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## POROUS SILICA MICROSPHERE COLUMN PACKINGS FOR HIGH-SPEED LIQUID-SOLID CHROMATOGRAPHY

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### SUMMARY

A new column packing for high-performance liquid chromatography, porous microspheres of silica produced by the agglutination of colloidal silica particles, has recently been introduced for use in adsorption chromatography. The narrow-size range, relatively homogeneous pore structure and short diffusion path lengths of these  $<10\text{-}\mu$  particles result in very high column efficiencies, and the relatively large, highly available surface area provides for high sample capacity. The microsphere packing displays retention and efficiency characteristics which are less dependent on water content than wide-pore silica gel. Columns of the microspheres may be prepared which are reproducible in chromatographic performance, using a simple high-pressure slurry-packing procedure. More than 10,000 theoretical plates have been obtained on a single 25-cm-long column of  $5\text{-}\mu$  microspheres at carrier velocities of about 0.7 cm/sec. Plate heights of about five particle diameters and more than thirty-six effective plates/sec have been demonstrated for solutes with capacity factors ( $k'$ ) in the 2-5 range. These columns may be connected in series using low-volume fittings with little loss in efficiency. Columns of the  $5\text{-}\mu$  particles appear to be limited by mobile phase mass transfer effects, contrasted to the stagnant mobile phase mass transfer limitations exhibited by similar 8- to  $9\text{-}\mu$  particles.

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### INTRODUCTION

Recent studies have confirmed that a substantial improvement in the efficiency of columns for liquid chromatography (LC) can be obtained by working with particles of  $<10\text{ }\mu$ , providing a regular packing structure is produced with these very fine materials<sup>1-5</sup>. The very high column efficiency resulting from the use of these very small particles is due to the more rapid equilibrium which can take place within the packed bed. Rapid mass transfer of solute molecules between the particles or in the stationary phase and/or stagnant mobile phase is excellent because of the short distances involved in such a packed bed. Studies to date have suggested that particles in the 5- to  $10\text{-}\mu$  range represent an excellent compromise between column efficiency and the pressure required to carry out separations<sup>2,5</sup>.

Previous papers have described the performance and some general properties of new totally porous silica microspheres of  $<10\ \mu$  which were developed especially for high-performance LC<sup>2,4</sup>. Since both the overall size and internal porosity can be selected and closely controlled, various forms of LC (liquid-liquid, liquid-solid, and exclusion) can be carried out with these new silica microspheres. The narrow-size range, relatively homogeneous pore structure and short diffusion path lengths involved with these  $<10\ \mu$  particles result in very high column efficiencies. In addition, the relatively large, highly available surface area of these microspheres provides for relatively high sample capacity.

This paper provides more details on the use of the porous silica microspheres for high-efficiency liquid-solid (adsorption) chromatography. Of particular interest in this study is the linear capacity of these particles, and the effects of water and other highly polar organic molecules used to control the activity of this adsorbent. Techniques for preparing columns with more than  $10^4$  theoretical plates have also been investigated, including the series-connection of these high-efficiency units. These studies also provide some additional insight into the limitations of mass transfer by mobile and stagnant mobile phase effects.

## EXPERIMENTAL

### *Apparatus*

The chromatographic apparatus and general technique used in this study have been previously described<sup>2,4,6</sup>. The pump was a special version of the Haskel pneumatic amplifier sample pump, Model No. 17082 (Haskel Engineering, Burbank, Calif., U.S.A.), modified with a third airhead. A Model 410 precision photometer (DuPont, Wilmington, Del., U.S.A.) was used to monitor the column effluent at 254 nm. An ultramicro ultraviolet (UV) absorption cell having an optical path of  $0.5\ \text{mm} \times 5\ \text{mm}$  was used. The optical path of this cell has a volume of about  $1\ \mu\text{l}$  and a total volume of  $3.2\ \mu\text{l}$  from the column outlet, including the connector<sup>4</sup>. Samples were introduced into columns by injection through a low-volume septum inlet, using a  $10\text{-}\mu\text{l}$  or  $25\text{-}\mu\text{l}$  series C-160 Pressure-Lok<sup>®</sup> microsyringe (Precision Sampling Corp., Baton Rouge, La., U.S.A.). At high inlet pressures, the "stop-flow" sample injection method was used.

The column tubes used in this study were 25-cm lengths of 0.25 in. O.D. and 0.125 in. I.D. precision-bore stainless-steel tubing manufactured by the Superior Tubing Co., Norristown, Pa., U.S.A., and obtained from Peter A. Frasse & Co., Philadelphia, Pa., U.S.A. This tubing is described as Type 316L Super Pressure tubing with a mirror inside finish and no radial cracks, carbide pits or scores. All fittings were of stainless steel (Swagelok<sup>®</sup>, Crawford Fitting Co., Salon, Ohio, U.S.A.). Column blanks were carefully cleaned before use as previously described.<sup>6</sup>

### *Chemicals*

Solvents were distilled-in-glass from Burdick & Jackson Lab., Muskegon, Mich., U.S.A. Dry solvents for use as mobile phases were prepared by passing the solvent through Davison Code 62 silica gel (W. C. Grace & Co., Baltimore, Md., U.S.A.). Water-saturated solvent was obtained by percolation through a column of 30% (by weight) water on silica gel, and then blending the water-saturated solvent

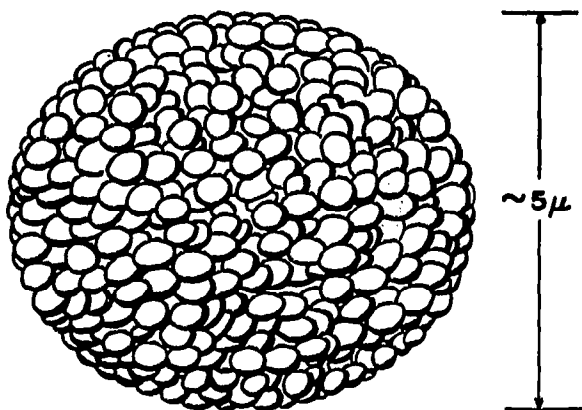


Fig. 1. Schematic of a porous silica microsphere adsorbent particle.

with dry solvent to give the desired percent water saturation<sup>7</sup>. Water levels were determined by conventional Karl-Fisher titrations.

The porous silica microspheres are produced by the agglutination of 50-Å silica microspheres to form spherical particles of uniform diameter<sup>8</sup>. Fig. 1 schematically shows the structure of such a particle, and Table I summarizes various physical characteristics of the 8- to 9- $\mu$  microspheres used in this study. The 4.6- to 5.6- $\mu$  particles used in certain experiments were similar in characteristics, but with a nitrogen surface area of 246 m<sup>2</sup>/g (dynamic method). Packed columns of similar microspheroidal adsorbent particles are available from the DuPont Instrument Products Division.

