Influence of Temperature and Nature of Hydrophobic Groups on Thermodynamic Parameters of Hydrophobic Bonding in a Model Polymeric System and Their Implications in Drug-Biopolymer Interactions

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Abstract □ By using a model polymeric system, the thermodynamic parameters of hydrophobic bonding between the alkyl groups as well as between two methylene groups were determined. This was accomplished by studying the binding of the anions of fatty acids, propionate through caproate, by the hexyl copolymer from 28 to 45°. The hexyl copolymer was synthesized by quaternizing with hexyl bromide the vinylpyridine units of a copolymer of an overall composition of 1 unit of vinylpyridine to 22 units of vinylpyrrolidone. The hexyl vinylpyridinium units of the copolymer are shown to constitute the definite sites for hydrophobic bonding. The results of the present study, together with those of a previous study, revealed the influence of temperature and the nature of hydrophobic groups on the thermodynamic parameters of hydrophobic bonding. The significance of these characteristic properties of hydrophobic bonding in drug-biopolymer interactions is discussed. A GLC method, developed for quantitative determination of the fatty acids, is described.

Keyphrases □ Hydrophobic bonding between alkyl and between methylene groups—temperature effect, implications in drug-biopolymer interactions □ Drug-biopolymer interactions—temperature and group effects on hydrophobic bonding □ Thermodynamic parameters, hydrophobic bonding—effects of temperature and length of alkyl group □ Temperature effect—thermodynamic parameters of hydrophobic bonding, alkyl fatty acids and vinylpyridine–vinylpyrrolidone polymer □ Vinylpyridine–vinylpyrrolidone copolymer—used to study hydrophobic bonding between alkyl and between methylene groups

In addition to the noncovalent forces such as ionic attraction and hydrogen bonding, the importance of hydrophobic bonding is well recognized in drug-receptor interactions (1). Hydrophobic bonding is the interaction between hydrophobic groups in an aqueous medium, and such an interaction is characteristically accompanied by positive enthalpy and entropy effects (2). In view of the biopharmaceutical significance of hydrophobic bonding phenomena, a model water-soluble polymeric system was devised and studied (3); it served to demonstrate certain properties of hydrophobic bonding between the phenyl and alkyl groups. An interesting feature noted in this study, carried out at 15, 22, 30, 37, and 45°, was that the binding between the hydrophobic groups increased up to 37° but decreased at 45°. Furthermore, when the corresponding binding constant was plotted against the alkyl copolymer of the homologous series, an ascending zigzag curve was obtained.

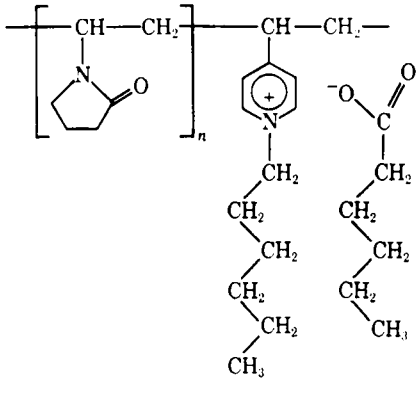
Based on a physical model, Nemethy and Scheraga (4) theorized that hydrophobic bond formation becomes stronger up to 42° between the aromatic hydrocarbons but that it becomes stronger up to 58° between the aliphatic groups. Therefore, the purposes of the present study were: (*a*) to determine the thermodynamic parameters of hydrophobic bonding (28–45°) between the alkyl groups, as well as between two methylene groups, using a model water-soluble alkyl copolymer containing known sites of hydrophobic bonding; and (*b*) to determine if the hydrophobic bonding between the alkyl groups in the model system continues to be stronger up to 45°, as well as with the increase in chain length at a given temperature.

MODEL SYSTEM

The present model system consists of the study of the binding of the fatty acid anions, propionate through caproate, by the hexyl copolymer. The procedures for synthesis and overall composition determination of the hexyl copolymer were previously reported (3). The overall composition of the copolymer was 1:23 (1 unit of quaternized vinylpyridine to 23 units of vinylpyrrolidone). The fatty acids (pKa 4.81–4.87) were expected to remain in solution in the ionized form since the interactions were carried out at pH 7.4. The binding energy involved in these interactions is due to both electrostatic binding and hydrophobic bonding. Scheme I depicts how the electrostatic binding facilitates the approach of the fatty acid anions sufficiently close to the quaternized units so that hydrophobic bonding can occur between the alkyl group of the carboxylate ion and the hexyl group of the hexyl copolymer. Evidence has been obtained (as explained later) to indicate that the sites on the alkyl copolymers for binding of fatty acid anions are indeed the vinylpyridinium units. The suitability of such a model system in determining the thermodynamic parameters of hydrophobic bonding between the interacting hydrophobic groups was explained previously (3).

EXPERIMENTAL

Materials—Hexyl copolymers (1:23, 1:27, and 1:42) were synthesized according to the method previously described (3). Sodium propionate[1] and sodium butyrate[1] were recrystallized from



Scheme I—Representation of the interaction of a caproate ion with a hexyl copolymer unit. The iceberg structures around the methylene and methyl groups are not shown. In the diagram, n represents the average 23 units of vinylpyrrolidone to 1 unit of quaternized vinylpyridine.

