in the method. We believe that the comparison of the relative position of the $1B^+$ transition in the hairpin and the all-trans polyenes, already discussed above, represents more convincing evidence in favor of the presence of transannular interactions in the former.

The energy calculated for the A_1^+ transition is somewhat too high but its shift with the increasing chain length is reproduced well. The calculation suggests that the observed second B_2 transition is not to be assigned as B_2^+ but as B_2^-, which cannot be described within the framework of the four-electron model, and this is the assignment indicated in the Figures 1-8. The change of the order of the $1A_1^-$ and $1B_2^+$ states in the heptaenes (**2**: A_1^- above B_2^+; **5**: A_1^- below B_2^+) may be due to stronger transannular interaction in **5**, which should increase the energy of the $1B_2^+$ state.

Conclusions

It has been possible to locate and assign the A_1^-, B_2^+, and A_1^+ excited states of hairpin polyenes, analogous to the A_g^-, B_u^+, and A_g^+ excited states of all-trans polyenes. In addition, an additional low-energy excited state of B_2 symmetry, presumably B_2^-, has been detected, whose analogue has apparently not yet been observed in the all-trans series. The energies of the transitions in hairpin polyenes differ in a characteristic way from those in all-trans polyenes and this variation, as well as trends in transition intensities, can be understood in terms of simple qualitative considerations.

In both series, the lowest A^- and B^+ states are nearly degenerate. In terms of the MO-CI description, the relatively low energy of the covalent A^- state is well-known to be caused by interactions with multiply excited configurations in the all-trans case. We now find that in the hairpin case it is due largely to interactions within the singly excited part of the CI space, although the interaction with multiply excited configurations also contributes as in all alternant π systems. We provide a simple qualitative explanation for the difference and point out the relation of hairpin polyenes to bridged $[4N + 2]$annulenes and acenes. The qualitative arguments used for the analysis of the dependence of transition energies on molecular geometry (as opposed to topology) have general applicability. For instance, they provide a simple explanation of the lower energy of the B^+ transition in *s-cis*-butadiene relative to *s-trans*-butadiene[38] (the difference is predicted to be smaller in the triplet manifold).

Numerical calculations in the π-electron approximation are in semiquantitative agreement with the experimental results. These calculations, and particularly the comparison of the hairpin with the all-trans polyenes, suggest that weak transannular interactions are present between the bridged carbon atoms in the hairpin polyenes **1-6**.

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Registry No. 1, 50785-96-1; **2**, 86846-94-8; **3**, 68539-85-5; 4, 4692-14-2; **5**, 86846-95-9; **6**, 86846-96-0; 1,6-diformylcyclohepta-1,3,5-triene, 28172-94-3; 3,5-diformylbicyclo[5.4.1]dodeca-2,5,7,9,11-pentaene, 55759-42-7; 5,7-diformyltricyclo[9.4.1.$1^{3,9}$]heptadeca-2,4,7,9,11,13,15-heptaene, 60237-65-2; triphenylmethylphosphonium bromide, 1779-49-3; sodium bis(trimethylsilyl)amide, 1070-89-9; trimethylene-1,3-bis(triphenylphosphonium)bromide, 7333-67-7.

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Evaluation and Perturbation of Micelle-Solute Interactions[1]

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Abstract: The interaction of seven compounds (i.e., naphthol green B, bromophenol blue, alizarin red S, 2-naphthol-6-sulfonic acid, ammonium thiocyanate, sodium 2-naphthalenesulfonate, and sodium nitroferricyanide) with sodium dodecyl sulfate (SDS) micelles was studied using LC and TLC. All seven compounds showed unusual chromatographic behavior in that their retention increased when the concentration of micelles in the mobile phase increased. Sometimes a compound's retention behavior was independent of the concentration of micelles in the mobile phase. Based on their chromatographic behavior, all solute-micelle interactions can be classified as *binding*, *nonbinding*, or *antibinding*. Relatively small changes in the micellar environment or the micelle itself can result in pronounced alteration of micelle-solute interactions.

