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Admicellar Chromatography: Separation and Concentration of Isomers Using Two-Dimensional Solvents

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Abstract

Immobilized surfactant aggregates at a solid/liquid interface can act as two-dimensional solvents to increase the interfacial concentration of organic compounds selectively. This phenomenon is the basis for a new separation process presented here, admicellar chromatography. The technique offers certain advantages over conventional chromatographic separations. Batch and column separations of isomers of heptanol are used to illustrate the concepts of the process. Equilibrium calculations with single component data are found to give reasonable predictions for the batch separation of the isomers.

INTRODUCTION

The advent of the biotechnology era has brought new emphasis on "low temperature" separation techniques such as chromatography. However, chromatographic methods still suffer from such drawbacks as downtime and expense to change packings, difficulty of scaleup, and the requirement to work with dilute solutions. Combined with techniques for continuous rather than batch chromatography, new methods might help

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meet the separation demands for genetic engineering ventures moving toward production (*1*, *2*). In this paper are presented the concepts of a new separation method, admicellar chromatography, as well as experimental evidence and equilibrium calculations for a model separation. Two appealing advances are available by this new technique: 1) Column "packing" may be changed simply by changing the feed stream, and 2) product streams may be concentrated and purified simultaneously.

Admicelles

Essential to understanding the basis for this approach is comprehension of certain surfactant properties. Under suitable conditions, surfactant molecules form bilayer aggregates at solid–liquid interfaces. These aggregates are similar to Langmuir-Blodgett films (*3*), but unlike Langmuir-Blodgett films these are stable, equilibrium structures. As such, they are easily formed on a wide variety of surfaces, even heterogeneous and porous materials. To emphasize their bilayer structure, the aggregates have been called admicelles (*4*). Figure 1 shows the formation of a sodium dodecyl sulfate (SDS) admicelle on the solid surface of alumina. By changing the experimental conditions, formation of these adsorbed aggregate structures may readily be reversed (*5–7*).

Separations Using Admicelles as an Adsorbed Two-Dimensional Solvent

At higher concentrations, surfactants form aggregates in solution called micelles so as to achieve segregation of hydrophobic portions

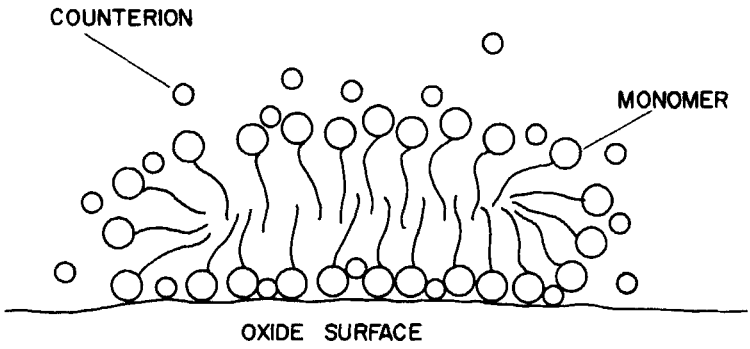

FIG. 1. An admicelle.

from the aqueous medium (*8*). Solubilization is a well-known phenomenon in which species which may be present in low concentrations in the aqueous solution dissolve within the organic interior of micelles (*9*). Because of different molecular interactions, the solubilization capacity of surfactants differs to a certain extent for different solubilizates (*10*). The possibility of a micellar separation process, based on this concept of selective solubilization, attracted several studies on mixed solubilization systems (*11–13*). Separation factors as large as seven have been reported for a binary system of solubilizates.

The analogous concentrating of an organic chemical species within an admicelle has been termed adsolubilization (*14*, *15*). In other words, the admicelle behaves as a two-dimensional solvent into which the lipophilic species preferentially partitions. This phenomenon forms the basis of the new separation technique. To demonstrate the concept, consider a binary mixture (say of molecular species A and B) which is introduced over admicelles on a solid support (Fig. 2). Surfactant and/or solvent are selected for the proposed separation so that Species A preferentially dissolves in the admicelle. The supernatant which is rich in B is then removed. Reversing formation of the bilayer yields a solution rich in Component A. Surfactant is readily removed and recycled. Figure 2 depicts the separation scheme in an equilibrium batch mode.

In practice, the process might begin by running a solution through a packed column under conditions which favor formation of admicelles (Fig. 3, Step 1). The mixture is introduced with additional surfactant to maintain the admicelle structure (Fig. 3, Step 2). When breakthrough of Species A begins to occur, the bed is backflushed under conditions which favor desorption of surfactant and release of the adsolubilizate (Fig. 3, Step 3). There are other modes possible, but when operated in this fashion the bed is ready for another cycle or even for a completely different separation after the third step.

Because of the considerable data available in our labs and in the literature concerning its behavior, sodium dodecyl sulfate (SDS) was selected as the surfactant for a preliminary separation study with aluminum oxide as the substrate. It has previously been demonstrated that admicelles may be formed reversibly on alumina (*4*, *6*). In other aspects of this work, aliphatic alcohols and their adsolubilization had been studied. Consequently, the model binary system chosen for separation was a mixture of heptanols.

EXPERIMENTAL

Two sets of experiments were performed in this study, batch adsorption isotherms and a fixed-bed column adsorption. In the batch isotherms the