[1] Matheson, Coleman and Bell, East Rutherford, N. J.

Table I—Retention Times Observed for the Fatty Acids[a]

Compound	Column Temperature	Retention Time, min.
Propionic acid	120°	6.0
Butyric acid	140°	4.5
Valeric acid	150°	5.0
Caproic acid	160°	5.0

[a] The conditions used were: sensitivity, range 10, attenuation 8–32×; injection port temperature, 210°; detector temperature, 210°; and helium flow rate, 30 ml./min.

an acetone–water (5:1) mixture according to Childers and Struthers (5). Sodium valerate and sodium caproate were prepared from *n*-valeric acid[2] and *n*-caproic acid[1], respectively, according to the methods described by Childers and Struthers (5). Other chemicals used were reagent grade.

Apparatus—The fatty acids were quantitatively analyzed with the aid of a gas chromatograph[3] equipped with a flame-ionization detector. The column employed was a 1.21-m. long and 0.63-cm. o.d. copper tube packed with 80–100-mesh diatomite[4] coated with 15% diethylene glycol adipate. Helium was used as the carrier gas. A 10-μl. standard syringe[5] was used for injecting the sample. A recording spectrophotometer[6] was used for the copolymer analysis. An expandomatic pH meter[7] was used for the potentiometric determination of bromide content of the hexyl copolymer and for pH determinations of the solutions. Plexiglas dialysis cells[8] of 2-ml. capacity were used in the equilibrium dialysis studies.

Solution of Hexyl Copolymer—A 4% (w/v) solution of hexyl copolymer (1:23) was prepared in a pH 7.4 phosphate buffer of 0.1 ionic strength. Similar solutions of hexyl copolymers (1:27 and 1:42) were also prepared. The ionic strength of all solutions was adjusted to 0.114 with sodium chloride.

Solution of Sodium Salt of Fatty Acid—The solutions of each acid (0.004–0.014 *M*) were prepared in pH 7.4 phosphate buffer. The ionic strength of each solution was adjusted to 0.114 with sodium chloride.

Dialysis Procedure—Each dialysis cell consisted of two Plexiglas halves, 5 × 5 × 2 cm., each with a cavity having 1-ml. capacity. The dialysis cells were set up in the same manner as previously described (3). The solutions were transferred into each cavity of the dialysis cell by means of 1-ml. tuberculin syringes. The cells were allowed to shake in a water bath maintained at 28, 32, 37, or 45 ± 0.3°. Although the system attained equilibrium in 60 hr., the cells were allowed to shake in the bath for 84 hr. To ensure accuracy of the results, the standard dialysis cells were set up along with the sample cells. The standard cells were set up in the same manner as the sample cells, except that 1 ml. solution of pH 7.4 phosphate buffer of appropriate ionic strength was used in place of 1 ml. solution of the hexyl copolymer.

After 84 hr., the cells were removed from the bath and promptly the contents from the nonpolymer side and polymer side of each cell were quantitatively transferred to 2.5-ml. graduated tubes by means of Pasteur pipets. The graduated tubes were stoppered and allowed to stand until the contents of the tubes attained room temperature. The volume of solution obtained from the nonpolymer side of each cell generally measured 0.9 ml. and that obtained from the polymer side of each cell generally measured 1.1 ml. Therefore, the appropriate correction was made for the concentration of hexyl copolymer in the polymer solution in each dialysis set-up. All studies were carried out in duplicate at each concentration of the fatty acid.

Quantitative Determination of Fatty Acids by GLC—The quantitative determinations of the fatty acids in the solutions obtained from the nonpolymer side of each cell were carried out by GLC, using 15% diethylene glycol adipate liquid phase coated on diatomite[4]. Immediately prior to GLC analysis, 0.01 ml. concentrated hydrochloric acid was added to each solution to convert the salt of the fatty acid to the free fatty acid. The pH of such solutions was found to be about 1. Four microliters of the acidified solution was then injected into the GLC column. The conditions for analysis are given in Table I. A typical chromatogram of fatty acids is shown in Fig. 1. The area under the curve of each chromatogram was calculated by the trapezoidal rule. The calibration curves relating the area under the curve to the concentration were prepared for each fatty acid by analyzing gas chromatographically the acid solutions of known concentration. A typical calibration curve obtained for valeric acid is shown in Fig. 2.

