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Steric Exclusion Behavior of Sodium Dodecyl Sulfate at Pressures up to 50,000 psi

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Abstract

The monomer–micelle equilibrium of sodium dodecyl sulfate (SDS) has been examined by steric exclusion chromatography as a function of pressure. Conditions for the high-pressure work were explored at atmospheric pressure using both frontal analysis and conventional chromatography. The variables included sample size and concentration, pore distribution in the packing, flow rate, and ionic background of the eluent. Dissociation of the SDS micelle in 0.1 *M* $NaNO_3$ or NaCl increased sharply above 30,000 psi, and it sorbed strongly on the column. While greater dissociation of the micelles was expected due to the smaller volume of water when oriented around the charged monomer species, the large increase in retention volume above 30,000 psi indicated strong adsorption. This has been attributed to hydrogen bonding to the sulfate end of the monomer, although the hydrophobic tail of the SDS anion may have been adsorbed because the relatively insoluble monomer concentration was greatly increased as a result of breakup of the micelles. The results of the high-pressure chromatography of micelles have been compared to results from high-pressure spectroscopy of inorganic complex ions and from the ultracentrifugation of proteins. Finally, high-pressure chromatography up to 20,000 psi has been done using Sephadex G-10 and G-25. Bed compression was minimal under our experimental conditions, and reproducible retention volumes using carbowax 20M and ethylene glycol were obtained.

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INTRODUCTION

The present chromatographic study on micelles was undertaken as part of a broad program to examine the scope of high-pressure liquid chromatography in the range of 5,000 to 50,000 psi (350 to 3500 kg/cm^2). Previous workers have used the same instrumentation in liquid–solid chromatography using silica gel (*1*, *2*) and in steric exclusion chromatography using porous glass (*3*).

In the present study we have examined the micelle–monomer equilibrium of sodium dodecyl sulfate (SDS) in order to determine chromatographically how pressure affected the equilibrium. The monomer has a molecular weight of 288 whereas, above the critical micelle concentration (CMC) of 0.008 *M* in water, many monomer units bind together to form a globular micelle having a molecular weight of about 18,000 (*4*). Steric exclusion chromatography should be ideal for the study of a monomer–micelle equilibrium because of the large difference in the molecular sizes.

Previous permeation work had been performed on SDS using small discrete samples on Sephadex G-25 (*5*) and on Corning porous glass (CPG) (*6*). Others have used the technique of frontal analysis on Sephadex G-50 (*7*, *8*) because of the tendency of small discrete samples, that originally contain micelles, to be diluted by the eluent to a concentration below the CMC before the sample emerges from the column (as a monomer). In addition, Giddings has shown that the equilibrium should shift so as to form more monomer (*8a*). The presence of a salt usually increases the repulsion between the hydrocarbon end of the monomer units and the solution, leading to both larger micelles and lower CMC values. For example, in 0.2 *M* NaCl the CMC drops to 0.0009 *M* SDS, and the micelle molecular weight rises to 29,000 (*4*). This is an important factor when small samples must be used, as on the high-pressure chromatograph. The literature also mentions a second critical micelle concentration for SDS at 0.02 *M* (*9*, *10*).

In the present study both frontal analysis and conventional elution chromatography have been used. We first familiarized ourselves with the SDS system by working near atmospheric pressure using Sephadex and then porous glass, which we thought would be more compatible with the high-pressure system. Next, conditions were explored that would stabilize the micelle enough to allow small discrete samples to be used on the columns without encountering significant dissociation of the micelles. Finally, behavior was observed using the high-pressure liquid chromatograph.

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An important feature of the high-pressure operation is that the columns themselves were operated under conditions which led to a small pressure drop, close to that which would have occurred in a normal operation near atmospheric pressure. Most of the pressure drop occurred across a valve located between the exit of the column and the detector.

EXPERIMENTAL

Reagents

Small quantities of pure SDS, synthesized from the n-C_{12} alcohol, were obtained from N. Muller of this Department. Commercial grades of SDS were purchased from M. Michel and Company, New York, New York (high purity 99% grade); Eastman Organic, Rochester, New York (Lot 701C); Sigma Chemical Company, St. Louis, Missouri ("approximately 95% pure"); Alcolac Incorporated, Baltimore, Maryland (98% Sipon grade); and Pierce Chemical Company, Rockford, Illinois (99% Sequanal grade). For frontal analyses, 0.04 F stock solutions of the above were made in deionized distilled water. The resulting solutions were clear, and no further purifications were attempted.

Blue dextran (Sigma Chemical Company) was used to obtain the void volume of permeation columns. Ethylene glycol was used to obtain the sum of the void and inner volumes of these columns. Analytical grade potassium nitrate, sodium nitrate, and sodium chloride (Mallinckrodt Chemical Works, St. Louis, Missouri) were used in aqueous solutions as eluents, and analytical grade potassium chloride (Mallinckrodt Chemical Works) was used to study ionic repulsion of small electrolyte samples.