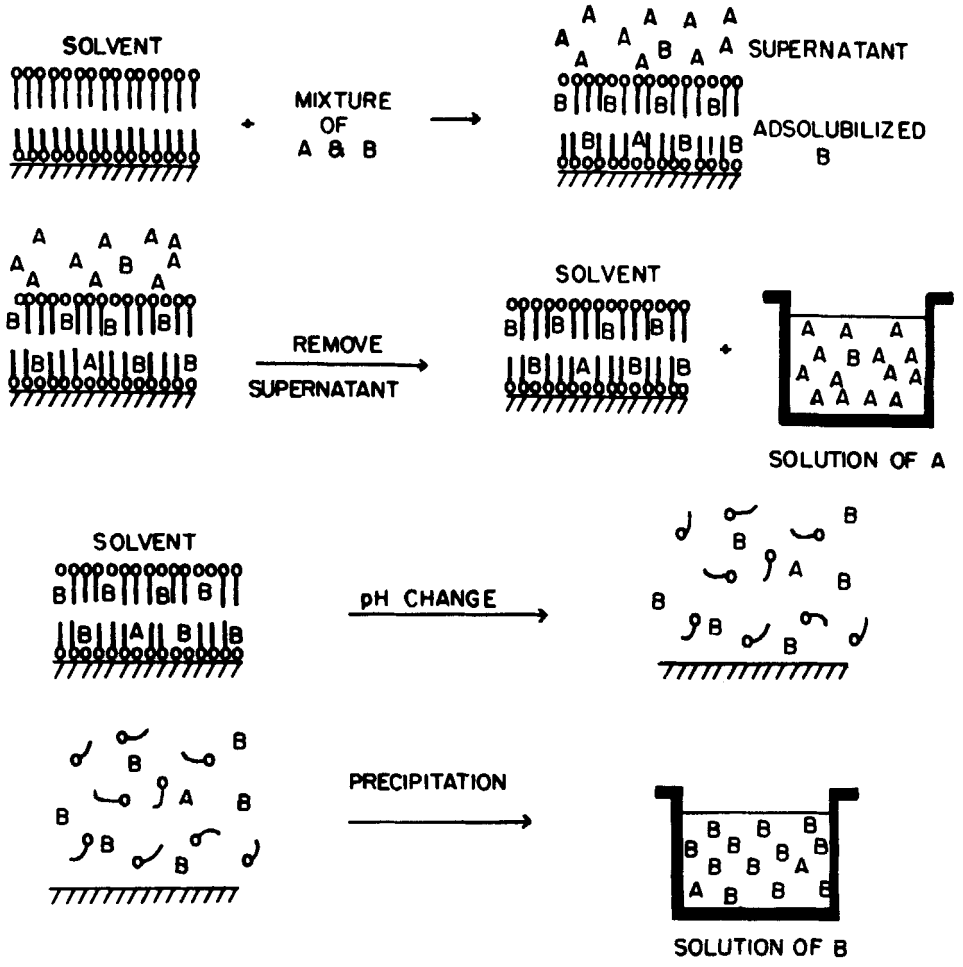

FIG. 2. A batch separation scheme using admicelles.

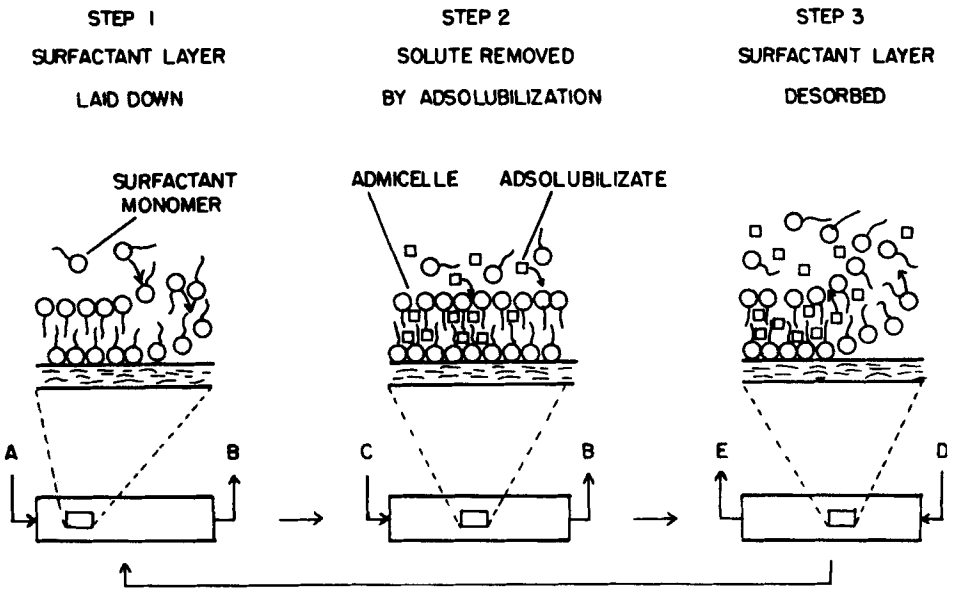

FIG. 3. Possible packed bed application of admicelles in a separation.

alumina used was Degussa Aluminum Oxide C, a primarily gamma alumina with a narrow particle size distribution and surface area of 100 ± 15 m^2/g, as determined by the manufacturer. The alumina powder was used as received. For the packed column experiment, a stainless steel High Performance Liquid Chromatography (HPLC) column (Alltech 9024D) packed with low surface area alumina (Alfa Products 87354) waas used. Sodium dodecyl sulfate (SDS) was purchased from Fisher Scientific. Reagent grade *n*-heptanol, 3-heptanol, and 2-methyl-2-hexanol were obtained from Aldrich Chemical Company and were used without further purification. Water used in the experiments was distilled and deionized.

Single-component batch isotherms were obtained for each of the three alcohols. In each case stock solutions of constant alcohol concentration but varying SDS concentration were prepared. All solutions contained 0.15 *M* NaCl. The pH values were adjusted to approximately 4.0 using 0.01 *N* HCl solution (Fisher Scientific). For the ratio of solution to solid used, the solutions equilibrated between pH 8 and pH 8.5. Aliquots (10 mL) of each of the solutions were added to separate 15 mL capped vials, each containing 0.5 g alumina. The solutions were allowed to equilibrate with the alumina for at least 3 days at ambient temperature (27°C). The

samples were then centrifuged for approximately 10 min at 1500 rpm, after which the supernatant solution was removed and stored in a separate capped vial.

Equilibrium SDS concentration in the supernatant was determined by HPLC using a Wescan conductivity detector. The column packing was a reverse phase silica with 5% loading. During the HPLC analysis a 25 vol% methanol solution was used as the mobile phase for 3 min at 4 mL/min, at which time the eluent was switched to pure HPLC grade methanol for 4 min. This procedure gave rapid quantitative determination of the SDS concentration. Samples of the supernatant were injected using an ISCO autosampler with three injections per sample. Concentrations were determined by comparing sample peak heights to peak heights of standard solutions with linear interpolation between the two nearest standard solutions. Standard solutions were reinjected for each sample run. Standard solutions all were at pH 4 and 0.15 *M* NaCl. SDS adsorptions were calculated from the known feed concentration and the final equilibrium concentration.

Alcohol concentration in the supernatant was determined by gas chromatography using a Varian 7000 GC with flame ionization detector. Standard solutions of the alcohols again were adjusted to pH 4 and 0.15 *M* NaCl and contained varying concentrations of SDS. GC peak heights were recalibrated using the stock solutions for each sample run. Four or five injections per standard and four or five injections per sample were required to obtain consistent peak areas.

No modifications of the above procedure were necessary to determine the SDS concentrations in the binary alcohol batch samples. The GC analysis had to be modified, however, by the introduction of a temperature gradient. The column was held at 140°C for 0.5 min after injection of the sample, then ramped to 200°C at 250°C/min and held at 200°C for 4 min. This procedure provided good peak separations for the alcohols. It was often necessary to inject numerous different standard alcohol solutions to determine the equilibrium concentrations of both components. A range of standards was injected to determine the equilibrium concentration of one alcohol component and then another set of standards had to be used to interpolate the other alcohol's equilibrium concentration.