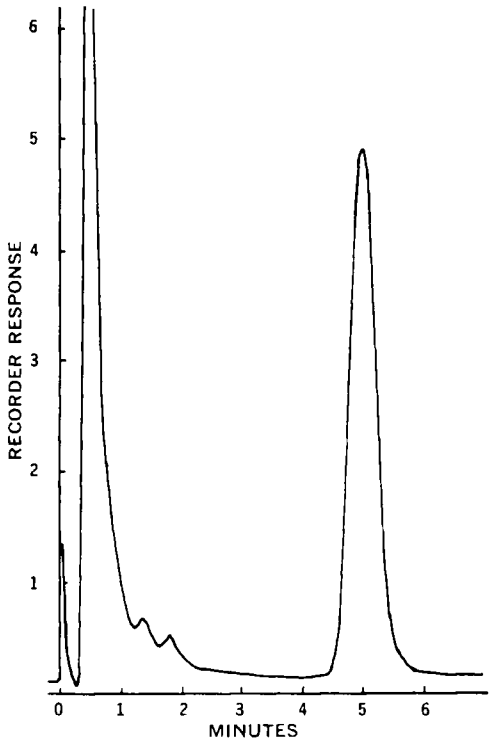


Figure 1—*A typical gas chromatogram obtained for valeric acid.*

Binding of Anions of Valeric Acid by 1:27 and 1:42 Hexyl Copolymers—To determine if binding of the fatty acid anions by the hexyl copolymer was solely due to the quaternized vinylpyridine units of the latter, the binding of the anions of valeric acid (0.006 *M*) by the hexyl copolymers (1:27 and 1:42) was studied at 37° and 0.114 ionic strength in the usual manner.

RESULTS AND DISCUSSION

Quantitative Determination of Fatty Acids by GLC—The use of 15% diethylene glycol adipate as the liquid phase in the quantitative GLC analysis of the fatty acids was found to be satisfactory (Fig. 2). With reference to such a standard plot as in Fig. 2, the amounts of the fatty acid contained in the solutions from the nonpolymer side of the sample cells were calculated. To ascertain the accuracy of such quantitative analyses, the standard plots were constructed for the fatty acid contained in the standard cells of each run. The amount of fatty acid bound by hexyl copolymer in each dialysis cell was calculated in the usual manner. It was generally observed that the difference between the quantity of fatty acid calculated from each duplicate sample was about 1–2%. None of the fatty acids was bound to the cellophane membrane or the dialysis cells.

Identification of Binding Sites in Hexyl Copolymer—As noted in Fig. 3, the extent of binding of valerate ions was directly proportional to the molar concentration of the hexyl vinylpyridinium bromide content of the hexyl copolymer, indicating that the hexyl vinylpyridinium groups constituted the binding sites for the fatty acid anions. Further support to this conclusion was obtained by demonstrating that a negligible amount of valerate ions is bound by nonquaternized 1:42 copolymer (4% w/v) at 37° and 0.114 ionic strength.

Binding Constants—The binding data obtained for the anions of

[2] Eastman Kodak Co., Rochester, N. Y.
[3] F&M model 810R-19.
[4] Diatoport S.
[5] Hamilton.
[6] Beckman model DK-2A.
[7] Beckman.
[8] The Chemical Rubber Co., Cleveland, Ohio.

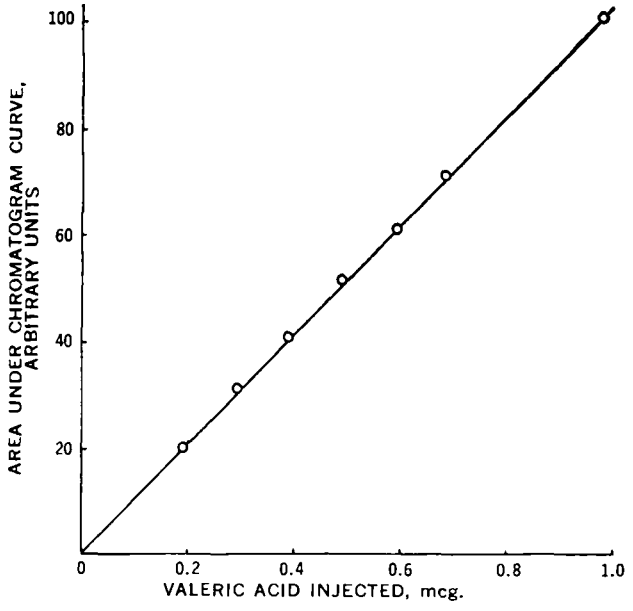


Figure 2—*Calibration curve for valeric acid.*

each fatty acid were treated according to the Klotz equation (6):

$$1/r = 1/n + 1/nKa \qquad \text{(Eq. 1)}$$

where r is the number of moles of fatty acid anion bound per mole of hexyl copolymer, n is the average number of binding sites per mole of the hexyl copolymer, a is the molar concentration of free fatty acid anion at equilibrium, and K is the binding constant (liters per mole). As shown in Fig. 4, typical Langmuir plots of $1/r$ *versus* $1/a$ were constructed for the binding data of the anion of each fatty acid, and the values of n and K were determined from the intercept ($1/n$) and the slope ($1/nK$) of the straight line obtained by the least-squares method. The binding constants are listed in Table II. The straight-line plots in Fig. 4 indicate that the fatty acid anions bind to identical sites present in the hexyl copolymer. Also, out of every 10 binding sites, the number of sites available on the hexyl copolymer for the binding of the fatty acid anion was about 7–9.7. This may be due to some coiling of the hexyl copolymer molecules, making a few sites unavailable for binding (3).

