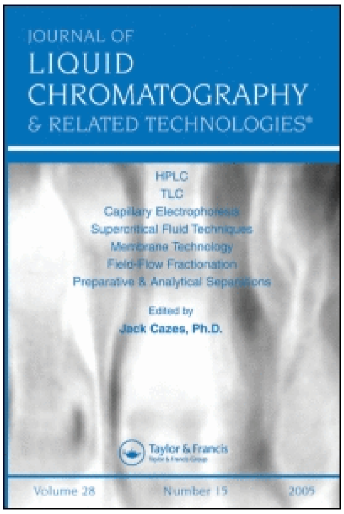

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SEPARATION PROPERTIES OF A POLYMERIC OCTADECYL SILICA GEL FOR LIQUID CHROMATOGRAPHY

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ABSTRACT.

The synthesis of a reversed phase material, based on polymeric bonding of octadecyltrichlorosilane to 5 um silica gel, is described. This phase, which exhibits unique properties with respect to the separation of polynuclear aromatic hydrocarbons (PAH), was tested and evaluated both in an analytical and an upscaled analytical mode. Effects of use of the phase over a long period of time are demonstrated and the material is characterized by surface coverage, resolution of critical isomers and separation capacity for a mixture of PAHs.

INTRODUCTION.

Following the introduction of organoalkylsilane modified silica gel introduced as packing material in

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HPLC (1,2), octadecylchlorosilane has been the most abundantly used reagent (3). Modification can be accomplished with mono-, di- or trichlorosilane (4). The choice of reagent together with variation in type of silica (structure, pore volume and surface area) will result in packing material having widely differing chromatographic properties. This has been well documented by several authors (5,6). However, this information can be very useful in the process of controlling the reaction in order to obtain column material of defined specificity. Thus, well defined silica gel and reaction conditions can be selected in order to achive a desired chromatographic behaviour.

Methods for synthesis of monomeric material, using chiefly a monochlorosilane, have been described in a large number of publications and have been reviewed (7). Polymeric material has usually been regarded as the result of uncontrolled reaction when a trichlororosilane together with unknown amounts of water have been utilized in the synthesis. However, the work of Sander and Wise has demonstrated the utility of controlled polymerisation of octadecyltrichlorosilane on silica, especially for the separation of the PAHs (8). For some commercial phases, exhibiting excellent properties in the separation of PAHs, it was demonstrated that the C_{18}-silane was polymeric bonded. In this case, it was shown that the pore volume is an important parameter to be considered in the chemical modification of silica gel with octadecylchlorosilane. If the volumes of the unmodified silica pores are too small, compared with the space occupied by the C_{18} chain molecules, the pores may become blocked and the separation efficiency be decreased (9). It is even more important to take into consideration the pore volume when a polymeric phase is synthesized. Water is added to a mixture of solvent, silane and silica gel, leading to a polymerisation of the silane, prior to binding to the silica gel (10). Thus, silica gels with large pore volume (30-50 nm) are considered to be the base material best suited for polymeric phases (10). The reproducibility of this type of phases has been discussed by several authors (8,11-15). In this article, the synthesis and performance of a particular polymeric ODS-phase, with particular separation properties, is described.

MATERIALS.

Chemicals

Octadecyltrichlorosilane (Merck) was used without futher treatment. Carbon tetrachloride, analytical grade (Merck), was distilled and stored over molecular sieves 4A. Tetrahydrofurane, methanol and acetone were all of analytical grade (Merck) and used as recieved. HPLC-grade acetonitrile (Rathburn) was used without further purification. Water used for HPLC was deionized and redistilled at the laboratory. The silica gel used for modification was Nucleosil 5u, 300Å (Macherey Nagel). Acid resistant stainless steel tubing and fittings (Valco or Svagelock) were used throughout the whole HPLC-column system.

Apparatus

Isocratic elutions were performed on a Spectra Physics HPLC instrument, model 3500 with a variable UV-adsorption detector. Solvent programmed chromatography was carried out on a Schimadzu Spectrophotometric LC-4A with an 8 ul variable UV-adsorption detector SPD-2AS. Column packing was effected by use of a Haskel pump. The detector signals from both instruments were A/D converted and registered by an ABC-800 microcomputer system, by which also all other signal treatment and plotting was performed.