TABLE I

CHARACTERIZATION OF POROUS SILICA MICROSPHERES FOR LIQUID-SOLID CHROMATOGRAPHY

Parameter	Value
Size (optical microscopy), $\mu$	8-9
Nitrogen surface area (B.E.T. method), m <sup>2</sup> /g	309
Volume of nitrogen for one monolayer, ml/g	70.6
Volume of nitrogen for saturation pressure, ml/g	400
Average pore diameter, Å	87.4
Pore diameter of highest population, Å	75
Pore volume (as nitrogen liquid), ml/g	0.620

*Data handling and calculations*

Column performance data (*i.e.*,  $H$ ,  $k'$ , etc.) were obtained with a supplementary program in the DuPont Experimental Station PDP-10 Chromatography Real-Time Computer System. Plate heights were derived from peak areas using the method suggested by James and Martin<sup>9</sup>.

RESULTS AND DISCUSSION

In liquid-solid chromatography (LSC), the water content of the adsorbent plays a critical role. Water is usually added to the adsorbent to optimize linear ca-

capacity and column efficiency<sup>7,10</sup>. The possibility of adsorbent-catalyzed reactions and irreversible sample adsorption also are reduced by the addition of water to the adsorbent. Most important, water composition must be held constant for reproducible separations, since the amount of water on the adsorbent significantly affects both absolute and relative capacity factor ( $k'$ ) values. The optimum amount of water in LSC has been stated to be 50–100% of a water monolayer, which corresponds to 0.2–0.4 g of water/100 m<sup>2</sup> of adsorbent surface<sup>7</sup>. This water may be added to the adsorbent before packing the column or may be adjusted by an *in situ* process after the column bed is formed.

Strongly polar organic modifiers, such as alcohols, have also been used for control of the activity of adsorbents in LSC<sup>11</sup>. In some studies, the use of methanol rather than water for adsorbent deactivation did not result in equivalent separations<sup>12</sup>.

Since the porous silica microsphere adsorbent has a different pore structure than the silica gels used for LSC, a series of experiments was carried out to determine the optimum conditions for modifying the activity of this adsorbent for high-performance LSC. As shown in Table II, five different systems were investigated. The first three systems involved the use of a water modifier in conjunction with mobile phases of low, intermediate and moderately high strength. The use of strongly polar alcohol modifiers was studied in two systems.

TABLE II

## EXPERIMENTAL CONDITIONS FOR MODIFYING THE ACTIVITY OF THE POROUS SILICA MICROSPHERES

<i>System</i>	<i>Modifier</i>	<i>Mobile phase</i>	<i>Solute</i>
A	Water	Hexane	Diphenyl ether
B	Water	Dichloromethane	3-Phenyl-1-propanol
C	Water	Diethyl ether	3-Methyl-3-(3,4-dichlorophenyl)-1,1-dimethylurea
D	2-Propanol	Dichloromethane	3-Phenyl-1-propanol
E	Methanol	Dichloromethane	3-Phenyl-1-propanol

*System A —Hexane modified with water*

In these studies, the *in situ* equilibration technique was used to establish an equilibrium amount of water modifier on the adsorbent<sup>7</sup>. Since hexane is capable of dissolving only a very small amount of water, the rate of equilibration of the adsorbent column packing with this solvent is relatively slow. To insure that the column was at true equilibrium, it was first necessary to determine how much of the water-containing hexane solvent had to be passed through the column before true equilibrium was obtained. Fig. 2 shows the relatively slow equilibration of water between dry hexane and a column initially equilibrated with 50% water-saturated hexane. About 400 ml of dry hexane (in this case also ~400 column volumes) were required to remove all of the water from the adsorbent, which had previously been equilibrated with 50% water-saturated hexane. Therefore, passing about 400 volumes of hexane equilibrated with another water content should be adequate to achieve equilibrium between the new solvent and the adsorbent. The volume of solvent required to equilibrate the porous silica microspheres is similar to that needed to equilibrate wide-pore silica gel<sup>7</sup>. As discussed later, there are other ways in which the slow equili-

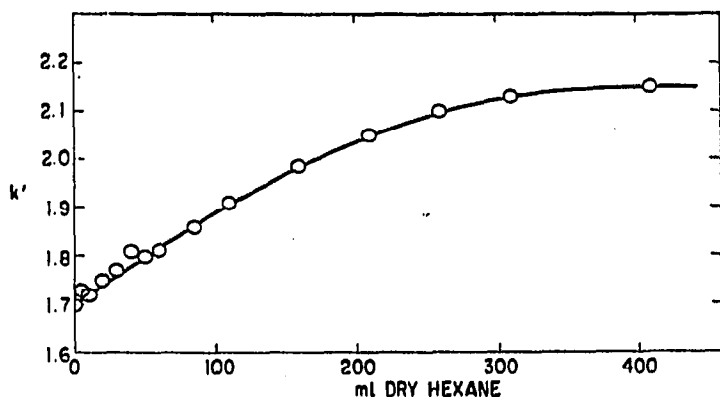


Fig. 2. Equilibration of water between dry hexane and a column of microsphere adsorbent initially equilibrated with 50% water-saturated solvent. Column, 25 cm  $\times$  3.2 mm, 8- to 9- $\mu$  porous silica microspheres, 75- $\text{\AA}$  pores; flow-rate, 2.0 ml/min; solute, 25  $\mu$ l of 0.025 mg/ml hexane solution of naphthalene.

bration of the water content between the solvent and the adsorbent can be accelerated, such as the use of a solvent with higher water solubility for initial column equilibration.

Fig. 3 shows the effect of the amount of water in the hexane mobile phase on  $k'$  and  $H$ , the plate height of the column for a test solute, diphenyl ether. A small amount of water in the hexane results in a rapid decrease in  $k'$ , compared to the starting dry hexane mobile phase. However, the curve is much flatter above about 10% water saturation, with much smaller changes observed in  $k'$  with increasing amounts of water. Contrary to results obtained with wide-pore silica gel<sup>12</sup>, very little change in plate height was seen with the porous silica microspheres as the amount of water was varied. Only about a 6% decrease in plate height was observed when the mobile phase was varied between 0% and 100% water-saturated hexane. This reduced effect of water content on plate height may be associated with the higher homogeneity of the porous silica microsphere adsorbent surface.

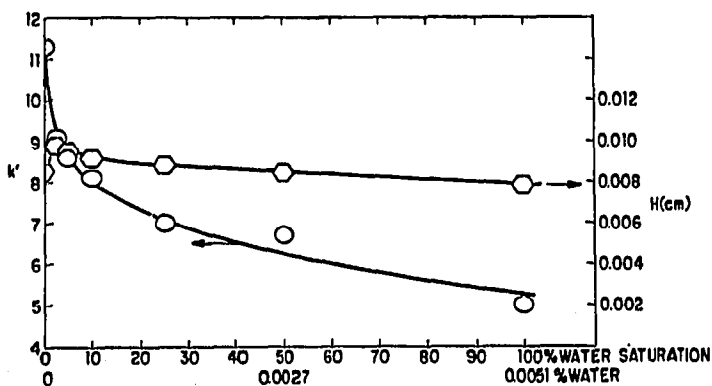


Fig. 3. Effect of water in hexane mobile phase on retention and efficiency of the microsphere adsorbent. Conditions same as in Fig. 2, except for the solute, which was 25  $\mu$ l of 0.5 mg/ml of diphenyl ether in hexane.

