of PVAc has a block-type distribution[2].

The effects of pH and temperature on the binding affinity were also examined in this paper. It is expected that such experiments will shed light on the role of the hydrophobicity of the complex.

EXPERIMENTAL

Cupric nitrate and AAB were obtained from Wako Chemicals and were used without further purification.

The concentrations of cupric nitrate and AAB were determined as shown in the previous paper[1].

Two commercial PVA samples obtained from Tokyo Kasei with a degree of saponification of 99% and 80% were used. The nominal degrees of polymerization of these samples were 1750 and 2000, respectively. The former is simply termed PVA. The latter may be regarded as a VA–VAc copolymer and is termed copolymer (C). Two other series of VA–VAc copolymers were prepared by the following procedures.

Copolymers (R). Copolymer (C) was dissolved in a water–acetic acid mixture and reacetylated in the presence of hydrochloric acid. Seven copolymers were obtained by changing the composition of the solvent mixture[4].

Copolymers (S). Commercial PVAc (Wako Chemicals, $P_n = 1400–1600$) was dissolved in a solvent mixture of methanol and water with a volume ratio of 9:1 and saponified by adding KOH solution. Five copolymers with different composition were obtained by controlling the amount of KOH[5].

The content of acetyl groups in these copolymers was determined by the KOH titration method.

Absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Measurements were carried out at 25°C in the high pH region above pH 8. A complex solution without the dye was taken as a reference.

RESULTS AND DISCUSSION

Analysis of dye binding by dual binding mechanism

In the previous paper[1], we found that the binding affinity of AAB to the Cu(II) complex prepared from copolymer (C) was larger than the binding affinity to the
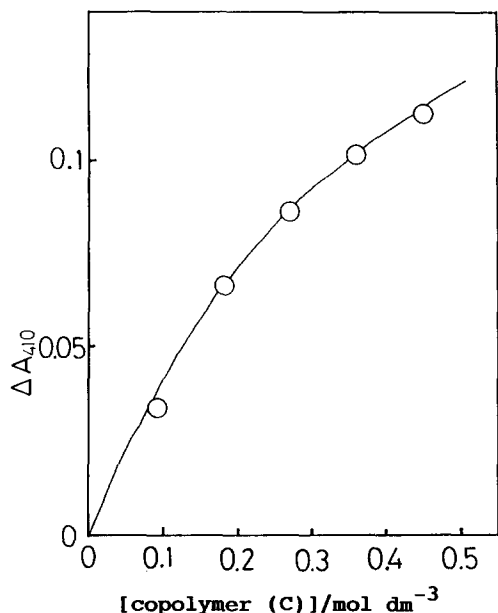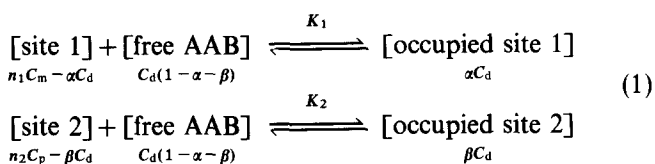

Figure 1 Plot of differential absorbance at 410 nm, ΔA_{410}, against the concentration of copolymer (C). Concentration of p-aminoazobenzene (AAB) was 2.68×10^{-5} mol dm^{-3}

complex prepared from PVA. The result seems to be natural because copolymer (C) has an ability to bind AAB even in the absence of Cu(II) ion. Thus, as a first approximation, we can suppose the existence of the two types of binding site as follows:

Site 1: this binding site appears only after complex formation; the concentration of the binding site would be proportional to the concentration of Cu.

Site 2: this binding site is inherent to VA–VAc copolymer; the concentration of the binding site would be proportional to the concentration of polymer.

The simultaneous equilibria between the above two types of binding sites and the dye may be formulated as:

$$[\text{site 1}] + [\text{free AAB}] \xrightleftharpoons{K_1} [\text{occupied site 1}]$$
$$\underset{n_1 C_m - \alpha C_d}{} \quad \underset{C_d(1-\alpha-\beta)}{} \qquad \underset{\alpha C_d}{}$$

$$[\text{site 2}] + [\text{free AAB}] \xrightleftharpoons{K_2} [\text{occupied site 2}]$$
$$\underset{n_2 C_p - \beta C_d}{} \quad \underset{C_d(1-\alpha-\beta)}{} \qquad \underset{\beta C_d}{}$$

(1)

where C_m, C_d and C_p are the concentrations of Cu, AAB and polymer based on the monomer unit, respectively; n_1 and n_2 are the numbers of sites 1 and 2 per monomer; α and β are the fractions of the bound dye on sites 1 and 2; and K_1 and K_2 are the binding constants for sites 1 and 2.

