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CHEMICALLY BONDED STATIONARY PHASES WITH VARIABLE SELECTIVITY

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SUMMARY

Polar chemically bonded phases with hydroxy, cyano and ester functional groups were prepared by the polymerization of trichloro-[3-(4-chloromethylphenyl)propyl]- and dichloromethyl-[3-(4-chloromethylphenyl)butyl]silanes on porous spherical siliceous beads and the subsequent selective modification of polymeric layers. Glycol phases were obtained by the oxidation of double bonds in bonded films of allylsiloxanes. The relative selectivities of the bonded phases for the nitroaniline isomers were measured and the chromatographic properties of substrates containing the same selective groups, but prepared from different monomers, were compared. The reproducibility of the preparation of cyano and hydroxy substrates was found to be better than that with the two-step modifications.

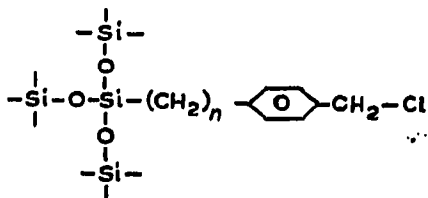
INTRODUCTION

Stable chemically bonded stationary phases are likely to have a major impact in future applications of modern liquid chromatography (LC). While the exact nature of solute interactions with chemically bonded phases is not yet known, their practical utility has been suggested¹. Among several approaches hitherto used for the preparation of bonded phases, the reaction of surface silanol groups with silanes seems to be the most realistic possibility. One of the most important features of such column substrates is that they are stable towards hydrolysis and are non-extractable and, therefore, suitable for gradient elution^{1,2}.

Silicone-based bonded phases can be prepared by surface reactions and polymerizations of chloro- or alkoxysilanes¹⁻⁸. Because of the variety of separation problems, it would seem necessary to have a number of substrates with different degrees of selectivity. However, only a limited number of silane monomers with selective properties is readily available. While the synthesis of novel silane compounds for

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specific use in bonded-phase LC would be highly desirable, it would also represent a tremendous amount of time-consuming work. A second approach consists of making suitable modifications of more readily available monomers. Ideally, the same initial monomers should be used for the preparation of substrates with different selectivities, as all reaction steps before the final selective modification can be more easily controlled. This would eliminate the problems due to different reactivity, degree of polymerization, crosslinking, etc., encountered with monomers of largely different natures. Chlorosilanes recently prepared by Parr and Grohmann⁹⁻¹¹ fulfill this requirement. Their reactions with a silica framework yield surface structures of the form



which can easily be modified⁸. Several modifications of the polymeric films prepared on the basis of this approach are reported in this paper. The selectivities of these packing materials are compared with those obtained by the polymerization of several alkoxysilanes as well as the glycol phases obtained by *in situ* oxidation of the double bond of allylsilicone layers. The chromatographic properties of selected substrates were further investigated.

EXPERIMENTAL

Preparation of chromatographic columns

The support material was Porasil C (37–75 μm) obtained from Waters Associates, Framingham, Mass., U.S.A. Its treatment and reactions with trichloro-[3-(4-chloromethylphenyl)propyl]silane and dichloromethyl-[3-(4-chloromethylphenyl)butyl]silane at 50° were described in an earlier paper⁸, except that the additional heat treatment at 100° was omitted. Exhaustive extractions of packings with different solvents were carried out before selective modifications. Similar conditions were also used when working with monochlorodimethyl-[3-(4-chloromethylphenyl)butyl]silane.

In order to introduce hydroxy groups into the surface structures, the terminal chlorine atoms were hydrolyzed in a slightly alkaline solution of sodium carbonate in a 1:1 mixture of tetrahydrofuran and water. To obtain an ester phase, the hydroxylated material was subjected to further reaction with benzoyl chloride in tetrahydrofuran in the presence of a small amount of pyridine as a catalyst. Cyano phases were prepared by the treatment of $\text{---C}_6\text{H}_4\text{---CH}_2\text{---Cl}$ residues with potassium cyanide in tetrahydrofuran and water.

