

Theoretical Considerations on Mononuclear Aggregations of Mixed Nucleosides in Dilute Aqueous Solutions Based upon Osmometric Measurements

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Abstract □ A theory is presented based on experimental work reporting shifts in the critical micelle-like concentrations of nucleosides and caffeine. An increase in these concentrations by multiple integers (called complexing capacity numbers) of the concentration of the vehicle compound, whose concentration was fixed, was observed for substances that tend to form complexes with each other and with some other compounds and to self-associate as well when their concentrations are varied. The physical model presented assumes the significance of the potential hydrogen bonding sites in determining the mode of aggregation as well as the magnitude of these complexing capacity numbers. This assumption was verified experimentally when reducing the hydrogen bonding by using 1,3-dimethyluracil. The mathematical model hypothesized has provided a means for calculating equilibrium constants for various consecutive processes as well as other quantities of theoretical interest.

Keyphrases □ Nucleosides, mixed—mononuclear aggregations, dilute aqueous solutions, osmometric measurements, theoretical considerations □ Aggregations, mononuclear—mixed nucleosides, dilute aqueous solutions, osmometric measurements, theoretical considerations □ Complexing capacity numbers—theoretical considerations, mononuclear aggregations of mixed nucleosides in dilute aqueous solutions based upon osmometric measurements

Goyan and Borazan (1) used an improved thermoelectric osmometer to study the colligative properties of dilute aqueous solutions containing caffeine and various substances of pharmaceutical interest. When a colligative property was plotted *versus* total molal concentration of caffeine (variable substance), pure or mixed with another substance kept at constant concentration (vehicle compound), two linear slopes could be used to represent all of the data. The points of break were referred to as the critical concentrations and were reported in each case.

The first slope of pure caffeine solution, expressed in terms of $\Delta R \Delta M^{-1}$, had a value similar to that determined for sucrose, indicating a monomeric behavior; a lower value was found for the second slope. Lower values were reported for both slopes of caffeine in the presence of a fixed concentration of a second substance which was used as a vehicle.

These critical concentrations were shifted to higher values on the molal scale of caffeine in the presence of a vehicle (1). When this increment in the critical concentration was divided by the vehicle concentration, a quotient approaching a simple integer was obtained. The investigators referred to these integers as the complexing capacity numbers; these numbers were assumed to be related to the stoichiometry of the complex. Later, the existence of the effect was confirmed with some biologically active nucleosides (2, 3), few of which were reported previously to form complexes and to self-associate (4, 5).

The purpose of this paper is to present theoretical,

physical, and mathematical considerations that shed some light upon the origin of the shift in these critical micelle-like concentrations and the mechanism of the formation of these complexing capacity numbers.

EXPERIMENTAL

Instrumentation—The vapor phase thermoelectric osmometer used was described previously (1, 6–8). The measured colligative property is expressed in terms of ΔR , the difference between the resistances of the measuring thermistor when in contact with pure solvent and when in contact with the sample under study for a given molal concentration.

Solutions—Solutions were prepared with distilled water on a molal basis. 2'-Deoxyguanosine¹, 1,3-dimethyluracil¹, and 2'-deoxyadenosine² were obtained commercially. An assay for the percentage of water was made by drying over phosphorus pentoxide in a vacuum drying apparatus at the boiling point of absolute alcohol. This percentage was used to correct the weight of solid chemicals when preparing various concentrations.

Reagent grade sucrose crystals³ were used to prepare the solutions used as the reference standard.

Standardization—The method was fully described previously (1–3). A value of 923.7 ohms/molal for the slope of the standard solution of sucrose is used for this work.

THEORY

To explain the origin of the shift in the critical micelle-like concentrations (1–3), the inhibition of self-association of the variable compound by a certain multiple integer of the vehicle concentration was investigated as a possible mechanism.

Pyrimidine Nucleosides—According to Table I, the self-association of pyrimidine nucleosides is modified by purine and pyrimidine nucleosides. When 2'-deoxyadenosine is used as a vehicle (3) (Table I), complexing capacity numbers of 6, 6, and 9 are obtained with thymidine, cytidine, and uridine, respectively, when they are used as variables. When 2'-deoxyguanosine is used as a vehicle, complexing capacity numbers of 7, 7, and 9 are obtained from these nucleosides, respectively, when they are used as variables.

A reasonable hypothesis is that these numbers represent the average number of molecules involved with one molecule of vehicle before the self-association takes place. The low bonding energy rules out firm complex formation and indicates that attachments must be regarded as arising from second-order effects. On the basis of this hypothesis, one can construct a model showing the possible orientation of these pyrimidine nucleosides relative to purine nucleosides. The model cannot represent a fixed structure but should show the most probable mode of aggregation having the lowest possible energy due to configuration. However, a comparison of the possible binding sites with the calculated complexing capacity numbers (Table I) provides clear evidence that there is some correlation between the possible potential hydrogen bonding sites and the arrangement of the molecules in solution.

Based on NMR data, Schweizer *et al.* (9) suggested the breakdown of purine and pyrimidine aggregates by pyrimidine nucleosides and/or their insertion between the purine stacks; a high concentration of purine was used. More recently, Raszka and Kaplan

¹ Nutritional Biochemicals.

² General Biochemicals.

³ ACS Code 2376.