Since the binding affinity of AAB to the uncomplexed PVA was confirmed to be negligible, the binding parameters calculated from the binding curve of AAB to the PVA–Cu(II) complex could be regarded as those of site 1. Thus using the data of our previous paper[1], the binding parameters $1/n_1$, K_1 and ε_1 for site 1 were determined to be 11.5, 5.19×10^3 and 6.90×10^3, where ε_1 is the molar extinction coefficient of the bound AAB, which is estimated from the asymptotic value of differential absorbance.

On the other hand, the absorption maximum of AAB at 375 nm shifted to longer wavelength on addition of uncomplexed copolymer (C) and an isosbestic point appeared in the vicinity of 373 nm. Therefore, copolymer (C) has an ability to bind AAB, probably due to its

hydrophobic character. *Figure 1* shows the plot of the differential absorbance at 410 nm, ΔA, against the concentration of the complex prepared from copolymer (C). Using the method described in our previous paper, the binding parameters could be determined by a non-linear least-squares fit of the curve shown in *Figure 1*. These are regarded as the binding parameters for site 2. The values determined for $1/n_2$, K_2 and ε_2 were 9.52×10^2, 2.22×10^3 and 8.43×10^3, respectively.
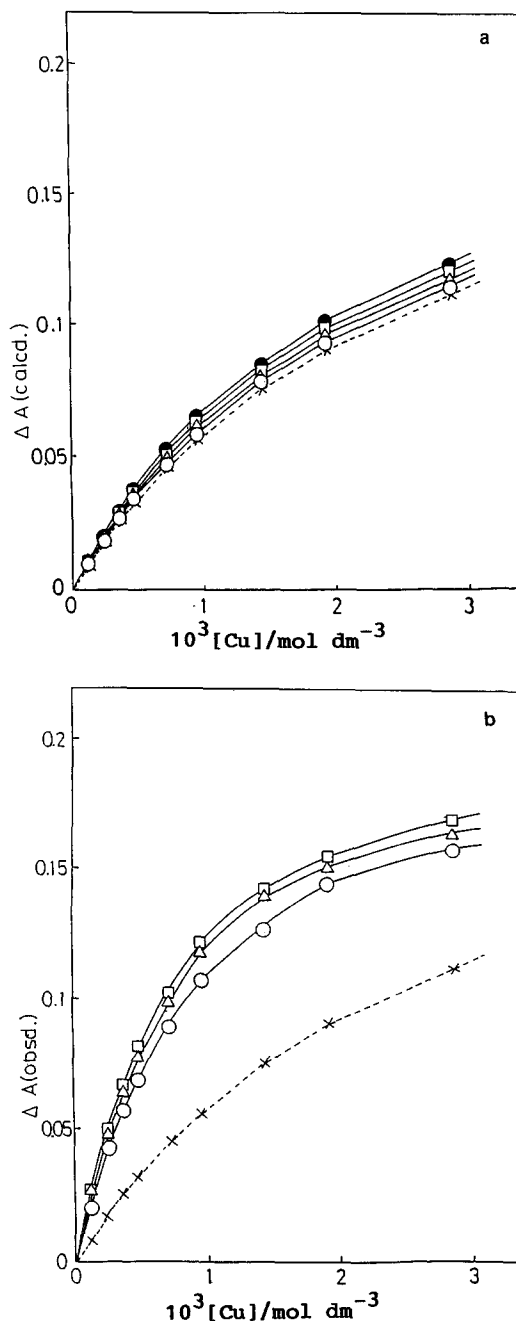
When simultaneous binding occurs, the following