In another series of experiments, a 1:1 mixture of allyltrichlorosilane and allylmethyldichlorosilane (5% of the weight of the support in tetrahydrofuran) was made to react with Porasil C at room temperature and the reaction product was extracted with a series of solvents of different polarity. The double bonds in the polymeric layer formed were further oxidized with a solution of 25% hydrogen peroxide in acetic

acid for 3 h. The reaction product was again purified with a series of solvents. According to Fieser and Fieser¹², such a modification should produce glycol structures. In a similar fashion, the oxidation of the double bonds was performed with the polymers obtained by the reaction with allyltriethoxysilane. N-(β -Aminoethyl)- γ -aminopropyltrimethoxysilane and β -cyanoethyltriethoxysilane were made to react with Porasil C at 50° for 20 h and extracted with the series of solvents. No further chemical modifications of these polymers were undertaken.

All chromatographic packings were dried after the extraction and packed into stainless-steel tubes (80 cm \times 2 mm I.D.) by a conventional dry-filling procedure and conditioned for 3 h at the flow-rate of 1.0 ml/min prior to chromatographic measurements.

LC measurements

All measurements were carried out on a Varian 4100 high-pressure liquid chromatograph with a UV detector and stop-flow injector. The mobile phase was a spectroquality *n*-heptane containing 1.5% of isopropanol as a "moderator". The chromatographic characteristics of Porasil columns with and without different bonded phases were measured with *o*-, *m*- and *p*-nitroanilines as standard solutes.

RESULTS AND DISCUSSION

Packings of different selectivities are needed for various separation problems. There are several general ways of preparing silicone polymeric layers of controlled selectivity. As shown by Novotny *et al.*⁸, the type of monomer, ratios of different monomers and the polymerization conditions may have considerable influence on the selectivity of such sorption materials through the change in polymer consistency. However, the selectivity given by the presence of certain functional groups within the polymeric film is a property that is more predictable and understandable. While it is likely that chemically bonded phases based on vastly different monomers may find applications in practical separation problems, more reliable information on the type of interaction between the solute and the stationary phase can be expected from the "tailor-made" modifications of the polymeric films (such as those obtained with chloro-[3-(4-chloromethylphenyl)alkyl]silanes). Table I shows average *k* values obtained with *o*-, *m*- and *p*-nitroanilines on bonded phases of different polarities obtained by the polymerization of both chloro- and alkoxy-silanes. As the retention of individual nitroaniline isomers on a polar stationary phase reflects to a large degree the extent of hydrogen bonding, the results obtained with hydroxy, ester and cyano phases are understandable. The substrate prepared by the polymerization of N-(β -aminoethyl)- γ -aminopropyltrimethoxysilane exhibits the greatest retention of the standard solutes of all of the studied monomers.

A different type of interaction is obtained with the glycol phases prepared from the allylchlorosilanes. While the retention of *p*-nitroaniline on these substrates is roughly comparable with that on the hydroxy phase, the selective retention of *m*-nitroaniline is noteworthy. No explanation, however, can be suggested at present for the drastically increased retention of *m*-nitroaniline on the phase prepared by polymerization of allyltriethoxysilane and subsequent oxidation. Here, obviously, factors other than polarity are significant.

TABLE I
AVERAGE RELATIVE SELECTIVITIES OF DIFFERENT PACKINGS OF CHLORO- AND ALKOXY SILANES

| Monomer | Modification of the stationary phase | Functional groups | <i>k</i> value | | |
|---|---|------------------------|------------------------|------------------------|------------------------|
| | | | <i>o</i> -Nitroaniline | <i>m</i> -Nitroaniline | <i>p</i> -Nitroaniline |
| None* | None | Surface silanol groups | 0.95 | 2.74 | 7.70 |
| Trichloro-[3-(4-chloromethylphenyl)propyl]silane and dichloromethyl-[3-(4-chloromethylphenyl)butyl]silane (1:1) | Hydrolysis with a slightly alkaline solution | Hydroxy | 0.98 | 3.01 | 8.62 |
| Same | Reaction with potassium cyanide | Cyano | 1.14 | 3.12 | 9.52 |
| Same | Hydrolysis and subsequent esterification of the hydroxy phase | Ester | 1.04 | 2.71 | 8.40 |
| Monochlorodimethyl-[3-(4-chloromethylphenyl)butyl]silane | Reaction with potassium cyanide | Cyano | 1.11 | 3.30 | 9.07 |
| Allyltrichlorosilane and allylmethyl-dichlorosilane (1:1) | None | Double bond | 1.00 | 3.16 | 7.40 |
| Same | Oxidation with H ₂ O ₂ in acetic acid | Glycol (epoxy?) | 1.09 | 5.35 | 8.95 |
| β -Cyanoethyltriethoxysilane | None | Cyano | 0.94 | 3.32 | 8.54 |
| N-(β -Aminoethyl)- γ -aminopropyltrimethoxysilane | None | Amino | 2.55 | 6.36 | 26.60 |
| Allyltriethoxysilane | Oxidation with H ₂ O ₂ in acetic acid | Glycol (epoxy?) | 0.83 | 24.40 | 9.15 |

