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SEPARATION OF C_6 CYCLIC HYDROCARBONS BEFORE THE INERT PEAK ON MICROPOROUS SILICA: A CASE OF NEGATIVE ADSORPTION

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SUMMARY

Pore size distributions and specific surface areas of microporous silicas were measured. It was demonstrated experimentally that more than half of the pore volume is blocked by the adsorbed dichloromethane eluent monolayer for an extremely non-polar and small sample molecule, *e.g.*, cyclohexane. If the eluent is "dry", C_6 hydrocarbons are separated from each other, and all of them are eluted before the deuterated eluent. This separation is due to differences in negative adsorption between the samples. With increasing water content of the dichloromethane the adsorbed eluent is gradually replaced with a monolayer of water. It was shown that this process is complete at a water content of 950 ppm. If the water content of the eluent is increased to 1600 ppm, then the available pore volume is decreased by a factor of 7 for the deuterated eluent. The application of size exclusion chromatography is limited because of negative adsorption. This is true whether the stationary phase is silica or a reversed phase. When determining the hold-up time of a column packed with silica or alumina, such as is used in routine liquid chromatography, the isotopically labelled eluent should be used as the inert peak. If other "inert" substances are used, as is sometimes recom-mended, then errors in the relative retentions can be intolerable. Negative adsorption may be of some interest in contact catalysis using microporous catalysts.

INTRODUCTION

In routine liquid chromatography (LC) silicas with mean pore diameters between *ca.* 60 and 200 Å and with specific surface areas between *ca.* 500 and 150 m²/g are typical. If the specific surface areas are smaller, the retentions and their differences become smaller. This is only desirable for simple and rapid separations, where the relative retentions are large enough. If, on the other hand, the pore diameters are smaller then unwanted (and sometimes unexpected) size exclusion effects can appear, and often the wall effects of the support also cannot be neglected.

Discussing the mechanism of adsorption chromatography, Snyder[1,2] assumed that retarded samples have to displace the eluent from the surface of the polar sup-port, *i.e.*, the eluent molecules in the monolayer are exchanged by the sample mole-cules. In the approach of Scott and Kucera[3–5] plurimolecular adsorption of the eluent

on the surface of the support is assumed. Samples with capacity ratios smaller than 10 interact only with this plurimolecular adsorbed layer, and do not interact with the surface of the polar support.

It is difficult to determine the pore size distribution of such microporous solids using conventional methods[6]. It is much simpler to determine this distribution by size exclusion chromatography (SEC), as described earlier[6–8]. The measurements are rapid, relatively simple and can be carried out under conditions analogous to those used in the application of solids, e.g., in catalysis or chromatography, extremely high pressures and low temperatures not being necessary. The SEC method, as used in this work to determine pore size distributions, enables us to cover the entire range of (so far) 8 to 4000 Å.

This study was designed to contribute to the understanding of the problems discussed above. However, the chromatographic separations obtained with micro-porous silicas (with average pore diameters smaller than 25 Å and with unusually high specific surface areas) were unexpected, so that with their help not only the mechanism of adsorption chromatography and SEC can be discussed, but also some problems important in the practical application of chromatography can be described and explained.

EXPERIMENTAL

Apparatus

The apparatus was constructed in the laboratory, the following components being set up in series: a high-pressure pump with a constant flow-rate (M 6000; Waters Assoc., Milford, MA, U.S.A.), a sample injector (Type 7120; Rheodyne, Kontron Technik, Eching bei München, G.F.R.), a stainless-steel separation column[9] with a drilled-out diameter of 4.2 mm, a UV detector of our own design (254 nm, cell volume 8 μl)[9] and a differential refractometer (R 401, Waters Assoc.).

The concentration of water in the eluent in the reservoir was kept constant with a closed-loop moisture control system (MCS)[10]. This inexpensive glass MCS can be inserted into any chromatographic equipment, between the detector outlet and the inlet of the reservoir. The MCS was filled with alumina (Type N-Super I, Woelm Pharma, Eschwege, G.F.R.) with a sieve fraction of 50–200 μm and was impregnated with different amounts of water. The water content and the temperature of the MCS determine the concentration of water in the eluent[10].

The temperature of the chromatographic column and the MCS were controlled at 24 $\pm$ 0.25°C with a water thermostat. The concentration of water in the eluent was measured by Karl Fischer titration using 10-ml samples.

Stationary phases and column packing

Two different microporous silicas were used: (1) Kugelgel (Dynamit Nobel, Troisdorf, G.F.R.) and (2) silica gel Typ 125 (Grace, Bad Homburg, G.F.R.). The original materials were ground with an agate mill. Sieve fractions of about 10 μm were obtained by sedimentation. The columns were packed using a suspending medium of isopropanol–methanol (7:3)[11].

