Theory of Capillary Analysis and its Application to Dyeing Chemistry*

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A general theory of the capillary analysis of solution was proposed. The ratio (R_h) of heights of the solute to the solvent front was related to the partition (or adsorption) coefficient (K) by the following expression:

$$\alpha K = \frac{1 - \ln 2}{R_h - \ln(1 + R_h)}$$

where α is the ratio of volumes of non-mobile and mobile phases. This equation was applied to the investigation of the interaction between several dyes and organic polar substances, as well as to the association of dyes in aqueous solutions. Complex formations between dyes and other substances such as several surfactants, polyvinylpyrrolidone and the deoxyribonucleic acid were examined and discussed.

INTRODUCTION

Gotoh and the present author used the paper-chromatography for the investigation of the interaction between dyestuffs and surfactants in aqueous solutions[1]. It was found that various dyestuffs spotted on strips of filter paper were developed by non-ionic surfactants and values of R_f increased with the increasing concentration of the surfactants. But ionic surfactants, both anionic and cationic, did not show such a developing power. Relations between R_f and the concentration of nonionic surfactants were discussed, the formation of complex compounds being assumed between dyestuffs and surfactants.

It was pointed out, however, that for such a theoretical, or quantitative, investigation the method of paper-chromatography was not adequate. This was because that numerical values of R_f were affected by the initial positions of dyes spotted and on developing the spot the dye was diluted, thus making the observation difficult.

Thus, the present author used the method of capillary analysis instead of the chromatography, and attempted to investigate the interaction between dyestuffs and various substances. The capillary analysis has been known as a method for qualitative analysis of various mixtures[2], and Goppelsroeder (1910) investigated the components of blood, milk, urine, bile and other physiological substances. Fichter and Saalbom also used this method for the

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analysis of colloidal solutions (1910). The experiments of capillary analysis has usually been carried out in open systems, and these are not preferable, of course, in accurate measurements.

Since no one has ever given the general quantitative theory of the capillary analysis, the present author proposes a theory of the capillary analysis and shows its applications to dyeing chemistry in this paper.

I. THEORY OF CAPILLARY ANALYSIS

Fig. 1 shows the principle of the capillary analysis. In a closed vessel, the bottom of a strip of filter paper is dipped vertically into a solution of a dyestuff. At the equilibrium state, the heads of the solvent and the solute come to the heights A and B, respectively. The ratio of BC to AC is defined as R_h, C being the initial level of the solution. As is shown later, the density of the color on the filter paper is almost homogeneous from the level C to the level B, and fades out rapidly above the level B over a short range of distance.
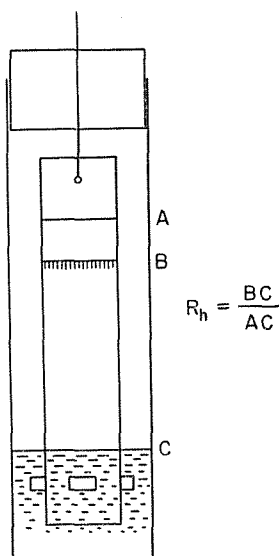

$$R_h = \frac{BC}{AC}$$

Fig. 1. Apparatus.

Plate theory of the capillary analysis : –

On the analogy of the plate theory of the liquid partition chromatography[3], the filter paper is regarded as being divided into successive layers of such a thickness that the solution issuing from each layer is in equilibrium with the mean concentration of the solute in the non-mobile phase throughout the layer. It is assumed that the diffusion from one plate to another is negligible and that the partition coefficient of solute between two phases is independent of its concentration.

It is assumed that during the rise of the solution the solute is in equilibrium at each plate and a fraction p of the solute in each plate is adsorbed

by the filter paper (the non-mobile phase), leaving a fraction q in the solution of the mobil phases. Accordingly

$$p+q=1. \tag{1}$$

The partition coefficient (K) is defined as follows:

$$K=\frac{p/V_s}{q/V_L}, \tag{2}$$

where V_s is the volume of liquid in the non-mobile phase and V_L the volume of the mobile phase. Hence, we have

$$\frac{p}{q}=K\frac{V_s}{V_L}. \tag{3}$$

When the solution is at the initial plate (the zeroth plate), the quantity of the solute in each plate is as follows:

  0th plate 1
  1st 〃 0
  2nd 〃 0.

When the solution rises to the first plate, a fraction p is adsorbed by the zeroth plate and a fraction q in the solution moves to the first plate. At the same time, the solution with a constant concentration is supplied to the zeroth plate; thus, the quantity of the solute in each plate is as follows:

  0th plate $1+p$
  1st 〃 q
  2nd 〃 0
  3rd 〃 0.

When it rises to the second plate, the distribution is given as

  0th plate $1+p+p^2$
  1st 〃 $q+2pq$
  2nd 〃 q^2
  3rd 〃 0
  4th 〃 0.

When it rises to the third plate, we have

  0th plate $1+p+p^2+p^3$
  1st 〃 $q+2pq+3p^2q$
  2nd 〃 q^2+3pq^2
  3rd 〃 q^3
  4th 〃 0
  5th 〃 0.

When it rises to the fourth plate, we have

  0th plate $1+p+p^2+p^3+p^4$
  1st 〃 $q+2pq+3p^2q+4p^3q$
  2nd 〃 $q^2+3pq^2+6p^2q^2$
  3rd 〃 q^3+4pq^3
  4th 〃 q^4
  5th 〃 0.

In the above-mentioned calculation, we find that the series of diagonal terms can be expressed as the binomial expansion of $(p+q)^n$. When the solution rises to the nth plate, the quantity of the solute in each plate is generally given as follows:

$$\left.\begin{aligned}
{}_nS_0 &= 1+p+p^2+p^3+\cdots\cdots+p^{n-1}+p^n \approx \frac{1}{1-p} \\
{}_nS_1 &= q+2pq+3p^2q+\cdots\cdots\cdots+np^{n-1}q \approx \frac{1}{1-p} \\
{}_nS_2 &= q^2+3pq^2+6p^2q^2+\cdots\cdots+{}_nC_2p^{n-2}q^2 \\
&\;\;\vdots \\
{}_nS_r &= q^r+(r+1)pq^r+\cdots\cdots+{}_nC_rp^{(n-r)}q^r \\
&\;\;\vdots \\
{}_nS_{n-2} &= q^{n-2}+(n-1)pq^{n-2}+{}_nC_2p^2q^{n-2} \\
{}_nS_{n-1} &= q^{n-1}+npq^{n-1} \\
{}_nS_n &= q^n
\end{aligned}\right\} \tag{4}$$

For a large value of n, ${}_nC_rp^{(n-r)}q^r$ has a maximum value at $r=r_m$, where $r_m = qn$. In the vicinity of r_m it can be written in the form:

$$ {}_nC_rp^{(n-r)}q^r = \frac{1}{\sqrt{2\pi npq}}e^{-\frac{(r-qn)^2}{2npq}} \tag{5}$$

Therefore, the total amount of the solute in the rth plate is given by

$$ {}_nS_r = \int_r^n \frac{1}{\sqrt{2\pi npq}}e^{-\frac{(r-qn)^2}{2npq}}\,dn. \tag{6}$$