As seen in Table II, not only the extent of binding of the fatty acid anion increased with the increase in the chain length at each temperature but also the extent of binding of the anion of each fatty acid increased with the increase in temperature from 28 to 45°.

Thermodynamic Parameters for Binding of Fatty Acid Anion—The free energy of binding (ΔF_b) of the fatty acid anion was calculated from the following equation:

$$\Delta F_b = -RT \ln K \qquad \text{(Eq. 2)}$$

where R and T have the usual meanings. Since the plots of log K *versus* $1/T$ (Fig. 5) were reasonably straight lines (least squares), the enthalpy of binding is considered to be constant over the 28–45° range. The enthalpy of binding (ΔH_b) was calculated from the slope of the straight line obtained according to the van't Hoff equation:

$$\log K = \frac{-\Delta H}{2.33RT} + \text{constant} \qquad \text{(Eq. 3)}$$

The values of entropy of binding (ΔS_b) were calculated from the following relationship:

$$\Delta S_b = \frac{\Delta H_b - \Delta F_b}{T} \qquad \text{(Eq. 4)}$$

The thermodynamic constants thus determined are listed in Table III.

Since the binding of the fatty acid anion by the hexyl copolymer was found to be associated with the positive enthalpy and entropy effects (Table III), it is concluded that the polymeric system used in the present study exhibits the properties of hydrophobic bond formation. Despite the fact that the positive enthalpy observed in the study is unfavorable for the binding process, the ΔF_b becomes increasingly negative (and, therefore, favorable) with the increase in temperature because of the gain in entropy. The gain in entropy occurs due to hydrophobic interactions in the aqueous medium between the alkyl groups of the hexyl copolymer and those of the fatty acid resulting in the release of water molecules from the "icebergs" around the alkyl groups and, consequently, in the release of configurational entropy of the alkyl groups (3).

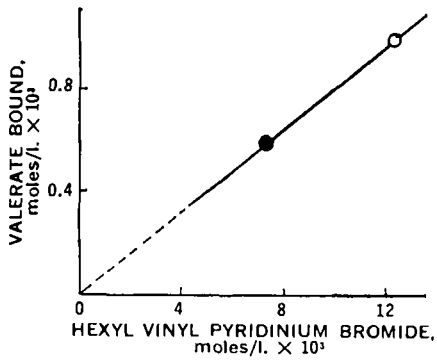


Figure 3—*Binding of valeric acid anion (6 μmoles/dialysis cell) by 1:42 (●) and 1:27 (○) hexyl copolymers at 37° and 0.114 ionic strength.*

The fact that the ΔF_b in the present system increased with the increase in chain length of the fatty acid further substantiates that the present polymeric system displays the characteristics of hydrophobic bonding.

Determination of Thermodynamic Parameters of Hydrophobic Bonding—Since the hexyl vinylpyridinium units of the hexyl copolymer constitute the sites for binding of fatty acid anions, it is apparent that the observed ΔF_b is mainly a combination of the free energy of binding due to electrostatic forces (ΔF_e) and the free energy of binding due to hydrophobic bonding between the side chains ($\Delta F_{H\phi}{}^S$):

$$\Delta F_b = \Delta F_e + \Delta F_{H\phi}{}^S \qquad \text{(Eq. 5)}$$

Furthermore, the ΔF_e of the anions of all fatty acids can be considered to be the same. Therefore, from the data in Table III, the $\Delta F_{H\phi}{}^S$ due to the alkyl groups of the copolymer and the corresponding fatty acid anions can be calculated by subtracting the ΔF_b of the appropriate fatty acid anion from those of the higher homologs at each temperature. It is recognized that there is a possibility

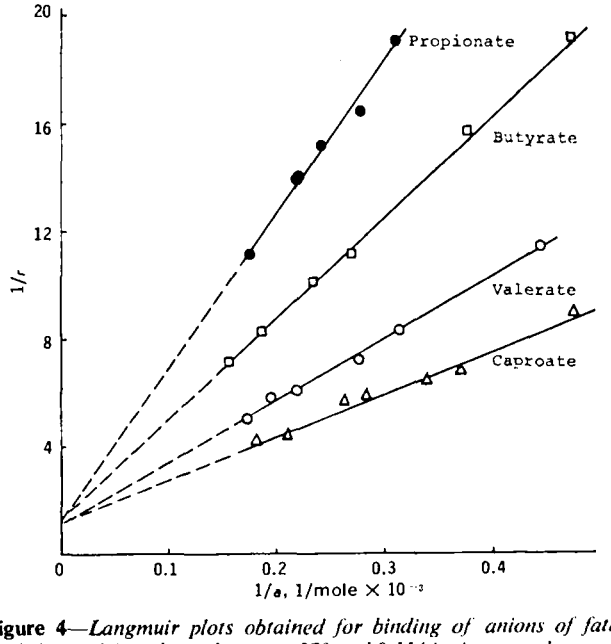


Figure 4—*Langmuir plots obtained for binding of anions of fatty acids by 1:23 hexyl copolymer at 37° and 0.114 ionic strength.*