Eluent

Before use the dichloromethane eluent was distilled in order to increase its UV transparancy. It then contained 50–220 ppm of water.

Samples

The samples were C$_6$ hydrocarbons and deuterated dichloromethane (Merck, Darmstad, G.F.R.) and Fluorinert, which is a perfluorinated hydrocarbon mixture (FC 78, 3M Deutschland, Neuss, G.F.R.) with an atmospheric boiling point of about 51°C and an average molecular weight of about 300 (ref. 12).

RESULTS AND DISCUSSION

Pore size distribution of the microporous silicas

Experience has shown that the pore size distribution of many silicas used in routine liquid chromatography may best be illustrated by a log–normal distribution curve. Fig. 1 shows a plot of the sum of the residues, R, as a function of log $\emptyset$ for the two microporous silicas. The sum of residues, R, for a given pore diameter, $\emptyset_t$, is defined as the fraction of the total pore volume formed by all the pores with a diameter greater than $\emptyset_t$. The pore size distribution and the mean pore diameters were calculated by the method discussed above[6–8]. The elution volumes of the polystyrene sample ($\bar{M}_w = 111,000$) correspond, to a first approximation, to the interparticle (interstitial) volumes of the columns if it is assumed that the volume of the pores with a diameter greater than *ca.* 600 Å (this is the exclusion value of our polystyrene[6]) is negligible or if the volume of these pores is taken as part of the interparticle volume. The totally
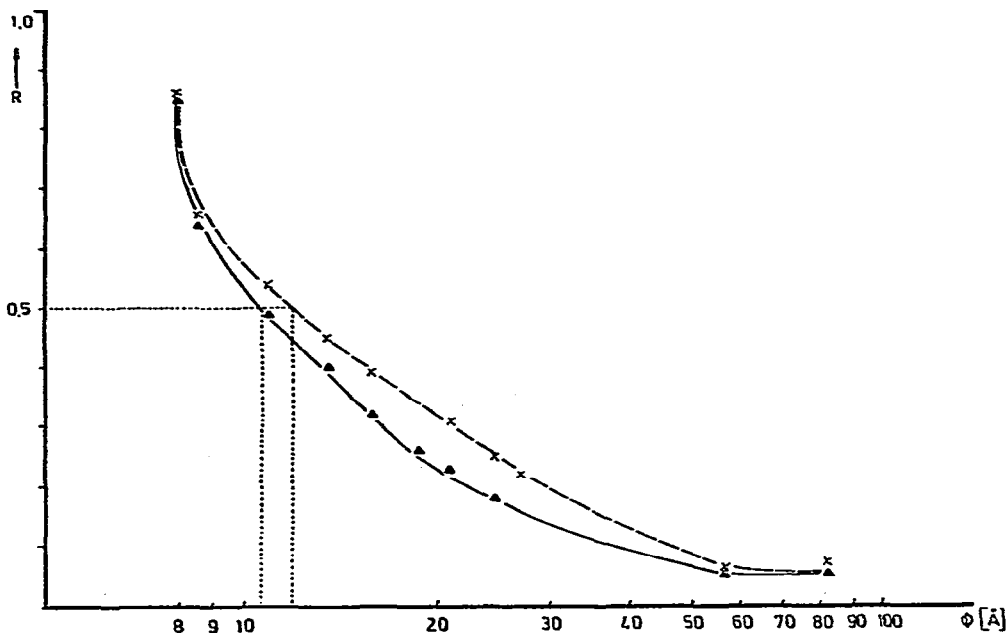

Fig. 1. Pore size distributions of microporous silicas. Eluent: "dry" dichloromethane with a water content <5 ppm. Silica: ×, Kugelgel; ▲, Typ 125.

permeating (inert) sample is the deuterated eluent, i.e., $C^2H_2Cl_2$. The total pore volume of the stationary phase is the difference between the elution volumes of the polystyrene and $C^2H_2Cl_2$ samples.

The R–ø curves for non-microporous silica are usually S-shaped. However, as shown in Fig. 1, these curves are concave in the whole pore size region for our microporous silicas. It will be assumed that the ø value corresponding to R = 0.5 is the mean pore diameter, i.e., 12 Å for Kugelgel and 11 Å for Typ 125.

It must be emphasized that the pore size distributions shown in Fig. 1 were obtained with "dry" dichloromethane (< 5 ppm of water). The problems caused by the water content of the eluent will be discussed later.