In equation (6), when the value of n varies in the neighborhood of the value of n satisfying the condition $r-qn=0$, the variation of $(r-qn)^2$ is much larger than that of $\sqrt{2\pi npq}$ or $2npq$. So equation (6) can be approximated as follows:

$$ {}_nS_r \approx \frac{1}{\sqrt{2\pi npq}}\int_{-\infty}^x e^{-\frac{x^2}{2npq}}\,dx \tag{7}$$

where

$$ x = r - qn. \tag{8}$$

In equation (7), ${}_nS_r$ varies most steeply when $x=0$, and then

$$ (d^2{}_nS_r/dr^2) = 0.$$

Accordingly, the number of plates in which the quantity of the solute shows a finite variation is given by

$$\left.\begin{aligned}
r_m &= qn, \\
n-r_m &= pn.
\end{aligned}\right\} \tag{8}$$

From equations (3) and (8), we have

$$ \frac{p}{q} = \frac{(n-r_m)}{r_m} = \frac{1-\dfrac{r_m}{n}}{\dfrac{r_m}{n}} = K\cdot\frac{V_s}{V_L}, \tag{9}$$

where r_m/n is the ratio of the plate numbers.

As shown above, ${}_nS_r$, that is the total amount of the solute in the plates

from 0th to the neiborhood of the rth, has a value approximately equal to $1/(1-p)$. It suggests that the concentration of the solute in the mobile phase is nearly equal to the initial concentration of the solute.

In equation (9), we get a relation between r_m/n and K. In the capillary analysis of solutions, it is necessary to know the relation between R_h and r_m/n.

It is usually assumed that the upward velocity of the front of the solution along the filter paper, v_l, is inversely proportional to its height; i.e.

$$v_l = k/l,$$

where k is a constant.

During the movement of the solution from the level of height l cm to that of m cm, the velocity of the solution varies from k/l to k/m. Hence the average velocity v_a at the level l during this period of time is given by

$$v_a = \frac{k}{2}\left(\frac{1}{l} + \frac{1}{m}\right).$$

Now, under the assumption that the adsorption equilibrium in each plate is attained in time τ, $v_a\tau$ gives the order of the plate thickness[*]. When the solution reaches the level m, the number of plates (r) over the distance form zero to l is given by

$$r = \int_0^l \frac{1}{v_a\tau}\,dl = \frac{1}{k\tau}\int_0^l \frac{lm}{l+m}\,dl = \frac{2m^2}{k\tau}\left\{\frac{l}{m} - \ln\left(1 + \frac{l}{m}\right)\right\}. \tag{10}$$

Similarly, the number of plates (n) from zero to m is as follows:

$$n = \frac{2m^2}{k\tau}(1 - \ln 2). \tag{11}$$

Hence, the ratio of these plate numbers is given from equations (10) and (11) as follows:

$$\frac{r}{n} = \frac{\dfrac{l}{m} - \ln\left(1 + \dfrac{l}{m}\right)}{1 - \ln 2}, \tag{12}$$

Since R_h is the ratio of the distance between the solution surface and the level l_h, at which the quantity of the solute steeply fades out, the following equation is obtained from equations (9) and (12)

$$\frac{r}{n} = \frac{R_h - \ln(1 + R_h)}{1 - \ln 2} = \frac{1}{1 + K\dfrac{V_s}{V_L}} \tag{13}$$

and

$$\alpha K = \frac{1 - \ln 2}{R_h - \ln(1 + R_h)} - 1, \tag{14}$$

where

$$\alpha = \frac{V_s}{V_L}.$$

Thus, we have a simple relation between R_h and the adsorption coefficient (K).

* This is the definition of "plate".

II. APPLICATIONS TO DYE CHEMISTRY

1. Interaction between Dyes and Non-ionic Surfactants

As has been mentioned in the introduction, in the chromatographic investigation several dyes spotted on a strip of filter paper can be developed by non-ionic surfactant solutions. This fact suggests that molecules of non-ionic surfactants form complexes with dye molecules, thus solubilizing these dyes.

Relation between R_h and complex formation. The method of the capillary analysis was applied to investigate the interaction between dyestuffs and surfactants. Now, it is assumed that there are equilibrium between dyes, surfactants and the filter paper as shown in Fig. 2.

MOBILE PHASE

$$[D_L] \qquad m[S_L] \qquad [C_L]$$

$$\text{Dyestuff} + m(\text{Surfactant}) \underset{K_2}{\rightleftarrows} \text{Complex}$$

$$\Updownarrow K_1 \qquad\qquad \Updownarrow \qquad\qquad \Updownarrow K_2$$

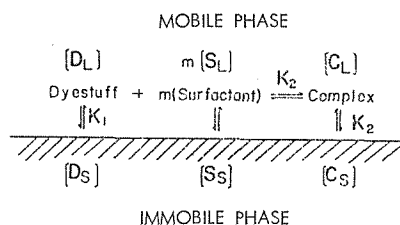$$[D_S] \qquad\qquad [S_S] \qquad\qquad [C_S]$$

IMMOBILE PHASE

Fig. 2.

The concentrations of the dyestuff, surfactants and the complex in the non-mobile phase are denoted by $[D_s]$, $[S_s]$ and $[C_s]$, and those in the mobile phase by $[D_L]$, $[S_L]$ and $[C_L]$, respectively. Then, we obtain the following three equations:

$$K_1 = \frac{[D_s]}{[D_L]}, \tag{15}$$

$$K_2 = \frac{[C_L]}{[D_L][S_L]^m}, \tag{16}$$

$$K_3 = \frac{[C_s]}{[C_L]}, \tag{17}$$

where K_1 is the adsorption coefficient of the dyes, K_2 the equilibrium constant of the complex formation and K_3 the adsorption coefficient of the complex. Then, the partition coefficient of the total dyes, K_r, is given as follows:

$$K_r = \frac{[D_s] + [C_s]}{[D_L] + [C_L]}. \tag{18}$$

From equations (15), (16), (17) and (18) we obtain

$$K_r = \frac{K_1 + K_2 K_3 [S_L]^m}{1 + K_2 [S_L]^m}. \tag{19}$$

If the concentration of dyes is much lower than that of the surfactant, the change in the concentration of surfactant by formation of complex is negligible. So, $[S_L]$ is nearly equal to the initial concentration. Using equations (14) and (19), we obtain

$$\alpha K_f = \frac{\alpha K_1 + \alpha K_2 K_3 [S_L]^m}{1 + K_2 [S_L]^m} = \frac{1 - \ln 2}{R_h - \ln(1 + R_h)} - 1. \tag{20}$$

In equation (20) the value of R_h for $[S_L] = 0$ is denoted by R_h^0, and then we have

$$\alpha K_1 = \frac{1 - \ln 2}{R_h^0 - \ln(1 + R_h^0)} - 1. \tag{21}$$

When $[S_L]$ is large and most of the dye molecules form complexes with surfactant molecules, R_h is denoted by R_h^∞. Then we have

$$\alpha K_3 = \frac{1 - \ln 2}{R_h^\infty - \ln(1 - R_h^\infty)} - 1. \tag{22}$$

Substituting equations (17), (21), (22) into (20) we have

$$\frac{[C_L]}{[D_L]} = K_2 [S_L]^m = \frac{\dfrac{1}{R_h^0 - \ln(1 - R_h^0)} - \dfrac{1}{R_h - \ln(1 - R_h)}}{\dfrac{1}{R_h - \ln(1 - R_h)} - \dfrac{1}{R_h^\infty - \ln(1 - R_h^\infty)}}. \tag{23}$$

$[S_L]$ and the right side of equation (23) are observable. Taking logarithms of equation (23), we can estimate the value of K_2 and m. Moreover, from the right hand side of the same equation we obtain the value of $[C_L]/[D_L]$, the concentration ratio of the combined to uncombined dyestuffs.