To ensure linear isotherm separation in LSC, sample size must be kept below some maximum value, which has been called the linear capacity of the adsorbent bed. Linear capacity,  $\theta_{0.1}$ , can be defined as the sample size (g of sample/g of adsorbent) which is just sufficient to cause a 10% change in  $k'$ , relative to the  $k'$  value at very small sample sizes, usually the linear isotherm  $k'$  value<sup>10</sup>. Fig. 4 shows the effect of sample size on the plate height and  $k'$  of porous silica microspheres equilibrated with hexane containing various levels of water. The plate height apparently is less affected by the amount of water on this adsorbent than has been previously reported for wide-pore silica gels<sup>12</sup>, although lowest plate heights were obtained with the hexane carrier fully saturated with water. Data were obtained on the 5% water-saturated hexane system to show effects in a region where small changes in water content result in larger effects from sample size. The  $k'$  values of the test solute are more affected by sample size when using dry hexane and hexane containing small concentrations of water than with 100% water-saturated mobile phase. The  $\theta_{0.1}$  value is highest for 100% water-saturated hexane, suggesting the highest homogeneity of the adsorbent surface in this system. At higher water content, the microsphere adsorbent shows  $\theta_{0.1}$  values which are comparable to those found with wide-pore silica gels<sup>3,12</sup>. It should be noted that the effect of water content on the  $\theta_{0.1}$  value is complicated by the fact that this parameter is influenced by the  $k'$  of the test solute, which in turn varies with a change in water content.

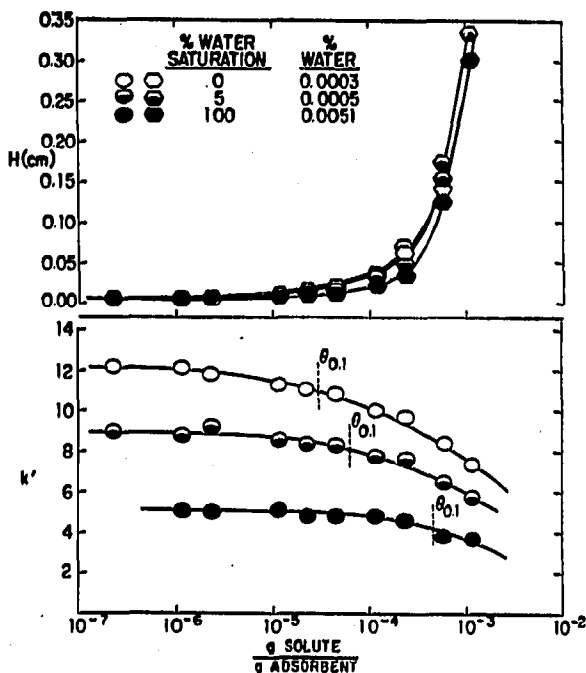


Fig. 4. Dependence of  $H$  and  $k'$  on sample size for the hexane-porous silica microsphere system. Column same as in Fig. 2; mobile phase, hexane; flow-rate, 2.0 ml/min; solute, 10  $\mu$ l of diphenyl ether solution in hexane.

### System B —Dichloromethane modified with water

The volume of solvent required to equilibrate the microsphere adsorbent with a change in carrier containing a different amount of water is less with a more polar mobile phase, because of its higher solubility for water. With dichloromethane, only about 40 column volumes of dry solvent are required to remove the water from a porous silica microsphere column which has been previously equilibrated with water-saturated dichloromethane.

The effect of the concentration of water in dichloromethane on  $k'$  and  $H$  values of a test solute, 3-phenyl-1-propanol, is shown in Fig. 5. In this system, both  $k'$  and  $H$  seem to be more affected by the level of water than with System A using hexane as the mobile phase. This effect may be associated with the significant difference in the test compounds, since the use of 3-phenyl-1-propanol in System B involves the possibilities of strong hydrogen bonding with the adsorbent surface.

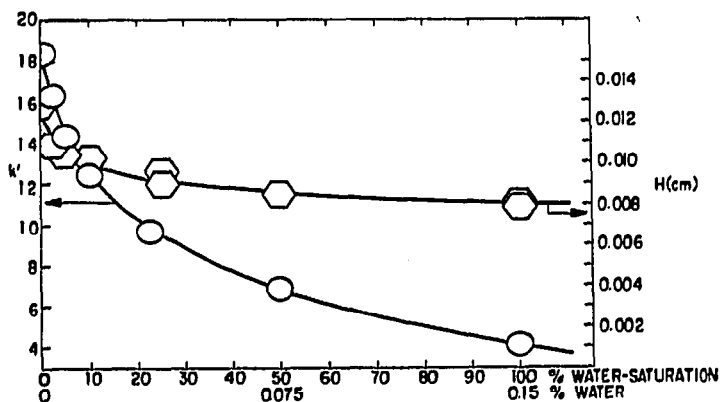


Fig. 5. Effect of water in dichloromethane mobile phase on retention and efficiency of the microsphere adsorbent. Conditions same as in Fig. 2, except for the solute, which was 10  $\mu$ l of 2 mg/ml 3-phenyl-1-propanol in dichloromethane.

The effect of sample size on  $H$  and  $k'$  for the dichloromethane/porous silica microsphere system is shown in Fig. 6. At higher sample sizes, water content has more of an effect on  $H$  in this dichloromethane system than in hexane. This effect could be a function of the different test solutes used.

As also found previously in System A involving hexane, larger  $\theta_{0.1}$  values are seen as the amount of water is increased in the carrier. At a higher water concentration, the linear capacity values are comparable with those others have found for wide-pore silica gels.

### System C —Diethyl ether modified with water

Adsorbents are quickly equilibrated with diethyl ether having varying degrees of water saturation because of the relatively high solubility of water in this solvent. Only 10–25 column volumes of dry ether are required to remove the water from a porous silica microsphere column which has been previously equilibrated with water-saturated diethyl ether. Because equilibration is so rapid with this solvent, it is very useful in making the initial adjustment of the adsorbent water content when using

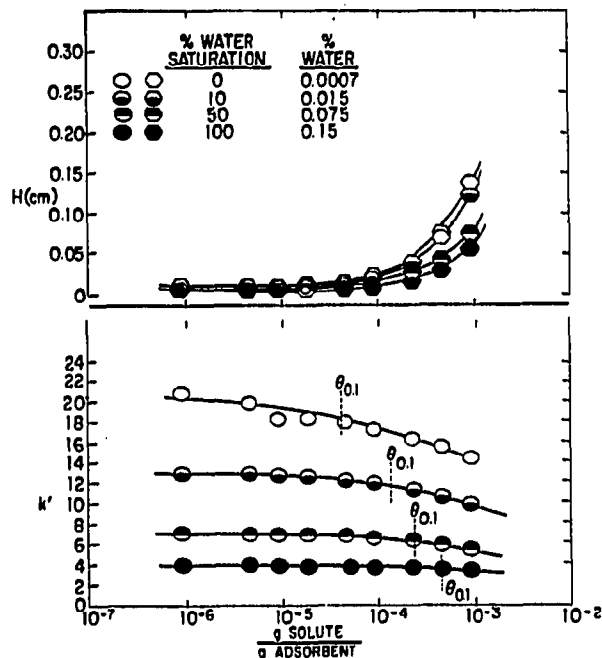


Fig. 6. Dependence of  $H$  and  $k'$  on sample size for the dichloromethane-porous silica microsphere system. Conditions same as in Fig. 4, except for the mobile phase, which was dichloromethane; solute, 10  $\mu$ l of 3-phenyl-1-propanol solution in dichloromethane.

organic carriers with much lower water solubility, which would normally involve a much longer conditioning process<sup>12</sup>.