METHODS

Modification of the silica gel

Reaction conditions outlined by Sander and Wise (8) for polymeric modification have been used with the following exceptions: 10 g of Nucleosil 5u, 300Å was treated with 33.5 ml octadecyltrichlorosilane and 2.5 ml water. After reaction, the modified silcia was washed with carbon tetrachloride followed by tetrahydrofurane, methanol, methanol/water (50/50) , methanol and tetrahydrofurane. The silica gel was transferred to a glass bottle fitted with a teflon seal. The various solvents were carefully mixed by shaking the sealed bottle by hand and then centrifuged to facilitate the removal of solvent. Finally, the material was dried to free flow under a stream of nitrogen and with careful heating.

Column packing

Columns of dimensions, 150 x 3 mm or 100 x 10 mm were packed by the slurry method. Modified silica was mixed with carbon tetrachloride during 30 seconds by ultrasonification, yielding a uniform and relatively stable slurry. 500 ml of acetone was pumped through the packing bomb followed by 500 ml of methanol (for the analytical column 250 ml portions were used). Each column was mounted in the HPLC instrument and equilibrated over night with acetonitrile or methanol.

RESULTS.

A number of ODS-phases based on the above outlined procedure were synthesized. The syntheses were performed with two different batches of base materials (silica gels), yielding two sets of columns with slightly different separation characteristics. Compared with other ODS materials (4), increased separation efficiencies and unique selectivities could be recorded, especially in the elution region for compounds containing up to five rings. In figure 1 is shown the separation of 10 PAHs on an analytical column (approximately 5 ng of each compound dissolved in methanol). Certain isomers, known as "critical isomeres" (16) were surprisingly well separated (for example benz(a)anthracene/chrysene, peaks 4 and 5).

In order to test reproducibility and batch to batch variations, a second set of phases, based on the same procedure of synthesis, but with another batch of silica gel from the same manufacturer, was synthesized. The separation of the same PAH mixture applied on the second column is shown in Fig. 2. A trend of decreasing capacity factors, but practically no change in selectivity factors, could be registered when compared to the first column material at the same chromatographic conditions (fig. 1). This discrepancy between the two ODS

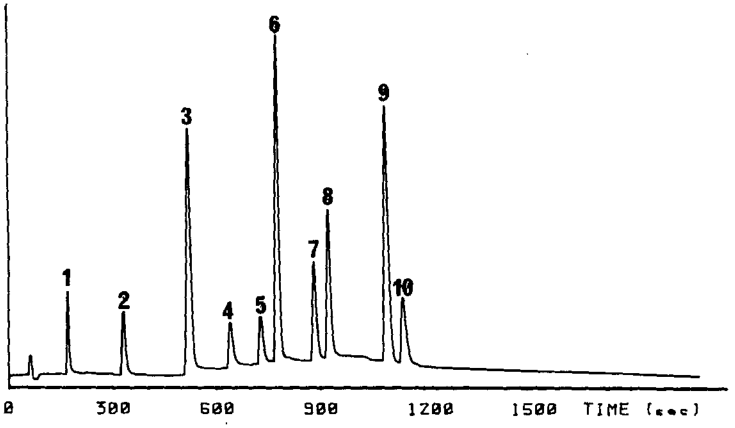

FIGURE 1. Separation of 10 PAHs on 150 x 3 mm analytical column with polymeric C_{18}. Separation of the 10-component mixture was performed by using gradient elution, 70-100% acetonitril in water over 30 min at 0.6 ml/min. Component identification : (1) phenanthrene, (2) anthracene, (3) benz(a)anthracene, (4) chrysene, (5) benzo(j)fluoranthene, (6) dibenz(a,c)anthracene, (7) benzo(k)fluoranthene, (8) benzo(a)pyrene, (9) dibenz(a,h)anthracene, (10) indeno(1,2,3-cd)pyrene.