Figure 2 (a) ΔA calculated by dual binding mechanism plotted against the concentration of Cu. The ratio of the concentrations of polymer to Cu, [polymer]/[Cu], was: (O) 7.27, (△) 14.5, (□) 21.8 and (●) 29.1. The concentration of the polymer is based on the monomer. Concentration of AAB was 2.98×10^{-5} mol dm^{-3}. The binding curve for PVA is represented by the broken curve. (b) Observed ΔA plotted against the concentration of Cu for copolymer (C). The ratio [polymer]/[Cu] was: (O) 7.27, (△) 14.5 and (□) 21.8 and 29.1. The binding curve for PVA is represented by the broken curve
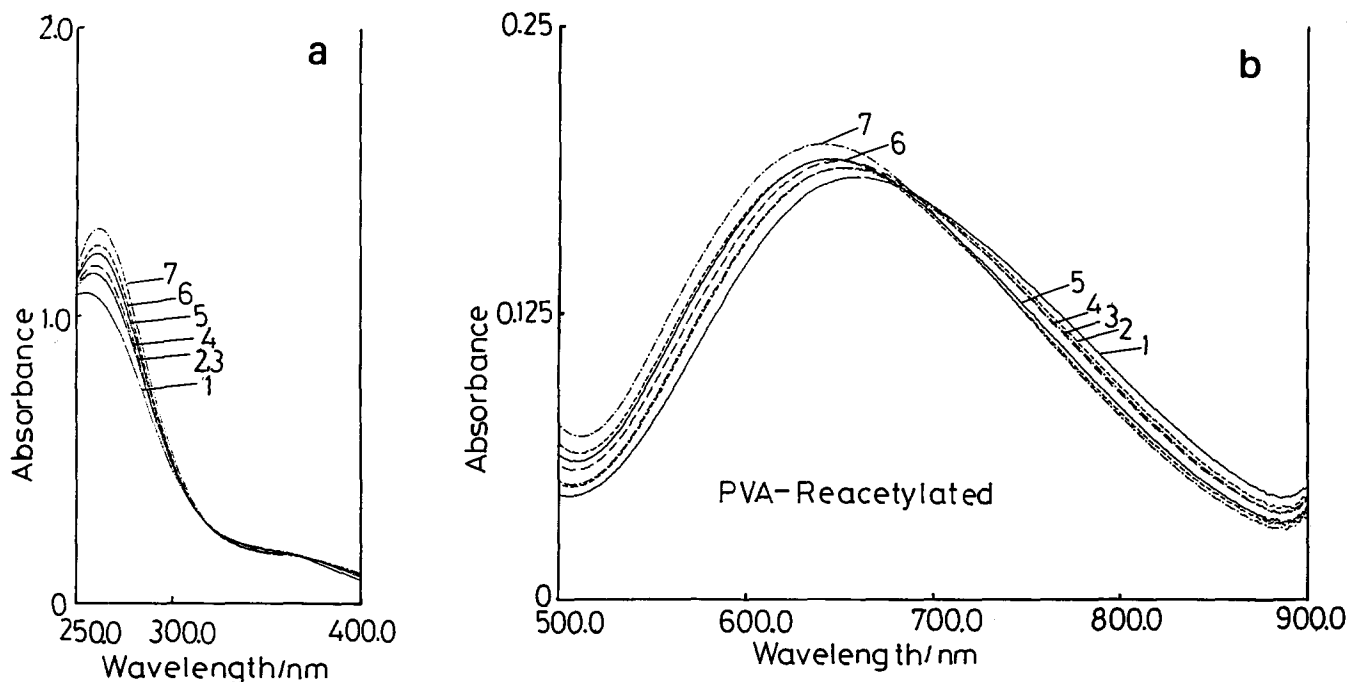
Figure 3 Absorption spectra of the copper complex of reacetylated PVA (copolymers (R)). The degree of saponification is: curve 1, 64; curve 2, 70; curve 3, 74; curve 4, 78; curve 5, 84; curve 6, 93; curve 7, 97. Concentration of Cu was 4.2×10^{-4} and 4.2×10^{-3} mol dm^{-3} for (a) and (b)
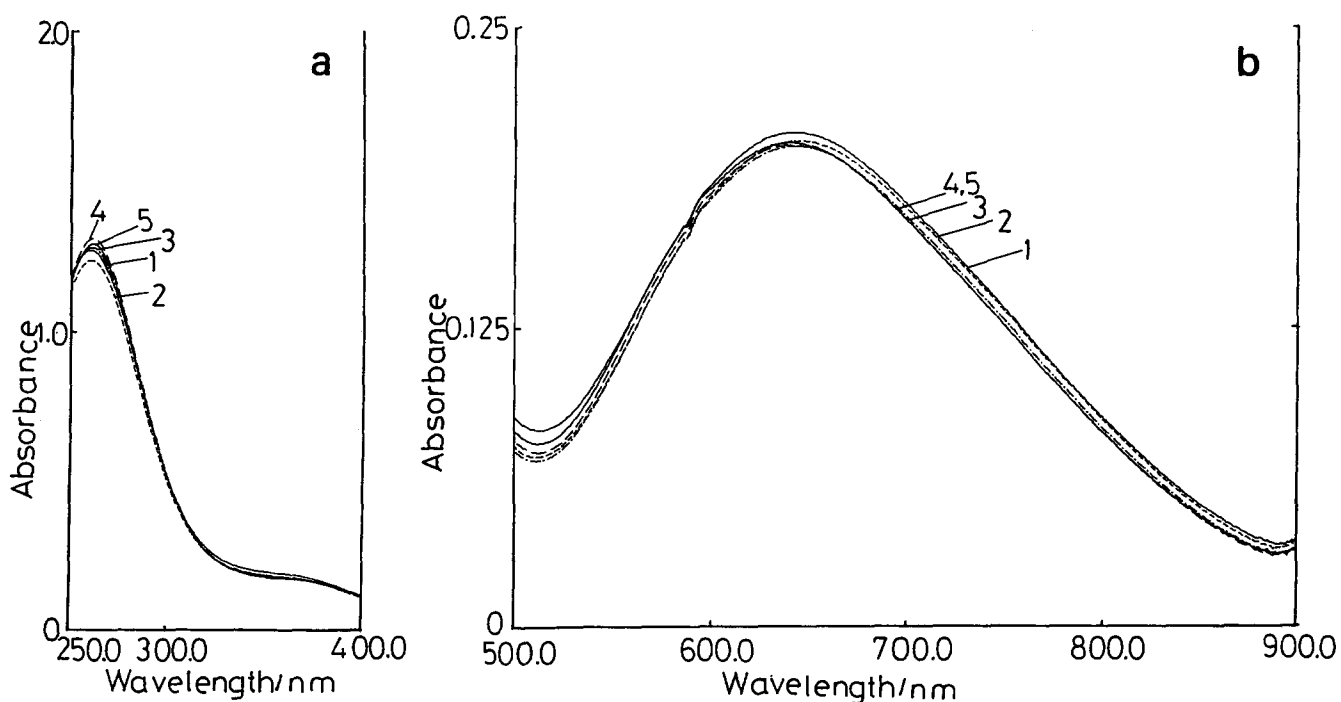