Table I—Comparison between the Number of Binding Sites on the Vehicle Molecule Relative to Variable Used and the Complexing Capacity Numbers with the Variable Molecule for Different Combinations of Nucleosides

Variable-Vehicle	Number of Binding Sites on Vehicle Molecule Relative to Variable Used	Complexing Capacity Numbers with Variables ^a
2'-Deoxyadenosine-thymidine (0.02 M)	4	1
2'-Deoxyadenosine-uridine (0.02 M)	4	1
2'-Deoxyadenosine-2'-deoxyguanosine (0.01 M)	7	4
2'-Deoxyguanosine-cytidine (0.003 M)	5	1
2'-Deoxyguanosine-2'-deoxyadenosine (0.003 M)	6	3
Uridine-2'-deoxyadenosine (0.015 M)	6	9
Uridine-2'-deoxyguanosine (0.012 M)	7	9
Cytidine-thymidine (0.03 M)	4	4
Cytidine-2'-deoxyadenosine (0.015 M)	6	6
Cytidine-2'-deoxyguanosine (0.012 M)	7	7
Thymidine-2'-deoxyguanosine (0.012 M)	7	7
Thymidine-2'-deoxyadenosine (0.015 M)	6	6
Thymidine-cytidine (0.02 M)	5	3
1,3-Dimethyluracil-2'-deoxyadenosine (0.015 M)	2	2
1,3-Dimethyluracil-2'-deoxyguanosine (0.012 M)	3	3

^a Figures are approximate and were rounded to integers. They were calculated according to data taken from Ref. 3 and Table III.

(10) obtained evidence for association by hydrogen bonding of mononucleotides in aqueous solution based upon high-resolution PMR spectroscopy (220 MHz). The absence of self-association in water of some purine and pyrimidine base derivatives also was reported (11). On these bases, it is proposed that a spherical orientation might occur in which a purine nucleoside serves as a template surrounded by the smaller pyrimidine nucleosides so that the surrounding nucleosides orient themselves in the least hindered way. This orientation possibly can be by way of partial parallel stacking of their bases, with the ribose or deoxyribose directed away from the template molecule.

This picture is somewhat similar to that proposed for the mode of stacking of pyrimidine nucleosides based upon their osmometric measurements (9), and it is well illustrated in the model⁴ shown in Fig. 1. The thymidine molecules are crowded around the template molecule and appear in a similar (but partial) way to the average parallel stacking of pyrimidine nucleosides (12). The potential hydrogen bonding sites also seem to play a role in directing the mode of aggregation of the nucleosides around the template at a distance of 3-4 Å (13) from each other.

Many factors seem to account for the smaller complexing capacity numbers associated with the inhibition of self-association of the pyrimidine nucleosides by other pyrimidines. These factors include the lack of aromaticity, the smaller size, the difference in steric effects, the smaller number of potential hydrogen bonding sites (Table I), and the differences in other physicochemical properties (14, 15).

Purine Nucleosides—The mechanism of inhibition of self-as-

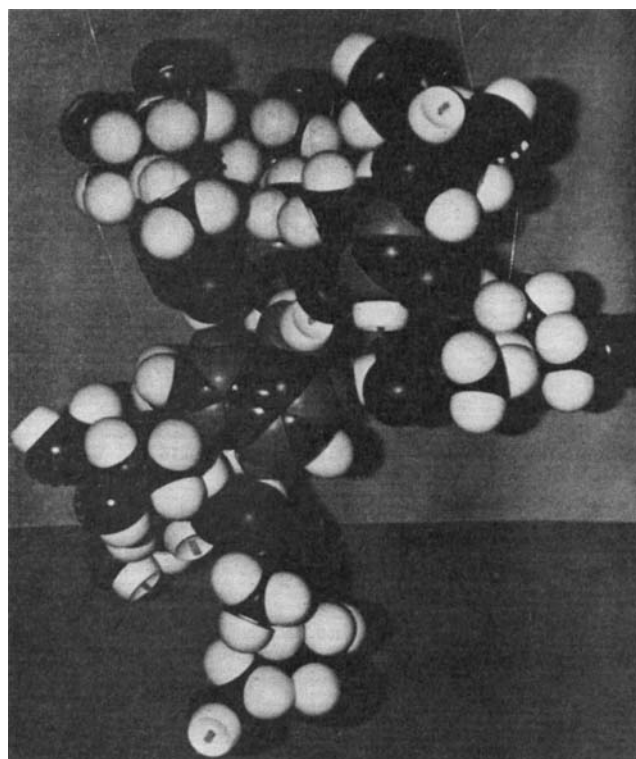


Figure 1—Model representing hypothetical aggregation: a pictorial representation of a space-filling model showing the way the potential hydrogen bonding directs the mode of aggregation most effectively around the template (2'-deoxyadenosine). In reality, it is not a fixed rigid structure but a rather dynamic situation. The surrounding molecules (thymidine) crowd around the template molecule in such a dynamic way that an average partial parallel stacking is implied from this dynamic state. Approximate overall dimensions are 28 × 21 Å.

sociation and the mode of aggregation of the purine nucleosides induced by other purine or pyrimidine nucleosides seem very similar to those described for pyrimidine nucleosides. The differences observed in the complexing capacity numbers can be accounted for on the same basis (Table I). The orientation of purine nucleosides around another purine nucleoside, which serves as a template, seems to be similar to that described previously. The partial average parallel stacking (16) is also proposed. Several investigators (5, 17, 18) confirmed that the stacking is similar to the π -complex of benzene in chloroform (19, 20). However, the picture is not simple; the model presented represents the orientation in an infinitely small amount of time. Rapid exchange of bases and nucleosides has been reported (5), in connection with the break and reformation of the stacks, because the free energy of self-association and of complex formation is of the order of magnitude of the thermal kinetic energy (4, 5, 15, 21-24).