As is widely known, we have
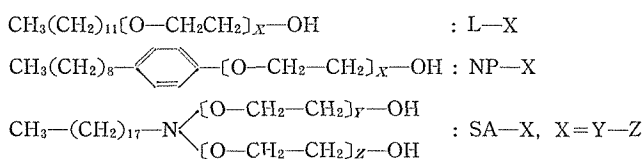
$$K_2 = e^{-\Delta\mu^0/RT}, \tag{24}$$

where $\Delta\mu^0$ is the standard chemical potential. Thus, we can estimate the value of $\Delta\mu^0$ from that of K_2 at various temperatures.

Experimental

Apparatus. The experimental apparatus is shown in Fig. 1. The chromatographic paper $(2 \times 40 \text{ cm}^2)$ was hung vertically in a closed glass tube which was kept in the air at various temperatures.

Procedure. About 20 ml. of the aqueous dye solution was poured into the glass tube. After the filter paper was kept in the glass tube for 2 hours, the lower part of the paper was dipped into the solution. The migration ratio of the dye R_h was observed after 2 hours.

Meterials. The dyestuffs were purified by the usual method. The nonionic surfactants were dissolved in xylene, and insoluble materials were removed by the filtration. These surfactants were dehydrated by the xylene-azeotrope process. The surfactants used were as follows:

$$CH_3(CH_2)_{11}[O\text{—}CH_2CH_2]_X\text{—}OH \qquad : L\text{—}X$$

$$CH_3(CH_2)_8\text{—}\langle\bigcirc\rangle\text{—}[O\text{—}CH_2\text{—}CH_2]_X\text{—}OH : NP\text{—}X$$

$$CH_3\text{—}(CH_2)_{17}\text{—}N\big\langle\begin{matrix}[O\text{—}CH_2\text{—}CH_2]_Y\text{—}OH\\ [O\text{—}CH_2\text{—}CH_2]_Z\text{—}OH\end{matrix} \quad : SA\text{—}X,\ X = Y\text{—}Z$$

The polyethylene glycol used was Carbowax 20 M. Polyvinyl pyrrolidone

(P.V.P.) used was of the commercial reagent grade (Plasdon C).

Results and Discussions

Interaction between dye and surfactant molecules. Since polyvinyl pyrrolidon (P.V.P.) has been known as a non-ionic decoloring agent, the interaction
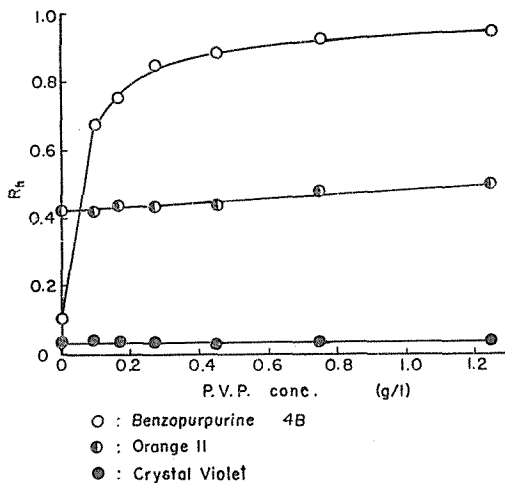

O : Benzopurpurine　4B
⊕ : Orange II
◉ : Crystal Violet

Fig. 3. Relationship between R_h and concentration of P.V.P. (30°C).

◉ : Benzopurpurine 4B　　△ : Orange II (equilibrium dialysis)
O : Orang II　　　　　　　 + : Crystal Vilet
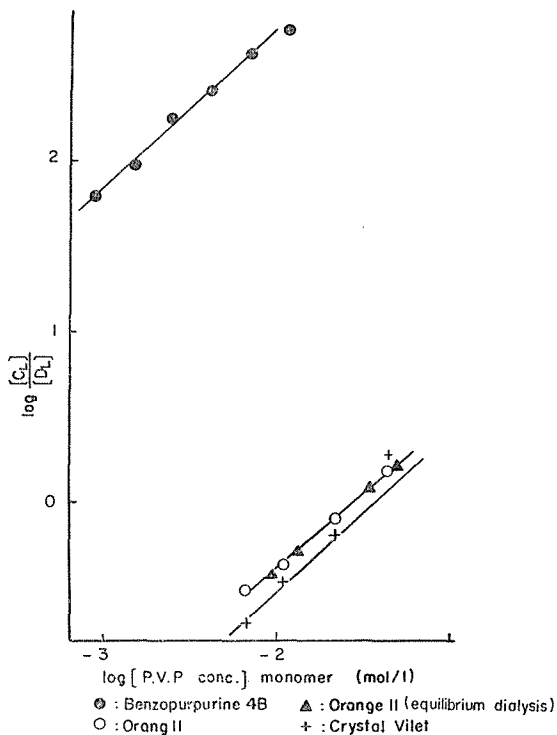
Fig. 4. Interaction between dyes and P.V.P. (30°C).

O : For capillary analysis
+ : For equilibrium dialysis
　Orange II : 5 × 10⁻⁵ mol/l 20°C
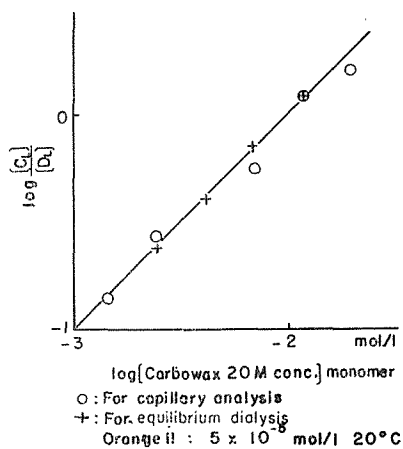
Fig. 5. Comparison between capillary analysis and equilibrium dialysis.

between P.V.P. and dyes was examined in comparison with surfactants. Fig. 3 shows the relation between the concentration of P.V.P. and R_h values for Benzopurpurine 4B, Orange II, and Crystal Violet. It is noticed that the R_h of Benzopurpurine 4B increases markedly with increasing P.V.P. concentration when the latter is small, but that neither of the remaining dyes shows such a marked increase. The linear relations between $\log(C_L/D_L)$ calculated by equation (23) and the logarithm of the P.V.P. concentration are shown in Fig. 4. The slope of lines m is unity, which is in good agreement with Scholtan's results obtained by the equilibrium dialysis[4]. In Fig. 4 the present author's values of $[C_L]/[D_L]$ for Orange II obtained by the equilibrium dialysis are also plotted, which come on the line obtained by the capillary analysis. The agreement between the capillary analysis and equilibrium dialysis carried out by the present author is also shown in Fig. 5, which gives values of $[C_L]/[D_L]$ obtained by both methods for the interaction between Carbowax 20 M and Orange II.