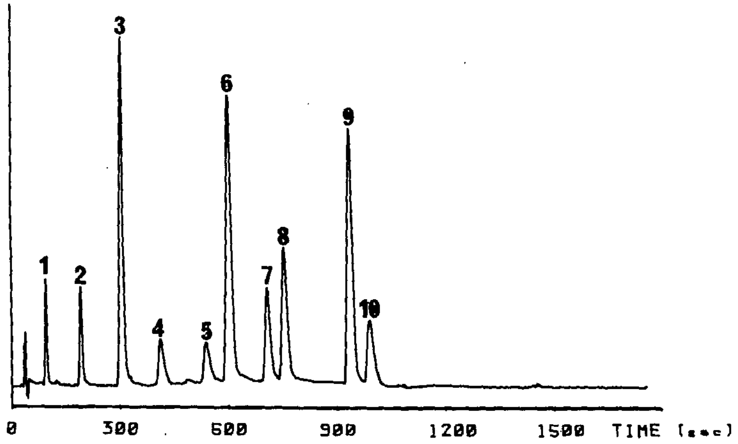

FIGURE 2. Separation ot 10 PAHs on a second batch of polymeric C_{18} phase. Chromatographic conditions and compounds as in figure 1.

batches is discussed in the following sections with respect to physical characteristics.

In addition to the chromatographic characterisation of a phase, elementary analysis and calculations of carbon coverage gives information of the degree of

TABLE 1.

Carbon content and surface coverage of the polymeric C-18 phase.

Batch	%C*	Coverage umol/m^2 **	
1	11.77 11.48	6.54	s=0.14
2	11.13 11.20	6.23	s=0.033

* Analysis was performed at NOVO microanalytical laboratory (Denmark)

** Coverage (17) = $$\frac{\%C \times 10^6}{((1200xN - \%C(M_w-1))S_{BET}}$$

N=18, M_w=331 (for polymeric C_{18} silane according to Sander and Wise (16))

S_{BET} = 100 m^2/g (according to the manufacturer).

polymerisation of modified silica. In Table 1 are presented the percentage of bonded carbon, measured by elementary analysis, and a calculation of surface coverage of the two batches listed.

A small difference, of surface coverage values of the two phases, can be discerned (significant on a 95%-level of confidience). However, even this difference is manifested in the chromatographic behaviour of the two batches of polymeric C_{18} phases.

Design and use of an upscaled analytical column.

In order to test the performance of the phase on a larger scale, a column of dimensions 100 mm x 10 mm was packed and evaluated. Sample capacity was tested by injections of up to 500ul of saturated solutions of PAHs. Overloading of the column with a mixture of pyrene/fluoranthene of various concentrations is illustrated in figure 3. A second column was packed with material from batch 2 and in the following discussion it is referred to as the new column. This column had a min HETP in the van Deemter plot of 18.83 um at a flow rate of 2.4 ml/min, measured for benz(a)anthracene. This is compared with the old column (HETP after use was 53 um) which has been used for two years and for several thousands of injections of crude synthetic mixtures. In these separations 100% methanol was principally used as eluent, due to solvent costs. Although acetonitrile usually is a better solvent for a polymeric column (with respect to chromatography). From time to time during a two year period, the column was rinsed with methylene chloride followed by methanol.

Linear capacity is calculated as the ratio of weights of the sample and the stationary phase when the k'-