* Unmodified Porasil used for comparative purposes.

Previously obtained results^{8,13} suggest that resinous films rather than "true liquids" are involved in bonded-phase chromatography. However, much work must be done in order to understand the nature of these substrates and the sorbent-solute interactions in such systems as well as to optimize the procedures for their preparation. As the penetration of the polar constituent of the mobile phase (isopropanol in this work) into the polymer layer is important for satisfactory chromatography, selective monomolecular layers prepared by the reaction of support materials with monochlorosilanes should be studied and compared with the "bulky" phases. The time of solvation of the polymeric films is usually long, which can present serious problems in gradient elution techniques. Three column materials were prepared with different silanes: β -cyanoethyltriethoxysilane, monochlorodimethyl-[3-(4-chloromethylphenyl)butyl]silane, and a mixture of trichloro-[3-(4-chloromethylphenyl)propyl]- and dichloromethyl-[3-(4-chloromethylphenyl)butyl]silanes. As the terminal chlorine atom in the side chain of the chlorosilanes was modified with potassium cyanide, all three

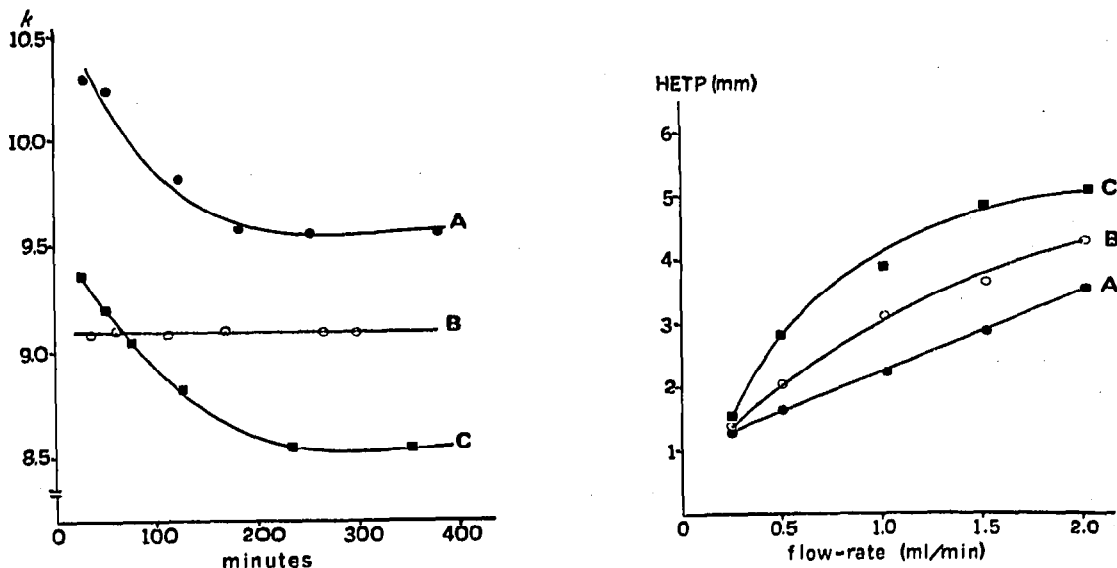


Fig. 1. Dependence of k values of *p*-nitroaniline on the equilibration time for different bonded phases possessing cyano groups. Conditions: columns (80 cm \times 2 mm I.D.) with cyano phases bonded to Porasil C (35–75 μ m); flow-rate 1.0 ml/min; room temperature. Monomers: A = 1:1 mixture of trichloro-[3-(4-chloromethylphenyl)propyl]- and dichloromethyl-[3-(4-chloromethylphenyl)butyl]-silanes; B = monochlorodimethyl-[3-(4-chloromethylphenyl)butyl]silane; C = β -cyanoethyltriethoxysilane.