These results are in agreement with those presented by Jardetzky (12) for bases of purine and pyrimidine derivatives: "... that the rate of exchange of bases between the stacks and the free state is of an order of magnitude comparable to the rate of diffusion. Thus, the forces responsible for 'hydrophobic' bonding of nucleic acid bases appear to be sufficient to produce some preferred orientation of the bases in solution, but insufficient to maintain stable rigid arrays." This conclusion, from NMR studies, is in agreement with the dynamic picture hypothesized here of the absence of stable discrete complexes and the presence of aggregates, spherically oriented, in a dynamic state.

According to suggested models (5, 25, 26) and theoretical calculations (27), all possible orientations could exist. The lifetime is so short for each, about 10^{-9} sec (12), that what one deduces from NMR or other physical measurements is an average phenomenon of the entire dynamic picture. The contribution of one form might

⁴ Overall dimensions were measured using CPK atomic models, Schwarz Bioresearch, Inc., Orangeburg, NY 10962

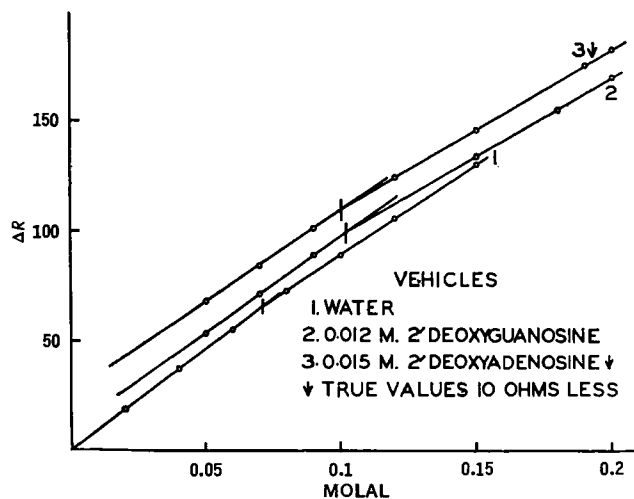


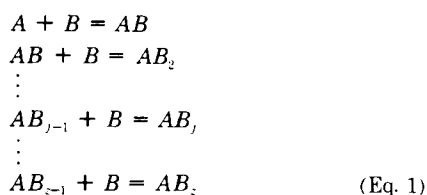
Figure 2—Plot of ΔR in ohms versus molal concentration of 1,3-dimethyluracil. Arrow indicates displacement by 10 ohms to avoid crowding.

be different from that of another because of various factors, including those that are inherent in the molecules themselves, such as size, aromaticity, polarizability, polarity, potential hydrogen bonding sites, and other physicochemical properties (14, 15).

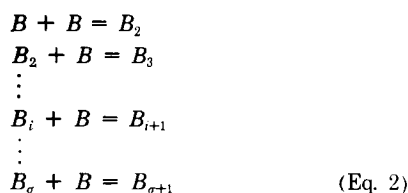
The concentrations used for the study of self-association were all 0.1 molal and lower; the nucleoside mixtures were all 0.2 molal and lower (2, 3). Very dilute solutions were preferred over concentrated solutions to trace this effect and also to obtain a better picture of the behavior. Use of concentrated solutions could put constraints on the system and force one process over the other with the result that the actual picture could be deformed.

The present dynamic theory of these complexes is not in conflict with previous work on stacking but merely suggests that the potential hydrogen bonding, if present, might play a role in directing the mode of stacks of these aggregates, which are in a dynamic state. However, it is a reasonable hypothesis that the magnitude of the number of the potential hydrogen bonding sites can determine the total number of monomers in each aggregate (including both the bonded and nonbonded species), based upon the complexing capacity numbers each has with other similar base derivatives, *i.e.*, purine-purine and pyrimidine-pyrimidine. On these bases, the numbers in Table II are assigned for the nucleosides studied.

Based upon the physical interpretations of the described effect, the following mathematical model is proposed for calculating equilibrium constants for the multiple processes of mononuclear aggregation around a solute molecule:



If the variable substance self-associates too, then:



In the presented models, it is assumed that no interactions among mixed and homogeneous associates, AB_j and B_i , respectively, take place; that the vehicle compound, A , does not self-associate; and that the binding sites on the nucleus molecule, A , for

heterogeneous association processes are dependent and nonequivalent, *i.e.*, the binding of a free B to A can influence the binding of another B to the same nucleus.