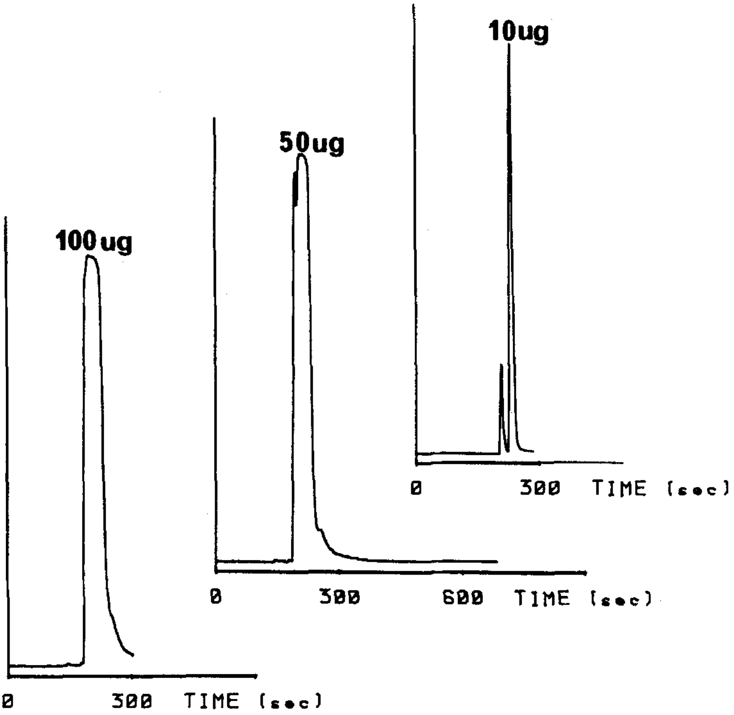

FIGURE 3. Separation of various concentrations of fluoranthene/pyrene on the "new" upscaled analytical column. Mobile phase was 80% acetonitrile in water.

value has been reduced by 10% (18). The value for the upscaled column was 10^{-5} g/g, which is a rather low value for a column used in a semi-preparative mode (19).

Table 2 is a comparison of the HETP values for the analytical columns and the upscaled columns. The higher values for the larger columns indicate that it is easier to pack a column with larger dimensions.

TABLE 2.

HETP values for the analytical and upscaled columns measured for B(a)A (k'= 3.40)

Batch	HETP(analytical) um	HETP(upscaled column) um
1	33	18.83
2	39	53*

* values after ageing.

Comparison of new and old polymeric C_{18} material.

Separation of critical isomers such as benz(a)-anthracene /chrysene and pyrene/fluoranthene was investigated on new and old materials, figures 4a,b and 5a,b. Separation could still be achieved on the old column, although the plate numbers had decreased considerably. Resolution between B(a)A/Chy is reduced on the old column and the k'-values are also decresed in spite of a heavier carbon load on this packing material. Sander and Weise states that k'-values for PAHs increase with increased carbon load of the polymeric phase (16). In agreement with this, k'-values are higher on the analytical column (batch 1, fig. 1) which has higher carbon load compared with batch 2, fig. 2. Futhermore, the used column exhibits decreasing

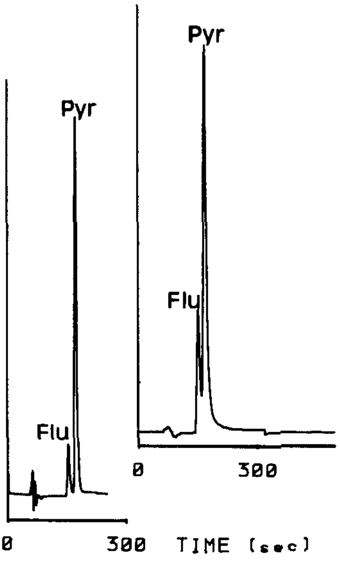

FIGURE 4. Separation of fluoranthene/pyrene (5ng/ul) on the "old" (a) and the "new" (b) 100 x 10 mm columns. Mobile phase was 80% acetonitrile in water.

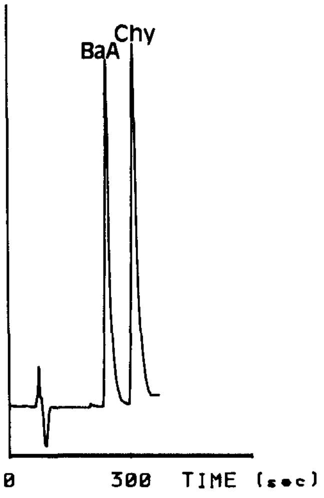

FIGURE 5a. Separation of benz(a)anthracene/chrysene (10 ng/ul) on the "old" column. Mobile phase 80% acetonitrile in water. R=2.86.