Alcohols for the standardization of the gas chromatographic column were n-dodecanol (Baker Chemical Co., Phillipsburg, New Jersey), n-tetradecanol (Eastman Organic, Rochester, New York), and n-undecanol (obtained from this Department).

The gas chromatographic packing consisted of 30/60 mesh Chromosorb P support, coated with either 20% by weight Carbowax 20 M or SF-96 (Analabs, North Haven, Connecticut). Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, New Jersey) with a 1000–5000 MW range was used in the 10–40 μ and 20–80 μ dry particle-size ranges. The G-50 (1,500–30,000 MW range) had a 100–150 μ dry particle-size range. Corning porous glass, Lot No. 9233 CPG–10–240 (10,000–95,000 MW range), was reported to have an average pore diameter of 274 Å and a size range of 36–75 μ.

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Apparatus

The high-pressure pump (S.C. Hydraulic, Los Angeles, California) produced pressures in excess of 50,000 psi. It utilized a small diameter hydraulic piston connected to a large diameter air piston capable of a 660-fold (hydraulic/air) increase in pressure. The high-pressure column (High Pressure Equipment Corp., Erie, Pennsylvania) used with this pump was 1/4 in. i.d. × 1-1/4 in. o.d. × 12 in. and was fabricated from 17-4PH stainless steel. Stainless steel frits of 10 μ porosity were placed at the ends of the column to support the bed. A full description of the apparatus is given elsewhere (*1*). A Sigmamotor peristalitic pump (Middleport, New York) was used for low-pressure work.

The low-pressure columns were made from borosilicate glass tubing of various lengths and diameters (see Table 1) using Beckman Teflon fittings (Beckman Instruments, Fullerton, California). Glass wool plugs supported the bed. Tygon tubing (1/32 in. i.d.) connected the columns to the detector. A Waters Associates R-4 differential refractometer was used as the liquid detector, and its output was connected to a Sargent SRL recorder.

An Aerograph Model 660 gas chromatograph was used with nitrogen carrier gas for the analyses of the hydrolysis products of the alkyl sulfates. A 1/4 in. × 6 ft stainless steel column was used. Hamilton (Reno, Nevada) syringes of various capacities were used for low-pressure on-column sample injection.

Procedures

The Sephadex packings were swollen overnight, and the fines were

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TABLE 1

Characteristics of Columns Used for Liquid Chromatography

Column	Packing	Particle size (μ)	i.d. (cm)	Length (cm)	V_o (ml)	$V_o + V_i$ (ml)
A	G-25	44–80	1.5	30	22.1	46.5
B	G-50	100–150	1	66	21.8	50.0
C	G-25	10–40	1	32	—	—
D	G-25	20–44	0.6	35	5.0	9.5
E	CPG-10-240	36–75	0.6	30	6.1	9.6
F	CPG-10-240	36–75	0.6	30	6.1	9.6
G	CPG-10-240	36–75	1	66	25.4	44.2
H	G-10	20–74	0.6	30	4.4	7.3
I	G-25	10–40	0.6	30	5.0	10.1

decanted off during several washings with distilled water. The Corning glass beads were covered with water and subjected to vacuum so as to draw air out of the pores.

All liquid columns were packed using a wet slurry technique. The columns were first filled with solvent, and the packing slurry was introduced through a funnel while the solvent eluted from the bottom of the column. In addition, for high-pressure work, the packed column was run at high pressure and flow, and more packing was added to the compressed bed. This was repeated until the bed stabilized.

For runs at low pressure, discrete samples were injected directly onto the column beds through Teflon (DuPont) T-fittings while eluent was flowing. For a frontal analysis the sample was placed directly on top of the support bed and throughout all of the pump lines leading to the column before flow was started. Thus sample entered the bed the moment the pump was turned on. Flow rates were approximately 1 ml/min.

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All of the permeation results are reported in terms of the distribution coefficient, K, where $K = (V_r - V_o)V_i$, where V_r is the retention volume of the sample, V_o the void volume determined using blue dextran, and V_i the inner volume of the porous particles, determined from the difference between the retention volumes for ethylene glycol and blue dextran. For frontal analyses, V_r is the volume corresponding to the half-height of the frontal break.

The gas chromatographic column was packed by introducing small amounts of coated support while continously tapping the column with a metal rod. Glass wool plugs and Swagelok fittings (Indiana Valve and Fitting, Inc., Indianapolis, Indiana) were used at both ends. The column was coiled after packing. Before starting an analysis, nitrogen was run through the column for 1 hr at 230°C (the working temperature) to insure a steady baseline.

The GC samples contained alcohols produced by hydrolysis of the alkyl sulfates using HCl. Those samples yielded chromatograms on the Carbowax 20M column. That column had previously been used to run the known alcohols so as to construct a plot of the logarithm of retention volume vs the carbon number. That plot was then used to determine the homologous impurities in the commercial SDS samples.