For the column runs, each run consisted of four steps. First, the packed bed was equilibrated with water at pH 8.2. Then, a 50,000 μmol/L SDS solution at pH 8.45 was injected into the column, and the effluent SDS concentration monitored. The column runs were made at a pH of approximately 8.4 since this was the approximate pH at which the batch adsolubilization solutions equilibrated and because the admicelle layer

can be removed in such a way as to produce a sharp chromatographic front at this pH. When the effluent concentration approached that of the inlet, this stream was switched off, and a new stream, at pH 8.35, containing SDS and the alcohols to be separated was injected. The outlet concentrations of the SDS and alcohols were monitored, and when breakthrough occurred for both of the alcohols, this stream was switched off. The last stream containing water at pH 12.4 was then injected. The alcohol and SDS concentrations at the column outlet were monitored again, and when they all reached zero, this stream was stopped. Burettes were used to record the amount of each stream injected, and in-line sampling valves allowed the effluent SDS and alcohol concentrations to be measured at regular intervals using the HPLC and GC procedures described above.

RESULTS AND DISCUSSION

The adsorption of sodium dodecyl sulfate and the formation of admicelles is shown in Fig. 4. Results are from experiments at room temperature and pH 4 with 0.15 *M* NaCl added. Increasing the concentration of SDS in the supernatant causes additional adsorption of surfactant until the plateau adsorption occurs. The plateau adsorption can occur not only from complete coverage of the surface but also from reaching the critical micelle concentration in the supernatant. Well above the CMC, competition between solubilization and adsolubilization would need to be considered.

In general, the adsorption of surfactant is seen to be enhanced in the presence of alcohols (0.01 *M* initial alcohol concentration); this is particularly significant in the case of normal heptanol. The additional adsorption with the alcohol provides indirect evidence for the interaction of the alcohols with the surfactant at the interface. Figure 5 shows the results for the adsolubilization for each of three heptanol isomers in admicelles. Detailed single-component findings for each of three sets of surfactant adsorption/alcohol adsolubilization experiments are given in Tables 1, 2, and 3. These results indicate that adsolubilization increases from 2-methyl-2-hexanol to 3-heptanol to *n*-heptanol. While this order reflects the well-known trends of branching on alcohol solubility in water, the differences might also arise from the steric constraints for the molecules to fit into an ordered admicelle (*10*).

From the single alcohol component data in Fig. 5, it would appear that *n*-heptanol could be separated from 3-heptanol or 2-methyl-2-hexanol at the lower end of the isotherm. At the upper end of the isotherm, 2-methyl-

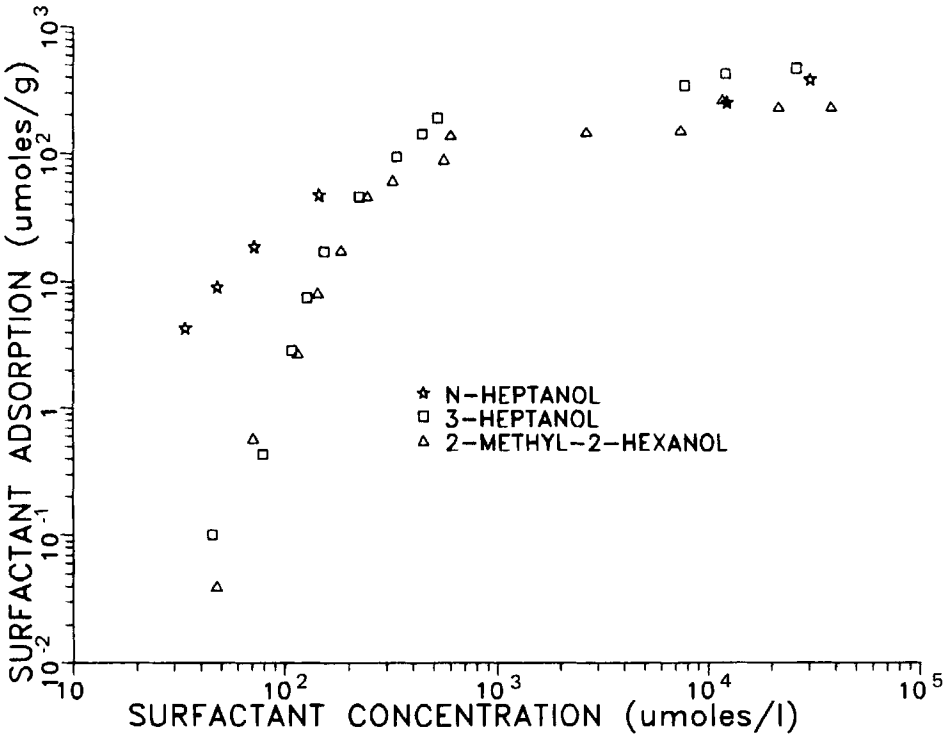

FIG. 4. Dodecyl sulfate adsorption isotherms in the presence of individual heptanol isomers.

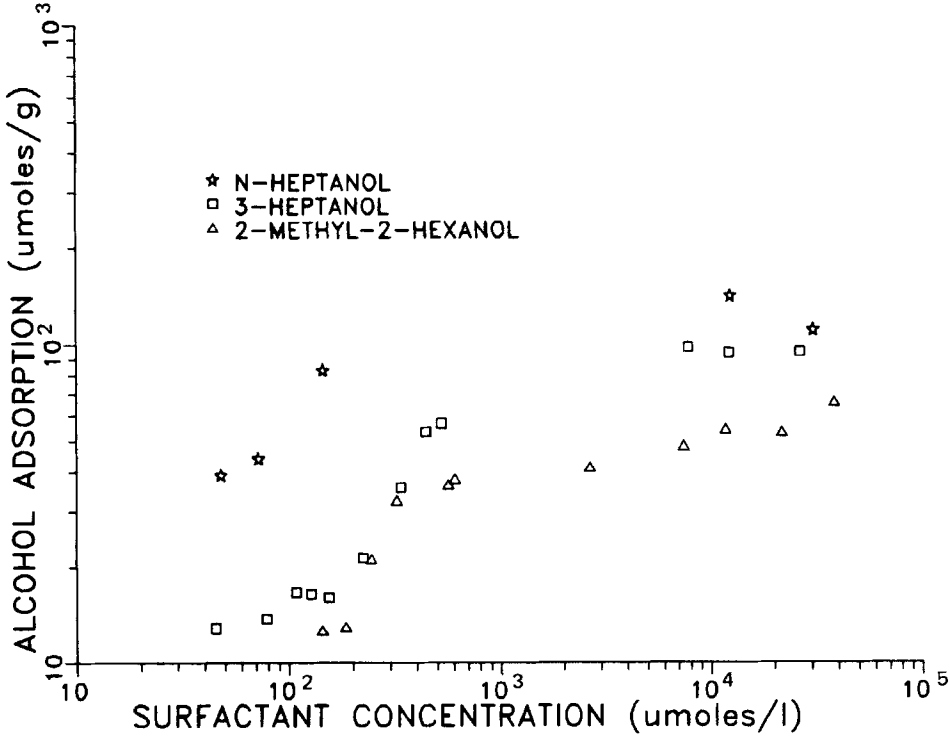

FIG. 5. Single component adsolubilizations of heptanol isomers in dodecyl sulfate admicelles.