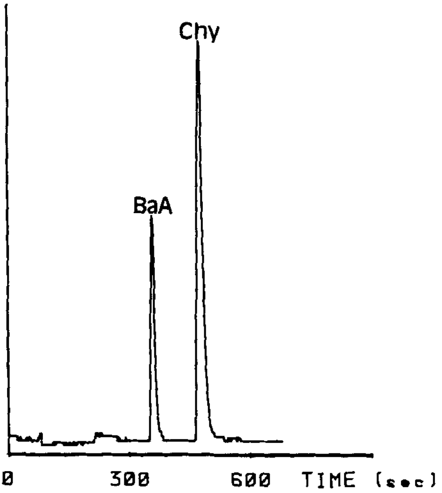

FIGURE 5b. Same chromatographic conditions as in 5a but on the "new" column. R=4.61.

k'-values, probably depending on a modification of the phase based on adsorption and reaction of the solute during two years.

In figure 6 is shown a plot of the number of plates versus the concentration of benzo(a)anthracene. The shape of the curve indicates that adsorption takes place for lower concentrations of solute for the used column. The number of theoretical plates for the used column has also decreased considerably.

The ageing characteristics can further be demonstrated by plotting the resolution of two solutes versus their concentration. In figure 7, this plot is shown for pyrene /fluoranthene, which demonstrates that the shapes of the curves are approximately the same, but the resolution is significantly lower for the older column.

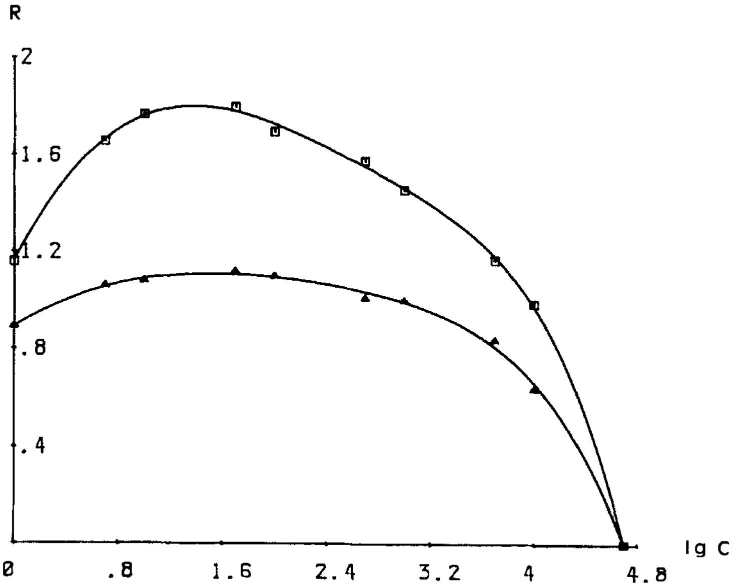

FIGURE 6. Resolution of fluoranthene/pyrene plotted against lg (conc). ◻ ="new" column, ▲ = "old" column.

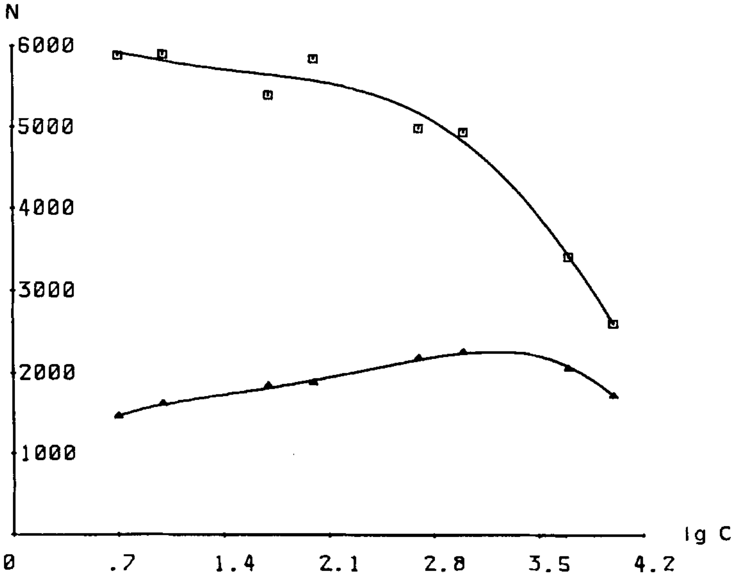

FIGURE 7. Variation in theoretical plate versus lg (conc.) for benz(a)anthracene. ◻ ="new" column, ▲ ="old" column.