The GC samples were prepared from the alkyl sulfates as follows: Exactly 0.68 ml of concentrated HCl was added to 50 ml of a 0.008-*F* SDS solution to give approximately 0.16 *M* HCl. The solution was then heated over steam for 3 hr. Then 50 ml of hexane were added to the slightly cloudy solution using vigorous agitation. Heating was continued for ½ hr,

after which two well-defined layers (aqueous and hexane) appeared. The hexane layer was retained, and most of the hexane was allowed to evaporate. A liquid solution of alcohols, corresponding to the original alkyl sulfate homologs, remained.

RESULTS

Frontal Studies

Table 2 shows the results of frontal analyses of Sigma SDS on Sephadex G-25 and G-50. Three important observations emerged from the data. First, the fact that there were several breaks suggested that significant amounts of impurities were present in the commercial SDS. This will be taken up in more detail below. Second, the fact that the K values invariably decreased with increased sample concentration suggested that all of the breaks were due to micellular species. This is understandable when one considers that the higher the sample concentration is above the CMC, the more stable the micelle will appear to be, and the sooner it will elute. Lower concentrations of sample will result in proportionately more monomer formation since the micelles are diluted below the CMC at the elution front. This, in turn, will result in later elution. Third, there was not much difference between the retention characteristics of the micelles for packings of different pore sizes. That was because the micelles were excluded from all of the G-25 pores and most of the G-50 pores, while the monomer was held up in all of the G-25 and G-50 pores, Hence it was the relative micelle–monomer stability which most strongly determined the elution volume of the micelle sample in aqueous solution at normal pressures.

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TABLE 2

Comparison of Distribution Coefficients from Frontal Analyses as a Function of Pore Size and Concentration using Sephadex Columns A and B

SDS conc, F	Distribution coefficient K					
	Break 1		Break 2		Break 3	
	A	B	A	B	A	B
0.0025	—	—	—	—	1.3	1.2
0.0050	0.77	0.70	1.0	0.89	1.2	1.1
0.0100	0.44	0.41	0.59	0.54	1.0	0.86
0.0200	0.17	0.29	0.27	0.38	0.57	0.61
0.0400	0.03	0.11	0.10	0.15	0.27	0.24

In contrast, when SDS solutions were run on G-10, where even the monomer would be unable to penetrate many of the pores, most of the sample unexpectedly eluted with $K > 2$. This meant that strong adsorption completely overrode the separation mechanisms that occurred on the gels which had larger pores.

As mentioned earlier, three breaks were observed where only one was expected. Previous work done with known mixtures of solutions of SDS and sodium tetradecyl sulfate (STS) (*8*) reported that maximal frontal resolution had occurred at 0.01 *F*. As Table 2 shows, our maximum ΔK between breaks 2 and 3 also occurred at 0.01 *F*. These results, coupled with the concentration effects, strongly suggested homologous micelle impurities in our sample.

To test further for the presence of discrete sample impurities, we twice repeated frontal analysis of 0.02 *F* SDS solution and each time collected the eluate range that corresponded to the first two breaks. This fraction was rechromatographed and gave frontal breaks at 25.6 and 29.8 ml, whereas the original sample had given breaks at 26.5, 28.8, and 35.6 ml. Since the species contained in the two earliest eluting fractions did not break down to give a third component, discrete impurities were present rather than another stable SDS species in an equilibrium favored by dilution.

The above frontal results were then compared with those obtained from 0.02 *F* solutions of Kodak, Alcolac, and Pierce samples of SDS, as well as 0.02 *F* solution prepared from SDS obtained from Dr. N. Muller. The Kodak sample gave three breaks that agreed closely with those from Sigma. The Alcolac and Pierce samples gave two breaks corresponding to the second and third breaks, whereas the Muller SDS gave a single break at the volume of the third break. This was clear evidence that the third break was SDS and that the first two breaks were impurities.

The gas chromatographic results in Table 3 indicate that steric exclusion can be a convenient means for qualitative analysis of alkyl sulfate mixtures, but can give misleading quantitative results. The retention volumes for the C_{12} and C_{14} standards agreed closely with two peaks of the extracts, and the third peak corresponded to the extrapolated value for C_{16} on the calibration curve. These results showed that two impurities in the commercial SDS were due to STS and sodium hexadecyl sulfate (SHS).

Those qualitative results are easy to explain. It is known that STS, with critical micelle concentration (CMC) of 0.002 *M*, elutes before SDS in frontal steric-exclusion chromatography (*8*). This is because the STS,

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TABLE 3

Comparison of Relative Gas Chromatographic Peak Areas with Relative Frontal Break Heights for SDS from Three Sources

	Source of SDS					
	Sigma, relative height		Kodak, relative height		Muller, relative height	
Homolog	GC[a]	GPC[b]	GC[a]	GPC[b]	GC[a]	GPC[b]
C-12	75	38.5	73	25.6	100	100
C-14	21.5	43.9	23	41.9		
C-16	3.5	17.6	3.6	32.6		

[a] Carbowax 20M column operated at 230°C.
[b] 0.02 *F* SDS on Column C.