Characterization of the microporous silicas

Some of the properties of the two silicas are summarized in Table I. The data were obtained from three different sources: (1) the manufacturers; (2) nitrogen adsorption isotherms (77°K), measured in the laboratories of Veba Chemie, Gelsenkirchen-Buer, G.F.R.; and (3) from our own SEC measurements. The chromatographic average particle sizes, d_p, were calculated from the specific permeabilities of the columns[13].

TABLE I

PROPERTIES OF THE MICROPOROUS SILICAS

Parameter	Kugelgel	Typ 125
Manufacturer's own data:		
Surface area (m²/g)	700	750–800
Mean pore diameter (Å)	—	21
Nitrogen adsorption:		
Surface area (m²/g)	685	550
Pore volume (cm³/g)	0.28	0.25
SEC measurements:		
Pore volume (cm³/g)	0.32	0.27
Mean pore diameter (Å)	12	11
Mean particle size (μm)	13	11

As can be seen from the non-chromatographic data in Table I, the specific surface areas of the supports are about twice those used in routine liquid chromatography.

The chromatographically determined pore volumes are in reasonable agreement with those calculated from the nitrogen adsorption isoterms; considering the difficulties involved in such measurements on microporous solids, this agreement is remarkable.

Chromatographic separations

In Figs. 2 and 3 the separation of polystyrene ($\bar{M}_w$ = 111,000), (2) cyclohexane, (3) cyclohexene, (4) cyclohexadiene and (5) $C^2H_2Cl_2$ using Kugelgel and Typ 125, respectively, are shown. The C_6 cyclic hydrocarbons separated in these figures are small molecules, with similar geometries, and all of them are eluted before the inert
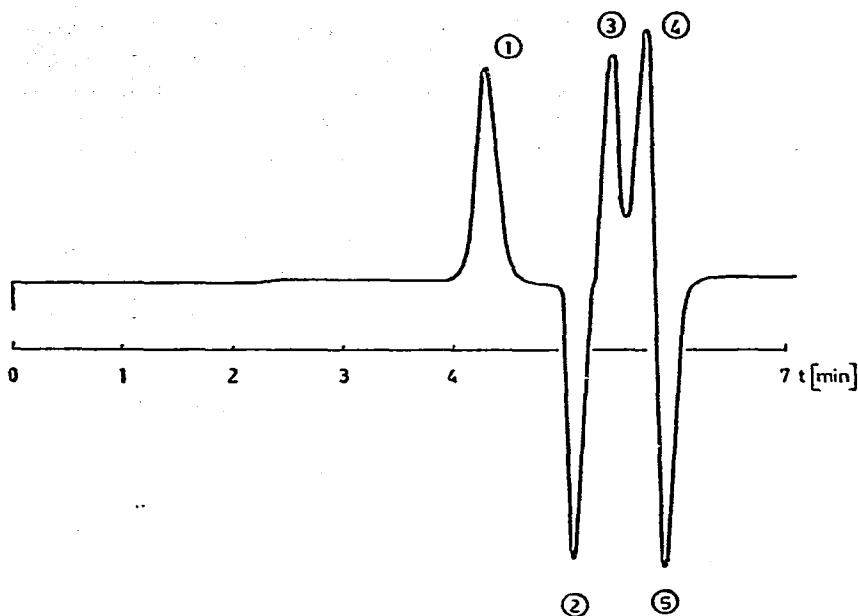
Fig. 2. Separation of "inert" samples on the microporous silica Kugelgel. Stationary phase: Kugelgel (d_p = 12.8 μm). Column: 25 cm long, 4 mm I.D. Packing density: 0.69 g/cm³. Temperature: 24°C. Eluent: dichloromethane with water concentration <5 ppm. ΔP: 5.8 bar. Flow-rate: 0.5 ml/min. Linear velocity: 0.66 mm/sec. Detector: refractive index. Samples: 1 = polystyrene ($\bar{M}_w$ = 111,000); 2 = cyclohexane; 3 = cyclohexene; 4 = cyclohexadiene; 5 = C²H₂Cl₂.
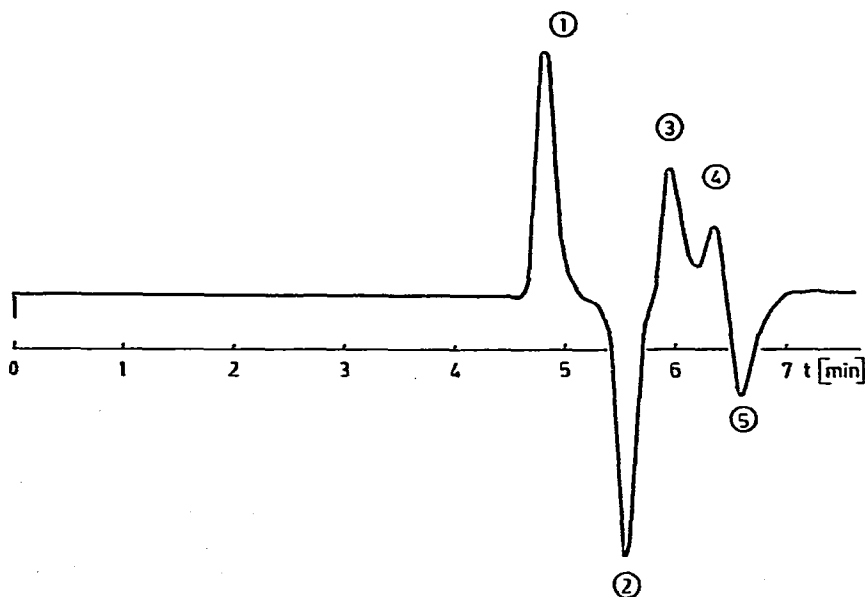
Fig. 3. Separation of "inert" samples on the microporous silica Typ 125. Experimental conditions as in Fig. 2, except the following. Column: 30 cm long, 4.2 mm I.D. Stationary phase: Typ 125 (d_p = 10.9 μm). Packing density: 0.67 g/cm³. ΔP: 8.3 bar. Linear velocity: 0.75 mm/sec.