Figure 4 Absorption spectra of the copper complex of saponified PVA (copolymers (S)). The degree of saponification is: curve 1, 70; curve 2, 77; curve 3, 82; curve 4, 94; curve 5, 99. Concentration of Cu was 4.2×10^{-4} and 4.2×10^{-3} mol dm^{-3} for (a) and (b)

quadratic simultaneous equations should be satisfied:

$$(n_1 C_m - \Delta A_1/\varepsilon_1)(C_d - \Delta A_1/\varepsilon_1 - \Delta A_2/\varepsilon_2) = \Delta A_1/\varepsilon_1 K_1$$
$$(n_2 C_p - \Delta A_2/\varepsilon_2)(C_d - \Delta A_1/\varepsilon_1 - \Delta A_2/\varepsilon_2) = \Delta A_2/\varepsilon_2 K_2 \qquad (2)$$

where $\Delta A_1 = \varepsilon_1 C_d \alpha$ and $\Delta A_2 = \varepsilon_2 C_d \beta$. Since n, K and ε values for sites 1 and 2 have already been determined, we can calculate ΔA_1 and ΔA_2 from equations (2). The differential absorbance ΔA is the sum of ΔA_1 and ΔA_2. *Figure 2a* shows the plots of ΔA against the concentration of copper based on the dual binding mechanism. The experimental curves are shown in *Figure 2b*. It should be

noted that the experimental degrees of binding are far higher than those calculated from the dual binding mechanism assuming the independent binding of the dye to both sites. Thus it has become apparent that the acetate groups on the PVA chain enhance the binding affinity of the dye in some cooperative manner.

The effect of the content and distribution of acetate groups

According to the foregoing experiments, the binding affinity of the dye is expected to be closely associated

Table 1 Binding parameters for VA–VAc copolymers

Degree of saponification (%)	Isosbestic point (nm)	LA	nK (dm³ mol⁻¹)
Copolymer (R)			
64	382	0.206	1.01×10^3
70	382	0.236	8.24×10^2
74	383	0.221	8.54×10^2
78	384	0.212	8.24×10^2
84	386	0.192	8.18×10^2
91	387	0.175	6.55×10^2
97	388	0.175	5.84×10^2
Copolymer (S)			
70	381	0.249	1.83×10^3
77	384	0.221	1.48×10^3
82	385	0.214	1.29×10^3
94	387	0.197	6.58×10^2
99	387	0.187	5.24×10^2
Copolymer (C)			
80	385	0.208	1.76×10^3
PVA			
99	387	0.215	4.03×10^2

with the content and distribution of acetate groups. The absorption spectra of the copper complexes of seven samples of the copolymer (R) series are shown in *Figures 3a* and *3b*. These samples have a random distribution of acetate groups. The absorption maximum around 650 nm, which is assigned to d–d transition of Cu, shifted to longer wavelength and the intensity of the maximum decreased with increasing content of acetate groups. On the other hand, the absorption maximum around 260 nm shifted to slightly shorter wavelength and the intensity of the maximum also decreased with decreasing content of acetate groups.

The absorption spectra of five samples of the copolymer (S) series are shown in *Figures 4a* and *4b*. These samples are known to have a block distribution of acetate groups. In contrast to the copolymer (R) series, no systematic change of the absorption maximum was observed when the content of acetate groups was changed.