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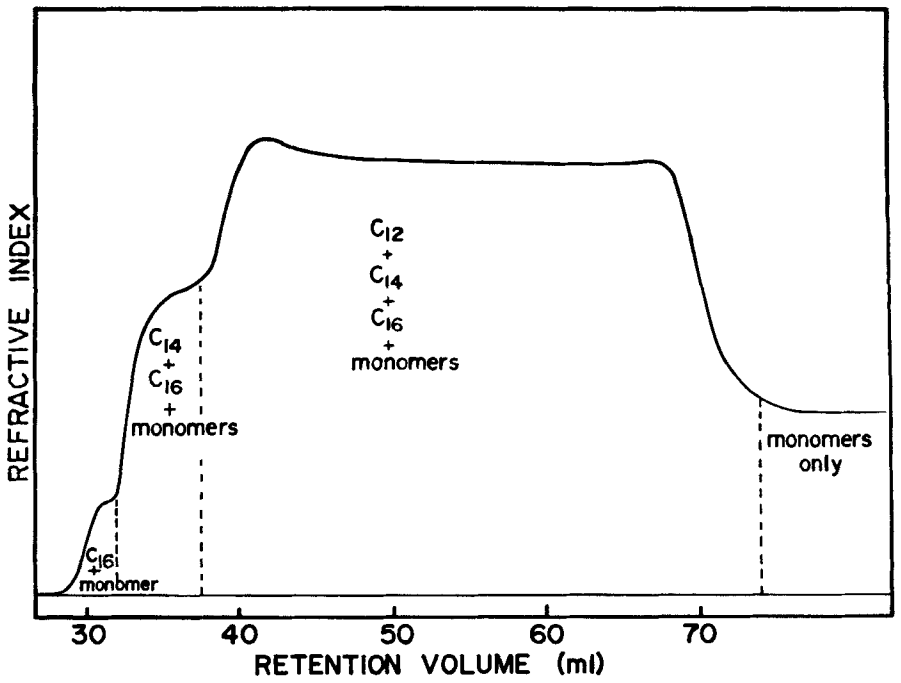

FIG. 1. Identification of the frontal breaks for 0.02 *F* Sigma SDS on Sephadex Column B using water as the eluent.

with its much smaller CMC, exists in the micelle form for a longer time at the sample front. The same argument may be used to identify the first break as that of SHS. Figure 1 shows the volumes that corresponded to the C_{16}, C_{14}, and C_{12} micelles, respectively. Sample introduction ended at 43.3 ml and the last of the micelles eluted from the column at 69.9 ml. The difference between these two volumes, 26.6 ml, was somewhat greater than the void volume of 22 ml due to breakup of some micelles. (A decrease in sample volume led to a narrowing of the highest plateau.)

The discrepancies between the relative amounts found by quantitative GC analysis and by frontal analyses of solutions can be explained as follows. Monomers must be present at a concentration equal to the CMC in order to stabilize the micelle species. Since the permeation column is constantly retaining the monomers, while excluding the micelles, some micelles must break down to monomers in order to maintain the CMC. The SDS micelle, being least stable, contributes the greatest amount on a relative basis to the background monomer concentration and to the final monomer plateau. As a result, the height of the break for its micelle is correspondingly lower than would be expected from the quantitative GC analysis of the original sample. Table 3 confirms this. Likewise, C_{16} breaks down the least on the column, so the relative height for its micelles is erroneously high. Commercial *n*-dodecanol, which is probably used by many SDS manufacturers, contains 68% C_{12}, 26% C_{14}, and 6% C_{16} (*11*). Our GC results are in surprisingly good agreement with those figures, whereas our frontal results are not. The SDS from M. Michel and Company proved to be chromatographically pure, and it was more plentiful than the pure sample supplied by N. Muller, so it was used for all of the later work, including the high-pressure studies.

The final frontal analysis on Sephadex was made so as to determine the effect of a modest change in the flow rate on the shape of the chromatogram. The results shown in Table 4 indicated that flow control should not be a critical factor in the high-pressure runs.

An attempt was made to do frontal analysis on porous glass beads (Column G) with water as a background using a 0.02-*F* Sigma SDS sample, but no homolog separation was observed. A K of approximately 0.5 was obtained for the break, a reasonable value for a species of MW 18,000 on CPG–10–240, according to manufacturer's specifications. There was an indication of ionic repulsion, however, since the back side of the chromatogram dropped off sharply. Such repulsion would inhibit entry of the monomers into the glass pores so that the normal homolog separation mechanism could not take place. For that reason, an experiment was run in 0.03

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TABLE 4

Effect of Flow Rate on Distribution Coefficients and Relative Heights of the Breaks on Sephadex Column A Using 0.02 *F* Sigma SDS

	0.27 ml/min		0.58 ml/min	
Homolog	*K*	Relative height	*K*	Relative height
C-12	0.57	35	0.57	35
C-14	0.25	55	0.27	50
C-16	0.20	10	0.17	15

M NaCl but with similar results. Only one break with $K = 0.5$ was obtained. Upon going to a more dilute sample, 0.005 *F* solution, which on Sephadex had been slightly adsorbed and gave a *K* of 1.2, a *K* of 0.77 was observed so some repulsion was still evident.