Table 1. Equilibrium Constant for Complex Formation between dyes and P.V.P.

Method Dye	Capillary analysis	Equilibrium dialysis	Conductivity[5]	Partition[4] equilibrium
Orange II	46	47	47	—
Benzopurpurine 4B	2.0×10^3	—	8×10^2	3.5×10^{12}

Table 1 shows that the equilibrium constant of the complex formation between Orange II and P.V.P. obtained by the capillary analysis agrees with that obtained by the equilibrium dialysis, while for the interaction between Benzo-purpurine 4B and P.V.P. equilibrium constant obtained by the present method lies between the scattered data obtained by the other authors. This difference may be due to the change in aggregation of Benzopurpurine 4B by changing
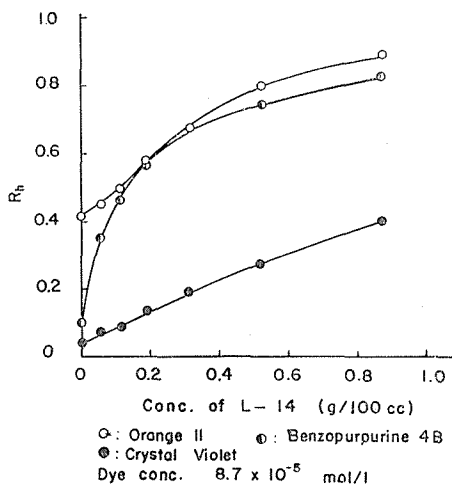

Fig. 6. Capillary analysis of dyes with aqueous solution of L-14 (30°C).

the concentration and also by the addition of electrolytes.

Values of R_h for various dyes are given as functions of the concentration of the nonionic detergent L-14 in Fig. 6. The R_h of Benzopurpurine 4B shows a marked increase with increasing L-14 concentration. Comparing Fig. 6 with Fig. 3, the change in R_h of Benzopurpurine 4B in the aqueous P.V.P. solution is larger, but that in R_h of Orange II or Crystal Violet is smaller, than in the L-14 solution.

Straight lines are obtained by plotting $\log [C_L]/[D_L]$ against $\log [S]$, the slope being the value of m, see Fig. 7 and 4. Fig. 4 shows at the same time the strong binding of Benzopurpurine 4B with P.V.P. and also the weak binding of Orange II or Crystal Violet with P.V.P. Considering the molecular structure of the dye, this was in good agreement with Scholtan's theory[4] ; dyes having N–H groups bind strongly with P.V.P.
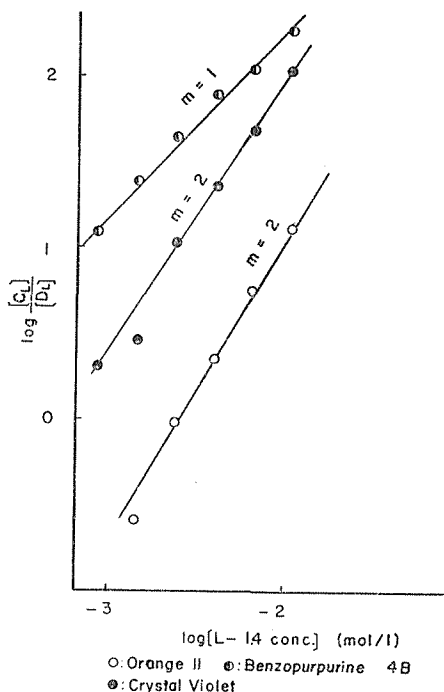
Fig. 7. Interaction between dyes and L-14 in aqueous solution (30°C).

In the case of P.V.P. it was found that the slope m for each dye was unity, and so the complex of dye with P.V.P. was of the 1 : 1 type. However, Fig. 7 shows that the slope for Benzopurpurine 4B was unity and that for Orange II or Crystal Violet was 2. The comparison of Fig. 7 with Fig. 4 shows that the binding of Benzopurpurine 4B with P.V.P. is stronger, and that of Orange II or Crystal Violet with P.V.P. is weaker, than with L-14. It seems, therefore, that the binding process with dye is a little different in the case of L-14 and P.V.P.

In Fig. 8 the value of $\log [C_L]/[D_L]$ obtained by the capillary analysis is
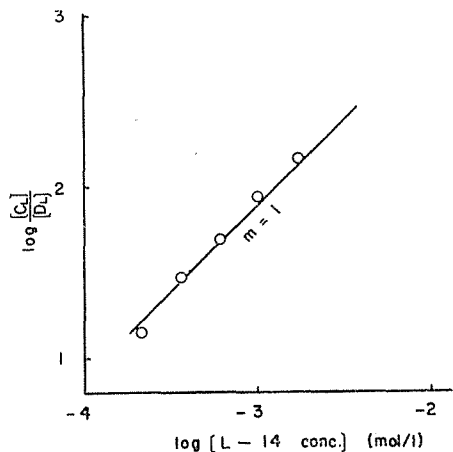
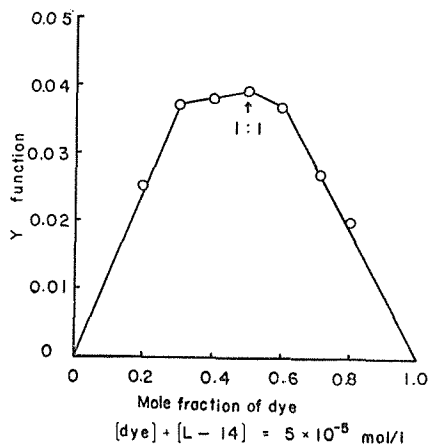Fig. 8. Interaction between Suminol Cyanine 5R and L614 (capillary analysis, 30°C).

Fig. 9. Interaction between Suminol Cyanine 5R and L-14 (optical density).

plotted against log $[S]$ for the combination of Solar Cyanine 5R and L-14, which shows that $m=1$ in this case. Fig. 9 shows the change in Vosburgh's Y function[6] with the mole fraction which was obtained by the optical density measurement. The composition of the complex is given by the mole fraction giving the maximum Y, which was $1:1$ in the present case. This was in good agreement with the result of capillary analysis obtained above.