Where z and σ are the maximum numbers of multiple equilibria, the corresponding equilibrium constants will be:

$$K_1 = (AB)/(A)(B) \quad (AB) = K_1(A)(B) \quad (\text{Eq. 3})$$

$$K_2 = (AB_2)/(AB)(B) \quad (AB_2) = K_2(AB)(B) \quad (\text{Eq. 4})$$

$$K_j = (AB_j)/(AB_{j-1})(B) \quad (AB_j) = K_j(AB_{j-1})(B) \quad (\text{Eq. 5})$$

$$K_z = (AB_z)/(AB_{z-1})(B) \quad (AB_z) = K_z(AB_{z-1})(B) \quad (\text{Eq. 6})$$

$$\beta_1 = (B_2)/(B)^2 \quad (B_2) = \beta_1(B)^2 \quad (\text{Eq. 7})$$

$$\beta_2 = (B_3)/(B_2)(B) \quad (B_3) = \beta_2(B_2)(B) \quad (\text{Eq. 8})$$

$$\beta_i = (B_{i+1})/(B_i)(B) \quad (B_{i+1}) = \beta_i(B_i)(B) \quad (\text{Eq. 9})$$

$$\beta_{\sigma} = (B_{\sigma+1})/(B_{\sigma})(B) \quad (B_{\sigma+1}) = \beta_{\sigma}(B_{\sigma})(B) \quad (\text{Eq. 10})$$

By substituting for (AB) of Eq. 3 into Eq. 4:

$$(AB_2) = K_1K_2(A)(B)^2 \quad (\text{Eq. 11})$$

If the same technique of substitution is carried out:

$$(AB_j) = K_1 \dots K_j(A)(B)^j \quad (\text{Eq. 12})$$

$$(B_{i+1}) = \beta_1 \dots \beta_i(B)^{i+1} \quad (\text{Eq. 13})$$

According to Eq. 1, the total concentration of the vehicle, (A_0) , can be expressed:

$$(A_0) = (A) + (AB) + (AB_2) + (AB_3) + \dots + (AB_j) + \dots + (AB_z) \quad (\text{Eq. 14a})$$

$$(A_0) = (A) + \sum_{j=1}^z (AB_j) \quad (\text{Eq. 14b})$$

By combining Eqs. 12, 14a, and 14b:

$$(A_0) = (A) + \sum_{j=1}^z \left(\prod_{j=1}^j K_j \right) (A)(B)^j \quad (\text{Eq. 15a})$$

$$(A_0) = (A) \left[1 + \sum_{j=1}^z \left(\prod_{j=1}^j K_j \right) (B)^j \right] \quad (\text{Eq. 15b})$$

where:

$$\prod_{j=1}^j K_j = K_1K_2 \dots K_j \quad (\text{Eq. 15c})$$

The total concentration of the variable, (B_0) , can be defined as follows:

$$(B_0) = (B) + (AB) + 2(AB_2) + \dots + j(AB_j) + \dots + z(AB_z) + 2(B_2) + 3(B_3) + \dots + (i+1)(B_{i+1}) + \dots + (\sigma+1)(B_{\sigma+1}) \quad (\text{Eq. 16})$$

$$(B_0) = (B) + \sum_{j=1}^z j(AB_j) + \sum_{i=1}^{\sigma} (i+1)(B_{i+1}) \quad (\text{Eq. 17})$$

Both (AB_j) and (B_{i+1}) can be substituted by their corresponding expressions (Eqs. 12 and 13, respectively) in Eq. 17:

$$(B_0) = (B) + \sum_{j=1}^z j \left(\prod_{j=1}^j K_j \right) (A)(B)^j + \sum_{i=1}^{\sigma} (i+1) \left(\prod_{i=1}^i \beta_i \right) (B)^{i+1} \quad (\text{Eq. 18})$$

Osmolal concentration, (\bar{M}) , can be measured experimentally and defined according to the model presented:

$$(\bar{M}) = (A) + (B) + (AB) + (AB_2) + \dots + (AB_j) + \dots + (AB_z) + (B_2) + (B_3) + \dots + (B_{i+1}) + \dots + (B_{\sigma+1}) \quad (\text{Eq. 19a})$$

$$(\bar{M}) = (A) + (B) + \sum_{j=1}^z (AB_j) + \sum_{i=1}^{\sigma} (B_{i+1}) \quad (\text{Eq. 19b})$$

Table II—Possible Number of Molecules in Each Aggregate

Nucleoside	Monomers in Aggregate
2'-Deoxyadenosine	4
2'-Deoxyguanosine	5
Thymidine	5
Cytidine	4

The experimental slopes reported previously (1-3) and in this work (Table II and Fig. 2), which are expressed in terms of $\Delta R/\Delta M$, can be converted to osmolal slopes by dividing by a slope of an ideal substance expressed in the same units. The sucrose slope is used for this purpose. It can be calculated from measurements of a colligative property, ΔR , at concentrations lower than 0.2 molal (2, 3, 8).