TABLE 1
Adsolubilization of *n*-Heptanol[a]

Initial SDS concentration (μM)	Initial C_7OH concentration (μM)	Equilibrium SDS concentration (μM)	Equilibrium C_7OH concentration (μM)	SDS adsorption (μmol/g Al_2O_3)	C_7OH adsorption (μmol/g Al_2O_3)
250	10,000	34	10,000	4.32	0
500	10,000	48	8,055	9.04	38.9
1,000	10,000	72	7,803	18.6	43.9
2,500	10,000	145	5,840	47.1	83.2
25,000	10,000	12,246	2,883	255	142
50,000	10,000	30,482	4,432	390	111

[a]All solutions: pH = 4.0; concentation of NaCl = 0.15 *M*.

TABLE 2
Adsolubilization of 3-Heptanol[a]

Initial SDS concentration (μM)	Initial 3-C_7OH concentration (μM)	Equilibrium SDS concentration (μM)	Equilibrium 3-C_7OH concentration (μM)	SDS adsorption (μmol/g Al_2O_3)	3-C_7OH adsorption (μmol/g Al_2O_3)
50	10,000	45.3	9,359	0.1	12.8
100	10,000	78.5	9,313	0.43	13.7
250	10,000	108	9,168	2.84	16.6
500	10,000	127	9,178	7.46	16.4
1,000	10,000	154	9,200	16.9	16.0
2,500	10,000	224	8,937	45.5	21.3
5,000	10,000	336	8,221	93.3	35.6
7,500	10,000	443	7,340	141	53.2
10,000	10,000	525	7,169	190	56.6
25,000	10,000	7,734	5,115	345	97.7
33,333	10,000	12,060	5,295	425	94.1
50,000	10,000	26,282	5,257	474	94.9

[a]All solutions: pH = 4.0; concentration of NaCl = 0.15 M.

TABLE 3
Adsolubilization of 2-Methyl-2-Hexanol[a]

Initial SDS concentration (μM)	Initial $2C2C_6OH$ concentration (μM)	Equilibrium SDS concentration (μM)	Equilibrium $2C2C_6OH$ concentration (μM)	SDS adsorption ($\mu mol/g\ Al_2O_3$)	$2C2C_6OH$ adsorption ($\mu mol/g\ Al_2O_3$)
50	10,000	47.8	10,000	0.04	0
99.95	10,000	70.9	10,000	0.58	0
249.8	10,000	115	10,000	2.70	0
549.5	10,000	143	9,369	8.13	12.6
1,049	10,000	185	9,355	17.3	12.9
2,547.5	10,000	245	8,942	46.1	21.2
3,380	10,000	322	8,380	61.2	32.4
5,045	10,000	563	8,192	89.6	36.2
7,542.5	10,000	603	8,110	138.8	37.8
10,040	10,000	2,629	7,940	148.2	41.2
15,035	10,000	7,370	7,589	153.3	48.2
25,025	10,000	11,674	7,279	267	54.4
33,350	10,000	21,564	7,335	235.7	53.3
50,000	10,000	38,167	6,693	236.7	66.1

[a]All solutions: pH = 4.0; concentration of NaCl = 0.15 M.

2-hexanol can be separated from either of the other two isomers. Each of the alcohols in a ternary mixture might be isolated in a two-stage process operated at opposing ends of the isotherm. For a test system, it was decided to try to split a 50:50 mixture of the *n*-heptanol and 2-methyl-2-hexanol at various points along the adsorption isotherm.

The initial batch experimental results are presented in Table 4. Separation factors (*16*) are seen to be as high as 4 ($\alpha = Y_1X_2/Y_2X_1$). If Y is defined as the mole fraction of alcohol in the admicelle (alcohol and surfactant only, neglecting any water present) and X as the mole fraction in the aqueous phase, then it is observed that the partition coefficient $K = Y/X$ keeps approximately constant for each alcohol except at the lower end of the isotherm where K varies with the equilibrium surfactant concentration. We believe this results from different structures of the admicelle when surfactant adsorption is sparse (patchwise adsorption) compared to higher, more tightly packed adsorptions. At the lower end of the isotherm, the surfactant concentration is so low that the cooperation of alcohols becomes crucial in the formation of admicelles. The constant K values are for *n*-heptanol, about 5.9×10^3; for 3-heptanol, about 1.9×10^3; and for 2-methyl-2-hexanol, about 1.6×10^3. From the single component data for *n*-heptanol and 2-methyl-2-hexanol, we would like to be able to predict the separation that can be attained using admicelles. By specifying the amount of surfactant adsorbed (i.e., the amount of the second phase), the relative adsolubilization of the alcohols can be calculated using the K values. For the following discussion, *n*-heptanol will be specified as Component 1 and 2-methyl-2-hexanol as Component 2. Hence, the equilibrium constant for heptanol will be K_1 and that for 2-methyl-2-hexanol will be K_2. The total number of moles in the admicelles N_{tot} is

$$N_{tot} = N_1 + N_2 + N_3 \tag{1}$$

where N_1 and N_2 are the moles of adsolubilized alcohols and N_3 is the number of moles of adsorbed surfactant (i.e., $\{[SDS]_0 - [SDS]_{eq}\} \times V$, where V is the volume of the aqueous phase). If $C_{1,0}$ is the initial concentration of Alcohol 1 and M_{sol} is the molarity of water, then at equilibrium

$$X_1 = \frac{VC_{1,0} - N_1}{VM_{sol}} \tag{2}$$

and

TABLE 4
Binary Separation: Adsolubilization of 2-Methyl-2-Hexanol and *n*-Heptanol[a]

Initial SDS concentration (μM)	Initial $2C2C_6OH$ and C_7OH concentration (μM)	Equilibrium SDS concentration (μM)	Equilibrium $2C2C_6OH$ concentration (μM)	Equilibrium C_7OH concentration (μM)	SDS adsorption (μmol/g Al_2O_3)	$2C2C_6OH$ adsorption (μmol/g Al_2O_3)	C_7OH adsorption (μmols/g Al_2O_3)
500	10,000	33.6	8,901	9,058	9.3	22.0	18.8
1,050	10,000	52.6	8,390	7,809	20	32.2	43.8
3,000	10,000	146	8,064	5,119	57	38.7	97.6
4,000	10,000	181	7,401	4,641	76	52.0	107
5,000	10,000	886	7,557	4,472	82.3	48.9	111

[a]All solutions: pH = 4.0; concentration of NaCl = 0.15 *M*.