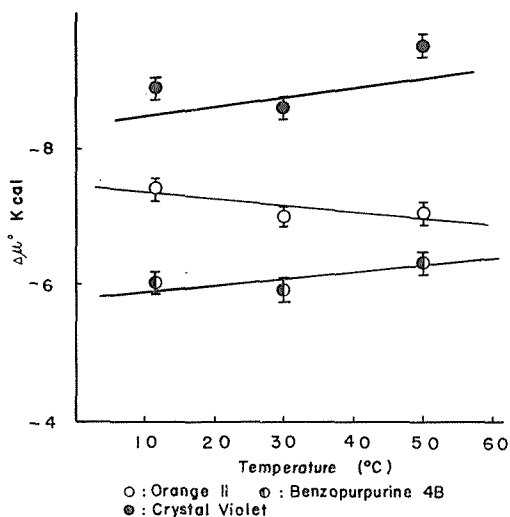Comparing Fig. 8 with Fig. 7 we find that Solar Cyanine 5R binds more

O : Orange II ◑ : Benzopurpurine 4B
◉ : Crystal Violet

Fig. 10. Effect of temperature on $\Delta\mu°$ for dyes-L-14 complex formation.
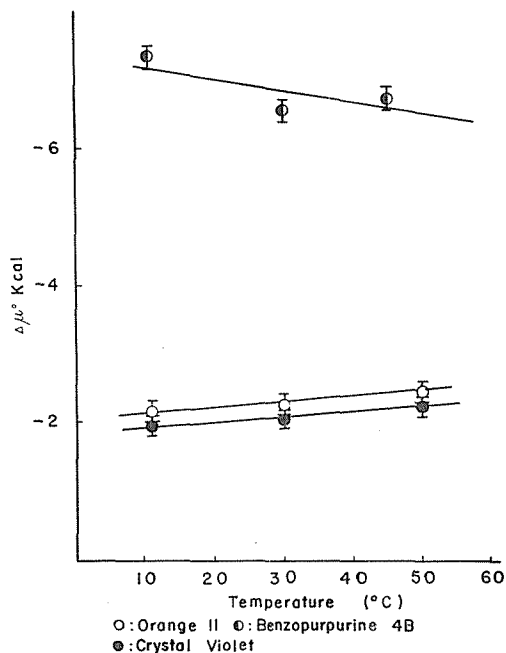
O : Orange II ◑ : Benzopurpurine 4B
◉ : Crystal Violet

Fig. 11. Effect of temperature on $\Delta\mu°$ for dye-P.V.P. complex formation,

strongly than Benzopurpurine 4B with L-14. It seems therefore that the complex formation of dyes with L-14 is independent of the number of N-H groups in the dye molecule. This is also shown by the fact that the Orange II and Crystal Violet which gave no N-H groups, combine strongly with L-14. It seems that the complex formation of dye with L-14 is due to the presence of the π-electron, which is common in dye molecules, rather than the H-bonding at NH groups.

There appears to be a relation between the size of dye molecules and the order of complex-formation (m). Large dye molecules, such as the Benzopurpurine 4B or Solar Cyanine 5R, form 1 : 1 complex, while small dye molecules, such as Orange II and Crystal Violet, form 1 : 2 complex.

The change in the standard chemical potential of the complex-formation ($\Delta\mu°_2$) was calculated by using equation (23), and was plotted against the temperature in Figs. 10 and 11. With the increase in temperature the $\Delta\mu^0$ of the complex-formation of Benzopurpurine 4B and Crystal Violet with L-14 increases, while that of Orange II decreases, see Fig. 12. Fig. 13 shows that the $\Delta\mu^0$ of the complex-formation of Benzopurpurine 4B with P.V.P. decreases with increasing temperature, while that of Orange II or Crystal Violet increases.

Table 2 gives values of ΔH^0 and ΔS^0 for the complex-formation.

Table 2.

	L-14		P.V.P.	
	ΔH (kcal)	ΔS (cal)	ΔH (kcal)	ΔS (cal)
Benzopurpurine 4B	− 3	10	−11	−15
Orange II	−10	−10	0.7	10
Crystal Violet	− 3.4	8	0.8	10

The Orange II-L-14 complex and Benzopurpurine-P.V.P. complex, which were found to have large negative ΔH values, have large negative values of the standard entropy change ΔS. It seems therefore that the strong binding of dyes with L-14 and P.V.P. reduces the internal degree of freedom of the complex. However, when heats of complex-formation ΔH are small, the standard entropy changes ΔS are found to have large positive values. Thus, it appears that structural changes probably occur upon binding in these cases. Now, in Figs. 12, 13, 14 and 15 the interaction of dyes with various surfactants is shown. For NP-10, NP-40, and SA-23, values of m of Crystal Violet at lower surfactant concentrations are larger than those at higher concentrations. The same tendency has been reported by Katsumi[7] for Solar Cyanine 5R. The value of m for Crystal Violet has the same value as Orange II at lower concentrations.

By comparing Fig. 13 with Fig. 14 we see that the value of m for Benzopurpurine 4B is smaller for the surfactant with a longer oxyethylene chain. The same tendency has been obtained by Nemoto for Solar Cyanine 5R[8,9].
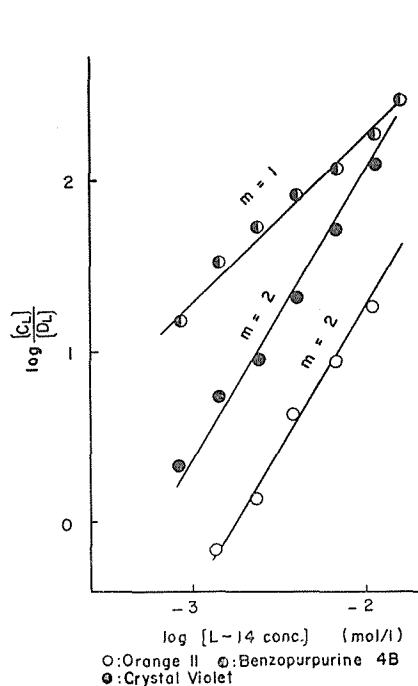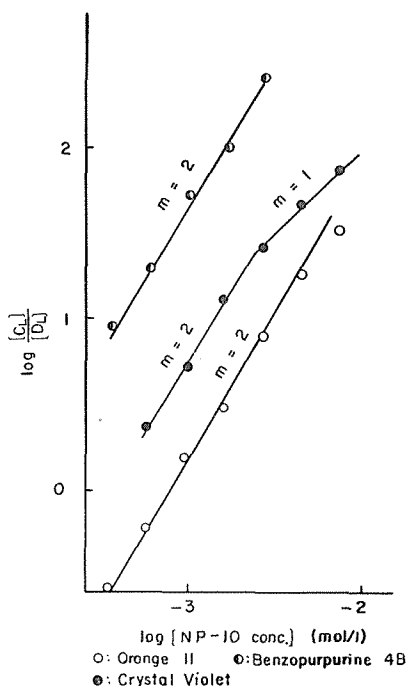
Fig. 12. Interaction between L-14 and dyes (20°C).

O:Orange II   ◑:Benzopurpurine 4B
◉:Crystal Violet

log [L-14 conc.] (mol/l)

Fig. 13. Interaction between NP-10 and dyes (20°C).