Introduction

The partitioning or binding of compounds to micelles is an important phenomena in many areas of study including membrane mimetic chemistry,[2] catalysis,[3-8] enzyme modeling,[9] chromatography,[10-14] tertiary oil recovery,[15] spectroscopic analysis,[16-18] emulsion polymerization,[19] and so on. There are a variety of

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methods to measure partition coefficients and/or binding constants[20] of solutes to micelles.[14] Much less attention is given to solutes that do not seem to interact with micelles or at least do not follow "models" that require a specific type or amount of interaction. The repulsion of a solute from a micelle is sometimes implied (the micellar inhibition of a reaction for example) but rarely treated in a rigorous quantitative manner. Part of the reason for this is the lack of experimental techniques which can measure zero or negative interactions. In addition, experimental phenomena which can be explained by the binding of solutes to micelles are often thought to be more relevant and interesting. In this report we hope to demonstrate that a complete understanding of micelle–solute interactions and their consequences is unlikely until all effects are taken into account. Using LC and TLC techniques one can detect and measure positive interactions between a solute and micelle (i.e., binding), negative interactions between a solute and micelle (i.e., antibinding), or zero interaction between a solute and micelle (i.e., nonbinding). Furthermore, one can monitor a solute's change in behavior (from binding to nonbinding to antibinding or vice versa) when small changes in the environment and/or micelle occur.

Experimental Section

Materials. Electrophoresis purity sodium dodecyl sulfate (SDS) was obtained from BioRad Laboratories. Naphthol green B was obtained from Allied Chemical; alizarin red S from Fisher; sodium 2-naphthalenesulfonate and 2-naphthol-6-sulfonic acid from Eastman; and bromophenol blue, ammonium thiocyanate, sodium nitroferricyanide, sodium chloride, and HPLC grade water from Baker. A 30 cm by 4 mm Varian Micropak CN, 10 μm, alkylnitrile column was used for all LC measurements. Polygram Polyamide-6 UV plates from Brinkman were used for all TLC measurements.

Methods. A Varian Model 5020 liquid chromatograph equipped with a UV 254-nm detector was used for all LC runs. The elution volumes of all solutes (vide supra) were determined at 22 °C using a pure aqueous mobile phase as well as at a variety of SDS concentrations (see Results and Discussion). Chromatographic "blanks" with aqueous NaCl solutions were run to show that the observed behavior was due to micelles and not to spurious salt effects. The total volume of the alkylnitrile column was 3.77 mL, the void volume (V_m) was 1.75 mL, and the volume of the stationary phase (V_s) was 2.02 mL.

All TLC developments were done in an $11^3/_4$ in. long, 4 in. wide, and $10^3/_4$ in. high Chromaflex developing tank. Development of all solutes was done using mobile phases consisting of pure water (or salt water) and at a variety of SDS concentrations (see Results and Discussion). All SDS mobile phase concentrations must be corrected as previously indicated because of adsorption of SDS on the TLC plate during development.[14] SDS critical micelle concentrations in the presence and absence of NaCl were measured using a Fisher (Model 20) surface tensiometer. All determinations were done at 22 °C on 20 mL of solution in a 15-cm diameter watch glass.[21]

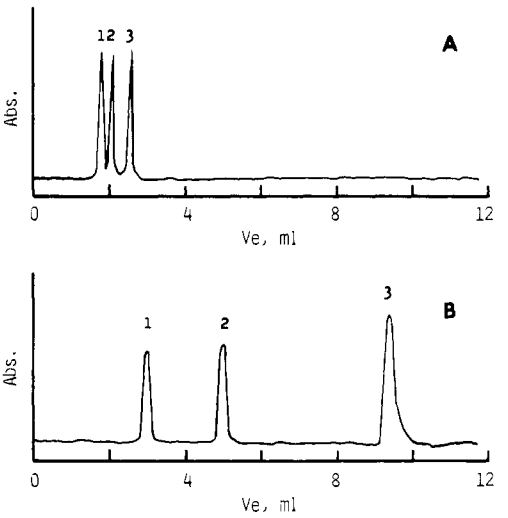

Figure 1. A comparison of the LC retention behavior of sodium nitroferricyanide (1), naphthol-6-sulfonic acid (2), and sodium naphthalenesulfonate (3), when eluted with a 0.025 M SDS mobile phase (A) and with a 0.4 M SDS mobile phase (B). Note that the retention of all compounds increases when the micelle content of the mobile phase is increased. This is the opposite of normally reported chromatographic behavior.[11-14]