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Elution Studies

When 20 μl of 1 *M* Michel SDS was injected onto Sephadex G-25 (Column D), complete micelle breakup occurred, as indicated by a peak that eluted at $V_o + V_i$. To stabilize the micelles of SDS, solutions were then made up in either 0.1 or 0.2 *M* NaCl, and the same NaCl concentration was used for elution. Table 5 shows that all samples eluted as a peak, at or near the void volume of 5.0 ml, followed by a monomer plateau. By increasing the sample size and/or the NaCl concentration, the relative amount of micelle significantly increased. Therefore, 30 μl of 1 *M* SDS in NaCl was selected for high-pressure work with discrete samples.

A similar study of ionic background was made using CPG because its use appeared to be more appropriate for high pressures because its bed might not compress so easily if the pressure drop were inadvertently made too large. In contrast to results obtained using a comparable Sephadex

TABLE 5

Effect of Background Salt Concentration on Retention Characteristics of Small Samples of 1 *M* SDS on Sephadex Column D

Sample size (μl)	Distribution ratio, *K*		Relative micelle peak area (%)	
	0.1 *M* NaCl	0.2 *M* NaCl	0.1 *M* NaCl	0.2 *M* NaCl
5	0.5	—	0.0	—
10	0.00	—	10	—
20	0.07	0.00	30	42
30	0.04	0.00	38	71

column, a 32-μl sample of 1 M SDS in water eluent showed no apparent breakup of micelle, as indicated by Peak 2 in Fig. 2A. However, a 32-μl sample of 0.01 M SDS gave Peak 1 in Fig. 2A. The results were anomalous since the lower concentration should have broken up readily in the water and eluted at or near $V_o + V_i$ rather than near V_o. Strong ionic repulsion by the glass was evident. This was verified by running small KCl samples as shown in Fig. 3. Figure 3A shows that a sample of 0.01 M KCl was eluted by water near V_o. However, a sample of 1.0 M KCl eluted much later, but its K value and front-side curvature indicated repulsion. By

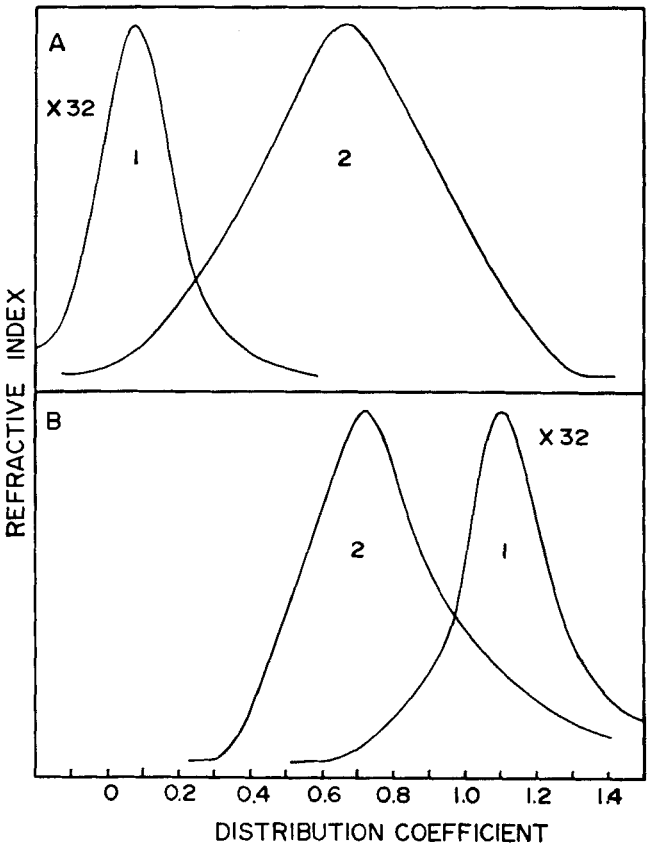

FIG. 2. Effect of an ionic eluent on the retention characteristics of small SDS samples on CPG Column E. A: H_2O eluent. B: 0.1 M NaCl sample eluent and solvent. 1: 32 μl 0.01 M SDS. 2: 32 μl 1.0 M SDS.