O: Orange II   ◑:Benzopurpurine 4B
◉: Crystal Violet

log [NP-10 conc.] (mol/l)
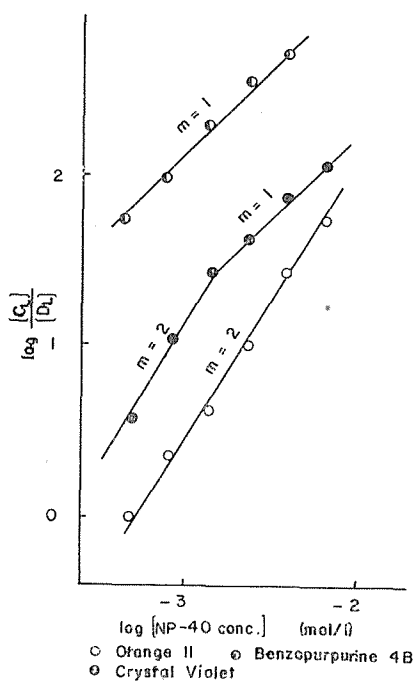
Fig. 14. Interaction between NP-40 and dyes (20°C).

O Orange II   ◑ Benzopurpurine 4B
◉ Crystal Violet

log [NP-40 conc.] (mol/l)
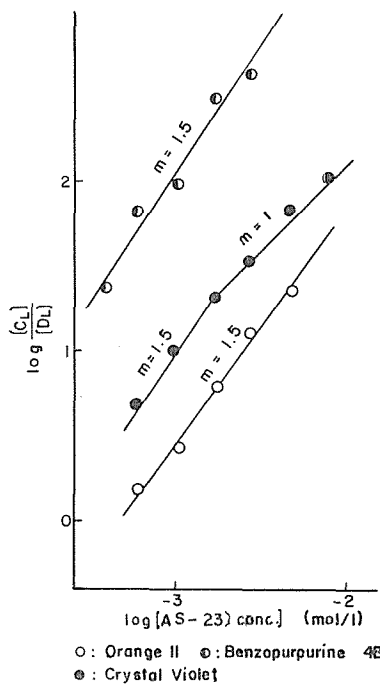
Fig. 15. Interaction between AS-23 and dyes (20°C).

O: Orange II   ◑:Benzopurpurine 4B
◉: Crystal Violet

log [AS-23] conc.] (mol/l)

2. Association of Dye Molecules in the Solution

The association of dye molecules in aqueous solution was investigated by the capillary analysis. It is assumed that associated molecules consist of a cluster of n single molecules. Let $[D_L]$ represents the concentration of single molecules, and $[A_L]$ that of associated molecules in the solution. Then we have the reaction

$$n[D_L] \rightleftharpoons [A_L],$$
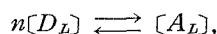
with an equilibrium constant K_A. Hence, we have

$$K_A = \frac{[A_L]}{[D_L]^n}. \tag{25}$$

Assuming that concentrations of single and associated molecules in the non-mobile phase are $[D_s]$ and $[A_s]$ respectively, and that the adsorption coefficients of the single and associated molecules are K_1 and K_2 respectively, we obtain following equations:

$$K_1 = \frac{[D_s]}{[D_L]}, \tag{26}$$

$$K_2 = \frac{[A_s]}{[A_L]}. \tag{27}$$

If c is the total concentration of dye molecules in the solution, and x the fraction of the molecules associated, we have

$$[A_L] = \frac{1}{n} xc, \tag{28}$$

$$[D_L] = c(1-x), \tag{29}$$

$$[A_s] = K_2 \frac{1}{n} xc, \tag{30}$$

$$[D_s] = K_1 c(1-x). \tag{31}$$

So, the apparent partition coefficient K_f of dye on the filter paper is as follows:

$$K_f = \frac{n[A_s] + [D_s]}{n[A_L] + [D_L]} = \frac{K_2 xc + K_1 c(1-x)}{c} = K_2 x + K_1 (1-x). \tag{32}$$

As was mentioned in the part of the theory of capillary analysis, the concentration of the solute in the mobile phase is almost equal to that in the solution except the vicinity of the solute front. Hence, an approximate relation is obtained from equations (14) and (32) as follows:

$$\alpha K_f = \alpha K_1 - \alpha(K_2 + K_1)x = \frac{1 - \ln 2}{R_h - \ln(1 + R_h)} - 1. \tag{33}$$

When R_h is R_h^0 at $x = 0$, we have

$$\alpha K_f = \alpha K_1 = \frac{1 - \ln 2}{R_h^0 - \ln(1 + R_h^0)} - 1. \tag{34}$$

When R_h is R_h^∞ at $x = 1$, we have

$$\alpha K_r = \alpha K_1 = \frac{1 - \ln 2}{R_h{}^\infty - \ln(1 - R_h{}^\infty)} - 1. \tag{35}$$

From equations (33), (34) and (35), we obtain

$$x = \frac{\alpha K_r - \alpha K_1}{\alpha K_2 - \alpha K_1} = \frac{\dfrac{1}{R_h - \ln(1 - R_h)} - \dfrac{1}{R_h{}^0 - \ln(1 - R_h{}^0)}}{\dfrac{1}{R_h{}^\infty - \ln(1 - R_h{}^\infty)} - \dfrac{1}{R_h{}^0 - \ln(1 - R_h{}^0)}}. \tag{36}$$

Using equations (25), (28) and (29), we have

$$cx = nK_A c^n (1 - x)^n, \tag{37}$$

and

$$\ln cx = \ln nK_A - n \ln c(1 - x). \tag{38}$$

When equation (38) holds, a straight line is obtained by plotting $\log cx$ against $\log c(1-x)$, the slope of the line being equal to n.

Results and Discussions

The relationship between values of R_h for Sky Blue 6G and the concentration of aqueous solution is shown in Fig. 16. The R_h approaches to a constant value with increasing concentration. The same results were also obtained for other reagents.
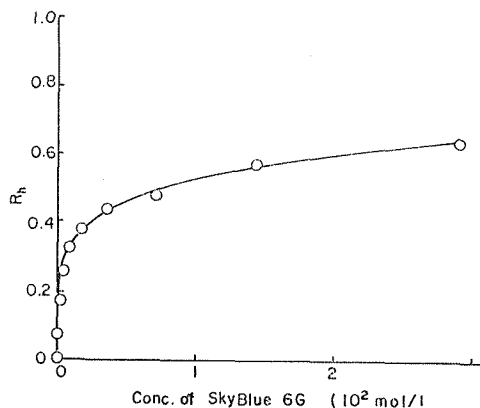

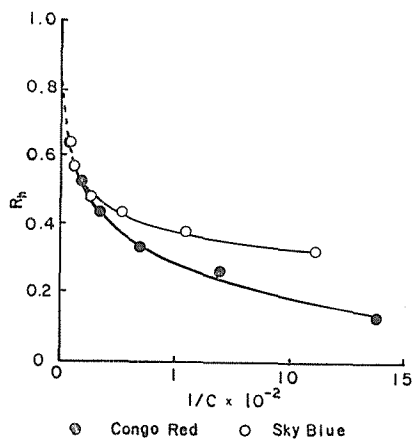
Fig. 16. R_h of Sky Blue at various concentrations (20°C).