Table II—Binding Constants Determined for the Interaction of the Fatty Acid Anion with the Hexyl Copolymer (1:23) at 28, 32, 37, and 45° and 0.114 Ionic Strength

Fatty Acid Anion	Temperature	Binding Constant, K, l./mole
Propionate	28°	15.92
	32°	16.67
	37°	23.57
Butyrate	28°	22.35
	37°	37.03
	45°	57.48
Valerate	28°	30.82
	32°	43.76
	37°	46.20
	45°	98.26
Caproate	28°	49.64
	32°	58.97
	37°	75.61
	45°	170.73

that the alkyl groups attached to the charged groups (such as the quaternary nitrogen in the hexyl copolymer and the carboxylate group of the fatty acid anion) may influence the structure of water around them differently than when they exist in water as aliphatic hydrocarbons. Therefore, in this context, the appropriate fatty acid anion in the homologous series refers to that anion of sufficient chain length whose end methyl group is just beyond the sphere of electrostatic effect of its charged group. This would then make it possible to determine the thermodynamic parameters of hydrophobic bonding between two methylene groups if, indeed, the iceberg structure around the methylene groups of the alkyl groups is uniform beyond the sphere of the electrostatic influence of the charged group. However, it is recognized that in a homologous series such uniformity in the iceberg structure around the methylene groups can be expected only up to a limited chain length of the alkyl group, since the alkyl groups beyond a certain chain length are subject to curling to reduce their surface exposure to water.

In view of the enthalpy of solution data in water reported by Snell and Greyson (7) for sodium formate (−0.269 kcal./mole), sodium acetate (−3.973 kcal./mole), sodium propionate (−3.015 kcal./mole), and sodium butyrate (−3.385 kcal./mole) at 25°, it

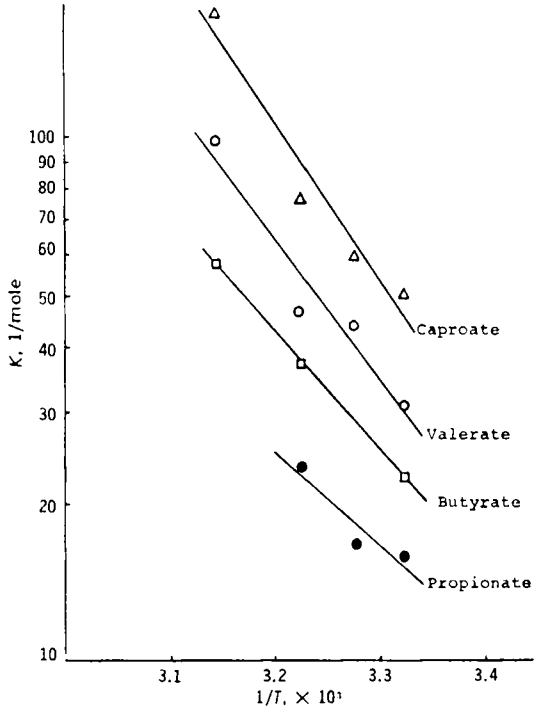


Figure 5—*Van't Hoff plots obtained for binding of anions of fatty acids by 1:23 hexyl copolymer over the temperature range of 28–45° and 0.114 ionic strength.*

Table III—Thermodynamic Parameters of Binding of the Anions of Fatty Acids by the Hexyl Copolymer (1:23) in pH 7.4 Phosphate Buffer at 0.114 Ionic Strength and 28–45°

Fatty Acid Anion	$-\Delta F_b$, cal./mole 28°	32°	37°	45°	ΔH_b, cal./mole	ΔS_b, e.u.
Propionate	−1655	−1705	−1946	—	8,211	32.65
Butyrate	−1858	...	−2225	−2560	10,560	41.25
Valerate	−2051	−2290	−2361	−2899	12,250	47.40
Caproate	−2336	−2471	−2665	−3247	13,817	53.40

becomes apparent that there exists a nonuniformity in the iceberg structure around the alkyl groups of short-chain fatty acid anions. Unfortunately, such thermodynamic data are not available for sodium salts of valeric and caproic acids to suggest that the nonconformity is minimized in the iceberg structure around the tail-end methylene groups of the alkyl chains of these salts. However, Everett (8) observed that the singly charged ions such as the quaternary nitrogen and carboxylate group exert a significant influence on the solvent (water) structure only over a distance of 5 Å. Since the C—C bond length is about 1.54 Å (8), this would then suggest that the influence of the singly charged groups of the copolymer and fatty acid anions on the structure of water around the methylene groups, which are separated from them by at least three methylene groups, should be minimal. Therefore, it is considered appropriate to obtain the data for free energy of hydrophobic bonding between the alkyl groups by subtracting the ΔF_b of butyrate ions from those of the higher homologs at corresponding temperatures. In this manner the free energy of hydrophobic bonding between the CH_2 group of valerate ion and the CH_2 group of the hexyl chain of the copolymer and between the CH_2—CH_2 group of caproate ion and the CH_2—CH_2 group of the hexyl chain of the hexyl copolymer can be estimated. The values of free energy of hydrophobic bonding thus obtained for the portions of the alkyl groups of the reactants are designated as $\Delta F_{H\phi}{}^{PA}$ (Table IV).