The effect of the concentration of water in diethyl ether on  $k'$  and  $H$  values of a test solute, 3-methyl-3-(3,4-dichlorophenyl)-1,1-dimethylurea, is shown in Fig. 7. In this system, both  $H$  and  $k'$  are grossly affected at very low concentrations of water.

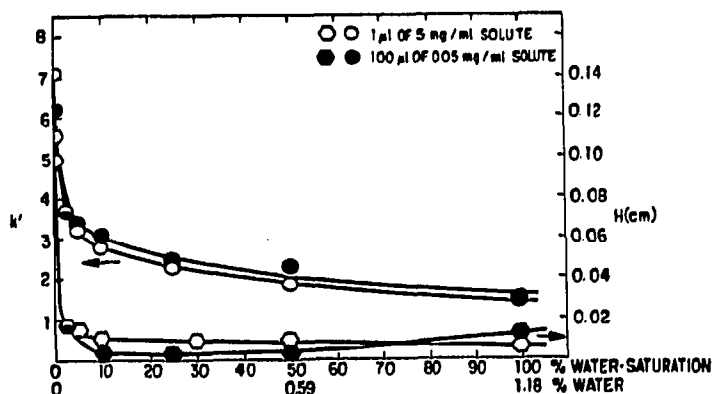


Fig. 7. Effect of water in ethyl ether mobile phase on the retention and efficiency of microsphere adsorbent. Conditions same as in Fig. 2, except for the solute, which was 3-methyl-3-(3,4-dichlorophenyl)-1,1-dimethylurea.



This may be a function of the test solute used; however, at levels above about 10% water saturation, effects on both parameters are minimal. The effect of sample volume is also shown in Fig. 7. The larger sample volume containing the same weight of solute shows slightly higher  $k'$  values and lower  $H$  values than smaller samples containing more concentrated solute. The apparent increase in  $H$  at 100% water saturation is an artifact caused by the emergence of an impurity peak on the leading edge of the solute band with these conditions.

Fig. 8 shows the effects of sample size on  $H$  and  $k'$  for the diethyl ether-microsphere adsorbent system. Unusually large sample size effects are seen with the dry diethyl ether system, including an unusual flattening-off of the  $k'$  curve at high sample weights. It is speculated that this levelling off of the  $k'$  vs. sample size curve might be due to a deactivation of the adsorbent by this relatively polar solute at high concentrations.

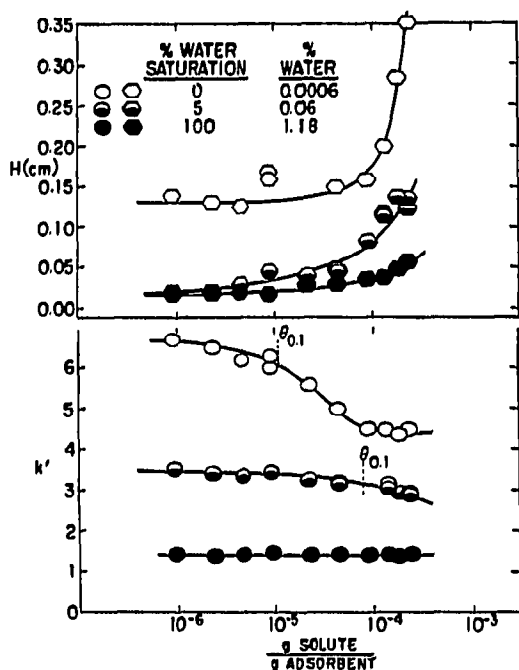


Fig. 8. Dependence of  $H$  and  $k'$  on sample size for the diethyl ether/porous silica microsphere system. Conditions same as in Fig. 4, except for the mobile phase, which was diethyl ether; solute, 100  $\mu$ l of 3-methyl-3-(3,4-dichlorophenyl)-1,1-dimethylurea in diethyl ether.

With at least 5% water-saturated diethyl ether, the sample size effects are grossly moderated. For 100% water-saturated diethyl ether, the  $k'$ -solute capacity curve is essentially linear for the amount of solute tested. It was not possible to estimate the  $\theta_{0.1}$  value for the solute in this system, since the limited solubility of the test compound did not permit the preparation of the higher concentrations needed for these data.  $H$  values at small sample sizes are somewhat higher than found for the hexane and dichloromethane systems because of the relatively large sample volume needed to obtain the desired sample sizes of this sparingly soluble test solute.

### System D—Dichloromethane modified with 2-propanol

For convenience, some workers have used polar organic modifiers (e.g., 2-propanol and methanol) in LSC systems<sup>11</sup>. Consequently, effects of the concentration of 2-propanol in dichloromethane on  $k'$  and  $H$  values were studied to determine the possible utility of this compound as a microsphere adsorbent modifier, and Fig. 9 presents data on this system. The discontinuities in both the  $k'$  and  $H$  curves are due to gross distortions in solute band shapes, which are discussed in a following section. It should be noted that at the 0.3% 2-propanol level,  $k'$  and  $H$  values for the solute were approximately those found in the 50% water-saturated dichloromethane system (see Fig. 5). The unusual trend in  $k'$  values beginning at 0% 2-propanol and increasing with the concentration of this modifier is probably also an effect associated with the unusual peak shapes which cause the discontinuity in these plots.

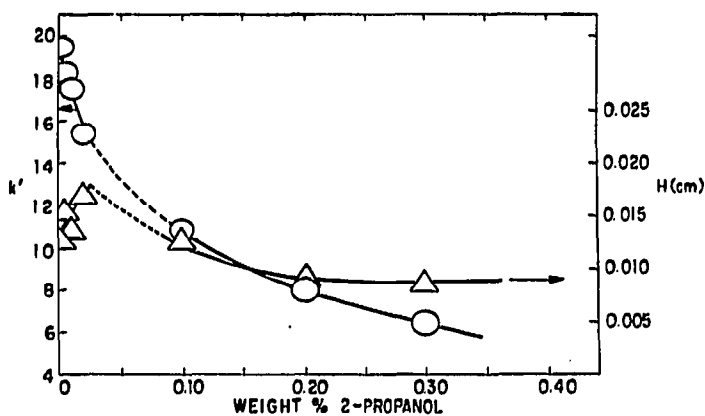


Fig. 9. Effect of 2-propanol in dichloromethane mobile phase on the retention and efficiency of the microsphere adsorbent. Conditions same as in Fig. 4, except for the mobile phase modifier.