Fig. 17. Plot of R_h against $1/c$ (20°C).

The relation between the value of R_h and the reciprocal of concentration is shown in Fig. 17. The intercept of the extrapolated line was used for the determination of $R_h{}^\infty$ in equation (36), and the value of R_h at the concentration 5×10^{-6} mol/1 was used for $R_h{}^0$ (which is nearly equal to 1). The fraction of associated molecules (x) can be calculated from equation (36).

Straight lines are obtained by plotting the logarithm of the concentration of associated molecules (cx) against the logarithm of the concentration of single molecules $c(1-x)$, see Fig. 18.
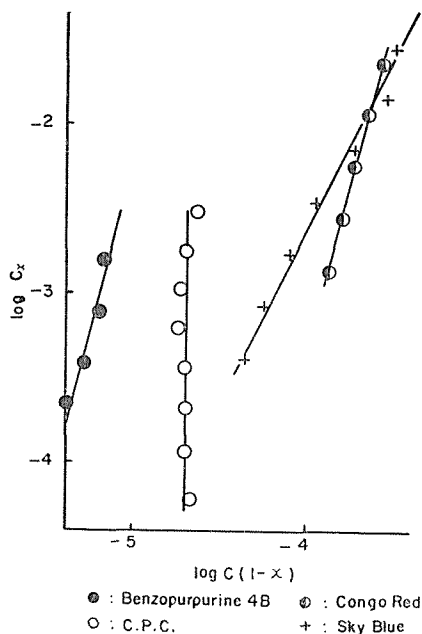
Fig. 18. Log c_x vs. $c(1-x)$ plots (20°C).

In Table 3 values of the degree of association of various dye molecules in aqueous solutions are given together with the data in literatures. The present data were in agreement with the lowest values in literatures, since the capillary analysis was carried out under the salt-free condition.

Table 3.

Method Dye	Osmotic[a] pressure	Diffusion[b]	Conductivity[b]	Capillary analysis
Congo Red	3.55	6 9	—	4.0
Benzopurpurine 4B	—	20 6 4	—	4.0
Sky Blue 6G	—	—	2.0	2.0

[a] W. Bilty, *Z. physik. Chem.* A77, 91 (1911).
[b] T. Vickerstaff, "The Physical Chemistry of Dyeing" London (1954).

In this case, R_h was determined by the color change of Congo Red solution sprayed on the filter paper. As is shown in Fig. 18 the slope m for CPC is very large. This result may be related to the micelle formation of the surfactant in the solution.

3. Interaction Between Dyes and Deoxyribonucleic Acid

It is well known that the deoxyribonucleic acid (DNA) is generally found in the chromosome of bio-cell and plays an important part in gene and multiplication. On the other hand, the mutation of bacteriophage by various dyes

and the developing of cancer have been observed. It is expected that a quantitative investigation of interactions of DNA with dyes will give some information concerning the macromolecular configuration of DNA in solution as well as the role played by the DNA in biological processes.

The interaction of DNA with acridine derivatives have been reported by various authors[10~13]. In this paper, studies on the interaction of various dyes with DNA by the capillary analysis and the infra-red spectroscopy are described.

Experimental

Materials. The dyes were purified by the usual method. The DNA used was of the commercial reagent grade. All solutions were made by using distilled water and kept at pH 7.0 with the 0.01 m phosphate buffer.

Results and Discussions

The R_h value of dyes obtained by capillary analysis are plotted against DNA concentrations in Fig. 19. While the value of R_h of anionic dyes (Orange II, Eosine, and Suminol Cyanine 5R) decreases with increasing DNA concentration, that of cationic dyes increases markedly.
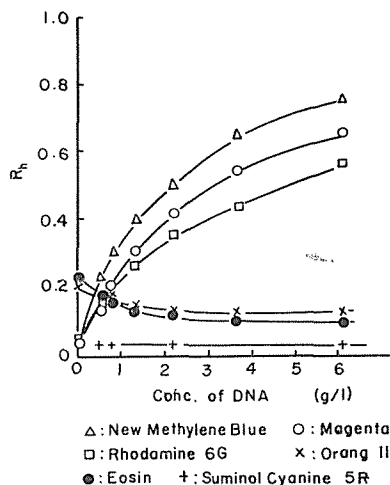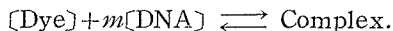

Fig. 19. Capillary analysis of dyes with aqueous solution of DNA (pH 7.0, 20°C).

Now, the following reaction is assumed to take place in aqueous solutions:

$$[Dye] + m[DNA] \rightleftarrows Complex.$$

Then, we obtain from equation (24)

$$\ln \frac{C_L}{D_L} = \frac{\Delta \mu^0}{RT} - m[DNA],$$

where [DNA] is the DNA concentration.

The data of Fig. 19 are plotted as $\log [C_L]/[D_L]$ against $\log [DNA]$ in Fig. 20 and 21, where $[C_L]/[D_L]$ is obtained from equation (23). It shows a good agreement with the theory, the slope of the line being the m value, and the
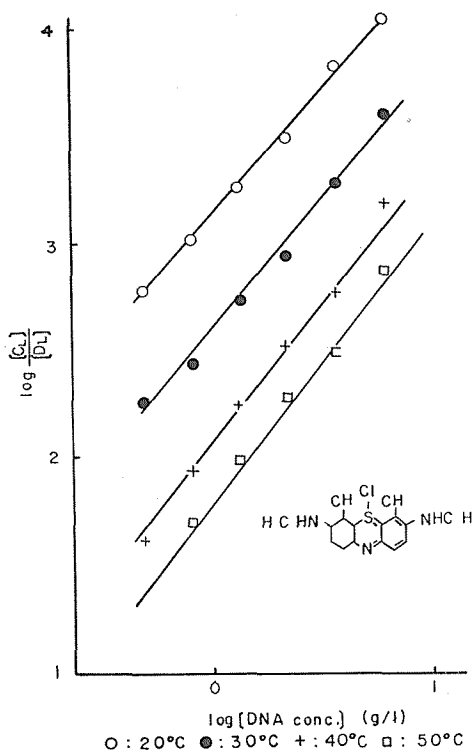
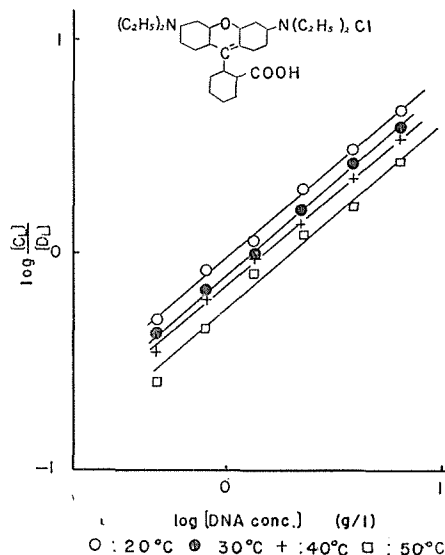Fig. 20. Interaction between New Methylene Blue and DNA.