These results suggest that the electronic character of the Cu complexes prepared from the copolymer (R) series depends on the content of acetate groups, while that of the complexes prepared from the copolymer (S) series is independent of the acetate content. The spectral shift of the complex was explained by Shirai and Hojo[6] from the standpoint of the distortion of the planar chelate structure of Cu. However, we cannot accept this explanation because of the serious flaws that were found in the chelate model[7,8].

It seems likely that the absorption spectrum of partially acetylated PVA–Cu(II) complex is influenced by the length of VA sequence on the chain. According to the cluster model, long VA sequences of copolymers (S) can increase the stability of the cluster. The cluster surrounded by copolymers (R), however, is not sufficiently stabilized because of the random distribution of the VA unit. Especially in the case of copolymer with low VA content, the VA sequence would be too short to stabilize the cluster.

The parameters for the binding of AAB to the complex are summarized in *Table 1*. The calculations have been carried out in a similar manner to our previous report[1]. The isosbestic point of the spectrum of AAB shifted to

shorter wavelength with increasing acetate group on the chain. LA is the asymptotic value of ΔA when all the dye molecules are assumed to be bound on the complex. LA has a tendency to increase with increasing content of acetate groups. nK may be regarded as the binding constant corresponding to one Cu atom, and a convenient parameter to compare the relative affinity of binding. *Figure 5* shows the plot of nK against the degree of saponification of three series of copolymers. The binding affinity of AAB to the complex increased with increasing content of acetate groups of the copolymer. The enhancement of binding by the acetate group for copolymers (S) and (C) was larger than for copolymers (R) in the whole range of VAc contents. The high binding affinity of copolymers (S) or (C) may be associated with the block character of comonomer sequence.

The spectral data of the complex from copolymers (S) and the binding experiments seem to be inconsistent with each other. The absorption maximum of the complex and its intensity were little affected by VAc content, while the binding affinity of AAB increased with increasing content of VAc. These conflicting results may be interpreted by the following assumptions. (i) The stability of the complex is only governed by the length of the VA sequence, not by the VA content. (ii) The cooperative enhancement of dye binding is possible only when the complex has a sufficiently long VAc sequence. These assumptions explain well the spectral shift with VAc content and the relatively low binding affinity of AAB in the case of the random copolymer (copolymer (R) series). It is not clear at present why the cooperative increase of the binding affinity is observed for the block type VA–VAc copolymer. One possible explanation is the presence of hydrophobic microdomains due to the long VAc sequence, which serves to stabilize the absorbed AAB on the surface of the cluster.

The effect of pH on the binding affinity

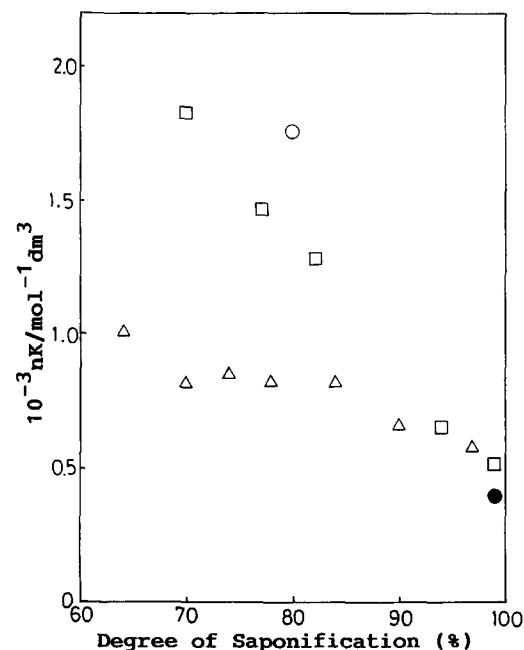The microenvironment of the PVA–Cu complex would be expected to change in the course of complex formation.