### System E—Dichloromethane modified with methanol

Fig. 10 shows the effect of methanol concentration in dichloromethane on  $k'$  and  $H$  of the test solute, 3-phenyl-1-propanol. Again, the discontinuities in the data are shown because of unusual band shapes which occurred at certain methanol concentrations. At 0.15% methanol, the  $k'$  values were essentially equivalent to the 50% water-saturated dichloromethane system; however,  $H$  values were about 20% higher.

The effect of sample size on  $k'$  and  $H$  for the two alcohol-modified adsorbent systems is shown in Fig. 11. The 2-propanol-modified system actually exhibits an increase in  $k'$  with increasing sample sizes to at least 1 mg of solute/g of adsorbent. This trend is contrary to that of the water-modified systems illustrated in Figs. 4, 6 and 8. The methanol-modified system does show an apparent decrease in  $k'$  due to increasing sample size, presumably because of the gross distortion of peak shapes which were found in both alcohol-modified systems.  $H$  values also increase with both systems as sample size is increased. The greater sensitivity of the methanol-modified system to sample size may be a function of the lower concentration of methanol which was used. It should be noted that the level of organic modifier was maintained in both systems to produce  $k'$  values approximating those of the 50% water-saturated dichlo-

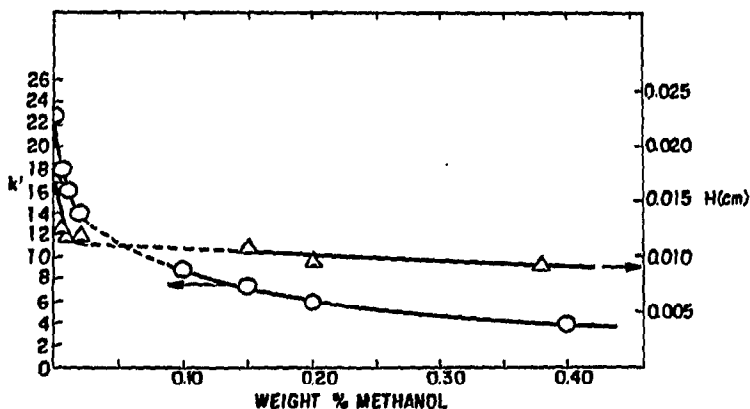


Fig. 10. Effect of methanol in dichloromethane mobile phase on the retention and efficiency of the microsphere adsorbent. Conditions same as in Fig. 4, except for the mobile phase modifier.

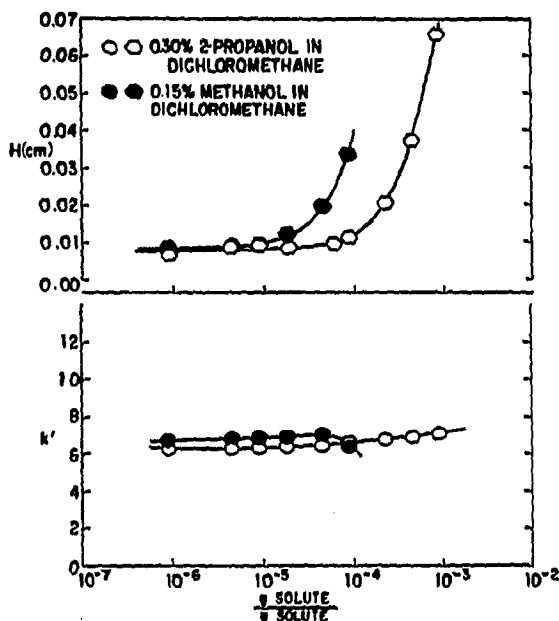


Fig. 11. Dependence of  $H$  and  $k'$  on sample size for alcohol-modified dichloromethane-porous silica microsphere system. Conditions same as in Fig. 6, except for the mobile phase modifier, as shown.

romethane/microsphere system. The  $k'$  values shown in the top plot of Fig. 11 illustrate the relative constancy of  $k'$  values which were obtained in these systems as sample size was increased. In general, however, it would appear that the linear capacity of alcohol-modified adsorbents might be less than that of water-modified systems, particularly where low concentrations of alcohol modifier are used.

The discontinuity in the plots obtained with the 2-propanol- and methanol-modified dichloromethane systems (Figs. 9 and 10) are the result of unusual band shapes which occur as the amount of alcohol modifier is increased to about 0.05%

for both systems. The shapes of the chromatographic peaks found for various concentrations of the organic modifiers are shown in Fig. 12. The test solute, 3-phenyl-1-propanol, exhibits a tailing peak at levels of 0.02% for both of the organic modifiers. At 0.05% of alcohol modifier, odd-shape peaks are formed in both systems, the envelopes appearing as the superimposition of two different chromatographic bands. Two peaks are particularly apparent in the 0.05% methanol curve. At the 0.1% level with both alcohol modifiers, the peak shapes change and now exhibit frontal asymmetry. This frontal asymmetry is significantly reduced at higher concentrations of alcohol, the minimum levels in this system apparently consisting of 0.30% 2-propanol and 0.15% methanol in dichloromethane.

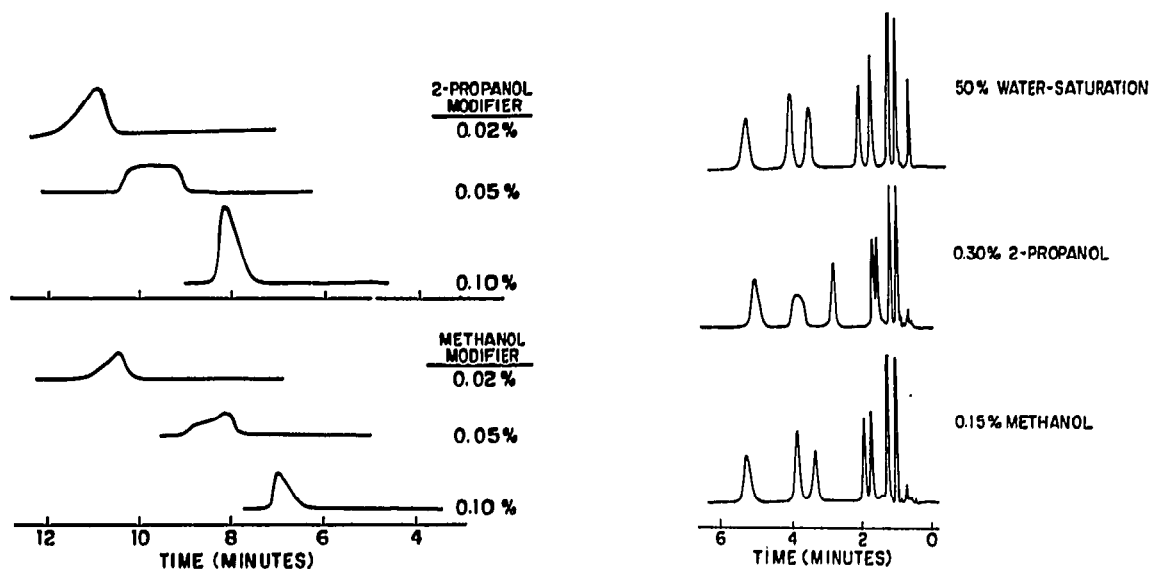


Fig. 12. Effect of alcohol modifier concentration on solute peak shape. Mobile phase, dichloromethane. Conditions same as for Fig. 4, except for the mobile phase modifier, as shown.