As shown for ΔF_b in Eq. 5, the two types of forces that contribute to the enthalpy of binding as well as to the entropy of binding of the fatty acid anions by the hexyl copolymer can be described as follows:

$$\Delta H_b = \Delta H_e + \Delta H_{H\phi}{}^{S} \qquad \text{(Eq. 6)}$$

$$\Delta S_b = \Delta S_e + \Delta S_{H\phi}{}^{S} \qquad \text{(Eq. 7)}$$

The contribution of ΔH_e and ΔS_e due to electrostatic interaction can be considered to be the same for all of the fatty acid anions. Therefore, the enthalpy ($\Delta H_{H\phi}{}^{PA}$) and the entropy ($\Delta S_{H\phi}{}^{PA}$) due to hydrophobic bonding between the portions of the alkyl groups of the reactants involved are calculated by subtracting the ΔH_b and ΔS_b of the butyrate ions from those of the higher homologs. The values thus calculated are presented in Table V.

It is interesting to note that the values of $\Delta H_{H\phi}{}^{PA}$ and $\Delta S_{H\phi}{}^{PA}$ (Table V) obtained for the interaction between CH_2—CH_2 (copolymer) and CH_2—CH_2 (caproate) are almost twice those obtained for the interaction between CH_2 (copolymer) and CH_2 (valerate). Although these data are limited, it appears that the enthalpy and

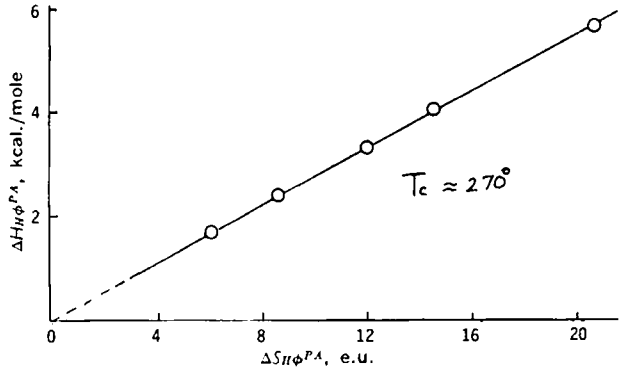


Figure 6—*Plot showing a linear relationship between* $\Delta H_{H\phi}{}^{PA}$ *and* $\Delta S_{H\phi}{}^{PA}$ *(from the data in Table V and that obtainable from Table III, as explained in the text).*

Table IV—Free Energy ($\Delta F_{H\phi}^{PA}$) Determined by Hydrophobic Bond Formation between the Corresponding Portions of the Alkyl Groups of the Hexyl Copolymer and the Fatty Acid Anions at 0.114 Ionic Strength

Temperature	Interacting Portions of Alkyl Groups of Hexyl Copolymer	Fatty Acid Anion	$\Delta F_{H\phi}^{PA}$, cal./mole
28°	CH_2	CH_2 (valerate)	−193
	CH_2—CH_2	CH_2—CH_2 (caproate)	−478
37°	CH_2	CH_2 (valerate)	−136
	CH_2—CH_2	CH_2—CH_2 (caproate)	−440
45°	CH_2	CH_2 (valerate)	−339
	CH_2—CH_2	CH_2—CH_2(caproate)	−687

Table V—Enthalpy ($\Delta H_{H\phi}^{PA}$) and Entropy ($\Delta S_{H\phi}^{PA}$) Determined for Hydrophobic Bonding between the Corresponding Portions of the Alkyl Groups of the Hexyl Copolymer and the Fatty Acid Anions at 0.114 Ionic Strength

Interacting Portions of Alkyl Group of Hexyl Copolymer	Fatty Acid Anion	$\Delta H_{H\phi}^{PA}$, kcal./mole	$\Delta S_{H\phi}^{PA}$, e.u.
CH_2	CH_2 (valerate)	1.69	6.15
CH_2—CH_2	CH_2—CH_2 (caproate)	3.26	12.15