By the application of simple analytical geometry, the first linear osmolal slopes of mixed nucleosides in water can be expressed:

$$S_1 = \frac{(\bar{M}) - (A_0)}{(B_0) - 0} = \frac{(\bar{M}) - (A_0)}{(B_0)} = \frac{\text{first experimental slope}}{\text{sucrose slope}} \quad (\text{Eq. 20a})$$

$$S_1 = \frac{(B) + \sum_{i=1}^{\infty} \left(\prod_{j=1}^i \beta_j \right) (B)^{i+1}}{(B) \left[1 + \sum_{j=1}^{\infty} j \left(\prod_{k=1}^j K_k \right) (A)(B)^{j-1} + \sum_{i=1}^{\infty} (i+1) \left(\prod_{k=1}^i \beta_k \right) (B)^i \right]} \quad (\text{Eq. 20b})$$

Then set:

$$\sum_{i=1}^{\infty} \left(\prod_{j=1}^i \beta_j \right) (B)^{i+1} = \Phi(\beta_i, B) \quad (\text{Eq. 21})$$

$$\sum_{i=1}^{\infty} (i+1) \left(\prod_{k=1}^i \beta_k \right) (B)^i = \frac{\partial \Phi}{\partial (B)} \quad (\text{Eq. 22})$$

$$1 + \sum_{j=1}^{\infty} \left(\prod_{k=1}^j K_k \right) (B)^j = \Psi(K_j, B) \quad (\text{Eq. 23})$$

and:

$$\frac{\partial \Psi}{\partial (B)} = \sum_{j=1}^{\infty} j \left(\prod_{k=1}^j K_k \right) (B)^{j-1} \quad (\text{Eq. 24})$$

Substituting for Φ of Eq. 21, $\partial \Psi/\partial (B)$ of Eq. 24, and $\partial \Phi/\partial (B)$ of Eq. 22 into Eq. 20:

$$S_1 = \frac{(B) + \Phi}{(B) \left[1 + (A) \frac{\partial \Psi}{\partial (B)} + \frac{\partial \Phi}{\partial (B)} \right]} \quad (\text{Eq. 25})$$

If one assumes that the self-association is inhibited at molal ratios of variable to vehicle of less than their corresponding complexing capacity numbers, according to the theory presented, then the function Φ and its derivative $\partial \Phi/\partial (B)$ should vanish; Eq. 25 then reduces to:

$$S_1 = (B)/(B_0) = \left[1 + (A) \frac{\partial \Psi}{\partial (B)} \right]^{-1} \quad (\text{Eq. 26})$$

By manipulating Eq. 26, the following can be written:

$$\frac{\partial \Psi}{\partial (B)} = \frac{1}{(A)} \left(\frac{1 - S_1}{S_1} \right) \quad (\text{Eq. 27})$$

Combining Eqs. 23, 15a, 15b, and 15c:

$$(A_0) = (A)\Psi \quad (\text{Eq. 28})$$

and substituting into Eq. 27:

$$\left(\frac{(A_0)S_1}{1 - S_1} \right) \frac{\partial \Psi}{\partial (B)} = \Psi \quad (\text{Eq. 28a})$$

Set:

$$\left(\frac{(A_0)S_1}{1 - S_1} \right) = \alpha_c = \text{constant} \quad (\text{Eq. 29})$$

Then:

$$\alpha_c \frac{\partial \Psi}{\partial (B)} = \Psi \quad (\text{Eq. 30})$$

This partial differential equation has the following solution:

$$\Psi = e^{(B)/\alpha_c} (\text{constant}) \quad (\text{Eq. 31})$$

While the constant has a value of 1, one may expand the function Ψ in the usual manner. By equating it with Eq. 23:

$$\Psi = 1 + K_1(B) + K_1K_2(B)^2 + \dots + K_1 \dots K_j(B)^j + \dots + K_1 \dots K_z(B)^z \quad (\text{Eq. 32a})$$

$$\Psi = 1 + \frac{(B)}{\alpha_c} + \frac{1}{2!} \left(\frac{(B)}{\alpha_c} \right)^2 + \dots + \frac{1}{j!} \left(\frac{(B)}{\alpha_c} \right)^j + \dots + \frac{1}{z!} \left(\frac{(B)}{\alpha_c} \right)^z + \dots + \frac{1}{\infty!} \left(\frac{(B)}{\alpha_c} \right)^{\infty} \quad (\text{Eq. 32b})$$

For large values of z and for low concentrations of B , the following can be set by excluding terms of higher order than z :

$$K_1 = 1/\alpha_c \quad (\text{Eq. 33a})$$

$$K_2 = 1/2\alpha_c \quad (\text{Eq. 33b})$$

$$K_j = 1/j\alpha_c \quad (\text{Eq. 33c})$$

$$K_z = 1/z\alpha_c \quad (\text{Eq. 33d})$$

Accordingly, the constant α_c will be referred to as the progression constant.

Calculations—The first slope, S_1 , and the predetermined concentrations can be used to calculate the concentrations of various molecular species, osmotic coefficients, according to the following calculations.

Free Vehicle Concentration—Substituting for (B) of Eq. 26 into Eq. 31:

$$\Psi = e^{(1-S_1)B_0/(A_0)} \quad (\text{Eq. 34})$$

Then by setting:

$$(B_0)/(A_0) = \gamma \quad (\text{Eq. 35})$$

and combining Eqs. 34 and 28:

$$(A) = (A_0)e^{-\gamma(1-S_1)} \quad (\text{Eq. 36})$$

Concentration of AB_j —The concentration of AB_j is found by using Eqs. 26 and 35:

$$(B) = S_1\gamma(A_0) \quad (\text{Eq. 37})$$

Then, according to Eq. 33:

$$\prod_{j=1}^j K_j = \frac{1}{j!} \left(\frac{1}{\alpha_c} \right)^j = \frac{1}{j!} \left(\frac{1 - S_1}{(A_0)S_1} \right)^j \quad (\text{Eq. 38})$$