$$Y_1 = \frac{VC_{1,0} - N_1}{VM_{sol}/K_1} \tag{3}$$

Noting that $Y_1 = N_1/N_{tot}$, one can show that

$$N_1 = \frac{VC_{1,0}N_{tot}K_1}{K_1N_{tot} + VM_{sol}} \tag{4}$$

with a similar expression for N_2. Substitution into Eq. (1) yields

$$N_{tot} = \frac{VC_{1,0}N_{tot}K_1}{K_1N_{tot} + VM_{sol}} + \frac{VC_{2,0}N_{tot}K_2}{K_2N_{tot} + VM_{sol}} + ([SDS]_0 - [SDS]_{eq})V \tag{5}$$

By specifying the amount of adsorbed surfactant, one may predict the amounts of adsolubilized alcohol from the single component experimental results and Eq. (5). Specifying the adsorbed surfactant indicates the extent of the second phase or the number of admicelles; this is similar to predetermining the amount of the vapor phase in a distillation or flash calculation. Though Eq. (5) may be rearranged to a cubic equation and solved explicitly for N_{tot}, it is easier to solve numerically. This equation was solved for the separation data of Table 4; results are compared with the observed values in Table 5. It is seen that the agreement is quite reasonable at the upper end of the isotherm (high surfactant adsorption), but not satisfactory at the other end. The poor prediction at the lower end is due to the variability of K at low surfactant concentrations and the different admicellar structure. It should be noted that the observed values for *n*-heptanol and 2-methyl-2-hexanol are comparable at the lower end of the isotherm and thus counter to expectations in the selectivity based on the single-component data. Thus separations at the lower end of the isotherm may not be feasible.

TABLE 5

N_{tot}		N_1		N_2	
Predicted	Observed	Predicted	Observed	Predicted	Observed
114	50	76	19	29	22
140	96	86	44	35	32
211	193	106	98	48	39
242	235	113	107	53	52
252	242	115	111	55	49

Figures 6 to 10 present the results of a packed column experiment in which an equimolar mixture of 2-methyl-2-hexanol and 1-heptanol are separated by the new separation process presented in this paper.

Figure 6 corresponds to Step 1 in Fig. 3, where the surfactant layer is formed by adsorption from the influent solution onto the bed material, in this case alumina. As shown in Fig. 6, breakthrough of the SDS during the admicelle layer formation step occurred between 1.2 and 1.5 pore volumes of injected surfactant solution, and reached half the plateau height at approximately 1.8 pore volumes. The effluent SDS concentration rose to near the inlet concentration, but did not fully reach it before the next step of the process was initiated. There was probably a second, slower surfactant wave which still had not reached the effluent. The adsorption of surfactant on the alumina is greatly affected by changes in pH. Although the pH of the various streams was kept approximately constant, the slight variations in pH between the streams may have been the source of this second surfactant wave.

Figures 7 and 8 show effluent histories for the SDS and the two alcohols during the adsolubilization step. This stage of the process corresponds to Step 2 of the schematic in Fig. 3. The SDS fell to its new inlet concentration after 1.5 pore volumes was injected, and remained there for the duration of this step. The inlet concentration of SDS during this step of the process was maintained at the critical micelle concentration of the surfactant. Previous work has shown that the adsorption of this anionic surfactant on alumina reaches a plateau value at the CMC of the surfactant (*6*). Thus, maintaining the surfactant concentration at the critical micelle concentration prevents premature desorption from the surfactant layer.

The effluent alcohol histories during the adsolubilization step are shown in Fig. 8. Though the alcohols show negligible adsorption on the bare alumina surface, complete removal of both alcohols occurs for two pore volumes in the presence of the adsorbed surfactant layer. The breakthrough of the 2-methyl-2-hexanol occurred at this point, with the *n*-heptanol breakthrough occurring a full pore volume later, as predicted by the adsorption isotherms. The isotherms indicate *n*-heptanol adsolubilizing preferentially over 2-methyl-2-hexanol in SDS admicelles (see Fig. 5). In a chromatographic system, preferential adsorption translates into a longer retention time for that particular solute. Effluent concentrations of both alcohols overshoot their inlet concentrations. This is a common occurrence in multicomponent adsorption systems where one component adsorbs more strongly than the other (*5*, *17*). It should be noticed that in an industrial application of this process, one would not necessarily wait for complete breakthrough of all components before