entropy of hydrophobic bonding between two methylene groups can be expected to be uniform as long as these groups of the alkyl chain are not influenced by the charged groups and are fully exposed to water. This finding also corroborates the observation made by Everett (8) that the singly charged ions significantly influence the structure of water only over a distance of 5 Å. This may also explain why the $\Delta H_{H\phi}^{PA}$ and $\Delta S_{H\phi}^{PA}$ values calculated by subtracting the ΔH_b and ΔS_b values of propionate ions from those of the higher homologs do not appear to be as uniform as those obtained by subtracting the ΔH_b and ΔS_b values of butyrate ions. This becomes evident by considering, for instance, the $\Delta H_{H\phi}^{PA}$ values of 2.34, 4.04, and 5.61 kcal./mole obtainable from the data in Table III (upon subtracting ΔH_b of propionate ions) for hydrophobic bonding between CH_2 (copolymer) and CH_2 (butyrate), CH_2—CH_2 (copolymer) and CH_2—CH_2 (valerate), and CH_2—CH_2—CH_2 (copolymer) and CH_2—CH_2—CH_2 (caproate), respectively. However, the extrathermodynamic analysis (Fig. 6) of the $\Delta H_{H\phi}^{PA}$ and $\Delta S_{H\phi}^{PA}$ data obtainable from Table III by subtracting the ΔH_b and ΔS_b values of propionate ion, or those data presented in Table V, reveals that a single interaction mechanism (in this case the hydrophobic bonding) is operative in the binding of the various alkyl groups of the anions of fatty acids with the alkyl group of the hexyl copolymer. This conclusion is drawn not only from the linear relationship observed between $\Delta H_{H\phi}^{PA}$ and $\Delta S_{H\phi}^{PA}$ according to the theory advocated by Leffler and Grunwald (9) but also from the compensation temperature (T_c) of 270° (10) noted from the slope of the straight line passing practically through the origin.

Comparison of Nature of Binding Constant Data Obtained for Hydrophobic Bonding between Phenyl and Alkyl Groups and between Alkyl Groups—It was observed in the present study that the binding constant of the fatty acid anions increased with the increase in the chain length. In the previous study (3), however, an ascending zigzag pattern in the binding constant was observed. The zigzag pattern was explained in terms of probable closer orientation of the terminal methyl group of the propyl and pentyl chain of the alkyl copolymers to the phenyl group of the target sulfonate ion than the terminal methyl group of the butyl and hexyl chain of the alkyl copolymers. In the present model system, both interacting groups involved in hydrophobic bonding are alkyl groups. Therefore, having brought these alkyl groups closer to each other through electrostatic forces, it does not seem unreasonable to expect the alkyl groups to align against each other in such a way that each methylene group and methyl group of the fatty acid would approach the methylene groups of the hexyl chain of the hexyl copolymer within the same van der Waals radius. Such alignment of the alkyl groups would result in the release of water molecules from the icebergs around the methylene groups in increasing numbers, thereby increasing the binding constants, with the increase in the chain length of the fatty acid anion.

Comparison of Temperature Effects of Hydrophobic Bonding between Phenyl and Alkyl Groups and between Alkyl Groups—In the previous study (3), it was observed that the binding of *p*-toluenesulfonate ions by the alkyl copolymers, ethyl through hexyl, increased with an increase in temperature up to 37° but decreased at 45°. However, it is noted in the present study that binding of fatty acid anions by the hexyl copolymer increased with an increase in temperature up to 45°. The decrease in binding of the sulfonate ions by the alkyl copolymers was attributed to the breakdown of the icebergs around these ions at 45°. Therefore, a gain in entropy due to hydrophobic interaction between the alkyl groups of the alkyl copolymer and the phenyl group of the sulfonate ions at 45° was substantially lower than that at 37°. The finding of the present study then suggests that the icebergs around the alkyl groups are not disrupted at 45°. Consequently, a gain in entropy due to hydrophobic interaction between the alkyl groups of the hexyl copolymer and those of the fatty acid anions would be expected to continue at 45°. These findings are in agreement with the theoretical expectation by Nemethy and Scheraga (4). It is, therefore, reasonable to conclude further that the polymeric system employed in the present study is successful in revealing experimentally the additional characteristic of hydrophobic bonding with regard to its temperature dependency.

SIGNIFICANCE

The interesting feature of the polymeric system employed in this study is that the hexyl copolymer contained the binding sites (to interact with fatty acid anions) whose chemical nature was known, thereby making it possible to determine the apparent thermodynamic parameters of hydrophobic bonding between two methylene groups.

The previous (3) and present studies, both of which involved essentially the same polymeric system, demonstrated that the strengths of hydrophobic bonds depend on the nature (aliphatic or aromatic) of the interacting hydrophobic groups. For instance, on comparison, it becomes clear that the hydrophobic bonding noted in the previous study between the toluyl group and the hexyl group is much weaker than that between the pentyl group and the hexyl group noted in the present study. It was also observed that the strength of the hydrophobic bond formed between the toluyl group and each of the alkyl groups (ethyl through hexyl) did not increase with the increase in chain length of the alkyl groups but varied in a zigzag manner, reflecting the importance of the odd or even number of carbon atoms contained in the alkyl groups. In the present study, however, the strength of hydrophobic bonding of the hexyl group of the hexyl copolymer with the alkyl groups (propyl through hexyl) of the fatty acid anions increased with the increase in chain length of the alkyl groups. Therefore, it is conceivable that these features of hydrophobic bonding can provide a useful basis for revealing the presence of polar or nonpolar (aliphatic or aromatic) groups in the vicinity of a binding site (or a receptor) of an enzyme or a similar biopolymer responsible for the action of a drug, by appropriately evaluating the thermodynamic parameters of the binding of a drug and its suitable homologs with the biopolymer. Such an approach has been the basis of the macromolecular perturbation theory of drug action developed by Belleau (11).