One may substitute for (A) of Eq. 36, (B) of Eq. 37, and the product of K 's to calculate the concentration of AB_j according to Eq. 12:

$$(AB_j) = (A_0) \frac{\gamma^j}{j!} (1 - S_1)^j e^{-\gamma(1-S_1)} \quad (\text{Eq. 39})$$

Calculated Osmolal Concentration—In the absence of self-asso-

Table III—Data Falling on Two Linear Slopes^a

Variable	Vehicle	Intercept ^b , ohms	First Slope, $\Delta R/\Delta M$	Critical Concentration	Second Slope, $\Delta R/\Delta M$
1,3-Dimethyluracil	Water	0.03	921.8	0.068	830.0
1,3-Dimethyluracil	2'-Deoxyadenosine (0.015 M)	13.86	859.5	0.0987	736.1
1,3-Dimethyluracil	2'-Deoxyguanosine (0.012 M)	11.06	866.0	0.1015	723.9

^a Measurements were at 25°. ^b The intercepts are not experimental points.

ciation, the calculated osmolal concentration can be obtained by substitution for (A) of Eq. 36, (B) of Eq. 26, and the concentration of AB_j of Eq. 39 into Eq. 19:

$$(\bar{M})_{\text{calc}} = S_1(B_0) + \sum_{j=0}^{\infty} (A_0) \frac{\gamma^j}{j!} (1 - S_1)^j e^{-\gamma(1-S_1)} \quad (\text{Eq. 40a})$$

$$(\bar{M})_{\text{calc}} = S_1(B_0) + (A_0)e^{-\gamma(1-S_1)} \sum_{j=0}^{\infty} \frac{\gamma^j}{j!} (1 - S_1)^j \quad (\text{Eq. 40b})$$

Osmotic Coefficient—The osmotic coefficient, ϕ , can be defined as the ratio of osmolal to the total molal concentrations. Thus:

$$\phi = (\bar{M})/[(A_0) + (B_0)] \quad (\text{Eq. 41})$$

The following relationship can be arrived at by substituting for each term in Eq. 41:

$$\phi = [S_1\gamma + 1]/[\gamma + 1] \quad (\text{Eq. 42})$$

RESULTS AND DISCUSSION

The experimental results obtained seem to fit the previous treatment for caffeine and nucleosides (1–3). Two linear slopes can also be used to express all of the data obtained, with ΔR as the ordinate and molal concentration as the abscissa.

Table III shows slopes, critical concentrations, and intercepts; the slopes pass through all points within the limits of experimental error and were calculated by the method of least squares. Table III also shows that these critical concentrations are shifted as a function of the nature and concentration of the vehicle used in much the same way as for caffeine and nucleosides (1–3).

Figure 2 shows clearly the shift along the variable molal scale. In each case, the amount of shift on the variable molal scale from that determined in pure water divided by the concentration of the vehicle used gave a certain number that also approaches an integer (Table I).

The importance of the potential hydrogen bonding sites in establishing the mode of aggregation is clearly indicated by the observation (Table I) that reducing the hydrogen bonding by using 1,3-dimethyluracil also reduces the complexing capacity numbers to 2 and 3 for 2'-deoxyadenosine and 2'-deoxyguanosine, respectively, when they are used as vehicles.

Borazan (2) and Borazan and Goyan (3) considered errors in standard and sample slopes which when combined introduced errors of about 1.0% in the osmotic coefficients. Accordingly, the pro-

Table IV—Data Falling on a Linear Slope of Aqueous Solutions of Caffeine^a

Variable	Vehicle	First Slope ^b , $\Delta R/\Delta M$	Progression Constant ^c , α_c (molal), %
Caffeine	<i>p</i> -Aminobenzoic acid (0.005 M)	743	0.0268 ± 9
Caffeine	<i>p</i> -Aminobenzoic acid (0.010 M)	659	0.0295 ± 6
Caffeine	<i>p</i> -Aminobenzoic acid (0.015 M)	605	0.0328 ± 4.5

^a Measurements were at 25°. ^b Data taken from Ref. 1. ^c Progression constant = (first slope × vehicle concentration)/(sucrose slope - first slope), according to Eq. 29. Sucrose slope has a value of 882 in connection with this work.

gression constants calculated according to Eq. 29 are reported with percentage uncertainty in each case.

Inspection of Table IV shows that by increasing the initial concentrations of the vehicle compound threefold, a slight increase in the values of the progression constant is produced. This increase is considered slightly above the values that can be accounted for on the basis of random error. A reasonable explanation for this finding is the various approximations made in the derivations such as the approximations made in deriving various equilibrium constants from Eq. 33. Also, empirical assumptions were made in the presented models, such as ignoring interactions among various associated forms. However, the results presented in Table IV are considered satisfactory and in agreement with the prediction of the equations.