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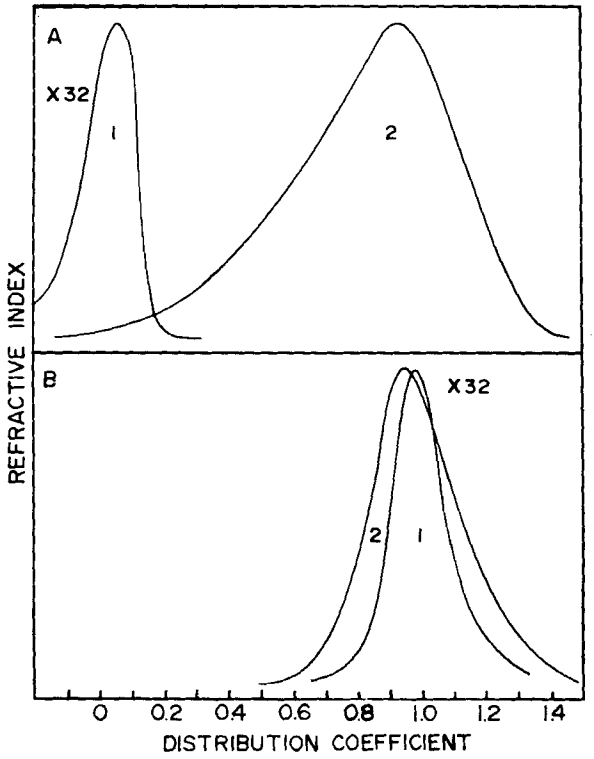

FIG. 3. Effect of an ionic eluent on the retention characteristics of small samples of electrolytes on CPG Column E. A: H_2O eluent. B: 0.1 *M* NaCl sample eluent and solvent. 1: 32 μl 0.01 *M* KCl. 2: 32 μl 1.0 *M* KCl.

going to a 0.1-*M* NaCl background as shown in Fig. 3B, the repulsion was overcome. The KCl eluted as expected near $V_o + V_i$, and retention was independent of sample concentration. The latter samples were made up in 0.1 *M* NaCl to avoid generating negative peaks.

The 0.1-*M* NaCl background had a similar effect on the SDS samples. As Fig. 2B indicates, there was a very large increase in the *K* of the 0.01-*M* SDS sample. Instead of being almost totally excluded, it was slightly adsorbed. The 1-*M* SDS sample, Peak 2, also showed a slight increase in *K* but, like the 1-*M* KCl sample, its high concentration made it less susceptible to ionic repulsion. Its front-side curvature did decrease, however. Thus, by using 1 *M* SDS with a 0.1-*M* NaCl background, small samples of stable micelles could be used with CPG for study at high pressures.

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High-Pressure Elution Studies

The high-pressure work was run on CPG Column F. Figure 4 shows that there was no observable pressure effect until 28,500 psi, at which pressure a monomer plateau appeared similar to the one seen on Sephadex at atmospheric pressures. By 32,000 psi the micelle peak had disappeared and it had been replaced by a low, broad, sloping plateau that began near a K value of about 0.5. Finally, at 53,500 psi, a break still appeared but the plateau was lower, and adsorption was still predominant since much of the sample eluted at a $K > 1$.

The breakup of micelles by pressure was reversible, as shown in Fig. 5.

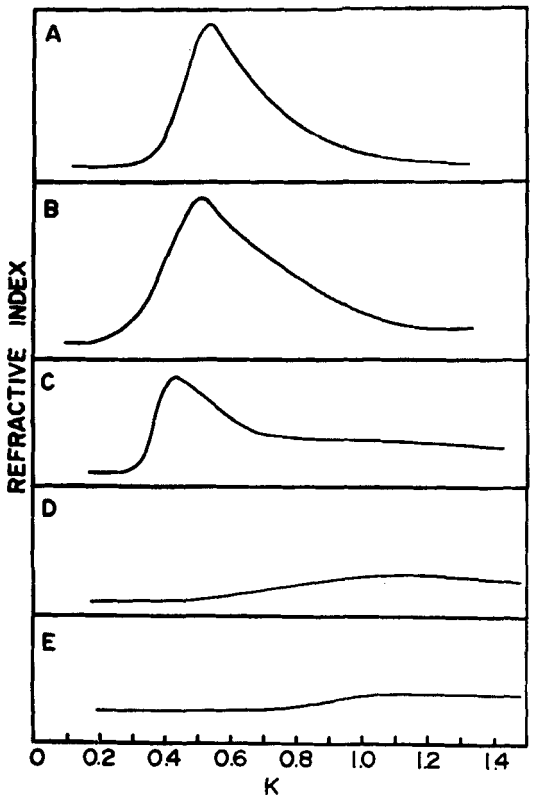

FIG. 4. Effect of pressure on SDS micellization using 0.1 M NaCl eluent and 32-μl samples of 1 M SDS on CPG Column F. A: 12,500 psi. B: 25,000 psi. C: 28,500 psi. D: 39,500 psi. E: 53,500 psi.