Fig. 2. HETP versus flow-rate for different bonded phases possessing cyano groups. Conditions as in Fig. 1.

packings possessed selective cyano groups. The k values of *p*-nitroaniline as a function of the equilibration time (the flow-rate of *n*-heptane with 1.5% isopropanol was 1.0 ml/min) are shown in Fig. 1, and suggest the different natures of the surface layers obtained with various monomers. The selectivity of the packing obtained with the monochlorosilane is different from unmodified Porasil and compares favorably with the remaining cyano phases. Furthermore, its k value does not change with time due to solvation. Similar results were obtained in the series of oxidized allylsilanes (allyltriethoxysilane, a mixture of allyltrichloro- and allylmethyldichlorosilanes, and allyldimethylchlorosilane). HETP versus flow-rate curves for the three cyano phases are shown in Fig. 2. This graph is used only for comparative purposes, and higher column efficiencies can be obtained with particles of different character and size. The effects of concentration of the "moderator" on efficiency and selectivity as well as the influence of temperature are reported elsewhere⁹.

Reproducibility in the preparation of chemically bonded phases is a common problem. Naturally, the considerations of the monomer type, reaction conditions, further modifications, etc., are all important. Table II shows k values of nitroanilines on separately made hydroxy and cyano packings (both are one-step modifications). The reproducibility was worse with the ester (two-step modification) and glycol (oxidized allylsiloxanes) phases. The approach of variable selectivity based on the modifications of the terminal chloromethyl groups in the packings obtained with the monomers studied in this work may cover the complete range of selectivities needed

TABLE II
REPRODUCIBILITY OF THE PREPARATION OF CYANO AND HYDROXY PHASES

| Bonded phase | <i>k</i> value | | |
|---|------------------------|------------------------|------------------------|
| | <i>o</i> -Nitroaniline | <i>m</i> -Nitroaniline | <i>p</i> -Nitroaniline |
| Hydroxy phase prepared from a 1:1 mixture of trichloro-[3-(4-chloromethylphenyl)propyl]-silane and dichloromethyl-[3-(4-chloromethylphenyl)butyl]silane | 0.95 | 2.96 | 8.45 |
| | 1.00 | 3.06 | 8.80 |
| Cyano phase prepared from a 1:1 mixture of trichloro-[3-(4-chloromethylphenyl)propyl]-silane and dichloromethyl-[3-(4-chloromethylphenyl)butyl]silane | 1.16 | 3.08 | 9.50 |
| | 1.14 | 3.06 | 9.50 |
| | 1.13 | 3.18 | 9.55 |

for different separations when other suitable modifications become available. In addition, this method still maintains relative simplicity and reproducibility.

ACKNOWLEDGEMENT

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REFERENCES

- 1 J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971.
- 2 R. E. Majors, *Anal. Chem.* 45 (1973) 4.
- 3 E. W. Abel, Z. H. Pollard, P. C. Uden and G. Nickless, *J. Chromatogr.*, 22 (1966) 23.
- 4 H. N. M. Stewart and S. G. Perry, *J. Chromatogr.*, 37 (1968) 97.
- 5 C. J. Bossart, *ISA Trans.*, 7 (1968) 283.
- 6 W. A. Aue and C. R. Hastings, *J. Chromatogr.*, 42 (1969) 319.
- 7 J. J. Kirkland and J. J. DeStefano, *J. Chromatogr. Sci.*, 8 (1970) 309.
- 8 M. Novotný, S. L. Bektesh, K. B. Denson, K. Grohmann and W. Parr, *Anal. Chem.*, 45 (1973) 971.
- 9 W. Parr and K. Grohmann, *Tetrahedron Lett.*, (1971) 2633.
- 10 W. Parr and K. Grohmann, *Angew. Chem., Int. Ed. Engl.*, 11 (1972) 314.
- 11 K. Grohmann, *Ph. D. Thesis*, University of Houston, 1972.
- 12 L. F. Fieser and M. Fieser, *Organic Chemistry*, Heath and Co., Boston, 3rd ed., 1956, Ch. 3, p. 67.
- 13 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1971) 206.