The data of Borazan and Goyan (3) and of Table III of this work provided information used to calculate progression constants, α_c , for the equilibrium constants (Table V). Equilibrium constants can be calculated from osmolal slopes, determined from experimental data, using a simple mathematical model:

$$A + B = AB \quad (\text{Eq. 43})$$

$$K = \frac{(AB)}{(A)(B)} \quad (\text{Eq. 44})$$

where (A) and (B) are the concentrations of the free reactants, and (AB) represents the concentration of the complex. The osmolal, (\bar{M}), and the total molal, (M), concentrations are then:

$$(\bar{M}) = (A) + (B) + (AB) \quad (\text{Eq. 45})$$

$$(M) = (A) + (B) + 2(AB) \quad (\text{Eq. 46})$$

By subtracting Eq. 45 from Eq. 46:

$$(AB) = (M) - (\bar{M}) \quad (\text{Eq. 47})$$

(\bar{M}) can be determined from experimental slopes (1–3) (Tables III and IV) after dividing by the sucrose slope to get the first osmolal slopes, S_1 , by the following relationship:

$$(\bar{M}) = S_1(B_0) + (A_0) \quad (\text{Eq. 48})$$

while:

$$(M) = (A_0) + (B_0) \quad (\text{Eq. 49})$$

By substituting for the osmolal and the total molal concentrations into Eq. 47:

$$(AB) = (B_0) - S_1(B_0) \quad (\text{Eq. 50})$$

The free reactant species can be expressed as:

$$(A) = (A_0) - (AB) \quad (\text{Eq. 51})$$

$$(B) = (B_0) - (AB) \quad (\text{Eq. 52})$$

By substituting for (AB) of Eq. 50 into Eqs. 51 and 52:

$$(A) = (A_0) + S_1(B_0) - (B_0) \quad (\text{Eq. 53})$$

$$(B) = S_1(B_0) \quad (\text{Eq. 54})$$

Thus, Eq. 44 can be expressed in terms of measurable quantities as follows:

Table V—Equilibrium Constants Calculated According to Eq. 55, for Different $(B_0)/(A_0)$ Ratios and the First Equilibrium Constants (K_1) Calculated from the Progression Constant^a (α_c) by Eq. 33, Together with the Standard Free Energies Based upon K_1 for Different Variable-Vehicle Nucleoside Combinations^b

$(B_0)/(A_0)$	K_1 , molal ⁻¹	$(B_0)/(A_0)$	K_1 , molal ⁻¹
Thymidine-2'-Deoxyadenosine (0.015 M)		Uridine-2'-Deoxyguanosine (0.012 M)	
0.1	12.25	0.1	7.80
0.5	13.07	5.0	13.43
1.0	14.23	$\alpha_c = 0.129 \pm 12.5\%$ molal; $K_1 = 7.75$ molal ⁻¹ ; and $\Delta G^0 = -1227$ cal/mole	
1.33	15.10	Cytidine-Thymidine (0.02 M)	
2.0	17.40	0.1	4.68
3.0	22.40	4.0	7.05
4.0	42.40	$\alpha_c = 0.215 \pm 12\%$ molal; $K_1 = 4.65$ molal ⁻¹ ; and $\Delta G^0 = -923$ cal/mole	
5.0	51.75	Cytidine-2'-Deoxyadenosine (0.015 M)	
6.0	146.30	0.1	10.10
$\alpha_c = 0.083 \pm 6.5\%$ molal; $K_1 = 12.04$ molal ⁻¹ ; and $\Delta G^0 = -1490$ cal/mole		5.0	28.46
2'-Deoxyadenosine-Thymidine (0.02 M)		$\alpha_c = 0.1005 \pm 8\%$ molal; $K_1 = 9.96$ molal ⁻¹ ; and $\Delta G^0 = -1380$ cal/mole	
0.1	17.35	Cytidine-2'-Deoxyguanosine (0.012 M)	
1.0	22.60	0.1	9.02
$\alpha_c = 0.0593 \pm 4\%$ molal; $K_1 = 16.84$ molal ⁻¹ ; and $\Delta G^0 = -1690$ cal/mole		5.0	17.40
2'-Deoxyadenosine-Uridine (0.02 M)		$\alpha_c = 0.1105 \pm 10.5\%$ molal; $K_1 = 9.04$ molal ⁻¹ ; and $\Delta G^0 = -1320$ cal/mole	
0.1	10.4	Thymidine-2'-Deoxyguanosine (0.012 M)	
1.0	12.3	0.1	11.86
$\alpha_c = 0.097 \pm 6\%$ molal; $K_1 = 10.30$ molal ⁻¹ ; and $\Delta G^0 = -1400$ cal/mole		5.0	30.40
2'-Deoxyadenosine-2'-Deoxyguanosine (0.01 M)		$\alpha_c = 0.0855 \pm 8\%$ molal; $K_1 = 11.7$ molal ⁻¹ ; and $\Delta G^0 = -1475$ cal/mole	
0.1	38.6	Thymidine-Cytidine (0.02 M)	
4.0	82.6	$\alpha_c = 0.2127 \pm 12\%$ molal; $K_1 = 4.7$ molal ⁻¹ ; and $\Delta G^0 = -927$ cal/mole	
$\alpha_c = 0.0267 \pm 3.5\%$ molal; $K_1 = 37.45$ molal ⁻¹ ; and $\Delta G^0 = -2170$ cal/mole		1,3-Dimethyluracil-2'-Deoxyadenosine (0.015 M)	
2'-Deoxyguanosine-Cytidine (0.003 M)		0.1	5.05
0.1	10.30	2.0	5.83
1.0	10.60	$\alpha_c = 0.201 \pm 14.5\%$ molal; $K_1 = 4.97$ molal ⁻¹ ; and $\Delta G^0 = -960$ cal/mole	
$\alpha_c = 0.097 \pm 40\%$ molal; $K_1 = 10.30$ molal ⁻¹ ; and $\Delta G^0 = -1400$ cal/mole		1,3-Dimethyluracil-2'-Deoxyguanosine (0.012 M)	
2'-Deoxyguanosine-2'-Deoxyadenosine (0.003 M)		0.1	5.60
0.1	38.0	3.0	7.03
1.0	40.2	$\alpha_c = 0.181 \pm 16\%$ molal; $K_1 = 5.52$ molal ⁻¹ ; and $\Delta G^0 = -1027$ cal/mole	
3.0	51.3	Uridine-2'-Deoxyadenosine (0.015 M)	
$\alpha_c = 0.0276 \pm 10\%$ molal; $K_1 = 36.50$ molal ⁻¹ ; and $\Delta G^0 = -2160$ cal/mole		0.1	10.5
Uridine-2'-Deoxyadenosine (0.015 M)		5.0	32.8
0.1	10.5	$\alpha_c = 0.0962 \pm 7.5\%$ molal; $K_1 = 10.40$ molal ⁻¹ ; and $\Delta G^0 = -1405$ cal/mole	
5.0	32.8		