sample. That is, the differences in elution volume are large enough to cause the separation of the "inert" C_6 hydrocarbons and the actually inert deuterated eluent $C^2H_2Cl_2$. Only half the pore volume of Kugelgel is accessible to cyclohexane. The mechanism of these separations will be discussed later in terms of negative adsorption[14], where the concentration of a sample is smaller in the adsorbed layer than in the bulk phase.

Effect of changing the water content of the eluent

If dichloromethane is actually adsorbed at the surface of the silica, it should be progressively removed on gradually increasing the concentration of a more polar substance in the eluent. To investigate this assumption we chose the microporous silica Kugelgel as the stationary phase and water as the polar moderator.

Freshly distilled dichloromethane normally has a water content of about 200 ppm. The separations illustrated in Figs. 2 and 3 were performed with "dry" dichloromethane (< 5 ppm of water).

The maximum pore volume (the difference between the elution volumes of $C^2H_2Cl_2$ and polystyrene) was measured in Figs. 2 and 3 using "dry" dichloromethane. In the following, θ is defined as the ratio of the pore volume available to a particular sample (with dichloromethane eluent with a particular water content) and of the maximum pore volume. These θ values with Kugelgel as the stationary phase are shown in Table II. The concentration of water in the eluent was increased to 1600 ppm. The samples include Fluorinert, which is even less polar than cyclohexane and may be a mixture of perfluorinated cyclohexane and other hexanes[15].

TABLE II

θ VALUES FOR "INERT" AND k' VALUES FOR RETARDED SAMPLES AS A FUNCTION OF THE WATER CONTENT OF THE CH_2Cl_2 ELUENT

Stationary phase: microporous silica Kugelgel. Flow-rate: 2 ml/min.

Sample	Water content (ppm)									
	<5	60	250	700	900	930	1050	1150	1300	1600
A*										
$C^2H_2Cl_2$	1.00	0.88	0.83	0.70	0.62	0.61	0.49	0.42	0.31	0.15
Benzene	0.85	0.75	0.68	0.56	0.48	0.47	0.38	0.32	0.22	0.10
Cyclohexadiene	0.84	0.75	0.68	0.55	0.48	0.47	0.37	0.32	0.22	0.09
Cyclohexene	0.64	0.59	0.54	0.45	0.42	0.40	0.32	0.28	0.16	0.06
Cyclohexane	0.45	0.45	0.42	0.38	0.37	0.36	0.29	0.24	0.13	0.05
Fluorinert	0.35	0.35	0.35	0.35	0.35	0.35	0.27	0.23	0.10	0.05
B*										
o-Nitroaniline	5.7	2.9	2.3	0.86	0.63	0.56	0.37	0.27	0.18	0.05
m-Nitroaniline	28	12	6.1	2.2	1.57	1.36	0.83	0.60	0.40	0.15

* In part A the θ values and in part B the capacity ratios (k') are given.

The columns in part A of Table II confirm that the accessible pore volume always decreases with decreasing sample polarity for a given water content.