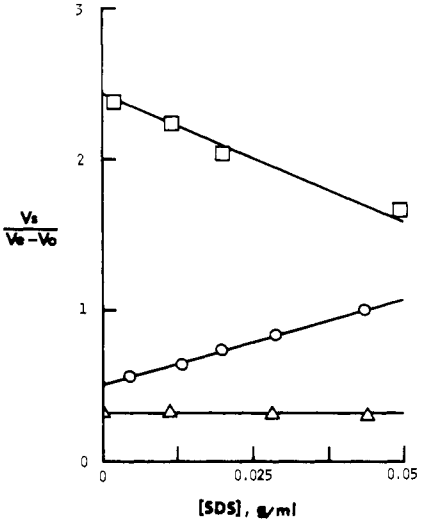

Figure 2. A plot of SDS micellar LC retention parameters illustrating *binding* (i.e., resorcinol (○)), *nonbinding* (i.e., bromophenol blue in 0.025 M NaCl (△)), and *antibinding* (i.e., 2-naphthol-6-sulfonic acid (□)) behavior. The partition equation plotted was $V_s/(V_e - V_m) = [(p(K_{MW} - 1))/K_{SW}]C_m + 1/K_{SW}$.[12,14]

Results and Discussion

The use of aqueous micellar and cyclodextrin solutions as mobile phases in LC and TLC (i.e., pseudophase liquid chromatography) has been recommended for a variety of reasons including unusual selectivities, lower detection limits, cost effectiveness, and safety among others. It is generally thought that the micelle acts much like an organic modifier or cosolvent when added to the mobile phase. Indeed, for many solutes (i.e., those which bind to micelles) this analogy may be appropriate. Consequently, when one increases the concentration of micelles in the mobile phase, the retention and capacity factors of many solutes decrease dramatically.[12] As a result of this behavior, one can calculate partition coefficients and/or binding constants of a wide variety of solutes to micelles.[12,14] There are, however, many solutes that do not bind to micelles. One might expect the chromatographic behavior of these "nonbinding" solutes to be uninteresting as one would see no change in retention behavior upon altering the micelle con-

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(20) Partition coefficients (which are dimensionless) are different from binding constants (K_b) which have units of M^{-1}. These two parameters are related by: $K_b = V(K_{MW} - 1)$, where V is the molar volume of the surfactant. See: Berezin, I. V.; Martinek, K. I.; Yatsimirskii, A. K. *Russ. Chem. Rev.* **1973**, *42*, 10, 787.
(21) When determining partition coefficients of solutes for ionic micelles in the presence of added salt, one must consider the fact that salt tends to lower the critical micelle concentration (cmc), increase the aggregation number, and slightly alter the partial specific volume of the surfactant. In this work all coefficients were calculated using individual cmc values determined at each salt concentration (see Experimental Section: Williams, R. J.; Phillips, J. N.; Mysels, K. J. *Trans. Faraday Soc.* **1955**, *51*, 728. Emerson, M. F.; Holtzer, A. *J. Phys. Chem.* **1967**, *71*, 1898). Partition coefficients can be reported per monomer or per micelle.[12] All coefficients in this work were reported per monomer so as to exclude effects due to the change in aggregation number with added salt. Changes in the partial specific volume with added salt were too small to affect the results and thus were neglected in the present work (see: Doughty, D. A. *J. Phys. Chem.* **1979**, *83*, 20, 2621).