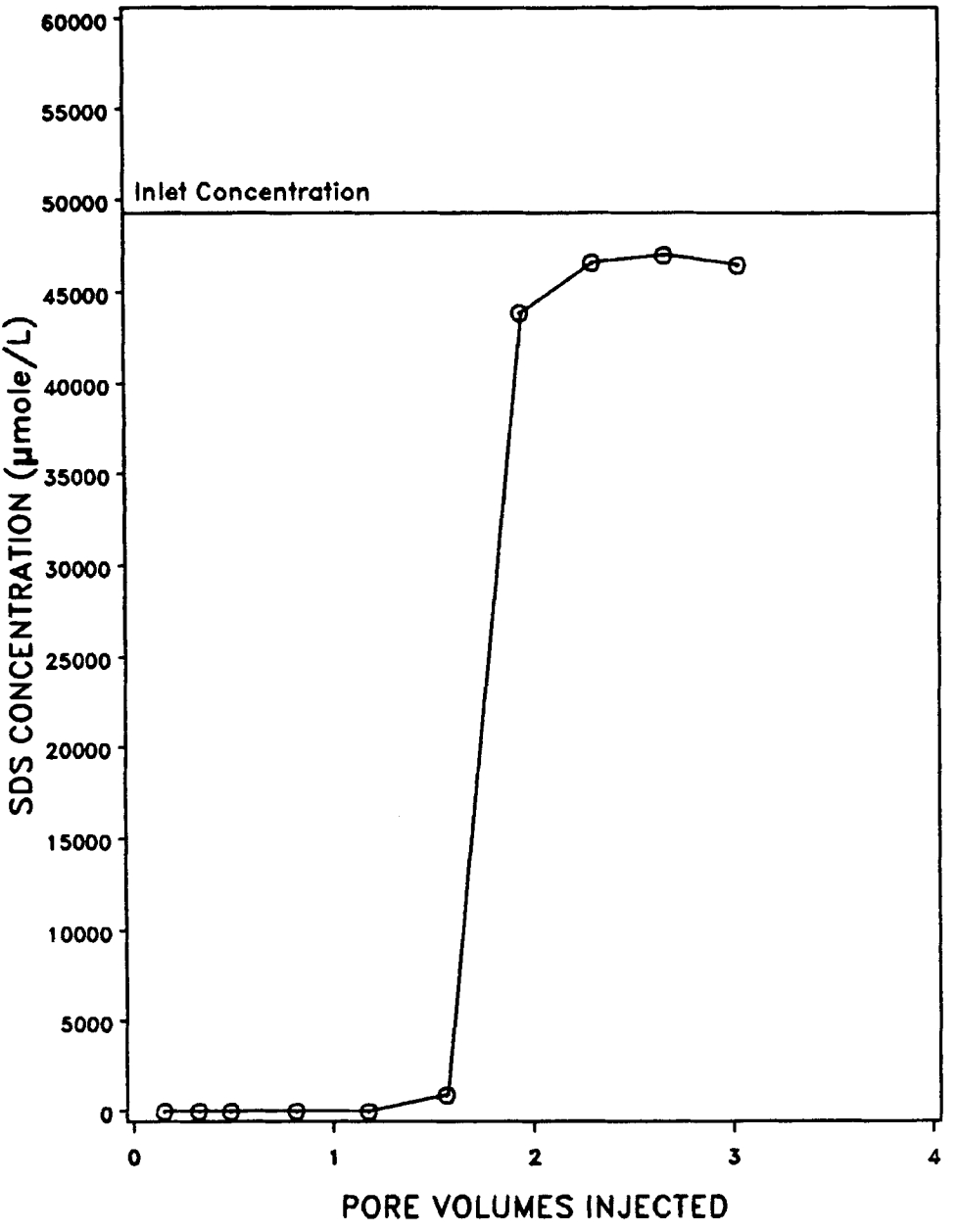

FIG. 6. Sodium dodecyl sulfate effluent history during formation of the surfactant bilayer in the packed column.

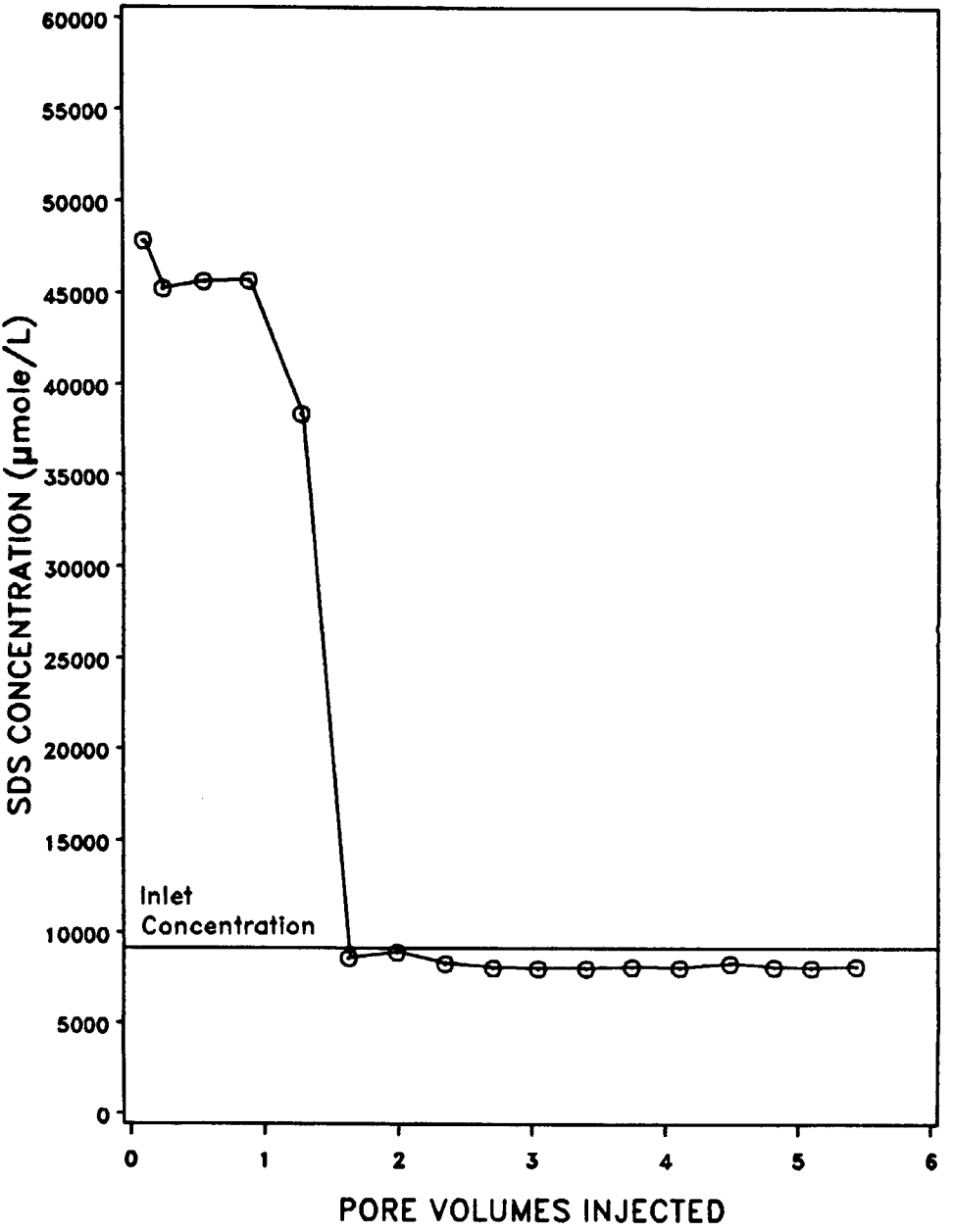

FIG. 7. Sodium dodecyl sulfate effluent history during adsolubilization step.