Fig. 21. Interaction between Rhodamine 6G and DNA.

position of the line giving the value of the equilibrium constant of the com-
plex formation (K_2).   Comparing Fig. 20 with 21, we find that for dyes which
strongly combine with DNA the slope m is larger.   A large influence of tem-
perature on the position of lines is also found for these dyes.   Values of K_2
and m at 20°C are shown in Table 4 together with apparent heats of complex
formation.   According to Table 4, it seems that the dye molecules which have
a small and planar structure show the tendency to combine strongly with
DNA.   In this connection, Lerman[13] has pointed out that, since dyes combine
at the interior of the double-helix structure of DNA, a large molecule connot
enter the interior and be intercalated.   Thus, such a large molecule is difficult
to combine with DNA.

   The influence of the thermal denaturation : It has been known that thermal
treatment changes the DNA structure from the double-helix to the random
coil[14].   In order to examine this, the interaction of the thermally treated (at
100°C) DNA in an aqueous solution with the New Methylene Blue was observed.

   The result is given in Fig. 22, which indicates that the value of R_h de-
creases with increasing times of the thermal treatment and that the interac-
tion of DNA with dye is weakened.   It seems that the interaction of the dye
with the DNA of double helix structure is stronger than with that of the
random coil structure.   Since the behaviors of basic parts of DNA molecules
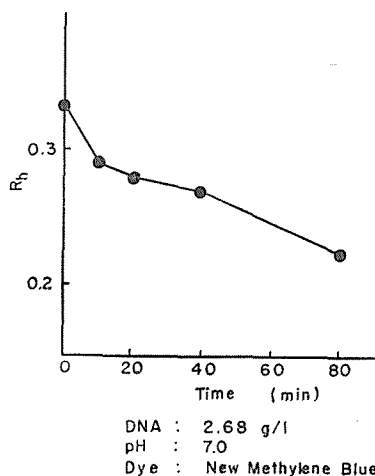
DNA : 2.68 g/l
pH : 7.0
Dye : New Methylene Blue

Fig. 22. Relationship between R_h of DNA and thermal treatment.

differ greatly, probably because of the degree of hydration and ionization, between the double-helix and random coil structures, the dye probably combine at the basic part of the double-helix structure.

The infrared absorption : The DNA were made into paste in liquid paraffin in which basic dyes were dissolved, and then the infra-red spectrum was observed. The infra-red absorption of the hydrogen-bonded base-pairs in DNA $(2680 \, cm^{-1})$[15] decreases by the addition of dyes such as New Methylene Blue and Crystal Violet. However, no such significant changes were found by the addition of dye which weakly combines with DNA, such as Bismark Brown. It seems that the base-pair formation in DNA is inhibited by binding with the dye.

The comparison with the structure units : In order to examine more about the binding of DNA with the dye, the interaction between the separate struc-
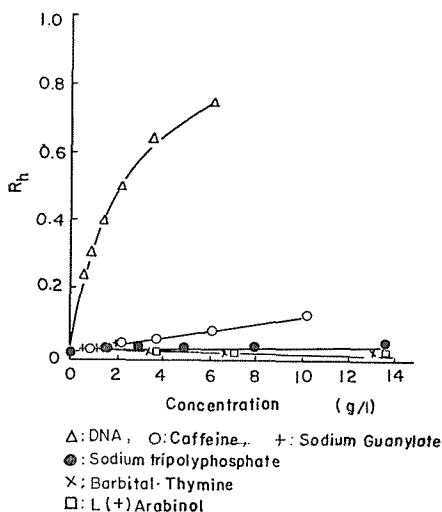

△: DNA , ○: Caffeine, +: Sodium Guanylate
◉ : Sodium tripolyphosphate
✕: Barbital·Thymine
□: L (+) Arabinol

Fig. 23. R_h of New Methylene Blue with unit material (20°C, pH 7.0).

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tural unit of DNA and the New Methylene Blue was investigated. The relation of the R_h of the New Methylene Blue to the concentration of various unit materials is shown in Fig. 23.

By adding purine derivatives, such as caffeine and sodium guanilate, R_h increases markedly with increasing concentration, while the addition of pyrimidine derivatives, such as thymine and barbital, does not show such singificant changes. The sodium tripolyphosphate also combines a little with New Methylene Blue, and $L(-)$ arabinose combines very little.

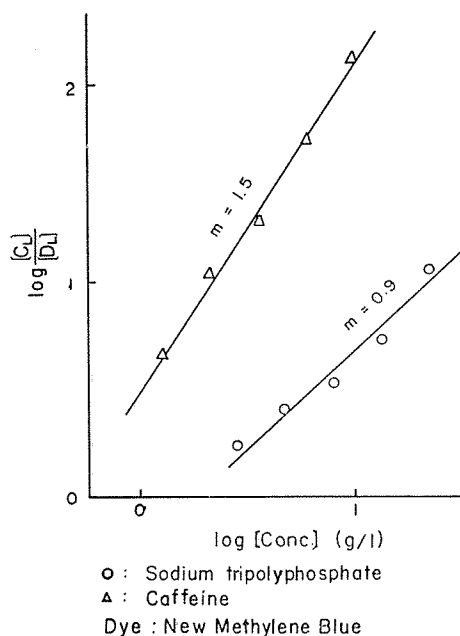The linear relation obtained from equation (23) is shown in Fig. 24. The

o : Sodium tripolyphosphate
△ : Caffeine
Dye : New Methylene Blue

Fig. 24. Interaction between New Methylene Blue and unit materials (20°C, pH 7.0).

Table 4.

Dye	K_2	m	E
New Methylene Blue	1.35×10^3	1.3	18 kcal
Methylene Blue	5.7×10^2	1.3	14
Acridine Orange NS	5.0×10^2	1.3	14
Safranin O	2.0×10^2	1.4	10.5
Methyl Violet	1.2×10^2	1.2	12.6
Basic Blue GO	1.0×10^2	1.4	10.1
Crystal Violet	6.3×10	1.3	5.6
Magenta	3.4×10	1.3	8.4
Rhodamine 6G	1.5×10	1.2	5.7
Primocyanine 6GX	1.1×10	1.2	5.4
Malachit Green	1.0×10	1.1	9.8
Rhodamine B	1.0	0.9	3.6
Bismarck Brown	0	—	—

value of m for caffeine is 1.5, and hence three caffeine molecules appear to combine with two dye molecules. The value of m for the sodium tri-polyphosphate is 0.9, and so it seems that one dye molecule combines with one phosphate.

The mode of binding: Summarizing the data in Table 4, the value of m for the weak binding of dye with DNA is 0.9, which is equal to the value for the binding of New Methylene Blue with phosphate, and value of m for the strong binding of dye with DNA is nearly equal to that for the binding of the New Methylene Blue with the purine base.

According to the Watson-Crick Model[14] the base-pair is composed of purine and pyrimidine bases. However, the strongly binding dyes combine only with the former. It seems that two dye molecules are intercalated between adjacent three purine bases of one of the DNA molecular chains, thus preventing the base-pair formation between the two chains.

ACKNOWLEDGMENTS

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