Fig. 13. Effect of adsorbent modifier on separation selectivity. Conditions same as Table III, except for the modifiers noted.

The reason for change in the peak shapes shown by the data in Fig. 12 is not understood, but the effect was reproducible and followed a regular pattern as the concentration of both alcohols was increased. Perhaps the phenomena can be explained by a change in the shape of the adsorption isotherm resulting from the amount of alcohol modifier associated with the adsorbent surface.

Some minor differences in selectivity ( $\alpha$  values) are found with the organic alcohol modifiers, as exhibited by the separations of aromatic alcohol mixtures in Fig. 13. The separation with methanol as modifier appears to be more like the water-saturated system than does the separation obtained with 2-propanol modifier. Of special interest is the middle chromatogram with a carrier of 0.30% 2-propanol in dichloromethane, which shows a distorted benzyl alcohol peak (second from last). The shape of this peak, together with the tailing and frontal asymmetry shown by some

of the other peaks in the bottom two chromatograms, indicates that a non-linear adsorption isotherm can occur when working with carrier systems modified with small concentrations of alcohol. If the solute compounds are very strongly adsorbed to the support, the possibility of these non-linear isotherms increases.

*Mass transfer effects in packed beds of the porous microsphere adsorbent*

In LC, band spreading is caused by eddy diffusion effects and mass transfer limitations in the mobile, stationary, and stagnant mobile phases<sup>13</sup>. It has been well documented that band broadening due to eddy diffusion can be reduced by packing homogeneous column beds. In the case of the porous silica microspheres, this can be accomplished with a high-pressure, stabilized slurry packing technique<sup>2,4</sup>. Use of columns with homogeneously packed, very small particles minimizes mobile mass transfer limitations. Further, the superficially porous particles in widespread use have been useful in reducing the limitations of mass transfer in the stagnant mobile phase within the particles. However, use of very small particles also can reduce stagnant mobile phase effects, and it has been speculated that if the particles are sufficiently small, stagnant mobile phase effects would be reduced to the point that use of superficially porous particles no longer would exhibit an advantage<sup>14,15</sup>. Data obtained during the study of the effect of water on the performance of the porous silica microspheres provide some insights regarding mass transfer effects with these particles.

The data given in Figs. 3 and 5 indicate that the plate heights for columns of the porous silica microsphere adsorbent are not greatly dependent on water content when used with non-polar or intermediate polarity mobile phases. The lower dependency of plate height on water content for these particles (contrary to that which has been reported for conventional silica gel adsorbents) suggests improved mass transfer within the microsphere particles, resulting from a relative lack of deep pools of stagnant mobile phase in relatively long, narrow pores. This trend is in keeping with what is known about the structure of the microspheres. Based on the method of preparation and characterizations by nitrogen adsorption studies, it is believed that the microsphere adsorbent is relatively free of deep, narrow pores, making undesirable deep pools of stagnant mobile phase less likely. As seen in Fig. 7, small changes in water content result in relatively large changes in the  $H$  values in the dry diethyl ether system. This trend may be due to the relatively polar test solute molecule used in these studies, which is tightly adsorbed to the silica surface. The subsequent significant lowering of plate heights with a very small amount of water in the ether suggests that a small population of high-energy sites still remain on the surface of the microsphere adsorbent. With the addition of a small amount of water, the surface apparently is made much more homogeneous, resulting in the relatively flat  $H$  and  $k'$  plots which are obtained. Thus, it appears that the porous silica microsphere adsorbent normally is not as dependent on water content as is silica gel for the maintenance of reproducible plate height and retention data. Nevertheless, the use of some water as a modifier apparently is desirable.

To clarify the question of whether stagnant mobile phase and/or stationary phase or mobile phase effects in LC dominate plate heights in the use of very small particles, some data were compiled from this study and previous work<sup>4</sup> and summarized in Fig. 14. The plots for the 8- to 9- $\mu$  LSC microspheres and the 5- to 6- $\mu$  porous silica microspheres used in a liquid-liquid chromatographic system (LLC)

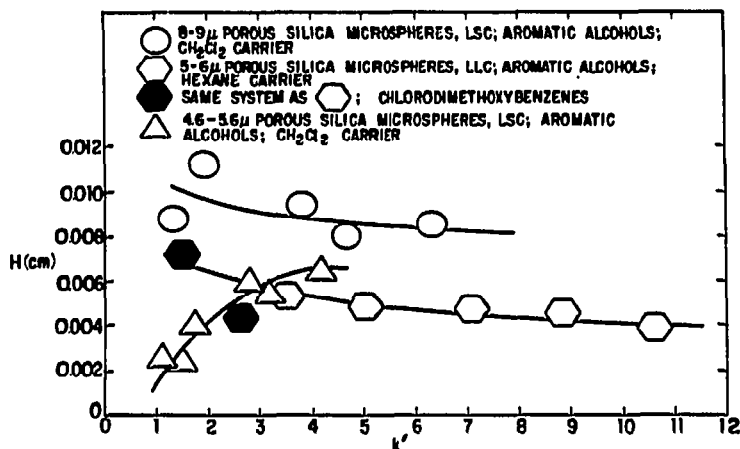


Fig. 14. Effect of  $k'$  on  $H$  for various porous silica microsphere systems. All columns were 25 cm  $\times$  3.2 mm I.D.; mobile phases were 50% water saturated; the aromatic alcohol mixture for LSC is given in Table III; the aromatic mixture for LLC is shown in Fig. 4 of ref. 2; chlorodimethoxybenzene is shown in Fig. 6 of ref. 2.

(350-Å pores with 30%  $\beta,\beta'$ -oxydipropionitrile as stationary phase) both show a decrease in  $H$  for solutes with increasing  $k'$  values ( $k' > 1$ ). This trend suggests that mass transfer for both of these systems is being limited by effects within the particles, and that the LSC system is stagnant mobile phase mass transfer limited, while band broadening in the LLC column is determined mainly by resistance to mass transfer within the stationary phase. Apparently, the pools of stagnant mobile phase in the 8- to 9- $\mu$  LSC particles are sufficiently deep so as to limit mass transfer. Even though the stationary liquid within the 5- to 6- $\mu$  (350-Å pores) LLC particles is in relatively shallow pools, mass transfer in this stationary liquid appears to be limiting, probably because of the higher viscosity of the stationary liquid (compared to the mobile phase existing as stagnant pools in the 8- to 9- $\mu$  particles).