As shown in the first line of Table II, the value of θ for the deuterated eluent decreases from 1 to 0.15 as the water content of the eluent is increased from *ca.* 5 to 1600 ppm. The available pore volume, for $C^2H_2Cl_2$ as the sample, is decreased by a factor of more than 6 on changing the water content of the eluent.

The results shown for Fluorinert in Table II are remarkable. Here the available pore volume is independent of the water content of the eluent up to 930 ppm ($\theta =$ 0.35). A further increase in the water content up to 1600 ppm results in a decrease in the available pore volume by a factor of 7 ($\theta = 0.05$). This effect is discussed later.

As demonstrated in part A of Table II, if the water content of the eluent is changed, the pore volumes for different samples can decrease by a factor of up to 20 (*i.e.*, from $\theta = 1$ to $\theta = 0.05$), although the samples are small enough to be "inert".

As can be seen in part A of Table II, the best separation of the "inert" samples is achieved if the eluent is dry (< 5 ppm of water).

In our experience, determining the capacity ratios, k', of retarded substances can be a more reliable method than Karl Fischer titration for determining small concentrations of water in a non-polar organic eluent. Part B (last two lines) of Table II shows the variation in the k' values of two nitroanilines as a function of the water content of the eluent.

Explanation of the results

The data in Table II are plotted in Figs. 4 and 5. The results for the cyclo-hexadiene as the sample are not shown in Fig. 4, because they are almost identical with those for benzene. In Fig. 4 the available pore volumes (as a percentage of the maximum available) are plotted as a function of the water content of the eluent. The slopes of these curves are constant up to *ca.* 950 ppm of water. The slopes decrease with decreasing polarity of the samples. For Fluorinert the line is horizontal. With an increasing water content of the eluent there is a sharp change (decline) in the slopes of
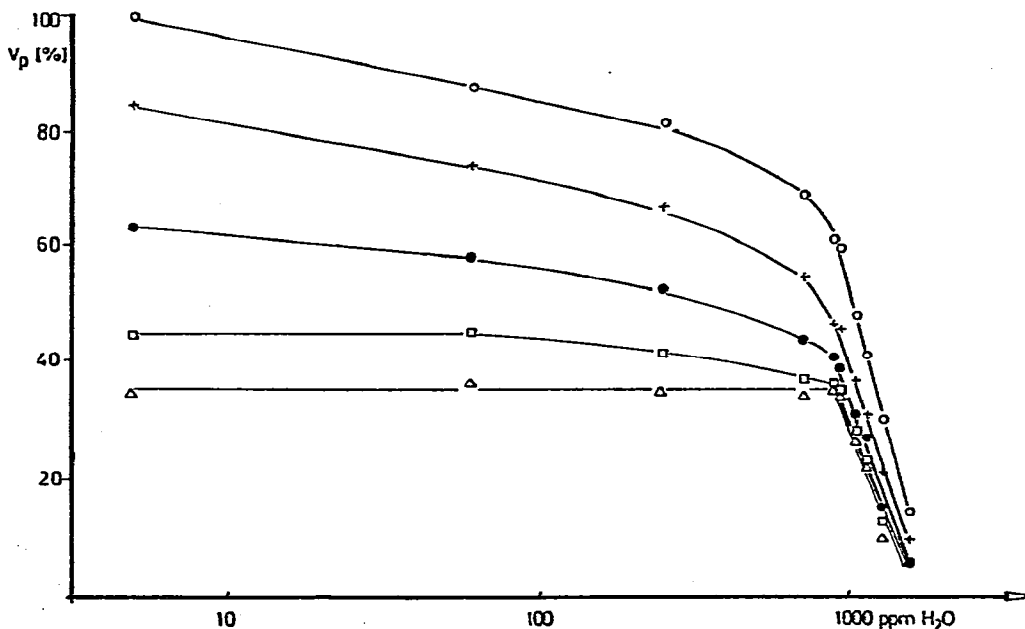

Fig. 4. Available pore volumes as a function of the water content of the eluent. Experimental conditions as in Fig. 2. Samples: O, C²H₂Cl₂; +, benzene; ●, cyclohexene; □, cyclohexane; △, Fluorinert.