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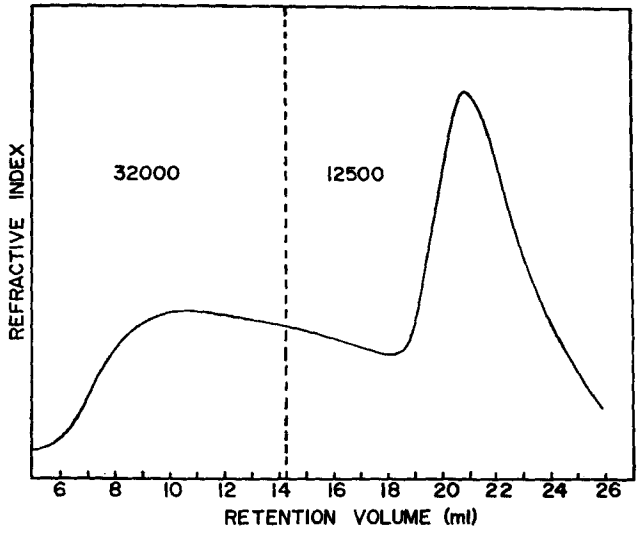

FIG. 5. Effect on micellization of lowering the pressure during an elution using CPG on Column F.

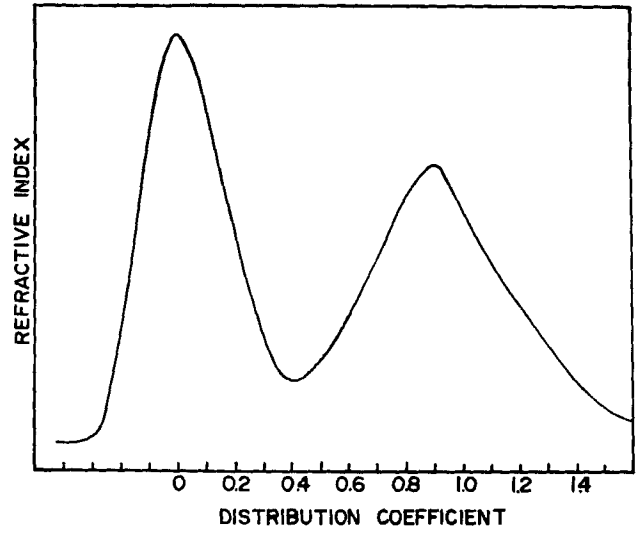

FIG. 6. Demonstration of a high-pressure separation on Sephadex G-10 Column H at 21,000 psi using 32 μl of 1:1:20 of ethylene glycol:Carbowax 20 M:H_2O to determine V_o and $V_o + V_i$ for the column.

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Instead of allowing the 32,000 psi plateau to reach the baseline, the pressure was decreased to 12,500 psi after 14.3 ml had eluted. A peak eluted 6.5 ml after the decrease. Apparently, due to very strong adsorption of the monomer at high pressures, the bulk of the SDS sample had traveled only 1.5 ml into the bed during the time that 14.3 of eluent had been collected. At lower pressures the sample readily desorbed and reformed the micelle which promptly eluted.

Though CPG was used for the study of pressure effects on micellization, successful preliminary runs were made on the high-pressure apparatus using Sephadex G-10 and G-25 in Columns H and I in Table 1. Volumes for V_o and $V_o + V_i$, which reproduced those obtained at atmospheric pressure, have been obtained as shown in Fig. 6. This means that these types of Sephadex, and possibly others, can be operated at high pressures. As a result, it will be easily possible to extend the studies of proteins and other large molecules to higher pressures.

It is important to note that the Sephadex columns, like those of CPG, were not operated under a pressure drop in the column of 21,000 psi but, instead, as close as possible to the same drop as in the runs near atmospheric pressure. That drop was primarily a function of particle size and linear flow rate. The key, of course, was in the use of a valve, at the exit of the column, to take up most of the pressure drop.

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DISCUSSION

The present study not only confirms that steric-exclusion chromatography can be done at pressures up to and beyond 50,000 psi, but also demonstrates the feasibility of using less rigid organic polymers. The latter opens up the possibility of extending steric exclusion studies of many species that have been examined at atmospheric pressure to high pressures by using porous organic polymers. In addition, the study demonstrates how one micellar species behaves under pressure.

In separations of micelles by frontal analysis, the relative CMC values are important. In general, the CMC decreases as the carbon chain becomes longer. For example, sodium octadecyl sulfate (SOS) has such a small CMC that it is difficult to measure by standard techniques (*12*). The smaller the CMC is, the more stable is the corresponding micelle to breakup by dilution on a column. Thus C_{16} elutes before C_{14}, and C_{14} before C_{12}.

The pore size of the column packing is not critical for micelle separation as long as the monomer is mostly retained by the column and the micelle

is mostly excluded, as shown by the results for G-25 and G-50. For example, others have seen differences in breakthrough volumes of micelles using single-component samples on G-75 (*7*). We attempted to extend this generalization to G-10 but strong interaction with the gel phase occurred, with most of the three-component sample eluting beyond $V_o + V_i$. Similar interaction was also present to a lesser degree on Sephadex G-25 since the monomer plateau for small samples invariably continued beyond $V_o + V_i$. Adsorption of alkyl sulfate on Sephadex has been mentioned in the literature (*5*).