Table I. A Comparison of "Coefficients" Obtained from LC and TLC Retention Data[12,14] for Solutes That Appear to be Excluded from SDS Micelles

compound	"coefficients" LC	TLC
naphthol green B	−13.5	−13.5
bromophenol blue	−8.2	−7.1
alizarin red S	−12.2	−12.6
2-naphthol-6-sulfonic acid	−9.6	−10.7
ammonium thiocyanate	−8.0	−10.5
sodium 2-naphthalene sulfonate	−8.2	−8.8

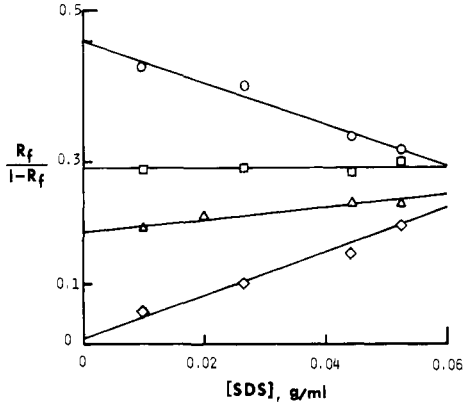

Figure 3. TLC retention plots showing the change in interaction of bromophenol blue with a SDS micelle as a function of added salt: 0.0 M NaCl, ○; 0.025 M NaCl, □; 0.05 M NaCl, (△); and 0.3 M NaCl, (◇). The partition equation plotted was $R_f/(1 - R_f) = (V_m/V_s)([(K_{MW} - 1)\bar{v}]/K_{SW})C_m + (V_m/V_s)(1/K_{SW})$.[12,14]

centration in the mobile phase. One must keep in mind that simply because a solute does not bind to a micelle does not mean that there are no interactions between that solute and a micelle (i.e., "negative" or repulsive interactions may be present). It appears that repulsive interactions can give rise to highly unusual chromatographic behavior (see Figure 1). For compounds such as sodium 2-naphthalenesulfonate, 2-naphthol-6-sulfonic acid, and potassium nitroferricyanide (Figure 1), there is a dramatic *increase* in retention upon increasing the micelle content of the mobile phase. This behavior cannot be explained by any ion-pairing mechanism as the stationary phase has been saturated with surfactant[12] and the charges of the solutes and surfactant are usually the same. It appears that the solutes are actively excluded from the mobile phase or more specifically by the micelles in the mobile phase. This type of behavior is referred to as *antibinding* and is easily distinguished from *nonbinding* behavior which manifests itself as a lack of change in retention with changing micellar mobile phase concentrations.

Using LC or TLC partition equations,[12,14] one can easily detect and measure different micelle–solute interactions (Figure 2). Lines of positive slope (i.e., resorcinol in Figure 2) indicate solute binding to micelles. Lines of negative slope (i.e., 2-naphthol-6-sulfonic acid in Figure 2) indicate that a solute is strongly excluded or repelled from the micelle. Lines of zero slope (bromophenol blue in Figure 2) indicate either that there are no attractive or repulsive forces between the solute and micelle or more likely that these forces are approximately equal and negate one another. While plots such as those for resorcinol can be used to calculate the partition coefficient (K_{MW}) of a solute between micellar and aqueous phases, plots such as that for 2-naphthol-6-sulfonic acid give negative "coefficients" which can be converted to negative "binding constants". Table I lists several compounds that show antibinding behavior with SDS micelles. Each compound has a characteristic negative "coefficient" which can be calculated using either LC or TLC retention data.[12,14]

The implications of micellar antibinding are not only important to LC as far as selectivity is concerned, but to other fields (i.e., membrane mimetic chemistry, catalysis, and inhibition, etc.) as