all of these curves. The same sharp decline in the capacity ratios at the same water content of the eluent can be observed in Fig. 5, where the capacity ratios of nitro-anilines are shown as a function of the water content of the eluent.
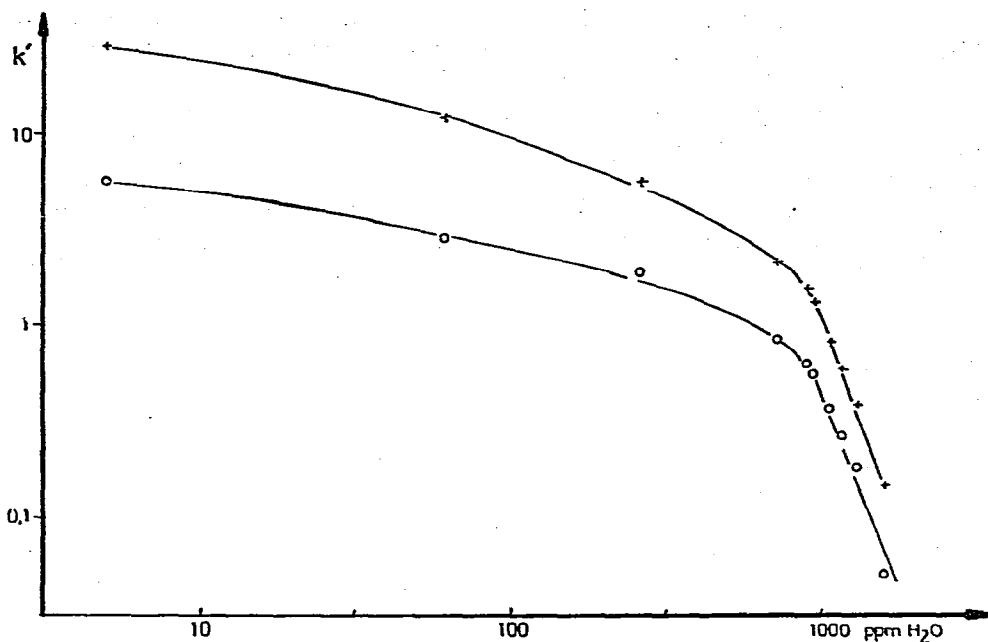

Fig. 5. Capacity ratios of nitroanilines as a function of the water content of the eluent. Experimental conditions as in Fig. 2. Samples: +, *m*-nitroaniline; ○, *o*-nitroaniline.

To explain the sharp changes in the slopes of the curves in Figs. 4 and 5, the adsorption isotherm of water from dichloromethane onto Kugelgel was measured at room temperature. The results are shown in Fig. 6. At a water content of 950 ppm *ca.* 70 mg of water is adsorbed per gram of silica. From Fig. 6 and Table I an average area requirement of about 30 $Å^2$ can be calculated for an adsorbed molecule. This area requirement seems to be reasonable for the localized adsorption of the polar water molecules on the surface of the polar silica. From this calculation and from the shape of the adsorption isotherm it is probable that the silica is covered with a mono-molecular layer if the water concentration in dichloromethane is around 950 ppm.

The results can be interpreted by using the following model. When the column is flushed with dry eluent, a monolayer of dichloromethane is adsorbed on the surface of the silica stationary phase. On increasing the water content of the eluent the dichloromethane molecules in the adsorbed layer are progressively replaced by the more polar water. At a water content around 950 ppm this exchange is complete and the silica surface is covered with a monolayer of water molecules. A further increase in the concentration of water in the eluent results in an increase in the thickness of the adsorbed layer and the micropores are gradually filled with water.

When the eluent is dry, the adsorbed dichloromethane layer becomes less accessible with decreasing sample polarity, the available pore volume decreasing with
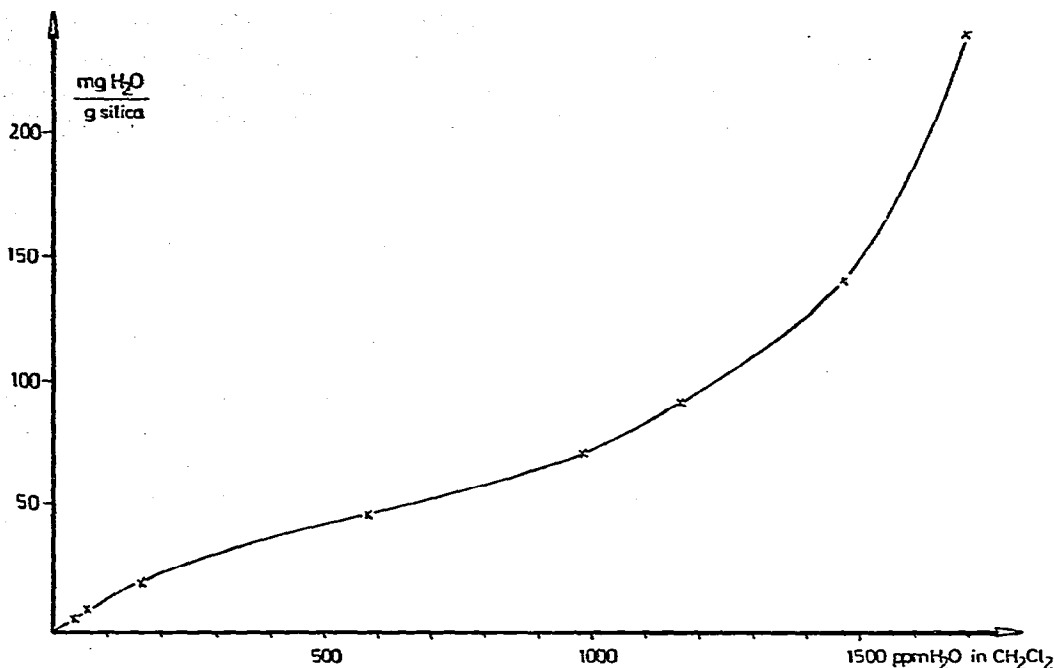
Fig. 6. Adsorption isotherm of water onto Kugelgel from dichloromethane at 24°C.