^a Calculated from data taken from Ref. 3 and Table III (for the combinations containing 1,3-dimethyluracil). ^b Measurements were at 25°.

$$K = \frac{(1 - S_1)}{[(A_0) + S_1(B_0) - (B_0)]S_1} \quad (\text{Eq. 55})$$

It is obvious from this relationship that, for slopes approaching unity and for low values of $(B_0)/(A_0)$, Eq. 55 reduces to:

$$K = \frac{(1 - S_1)}{(A_0)S_1} \quad (\text{Eq. 56})$$

This is essentially the same result obtained in calculating the first equilibrium constant from the model presented in Eq. 1. Therefore, one would not be surprised when getting different values of K from Eq. 55 along the molal scale of the variable compound (Table V). The magnitude of the K values increases with the increase in the total molal concentration of the variable compound, (B_0) , while approaching K_1 values calculated from Eqs. 29 and 33 at a molal ratio of $(B_0)/(A_0)$ of less than 0.1.

These results indicate the significance of a simple equilibrium situation at low ratios of $(B_0)/(A_0)$. Thus, the linear experimental slopes indicate a rather complex equilibrium and a formation of complexes having stoichiometry different from 1:1.

One can also verify the validity of the derived equations and their consistency with experimental results by considering Eq. 40. For large values of z , the summation of terms in this equation approaches $e^{\gamma(1-S_1)}$; thus:

$$e^{-\gamma(1-S_1)} \sum_{j=0}^z \frac{\gamma^j}{j!} (1 - S_1)^j = 1 \quad (\text{Eq. 57})$$

and $(\bar{M})_{\text{calc}}$ equals osmolal concentration determined from the first osmolal slopes by the process of application of simple analytical geometry, i.e., $S_1(B_0) + (A_0)$.

Another test for the validity of the derived relationships, α_c (Table V), is found to have approximately the same values in most cases studied, within the limits of accuracy, for the same combination when each is used as a variable and a vehicle.

According to the equilibrium constants calculated (Table V), the order of nucleoside heterogeneous association is as follows: purine-purine > purine-pyrimidine > pyrimidine-pyrimidine. This order is in agreement with previous findings (4, 13, 21).

Based upon configurational probabilities, Borazan (28) demonstrated that the self-association processes can induce identical, in-

dependent, and equivalent binding sites and that the overall equilibrium constants are all related to an intrinsic binding constant such that the following holds:

$$\beta_i = \left(\frac{i}{\sigma - i + 1} \right) k \quad (\text{Eq. 58})$$

where β_i is the overall equilibrium constant for the i th process, k is the intrinsic binding constant, and σ is the maximum number of association processes. Experimental curves were all generated according to the model.

The first equilibrium constants were smaller than the last in all cases studied and were the smallest constants for the heterogeneous association processes, based upon the assumption that the complexing capacity numbers determine the maximum number of multiple association processes. These results are consistent with the inhibition of the normal pattern of self-association hypothesized below a certain threshold concentration.

CONCLUSION

The theory presented in this paper gives physical and mathematical interpretations concerning the origin and the mechanism of the shift in the critical micelle-like concentrations of the nucleosides, whose concentrations are varied in the presence of a fixed concentration of another reacting nucleoside.

The theory was verified experimentally and found to be consistent with the inhibition of self-association of the compounds used in excess at variable-vehicle molal ratios equal to or less than their corresponding complexing capacity numbers.

A relationship was found between the number of the binding sites on the vehicle molecules and the magnitude of these numbers. Equilibrium constants for various mononuclear association processes are all related to one constant and their corresponding coordination number.

The present work has provided a tool for calculating concentrations of various molecular species which might otherwise be almost impossible to determine for such weak complexes. Other functions of theoretical interest can be determined from a measurable quantity, the slope of the curve when plotting osmolal versus molal concentrations, and predetermined concentrations.

Finally, it is hoped that the concept of the complexing capacity numbers and the determination of these numbers will add another thermodynamic parameter to the dimension of weak complexation problems.

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