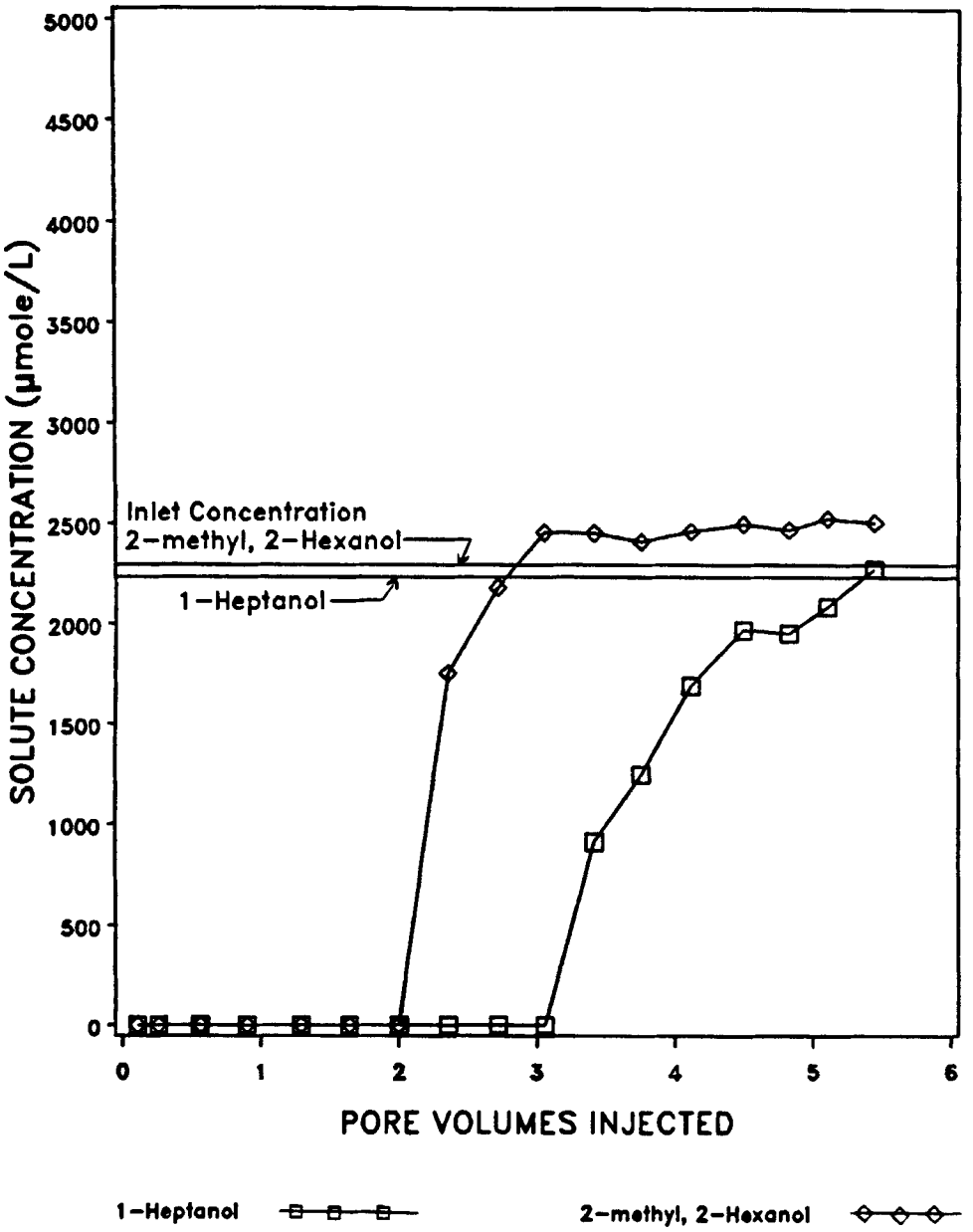

FIG. 8. Alcohol effluent history during adsolubilization step.

backflushing the bed (Step 3 of Fig. 3). The operating scheme chosen here does, however, make the chromatographic behavior more apparent.

The effluent SDS profile during the bed stripping step (Fig. 3, Step 3) is shown in Fig. 9. As was anticipated, the SDS began eluting after one pore volume of the stripping solution (H_2O at pH 12.4) was introduced into the column. After one more pore volume, the concentrated wave of SDS had almost completely eluted, with the outlet SDS concentration falling to zero after four pore volumes.

Figure 10 shows the effluent alcohol profiles during the bed stripping step. As seen from the graph, the 2-methyl-2-hexanol concentration has fallen to zero at 2.5 pore volumes, while the 1-heptanol is at its highest concentration. The 1-heptanol has been effectively separated from the 2-methyl-2-hexanol in a single pass. The 2-methyl-2-hexanol began eluting after less than one pore volume of pH 12.4 water was injected. With the outlet concentration peaking at 2644 μmol/L, the 2-methyl-2-hexanol eluted out slightly more concentrated than its initial 2294 μmol/L, for a 15% increase in concentration. The 1-heptanol began eluting after one pore volume. Its concentration rose from 2234 to 3169 μmol/L, an increase of 42%.

The concentration increases observed here were smaller than those observed in experiments reported elsewhere (*14*) which involved a single adsolubilizate; over 225% increases in concentration have been achieved in a single pass for single adsolubilizate experiments. Nevertheless, the alcohols were effectively separated from each other in a low energy process using common commercial materials. Even better results could be obtained by making this a multipass operation instead of a single pass process. During the bed stripping step, for example, the effluent coming out of the column at 2.0 to 2.5 pore volumes contains little 2-methyl-2-hexanol, and significant amounts of 1-heptanol (see Fig. 10). If this stream was introduced into a fresh bed, the alcohols could conceivably be separated further and concentrated even more.

Mass balances were performed on each component in the process. The SDS balance closed within 7.65%, the 2-methyl-2-hexanol balance within 0.90%, and the *n*-heptanol balance within 16.27%. This gives support to the claim made earlier that the adsorption process is reversible, with little loss of either surfactant or solute.

CONCLUSIONS

Surfactant admicelles physically adsorbed on the surface of inorganic packing can be used to separate isomers of organic compounds. A new