CPG gave a very poor frontal separation, apparently due to repulsion of the monomer by the glass pores. Repulsion of ionic species by membranes is well-documented on Sephadex (*13*) and on certain types of porous glass filters (*14*). It is believed that there is a layer of charge along the pore walls that repels the ions (*15*). Those charges on CPG may be due in part to the presence of ionizable Brønsted acid sites (*16*). Since the monomer is not held up on the glass bed, there is no tendency for the mixed micelle to break down by dilution into its homolog components. Thus there was no separation.

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The repulsion phenomenon makes a background electrolyte essential when using small SDS samples. Fortunately, while overcoming repulsion, the electrolyte also lowers the CMC of the SDS, thereby preventing total breakdown to the monomer. However, care must be taken in choosing the background electrolyte. While NaCl and $NaNO_3$ both performed satisfactorily, KNO_3 provided almost no micelle stabilization on Sephadex Column D. Sodium is known as a structure-maker, while potassium is known as a structure-breaker in aqueous solutions. This is typified by a decrease in solution viscosity when potassium salts are added to pure water (*17*). As a result, Sephadex G-25 has been shown to be a good medium for the study of micelles using an electrolyte background and small discrete samples in the microliter range. Under the same conditions, CPG was also satisfactory, and it was less prone to compression.

Our high-pressure chromatographic results compare favorably with those done on other equilibrium systems using high-pressure spectroscopy and ultracentrifugation. It is known that there is a tendency for weak electrolytes and complex ions to ionize under pressure (*18*). For example, the $CuCl_4^{2-}$ dissociation to Cu^{2+} and $4Cl^-$ is doubled at a pressure of about 20,000 psi, as determined spectroscopically. This can be attributed to an overall decrease in the volume of the system, due to the greater ability of the compressed water structure to fit more closely to the in-

dividual ions rather than to the bulk complex. Further volume decrease due to electrostriction by the ions is also important.

Similar dissociation effects under pressure have been found using the ultracentrifuge. At atmospheric pressure many proteins undergo rapid reversible associations that have been characterized by frontal analyses using Sephadex (*19*, *20*). Those results are strikingly similar to ultracentrifuge sedimentation patterns, and they also parallel closely the micelle elution pattern shown in Fig. 1. Recently, myosin, a protein which undergoes rapid and reversible association, has been shown to shift its equilibrium toward the monomer with an increase in the spin rate of the ultracentrifuge (*21*). Such an effect may explain the discrepencies between low-pressure Sephadex frontal analysis (*19*) and earlier ultracentrifuge results with α-chymotrypsin (*22*), where a somewhat larger peak of slow-moving monomer appeared when using an ultracentrifuge. Pressures in modern ultracentrifuges can be as high as 7000 psi and can help or hinder molecular dissociation, depending on whether ΔV is negative or positive (*23*). The SDS micelle behaves similarly to myosin with pressure, though the actual transition to the monomer occurs at much higher hydraulic pressures (30,000 psi vs ~4,000 psi). It has been determined that the transfer of hydrocarbons from an aqueous solvent to a nonpolar solvent results in a positive entropy effect (*25*). This is in line with the fact that large anions are known to intrude into the water structure (*26*). Though they do not bind the water, they do have an organizing effect. When micellization occurs, this organizing effect is lost as the hydrocarbon chains turn in on themselves, and the randomness of the system increases. Under pressure the positive ΔS of micellization is overridden by the negative ΔV of dissociation which results when the water is again restructured by the exposed hydrocarbon chain of the monomer. In addition, compression of the micelles may result in greater repulsion between the anionic sites of micelles and thereby favor dissociation.

It is interesting to note that at 28,500 psi (Fig. 4C), where micelle breakup was first apparent, the chromatogram was similar to those obtained on Sephadex using 0.1 *M* NaCl background. There was strong adsorption of the trailing monomer plateau. Adsorption was very strong above 30,000 psi, with most of the sample eluting beyond $K = 1$. This may have been due to increased ability of the acidic groups on the surface to hydrogen bond with the sulfate end of the monomer. It is also possible that the hydrocarbon chains may have been adsorbing onto the surface, since the concentration of relatively insoluble monomer was then very

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high. The early literature mentions that glass is reversibly permeable to water at pressures around 140,000 psi (*27*). It is interesting to speculate on the possibility than an increase in permeability of the eluent and solute molecules may have contributed to the increased retentions and adsorption, though we were working at much lower pressures.

Finally, it is apparent that most commercial samples of SDS are mixtures of homologs. Since other workers have used SDS without mention of these impurities (*28–30*), greater care should be exercised in future studies. A simple qualitative test using frontal analysis of the sample on Sephadex G-50 is suggested. While gas chromatography will be required for better quantitative results, it requires a tedious pretreatment of the alkyl sulfates.

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