A similar approach can also be useful in detecting the hydrophobic groups present in the vicinity of the charged binding site(s) of the plasma proteins for a variety of drugs that remain in the charged form in the plasma. Such approaches will by no means provide instant knowledge of the chemical nature of the binding or receptor sites on the macromolecules, but they will serve to focus attention on the more likely chemical entities or residues of macromolecules that possess positive or negative charges in the plasma. For instance, one would consider evaluating the amino acid residues such as lysine and arginine as the possible binding sites if the binding of anionic drugs to proteins is being investigated, or one would evaluate the amino acid residues such as aspartic acid and glutamic acid as the possible binding sites if the binding of cationic drugs to proteins is concerned.

The effect of temperature on the icebergs around the phenyl group as noted in the previous study (3), in contrast to those around the alkyl groups in the present study, is noteworthy. This phenomenon, in case of hydrophobic bonding between a drug molecule and a biopolymer, should offer additional means of revealing if the hydrophobic bonding is due to the interaction between the phenyl

groups, between the alkyl groups, or between the alkyl and phenyl groups of the interacting species. Also, in this context, the degree of effect of higher body temperature (fever) on the tertiary structure of vital biopolymers (enzymes), and consequently on their physiological activities, could be expected to depend on the nature of hydrophobic groups involved in hydrophobic bonding responsible for maintaining the tertiary structure of the biopolymers.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 7, 1972, from the *College of Pharmacy, Wayne State University, Detroit, MI 48202*

Accepted for publication April 12, 1973.

Abstracted in part from a thesis submitted by N. Muangnoicharoen to the College of Pharmacy, Wayne State University, in partial fulfillment of the Master of Science degree requirements.

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Preparation and *In Vitro* Evaluation of Cellulose Acetate Phthalate Coacervate Microcapsules

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Abstract □ Microcapsules of phenacetin were prepared by coacervation of aqueous cellulose acetate phthalate solutions. Appropriate solutions were made by dissolving cellulose acetate phthalate in an equivalent concentration of disodium hydrogen phosphate. A triangular phase diagram of the coacervation system was elaborated by using sodium sulfate as the coacervating agent, and the coacervation and encapsulation conditions were optimized. The amount of drug encapsulated has no significant effect on the particle-size distribution of the capsules; however, it influences the release rates of the drug, indicating that drug diffusion through the shells is the controlling step. When the shells are plasticized by imbibition with glycerol, the release rate is no longer controlled by drug diffusion through the shells but by the dissolution of phenacetin in the microcapsules.

Keyphrases □ Cellulose acetate phthalate coacervate microcapsules—formulation, *in vitro* drug (phenacetin) release rates, effect of glycerol plasticizer □ Encapsulation—formulation and *in vitro* testing of cellulose acetate phthalate coacervate microcapsules of phenacetin □ Coacervation of cellulose acetate phthalate—formulation of microcapsules, *in vitro* phenacetin release rates, plasticizer effect □ Microcapsules, phenacetin- formation from cellulose acetate phthalate coacervate system, release rates, plasticizer effect

The preparation of microcapsules by coacervation can be applied to various products (1). Different coating materials, suitable for coacervate encapsulation, were reported by Ranney (2). In pharmacy, however, the most common preparation technique is either simple coacervation of gelatin with ethanol or sodium sulfate as dehydrating agents (3–9) or complex coacervation of gelatin-acacia mixtures (10–12). Coacervation methods with pure gelatin and mixtures are rather complicated and difficult to keep under control, particularly with regard to the hardening of shells and the recovery technique of the microcapsules. The suitability of other coacervation systems for pharmaceutical purposes has not yet been studied.

Cellulose acetate phthalate was used by Jensen and Wagner (13) as a film-forming agent for encapsulation of a herbicide by simple coacervation. However, the encapsulation procedure presented in this patent is not practicable, owing to the insolubility of the cellulose ester in pure water and its total hydrolysis at pH 9.7, which is proposed as the encapsulation pH. Due to acidic groups, the cellulose ester is insoluble in strong acids but soluble in weak acids (>pH 5.5), neutral electrolytes, and bases. It is a suitable encapsulation material for timed-release dosage forms. Due to its very low acute and chronic toxicity, cellulose acetate phthalate was chosen as the encapsulating material in this study.

The present article reports a relatively uncomplicated process for the encapsulation of solid drugs by the simple coacervation of the cellulose ester, which is dissolved in appropriate disodium hydrogen phosphate solutions. The coacervation is induced by the dehydrating effect of the added sodium sulfate solution. Furthermore, the optimal coacervation and encapsulation conditions and the properties of the resulting microcapsules are examined with regard to drug content, size distribution, and release and release mechanisms of drugs.

EXPERIMENTAL

Materials and Methods—The cellulose acetate phthalate used was of Ph. Helv. VI quality, containing 34.1% phthalic acid and 2.7% free acid, calculated as phthalic acid.

Phenacetin (*N*-acetyl-*p*-aminophenetidin), of Ph. Helv. VI quality (m.p. 134–135°), was chosen as the drug model for the encapsula-