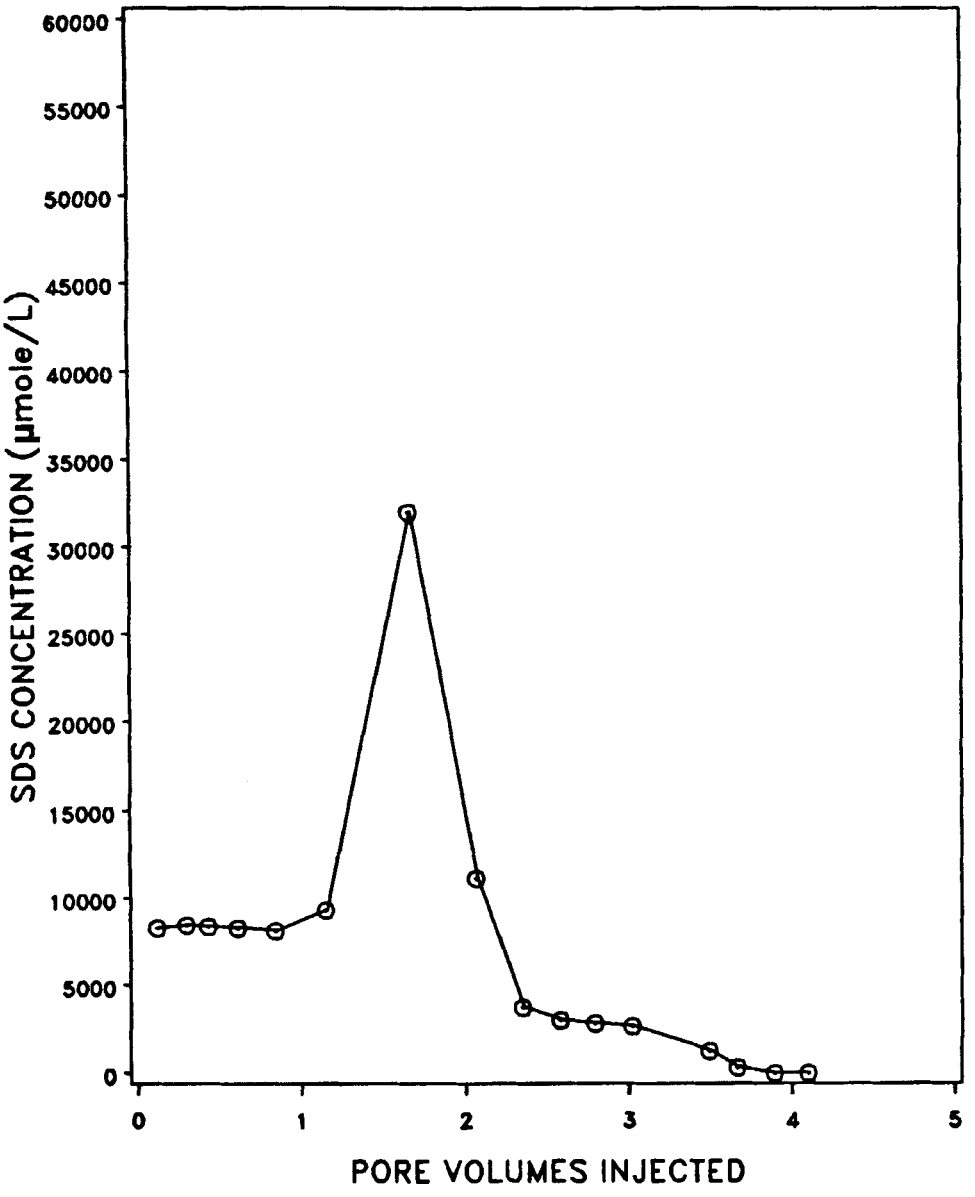

FIG. 9. Sodium dodecyl sulfate effluent history during bed stripping step.

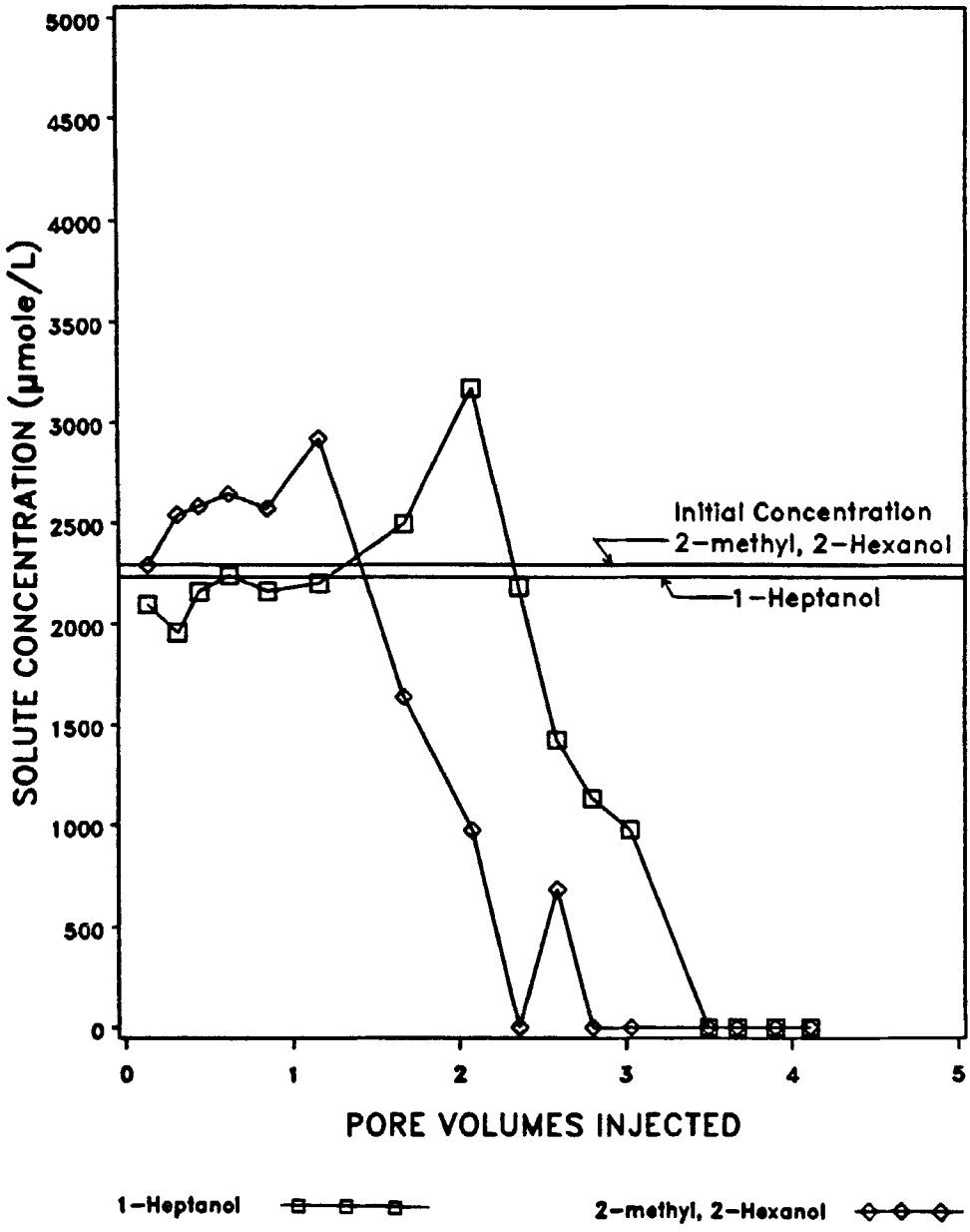

FIG. 10. Alcohol effluent history during bed stripping step.

process, admicellar chromatography, based on the phenomenon of adsolubilization, has been proposed and demonstrated to be feasible for the separation of isomers of heptanol. One important area of potential application of admicellar chromatography is in bioseparations (*18*), where it may have important advantages over conventional chromatographic separations.

SYMBOLS

$C_{1,0}$	initial concentration of Alcohol 1 (*n*-heptanol)
$C_{2,0}$	initial concentation of Alcohol 2 (2-methyl-2-hexanol)
K_1	adsolubilization constant for Component 1
K_2	adsolubilization constant for Component 2
M_{sol}	molarity of water
N_1	number of moles of adsolubilized Component 1
N_2	number of moles of adsolubilized Component 2
N_{tot}	total number of moles of adsolubilized alcohols and adsorbed surfactant
$[SDS]_0$	initial concentration of SDS
$[SDS]_{eq}$	equilibrium concentration of SDS
V	volume of aqueous phase
X_1	mole fraction of Component 1 in the admicelle (N_1/N_{tot})
Y_1	mole fraction of Component 1 in the aqueous phase
α	separation factor

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