A decrease in  $H$  with increasing  $k'$  is a pattern similar to that found in most packed gas chromatography (GC) columns, and is typical of stationary phase (and/or stagnant mobile phase) mass transfer-limited systems. On the other hand, the 4.6- to 5.6- $\mu$  LSC particles show an increase in  $H$  with increasing  $k'$ , suggesting that peak broadening within this column is dominated by mobile phase mass transfer effects. Apparently, the stagnant mobile phase within these smaller particles does not significantly contribute to the band broadening process. An increase in  $H$  with  $k'$  also is typical of superficially porous particles, which are known to produce columns which are mobile phase (not stationary phase or stagnant mobile phase) mass transfer limiting. Contrary to that predicted by theory, the  $H$  values at  $k' > \sim 4$  for the 4.6- to 5.6- $\mu$  LSC particles appear slightly larger than the  $H$  values for the 5- to 6- $\mu$  LLC column. This effect could be associated with experimental variations. However, as shown below, data suggest that it is more difficult to pack the smaller particles into homogeneous beds.

Table III summarizes the data from the 4.6- to 5.6- $\mu$  porous silica microsphere LSC adsorbent column, part of which is plotted in Fig. 14. Values for solutes with  $k' < 1$  were not plotted in Fig. 14, since sample injection effects result in extra-column

TABLE III

DATA FROM SEPARATION WITH 4.6 TO 5.6- $\mu$  POROUS SILICA MICROSPHERE ADSORBENT\*Column, 25 cm  $\times$  3.2 mm I.D.; mobile phase, 50% water-saturated dichloromethane; mobile phase velocity, 0.64 cm/sec; pressure, 2000 p.s.i.; flow-rate, 2.0 ml/min.

Peak No.*	Peak identity	$k'$	Plate number, $N$	$H$ (cm)	Effective plates, $N_{eff}$	$N_{eff}/t^{**}$
1	Carbon tetrachloride ( $r^\circ$ marker)	0.00	2,290	0.01090	0	0
2	2,3-Dimethylphenol	0.44	2,960	0.00846	277	4.9
3	Impurity	0.70	9,915	0.00252	1,690	25.4
4	Impurity	1.28	10,340	0.00242	3,265	36.7
5	Benzhydrol	1.64	6,320	0.00396	2,440	23.7
6	$\alpha,\alpha$ -Dimethylbenzyl alcohol	3.24	4,135	0.00605	2,415	14.6
7	Benzyl alcohol	3.88	4,725	0.00529	2,990	15.7
8	3-Phenyl-1-propanol	5.40	3,915	0.00638	2,790	11.2

\* Same mixture as shown in Fig. 13.

\*\* Effective plates per second.

peak broadening for solutes with  $k' < 1$  with this very high-efficiency column<sup>4</sup>. The data in Table III show that more than  $10^4$  theoretical plates were obtained on this 25-cm long column of  $\sim 5\text{-}\mu$  microspheres (mobile phase velocity of 0.64 cm/sec). This performance results in a reduced plate height of about five particle diameters. More than 36 effective plates per second are demonstrated for the solute with a  $k'$  of  $\sim 1.3$ .

The data in Table III were obtained on a carefully constructed column by injecting the sample directly into the top of the column bed in a flowing system. Injection of the sample into the column packing is not a preferred routine procedure, since it disturbs the homogeneity of the bed. Instead, a thin, porous stainless-steel screen or a porous Teflon<sup>®</sup> wool plug is usually preferred to retain the packing in the inlet of the column<sup>4</sup>. However, putting an inlet plug into a carefully constructed column of 4.6- to 5.6- $\mu$  microspheres usually results in a one-third to one-half decrease in the plate number of the column with solutes of about  $k' < 2$ . The  $H$  values of solutes with  $k' > 2$  do not seem to be significantly affected. With columns of the larger microsphere particles (*i.e.* 8–9  $\mu$ ) and presumably other particles of the same size, extra-column band broadening effects are less critical but still significant. Therefore, it is apparent that extra-column effects are especially significant in high-performance columns containing particles of about 5  $\mu$  (see also ref. 5).

#### *Very high-performance LC using series-connected columns*

Even though a single 25-cm column of the porous silica microsphere adsorbent exhibits good efficiency, separations of very complex mixtures or pairs of compounds with  $\alpha$  separation factors approaching unity require a very large number of theoretical plates. Therefore, it is of interest to know whether or not, in practice, one can connect individual lengths of these 25-cm columns to produce longer columns with the expected additive number of theoretical plates. To test the concept experimentally, four 25-cm columns of the 4.6- to 5.6- $\mu$  porous silica microsphere adsorbent were

prepared by the high-pressure aqueous slurry technique. These columns were then individually tested at two mobile phase velocities using two solutes, benzhydrol and 3-phenyl-1-propanol, having  $k'$  values of 1.6 and 5.3, respectively. The plate height data obtained on these individual columns are shown in Table IV, together with the pressures needed to achieve the mobile phase velocities used. The number of plates of these columns are reasonably comparable, although more variation was found with columns of these 5- $\mu$  particles than was previously obtained with 8- to 9- $\mu$  porous silica microsphere adsorbent<sup>4</sup>. As might be anticipated, the smaller particle sizes appear to pack less reproducibly into homogeneous beds.

TABLE IV

## CHARACTERISTICS OF INDIVIDUAL POROUS SILICA MICROSPHERE COLUMNS

Columns, 25.0  $\times$  3.2 mm I.D.; mobile phase, 50% water-saturated dichloromethane; temperature, 27°.

Column designation	Number of theoretical plates*, $N$			
	Benzhydrol ( $k' = 1.6$ )		3-Phenyl-1-propanol ( $k' = 5.3$ )	
	0.16 cm/sec	0.62 cm/sec	0.16 cm/sec	0.62 cm/sec
A	6,710 (450)**	4,320 (1,810)	6,080	3,530
B	5,360 (475)	4,260 (1,900)	3,680	3,420
C	5,900 (500)	5,270 (2,000)	3,560	3,510
D	5,420 (450)	3,360 (1,950)	4,910	3,410

\* Average of duplicate runs.

\*\* The figures in parentheses denote pressure (p.s.i.g.).

The individual columns described in Table IV were series-connected into 50-, 75-, and 100-cm total lengths, using low-volume modified Swagelok<sup>®</sup> connectors (0.01 in. I.D.). The data in Table V show the number of theoretical plates obtained for the series-connected columns, and the pressures required to operate these combinations at the indicated mobile phase velocities. A significant loss in the expected number of theoretical plates occurred with the test solute having a  $k' = 1.6$ . This less-than-theoretical result probably can be explained by extra-column peak broadening effects, *e.g.*, unwanted diffusion in the inlet and outlet plugs and the connecting fittings between the columns. However, for the solute having  $k' = 5.3$ , essentially no loss in theoretical plates was found at the lower mobile phase velocity. At the higher velocity, somewhat less than theoretical efficiency was observed, the loss averaging less than 10% per length for these series-connected systems. It is interesting to note that 18,800 theoretical plates were obtained for 3-phenyl-1-propanol with the four series-connected (1-m) columns operated at 0.16 cm/sec mobile phase velocity. Under these conditions, this column exhibits more than 13,400 effective plates for this solute. Fig. 15 shows a separation of a test mixture of phenols and aromatic alcohols obtained with the 1-m column of the 4.6- to 5.6- $\mu$  porous silica microsphere adsorbent.