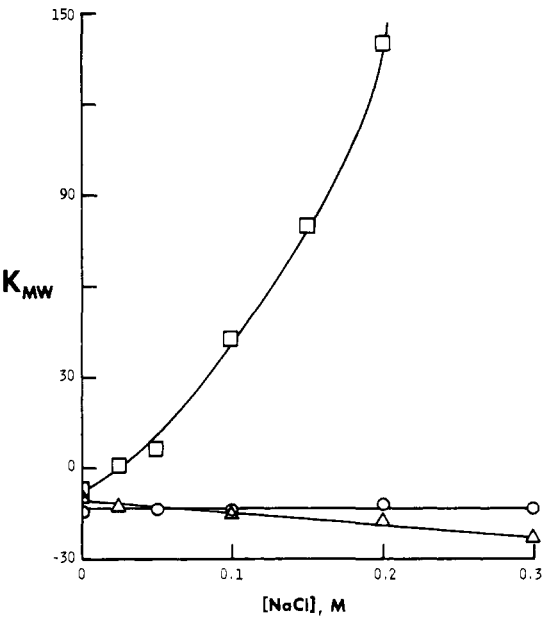

Figure 4. A plot of the apparent coefficients of three different compounds to SDS micelles as a function of sodium chloride concentrations: bromophenol blue, □; naphthol green B, ○; and ammonium thiocyanate, △.

Table II. Data Showing the Effect of Added Salt on Solute "Coefficients" between Micelle and Bulk Aqueous Solution

compound	"coefficients" NaCl concentration, M 0.0	0.025	0.05	0.10	0.20	0.30
naphthol green B	−13.5	−12.6	−13.5	−11.8	−10.6	−13.3
bromophenol blue	−7.1	0	+6.4	+40.2	+143.1	+491.6
alizarin red S	−12.6	−10.4	−12.1	−3.2	+0.4	0
2-naphthol-6-sulfonic acid	−10.7	−8.1	−8.5	−5.7	−5.3	−6.8
ammonium thiocyanate	−10.5	−12.6	−14.5	−14.9	−15.7	−22.5
sodium 2-naphthalene sulfonate	−8.8	−7.3	−2.5	−2.8	+8.9	+9.6

well. For example, an intriguing and important possibility is that small changes in the micellar environment (e.g., pH, ionic strength, buffer type, etc.) and/or micelle properties (e.g., fraction of charge, aggregation number, etc.) might be sufficient to completely change the solute–micelle interaction. A good example of this is illustrated in Figure 3. Bromophenol blue shows pronounced antibinding to SDS micelles in pure water. In the presence of as little as 0.02 M NaCl, bromophenol blue appears to be nonbinding. At slightly higher salt concentrations, bromophenol blue binds strongly to SDS micelles. In fact, the binding of bromophenol blue to SDS micelles increases substantially more than might be expected from the linear increase in the concentration of sodium chloride (Figure 4). Not all compounds show this type of behavior. For example, the interaction of naphthol green B with SDS micelles appears to be largely unaffected by adding salt (Figure 4). Conversely, thiocyanate ion becomes even more antibinding when salt is added (Figure 4). The data in Table II show the variety of changes that can occur in solute–micelle interactions when even small variations are made in the system. Note that not only does each compound have a distinct "coefficient", but the change in each "coefficient" as a function of added salt can also be unique.[21]

In this study identical micellar systems were perturbed by the addition of identical amounts of sodium chloride. Only trace amounts of solute (which did not measurably alter the CMC) were present in all studies. Consequently, at any given salt concentration both the micelle and bulk aqueous solution were identical for all solutes studied. Yet the behavior of similarly charged solutes to identical negatively charged micelles can be completely different (Table II and Figure 4). Thus one must assume that each free solute perceives the same micellar solution differently and that

simple electrostatic arguments may not totally explain the observed phenomena.

One might consider antibinding to simply be an excluded volume effect based on the repulsion of a charged solute from a similarly charged micelle. As a result of this repulsion, a solute would not only be excluded from the volume of the micelle but also from much of the volume of the double layer surrounding the micelle (which can be appreciable). Theories have been derived for charge–charge interactions in aqueous solution as well as for the effect of added salt on these interactions. For example, both the Debye length for simple ions (i.e., the distance over which the electrostatic field extends) and the thickness of the electric double layer of charged lyophobic colloids are proportional to the inverse of the square root of ionic strength.[22,23] In this work the interaction of organic anions with negatively charged association colloids is analogous to the previous examples, yet there are fundamental differences. From strictly electrostatic arguments (vide supra) one would expect solutes to show decreased micellar antibinding with increasing ionic strength. In fact, this occurs for the majority of solutes studied. Sodium 2-naphthalenesulfonate, alizarin red S, and 2-naphthol-6-sulfonic acid show a linear increase in the value of their measured "coefficients" with increasing salt concentration (Table II). This behavior is typical of many organic anions with SDS micelles. Bromophenol blue (Figure 4) is the only compound found, thus far, in which the relationship is not linear.