Figure 5 Relationship between nK and the degree of saponification: (●) PVA, (○) copolymer (C), (△) copolymers (R), (□) copolymers (S)
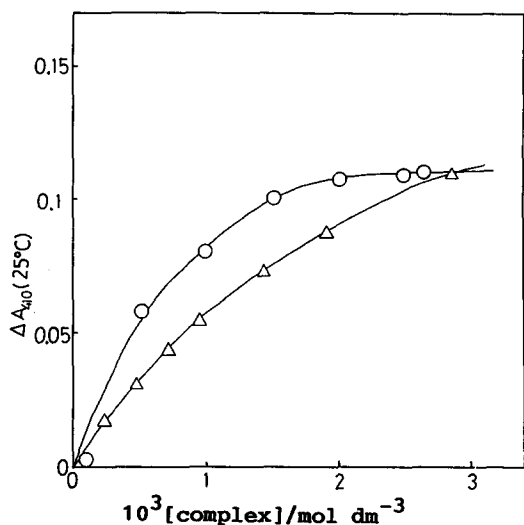
Figure 6 Effect of pH on the binding curve for PVA. The ratio [PVA]/[Cu] was 9.32, and the concentration of dye was 3.05×10^5 mol dm^{-3}. (O) Observed curve obtained under pH variation. (△) Calculated curve using binding parameters $1/n = 11.5$, $K = 5.19 \times 10^3$ and $\varepsilon = 6.90 \times 10^3$
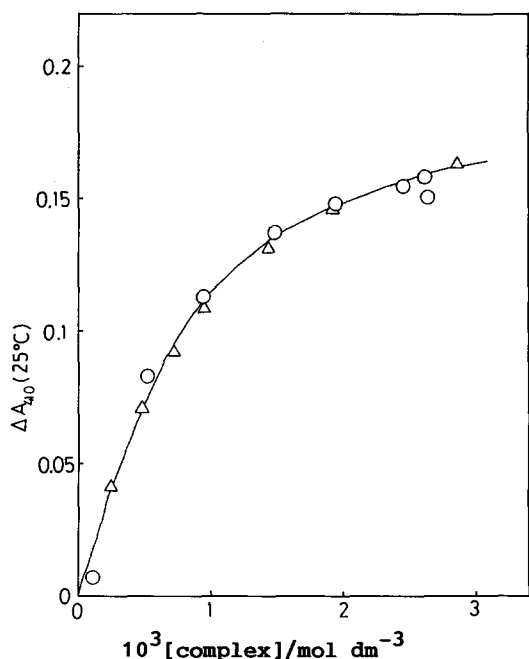

Figure 7 Effect of pH on the binding curve for copolymer (C). The ratio [PVA]/[Cu] was 8.59, and the concentration of dye was 3.05×10^5 mol dm^{-3}. (O) Observed curve obtained under pH variation. (△) Calculated curve using binding parameters $1/n = 8.43$, $K = 1.09 \times 10^4$ and $\varepsilon = 6.95 \times 10^3$

Since the complex is formed in the relatively narrow pH range of pH 5–7 by the addition of KOH to an aqueous mixture of PVA and cupric nitrate, it is of considerable interest to examine the effect of pH on the binding affinity of AAB.

Figure 6 shows a plot of ΔA against the concentration of the complex, which was determined by absorption spectra. The concentration was varied by changing the pH, under a constant concentration of PVA and Cu. If the binding constant is unchanged during complex formation, the relationship between ΔA and the concentration of the complex can be calculated using the binding parameters determined in the vicinity of pH 8. This is shown by open triangles in *Figure 6*. The observed ΔA was far higher than the calculated value as shown in

Figure 6. Thus the binding affinity of AAB to the complex formed in the first stage is higher than that expected from the binding data of the complex formed around pH 8.

On the other hand, the binding behaviour to the complex prepared from copolymer (C) is completely different. *Figure 7* shows a similar plot to *Figure 6* for the complex from copolymer (C). In this case, we found that the binding affinity was unaffected by the change of pH and that the observed ΔA coincided completely with the calculated value.

It is not clear at present why the pH effect on the binding behaviour is different for the PVA and the copolymer (C). A possible explanation is that the complex from copolymer (C) has a strong binding site for the dye, which is insensitive to a change in the environment. On the other hand, the binding site of the PVA complex would be easily influenced by the environment, such as the chain conformation of PVA.

The effect of temperature on the binding affinity

The temperature dependence of the binding affinity was investigated using PVA and copolymer (C). *Figures 8a* and *8b* show the differential absorbance spectra of the
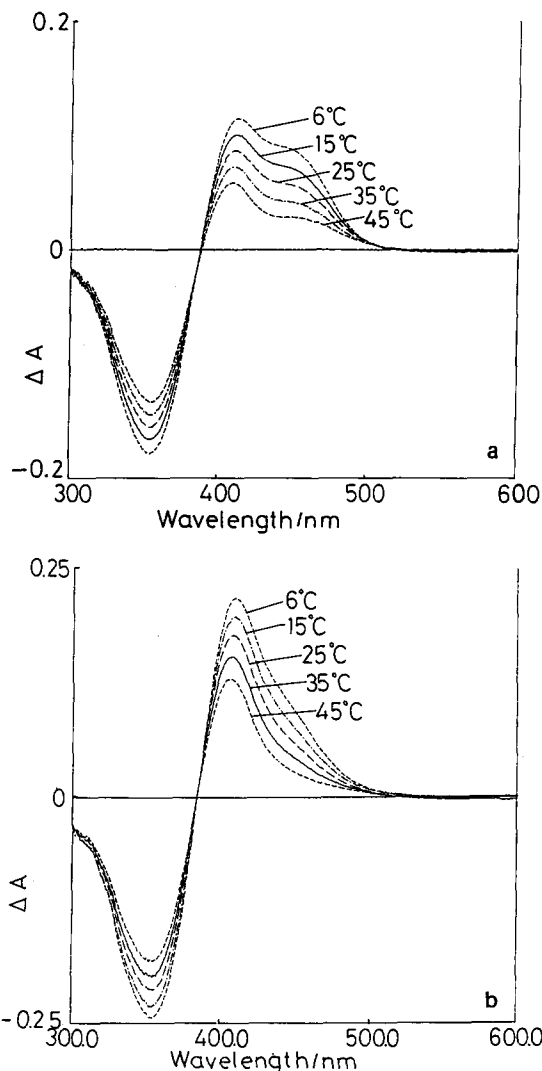