decreasing polarity. This availability is diminished even more when the dichloromethane eluent monolayer is displaced by a much more polar water layer.

The extremely non-polar Fluorinert is able to displace neither the dichloromethane nor the water molecules and cannot reach the surface of the silica. Therefore, the available pore volume is independent of the water content of the eluent, as long as the silica is covered only with a monomolecular adsorbed layer. The available pore volume for Fluorinert starts to decrease when the pores are filled with water, *i.e.* if the water content of the eluent is higher than 950 ppm.

Possible mechanism of separation

The main mechanism of separation shown in Figs. 2 and 3 cannot be conventional adsorption, because the C₆ hydrocarbons are eluted before the deuterated eluent sample. Size exclusion effects due to the different sizes of these molecules are also very improbable.

The separation can be explained if it is postulated that a monomolecular layer of eluent, which is in equilibrium with the bulk eluent, is adsorbed on the surface of the microporous silica. As the average pore diameters of the silicas are smaller than 12 Å, the "volume" of the monomolecular layer is not negligible compared with the whole pore volume. In order to be able to penetrate the whole of the pore volume a sample molecule must be able to displace eluent molecules from the monomolecular layer. Of the injected samples, only the deuterated eluent has the same chance as the eluent to occupy the whole pore volume, *i.e.*, to reach the silica surface. The probability of a sample molecule displacing an eluent molecule at the surface of the polar silica

increases with increasing polarity or polarizability of the sample. As can be seen in Figs. 2 and 3, cyclohexane, the least polarizable sample, is eluted first, *i.e.*, it is more excluded than the more polarizable cyclohexene, which in turn is eluted before the even more polarizable cyclohexadiene. This behaviour may be described as negative adsorption[14].

Separation of small molecules by SEC

In recent years attempts have been made to extend the applicability of SEC to smaller and smaller molecules. Majors and Pecsar[16] separated C_6–C_{28} alkanes on polystyrene gels using SEC.

If one wishes to use SEC with silica as the stationary phase for the separation of substances with molecular weights less than 1000, then the mean pore size of the silica has to be smaller than 30 Å, *i.e.*, microporous materials have to be used. The peak capacity[17] achievable with this method depends on the maximum available pore volume of the silica. However, with microporous stationary phases, as shown in Figs. 2 and 3, the pore volume available to the sample depends not only on its size, but also on its polarity or polarizability. The peak capacity becomes very small for non-polar samples, because of the (to them) inaccessible adsorbed eluent layer. Further, the specific pore volumes for microporous silicas are small, as shown in Table I.

This is also true for non-polar stationary phases, the effects of polarity or polarizability of the sample being, of course, reversed.

Inert samples in routine retentive chromatography

The totally permeating sample in SEC corresponds to the inert sample in "retentive" chromatography. It is the last sample for the exclusion mode and at the same time the first sample for the retentive mode. An inert sample must not be adsorbed at the surface of the stationary phase and must be able to penetrate the whole of the pore volume. In routine chromatography with polar stationary phases (*e.g.*, silica, alumina, chemically bonded polar phases) it is usually assumed that small molecules which are less polar than the eluent will be inert. The three C_6 hydrocarbons in Figs. 2 and 3 all fulfill the above conditions and yet they are separated from each other and from a fully inert peak, the deuterated eluent. As the pore diameter increases, the problem of the choice of the inert peak becomes less difficult. It is demon-

TABLE III

θ VALUES FOR "INERT" SAMPLES ON COMMERCIALLY AVAILABLE SUPPORTS

Eluent: "dry" dichloromethane (<5 ppm of water). Flow-rate: 2 ml/min. Stationary phases: LiChrosorb SI 100 silica (Merck, Darmstadt, G.F.R.), 10 μm; and Spherisorb A5Y alumina (Kratos, Trappenkamp, G.F.R.), 5 μm.