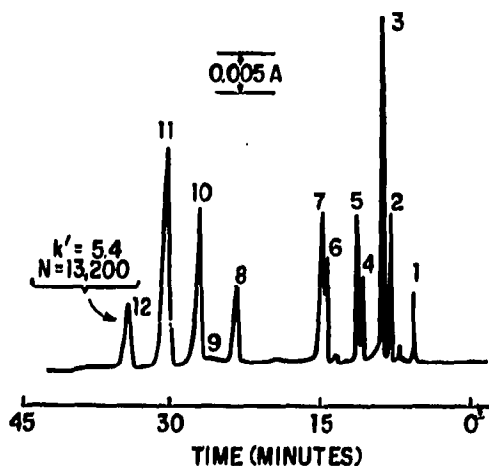


Fig. 15. High-efficiency LSC separation with porous silica microspheres. Column, 1 m (four 25-cm connected)  $\times$  3.2 mm I.D., 5- $\mu$  porous silica microsphere adsorbent; mobile phase, 50% water-saturated dichloromethane; pressure, 6000 p.s.i.; flow-rate, 1.45 ml/min; temperature, 27°; 5- $\mu$ l sample aliquot. Peak identification: 1 = carbon tetrachloride ( $t^0$  marker); 2 = 2,6-dimethylphenol; 3 and 4 = impurities in standards; 5 = 3,4-dimethylphenol; 6 = benzhydrol; 7 = *p*-cresol; 8 =  $\alpha,\alpha$ -dimethylbenzyl alcohol; 9 = *p*-methoxyphenol; 10 = benzyl alcohol; 11 = 2-phenyl ethanol; 12 = 3-phenyl-1-propanol; the remainder of peaks are impurities.

## CONCLUSIONS

The performance of the porous silica microsphere adsorbent appears to be less affected by the level of water adsorbed than conventional wide-pore silica gel, particularly with non-polar mobile phases and solutes. Mobile phases which are more than 10% water-saturated appear to be suitable for use with the microsphere adsorbent.

The linear capacity,  $\theta_{0.1}$ , of the microsphere adsorbent is equivalent or exceeds that of wide-pore silica gels of the same surface area. However, the linear capacity is influenced by the strength (polarity) and % water saturation of the mobile phase. With dry solvents, lower linear capacities are demonstrated for polar solutes.

The microsphere adsorbent may be modified with organic alcohols, such as 2-propanol and methanol. However, these solvents should be used at concentrations exceeding 0.3% and 0.15%, respectively, to eliminate non-Gaussian peak shapes at low modifier concentrations for some solutes. These levels of organic modifiers result in solute  $k'$  factors which are comparable to those of 50% water-saturated adsorbent systems. At 0.3% and 0.15%, respectively, for 2-propanol and methanol, the plate heights of microsphere adsorbent columns are comparable to water-modified adsorbent only at small sample weights, the linear capacity of alcohol-modified systems generally being smaller than for water-modified adsorbent. Water is still preferred as the adsorbent modifier when conditions allow its use.

The plate heights of water-modified microsphere adsorbent columns are slightly lower for larger sample volumes of more dilute solute than for smaller volumes of more concentrated solute, probably as a result of the reduced likelihood of localized overloading during sample injection.

The efficiency of columns containing microsphere adsorbents (75-Å pores) of

TABLE V  
RESULTS FROM SERIES-CONNECTED POROUS SILICA MICROSPHERE COLUMNS  
Same columns and conditions as Table IV.

Column in series	Total column length (cm)	Number of theoretical plates*, N											
		Benzhydrol ( $k' = 1.6$ )			3-Phenyl-1-propanol ( $k' = 5.3$ )								
		0.16 cm/sec		0.62 cm/sec	0.16 cm/sec		0.62 cm/sec						
	Theory	Actual	%	Theory	Actual	%	Theory	Actual	%				
A + B	50	12,100 (925)**	9,200 (925)	76	8,600 (3,710)	6,400 (3,900)	75	9,800	8,800	90	7,000	5,800	83
A + B + C	75	18,000 (1,425)	12,200 (1,450)	68	13,800 (5,710)	7,200 (6,000)	52	13,300	13,200	99	10,500	9,100	87
A + B + C + D	100	23,400 (1,875)	12,800 (2,000)	55	17,200 (7,710)	12,400 <sup>§</sup> (8,000) <sup>§</sup>	72	18,200	18,800	103	13,900	10,300 <sup>§§</sup>	74

\* Rounded off to nearest 100 plates.

\*\* The figures in parentheses denote pressure (p.s.i.g.).

§ Extrapolated N for 0.62 cm/sec; actual value measured, 13,700 plates at 0.43 cm/sec for 6,000 p.s.i.g.

§§ Extrapolated N for 0.62 cm/sec; actual value measured, 11,700 plates at 0.43 cm/sec for 6,000 p.s.i.g.

very small size ( $5\ \mu$ ) appears to be limited by mobile phase mass transfer effects, contrasted to the stagnant mobile phase mass transfer limitations exhibited by microspheres of larger size ( $8\text{--}9\ \mu$ ). Wider-pore microspheres ( $350\ \text{\AA}$ ) of  $5\text{--}6\ \mu$  filled with  $\beta,\beta'$ -oxydipropionitrile stationary liquid appear to be stationary phase mass transfer limiting.

These and other studies suggest that particles of about  $7\text{--}8\ \mu$  might represent a practical compromise between column efficiency on one hand, and column permeability and packing reproducibility on the other.

Columns (25 cm long) of  $5\text{-}\mu$  microsphere adsorbents can exhibit  $>10^4$  theoretical plates at a carrier velocity of 0.7 cm/sec when used in the on-column sampling mode. However, the use of inlet frits in the column for practical operation results in a one-third to one-half decrease in theoretical plates for solutes with  $k' < 2$ , due to extra-column band broadening effects. No significant loss in column efficiency occurs for solutes with  $k' > \sim 2$ .

Using low-volume fittings, 25-cm lengths of the microsphere columns may be connected with little loss in expected column efficiency for solutes with  $k' \sim 5$ . However, a significant loss in efficiency for solutes with  $k' \leq 1.5$  is observed in these connected columns, presumably due to extra-column band broadening. A 1-m column of  $5\text{-}\mu$  porous silica microspheres made by connecting four 25-cm columns has demonstrated  $>18,800$  theoretical plates ( $>13,400$  effective plates) when operated at a carrier velocity of 0.16 cm/sec.

Although these conclusions are based on data obtained with columns of the porous silica microsphere adsorbent, it is believed that some also may be valid for columns prepared with very small particles of wide-pore silica gel.

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