Figure 8 Effect of temperature on the differential absorbance spectra of AAB in the complex solution with reference to the spectrum in pure water. (a) PVA: concentration of dye was 2.71×10^{-5}; [PVA]/[Cu] was 18.7. (b) Copolymer (C): concentration of dye was 2.98×10^{-5}; [copolymer (C)]/[Cu] was 34.6

dye in the complex solution with reference to the spectrum in pure water at various temperatures. The intensity of the spectra decreased with increasing temperature. It should be noted that the variation of the spectra was reversible with respect to temperature. Moreover, the absorption spectra of the complex and the dye were unaffected by temperature changes.

The binding curves of AAB to the Cu complex are shown in *Figures 9a* and *9b*. Binding parameters n, K

and LA were calculated from these curves and are summarized in *Table 2*. If ΔA is determined only by the quantity of bound dye, the asymptotic value of ΔA (LA) should be independent of temperature. *Figure 9* shows, however, that this is not the case. Evidently LA decreases with increasing temperature. This result means that the spectrum of the bound dye depends on temperature, while that of the free dye was confirmed to be independent of temperature. Thus the binding parameters n and K are considered to be approximate values.

It would be of value, however, to derive several thermodynamic parameters from these binding parameters. Since the accuracy of the estimation of nK is relatively high, we calculated the thermodynamic parameters from nK according to the following equations[9]:

$$\Delta F = -RT\ln(nK) \tag{3}$$

$$\Delta H = -[d\ln(nK)/d(1/T)] \tag{4}$$

$$\Delta S = (\Delta H - \Delta F)/T \tag{5}$$

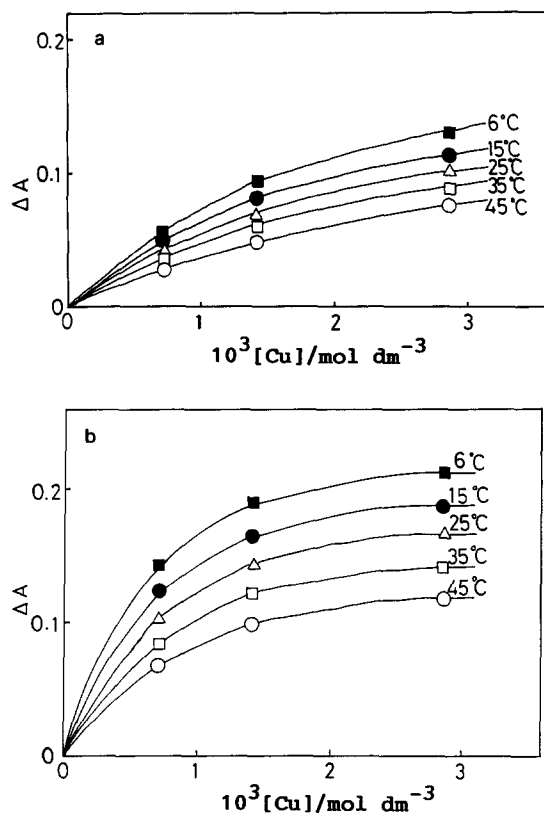where ΔF, ΔH and ΔS are the standard free energy,

Figure 9 Effect of temperature on the binding curves for (a) PVA and (b) copolymer (C). The concentration of dye and [polymer]/[Cu] ratio were the same as those of *Figure 8*

Table 2 Temperature dependence of binding parameters

(a) PVA

Temperature (°C)	LA	$1/n$	K (dm³ mol⁻¹)
6	0.219	10.2	5.60×10^3
15	0.193	10.4	5.58×10^3
25	0.187	11.5	5.19×10^3
35	0.160	11.4	5.06×10^3
45	0.163	14.6	4.62×10^3

(b) Copolymer (C)