Sample	Stationary phase	
	Silica	Alumina
$C^2H_2Cl_2$	1.00	1.00
Benzene	0.97	0.94
Cyclohexane	0.90	0.90
Fluorinert	0.88	0.87

strated in Table III, however, that this problem is definitely not negligible if commercially available supports with mean pore diameters between 60 and 200 Å are used.

The silica SI 100 in Table III has a mean pore diameter of around 100 Å. All of the "inert" samples listed in Table III are eluted before the deuterated eluent. If benzene or Fluorinert is used to determine the retention time of the inert peak on a column packed with this silica, then the real hold-up time will be underestimated by 3% or 12%, respectively. This effect results in an intolerable error in calculating relative retentions, especially for samples with small capacity ratios.

As is demonstrated in the last column of Table III, very similar problems are encountered if the stationary phase is a commercially available alumina. It is always safest to use the isotopically labelled eluent for the inert peak in retentive chromatography, no matter whether the support is polar or a reversed phase.

Catalysis

The negative adsorption effects caused by varying the water content of the solvent could, under certain circumstances, be of some interest in contact catalysis, if polar microporous catalysts were to be used, the portion of the catalyst surface available for the compounds in the fluid reaction mixture being influenced by the varying water content of the medium.

REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1958.
2 L. R. Snyder, *Anal. Chem.*, 46 (1974) 1384.
3 R. P. W. Scott and P. Kucera, *Anal. Chem.*, 45 (1973) 749.
4 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 149 (1978) 93.
5 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 171 (1979) 37.
6 I. Halász and K. Martin, *Angew. Chem.*, 90 (1978) 954; *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 901.
7 I. Halász and K. Martin, *Ber. Bunsenges. Phys. Chem.*, 79 (1975) 731.
8 I. Halász, P. Vogtel and R. Groh, *Z. Phys. Chem., Neue Folge*, 112 (1978) 235.
9 J. Asshauer and I. Halász, *J. Chromatogr. Sci.*, 12 (1974) 139.
10 W. Böhme and H. Engelhardt, *J. Chromatogr.*, 133 (1977) 67.
11 H. Elgass, H. Engelhardt and I. Halász, *Z. Anal. Chem.*, 294 (1979) 97.
12 B. Bogdoll, W. Böhme, H. Engelhardt and I. Halász, *Z. Anal. Chem.*, 298 (1979) 349.
13 R. Endele, I. Halász and K. Unger, *J. Chromatogr.*, 99 (1974) 377.
14 S. J. Gregg and K. S. W. Sing, *Adsorption, Surface Area and Porosity*, Academic Press, New York, 1967.
15 B. Bogdoll, *Diplomarbeit*, Universität Saarbrücken, 1978.
16 R. Majors and R. Pecsar, *Varian Instrument Applications*, 11, No. 3 (1978).
17 J. C. Giddings, *Anal. Chem.*, 39 (1967) 1027.

EDITOR'S NOTE

This paper was reviewed by three referees, who pointed out that deuterated dichloromethane may behave quite differently compared to ordinary dichloromethane, due to a change in dipole moment. They also mentioned that several gas chromatographic separations of CH_2Cl_2 and $C^2H_2Cl_2$ have been reported in the literature and that deuterated and non-deuterated compounds have also been separated by thin-layer chromatography. One referee suggested an elegant method for checking whether $C^2H_2Cl_2$ can really be used as an inert marker:

"If the authors want to check on this, they might equilibrate a portion of the two solvents to obtain CH^2HCl_2 and inject this with $C^2H_2Cl_2$, to see if a single peak is obtained."

Also it was suggested that it might be useful to show a calibration curve of polystyrols of various molecular weights and then insert the three cyclohexanes to illustrate the "negative adsorption" visually.

Editor J. Chromatogr.

AUTHORS' REPLY

The probability that the retention of CH^2HCl_2 and $C^2H_2Cl_2$ differ is not likely to be outside the experimental error.

The idea of the referee is excellent and seems simple to realise. Fig. 1 is already the differential of the calibration curve as proposed by one of the referees.