DISCUSSION

It has been demonstrated that a fairly reproducible polymeric octadecyltrichlorosilane phase with particular chromatographic charateristics can be synthesized. If silica gels with better defined surface area, pore volume and particle size were obtainable from the manufacturers, the reproducibility of the phase could be even more increased. The efficiency of the phase very much depends on the physical dimensions and homogeneity of the base material. Upscaling of the column proved to give a better HETP, which can be explained by a better and more uniform packing performance of the larger column. The particular phase, decribed in this paper exhibited high separation efficiency with respect to PAHs but a rather poor linear capacity factor.

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REFERENCES

1. Stewart, H. N. M., Perry, S. G., A New Approach to Liquid Partition Chromatography, J. Chromatogr., 37, 97, 1968.

2. Locke, D. C., Chemically Bonded Stationary Phases for Liquid Chromatography, J. Chromatogr. Sci., 11, 120, 1973.

3. Majors, R.E., Barth, H.G., Lochmüller, C.H., Column Liquid Chromatography, Anal. Chem. 54, 323R, 1982.

4. Snyder, L.R., Kirkland, J.J. ed., Introduction to Modern Liquid Chromatography, John Wiley & Sons INC, 1979, p272.

5. Katz, E., Ogan, K., Selectivity Factors for Several PAH Pairs on C-18 Bonded Phase Columns, J. Liq. Chromatogr., 3, 115, 1980

6. Colmsjö, A., MacDonald, J., Column-Induced Selectivity in Separation of Polynuclear Aromatic Hydrocarbons by Reversed-Phase High-Performance Liquid Chromatography, Chromatographia, 13, 349, 1980.

7. Engelhardt, H., Ahr, G., Properties of Chemically Bonded Phases, Chromatographia, 14, 227, 1981.

8. Sander, L.C., Wise, S. A., Synthesis and Characterization of Polymeric C_{18} Stationary Phases for Liquid Chromatography, Anal.Chem., 56, 504, 1984.

9. Berendsen, G.E., Pikaart, K.A., de Galan, L., Preparation of various Bonded Phases for HPLC using monochlorosilanes., J. Liq. Chromatogr., 3, 1437, 1980.

10. Sander, L. C., Wise, S. A., The Influence of Substrate Parameters on Column Selectivity with Alkyl Bonded-Phase Sorbents, J. Chromatogr., 316, 163, 1984.

11. Kirkland, J. J., DeStefano J. J., Controlled Surface Porosity Supports with Chemically-Bonded Organic Stationary Phases for Gas and Liquid Chromatography, J. Chromatogr. Sci., 8, 309, 1970.

12. Kirkland, J. J., High Speed Liquid-Partition Chromatography With Chemically Bonded Organic Stationary Phases, J. Chromatogr. Sci., 9, 206, 1971.

13. Novotny, M., Bektesh, S. L., Grohmann, K. L., Chemically Bonded Stationary Phases with Variable Selectivity, J. Chromatogr., 83, 25, 1973.

14. Majors, R. E., Hopper, M. J.,Studies of Siloxane Phase Bonded to Silica Gel for Use in High Performance Liquid Chromatography, J. Chromatogr. Sci., 12, 767, 1974.

15. Verzele, M., Mussche, P., Monomeric and Polymeric Derivatization in Reversed Phase High-Performance Liquid Chromatogaphic MaterialsJ. Chromatogr., 254, 117, 1983.

16. Sander, L. C., Wise, S.A., Investigation of Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons, Advances in Chromatography, Giddings, Brown and Grusha, eds, 26, 1986, in press.

17. van der Venne, J. L. M., Rindt, J. P. M., Coenen, G. J. M. M., Cramers C. A. M. G., Chromatographia, 13, 11, 1980.

18. Snyder, L.R., Kirkland, J.J., ed, Introduction to Modern Liquid Chromatography, John Wiely & Sons INC, 1979, p26.

19. Farmakalidis, E., Murphy, P.A., Semi-preparative High-Performamce Liquid Chromatographic Isolation of Soya-bean Isoflavons, J. Chromatogr., <u>295</u>, 510, 1984.