In lyophobic colloidal systems, the addition of sufficient salt generally brings about flocculation. In the present system, the addition of sufficient salt can cause organic ions to go from antibinding to binding. It is tempting to draw an analogy between colloidal flocculation values and the nonbinding values observed in this study. In order for the transition from antibinding to nonbinding to binding to occur, the solute ion of interest must have sufficient hydrophobic character to associate with the nonpolar portion of the micelle once electrostatic repulsions have been minimized.

The behavior of ammonium thiocyanate (Table II and Figure 4) is difficult to explain in terms of the aforementioned electrostatic criteria. However, it may be possible to rationalize the increased antibinding of thiocyanate ion with added salt by considering the equilibrium constant of thiocyanate between the bulk aqueous solution and the micellar phase as well as the constant between the micellar and stationary phase. Thiocyanate ion has little hydrophobic character compared to the other solutes in this study. Consequently, when salt is added, the predominate factor may be the ions increased affinity for the bulk solution or for the stationary phase. In contrast, many organic ions tend to be salted into the micelle.

The apparent lack of variation in antibinding of naphthol green B with added salt is a bit of an enigma (Table II and Figure 4). This compound seems to be oblivious to changes in the micelle and in the bulk aqueous solution. It is possible that changes too small to detect by chromatographic techniques are occurring. It may also be possible (although not probable) that the change in the excluded volume effect of the micelle is exactly counterbalanced by an increase in the solution or stationary phase affinity of naphthol green B.

The antibinding phenomena is useful in liquid chromatography because it produces unusual selectivities (Figure 1). It may also be useful in studies involving micelles, liposomes, vesicles, and even membranes. Indeed, unusual salt effects have been noted in kinetic studies in micellar media.[24]

Antibinding and nonbinding phenomena are by no means restricted to negatively charged micelles and solutes. They are easily observed with cationic micelles and positively charged solutes. Indeed, some uncharged solutes behave in an analogous manner with charged micelles. Antibinding behavior has also been observed between micelles composed of amphoteric surfactants and some solutes. These and other studies on this phenomenon, its control, and use are in progress and will be reported subsequently.

Registry No. SDS, 151-21-3; naphthol green B, 19381-50-1; bromophenol blue, 115-39-9; alizarin red S, 130-22-3; 2-naphthol-6-sulfonic acid, 93-01-6; ammonium thiocyanate, 1762-95-4; sodium 2-naphthalenesulfonate, 532-02-5; sodium nitroferricyanide, 14402-89-2.

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Solvent and pH Dependence of Absorption and Fluorescence Spectra of 5-Aminoindazole: Biprotonic Phototautomerism of Singly Protonated Species

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Abstract: Absorption and fluorescence spectra of 5-aminoindazole have shown that the amino group acts as a hydrogen-bond acceptor in S_0 and hydrogen-bond donor in S_1. pH studies have indicated that the monocation and the monoanion of 5-aminoindazole are different in S_0 and S_1 states, respectively, i.e., species II and IV in the ground state and species V and VI in the excited singlet state. Solvent studies have also shown that there exists a biprotonic phototautomerism in the singly protonated species.

Introduction

Excited singlet-state proton-transfer reactions of several bifunctional molecules such as quinoline carboxylic acids[1] and hydroxy aromatic acids[2,3] have been studied. In the ground state of these molecules the electron-donating (OH, NH_2) and electron-withdrawing groups (COOH, pyridinic nitrogen atom) behave in a similar manner to that in monofunctional molecules. But it has been observed that the gain in acidity of an electron-donating

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