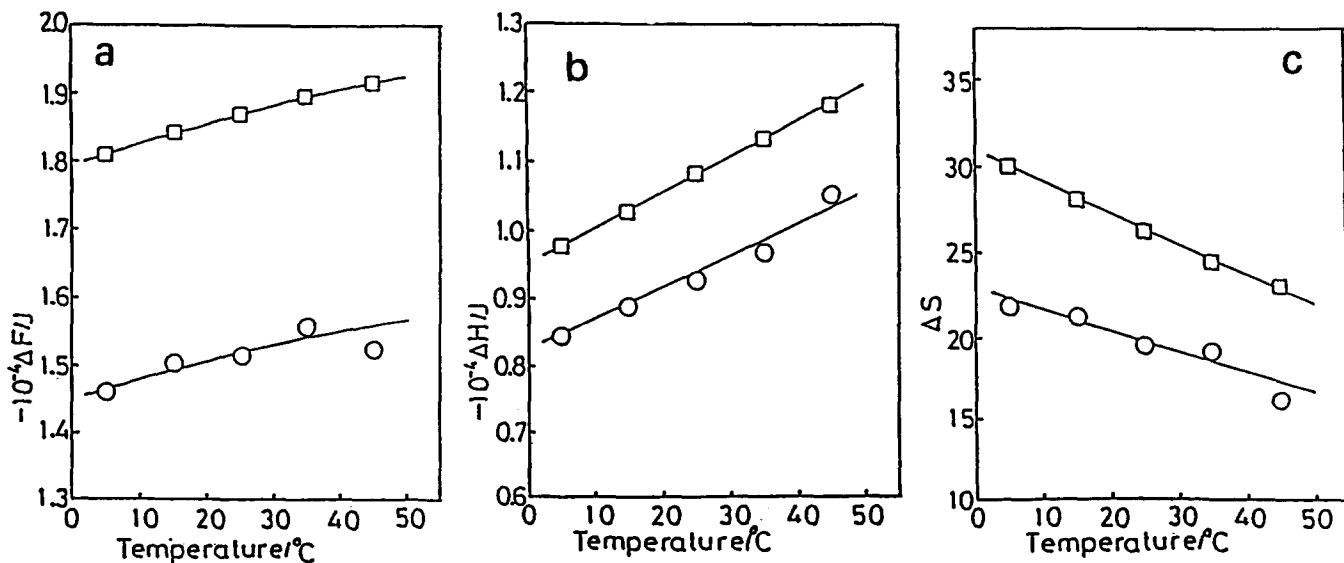| Temperature (°C)	LA	$1/n$	K (dm³ mol⁻¹)
6	0.246	8.10	2.03×10^4
15	0.222	8.33	1.84×10^4
25	0.201	8.42	1.59×10^4
35	0.175	9.28	1.53×10^4
45	0.151	9.15	1.28×10^4

Figure 10 Temperature dependence of the thermodynamic parameters: (a) standard free energy change; (b) standard enthalpy change; (c) standard entropy change. □, copolymer C; ○, PVA

enthalpy and entropy of binding. These thermodynamic parameters are plotted as a function of temperature in *Figures 10a, 10b* and *10c*. As shown in *Figure 10*, $-\Delta F$ and $-\Delta H$ increased and ΔS decreased with increasing temperature. This means that the unfavourable entropy change accompanying the increase of temperature is compensated by the more favourable enthalpy change. The net effect of the increase of temperature is a favourable free energy change for dye binding.

The above effect of temperature is very similar to that of a hydrophobic interaction[10]. A hydrophobic interaction is considered to be the association of non-polar solutes to diminish the cluster structure of water. At higher temperatures, the cluster structure of water would be destroyed. This effect results in the decrease of ΔH and ΔS. As shown in *Figure 10*, the effects of experimental temperature on thermodynamic parameters is in agreement with that expected from a hydrophobic interaction. Hence, it can be presumed that a hydrophobic interaction plays an important role in the binding of AAB to the PVA–Cu(ii) complex. It should be noted that the decrease of ΔH and ΔS with increasing temperature was larger for copolymer (C). This decrease implies a larger contribution of the hydrophobic effect in the complex from copolymer (C), which is consistent with the enhancement of the binding affinity of the dye to this complex. Thus the long VAc sequence of this polymer would participate cooperatively in the stabilization of the dye adsorbed on the surface of the cluster.

CONCLUDING REMARKS

The microstructure of partially acetylated PVA chains has a great influence on the binding affinity of AAB to the PVA–Cu(ii) complex. It was found that a long VAc sequence greatly enhances the binding ability of the dye. The enhancement could not be explained by the simple sum of the contributions from the complex and the VAc sequence, and some cooperative effect seemed to be operative.

The apparent thermodynamic parameters for the binding have been determined. The change of these parameters with temperature seemed to be characteristic of a hydrophobic interaction. This means that the binding would occur on the hydrophobic surface of the cluster, which appears only after the complex is formed. Thus the hydrophobic probe AAB provides a clue to investigate the structure and properties of the copper cluster.

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