Journal of Chromatography, 125 (1976) 115–127

CHROM. 9566

RECENT DEVELOPMENTS IN THE EVALUATION OF CHEMICALLY BONDED SILICA PACKINGS FOR LIQUID CHROMATOGRAPHY

K. K. UNGER, N. BECKER and P. ROUMELIOTIS
Fachbereich 8, Technische Hochschule, 6100 Darmstadt (G.F.R.)

SUMMARY

The basic variables and conditions in the synthesis of chemically bonded silica packings (CBSPs), obtainable by means of a surface reaction between porous silica and an organochlorosilane or organoethoxysilane as a modifier, yielding a dense monolayer of bonded functional groups, are discussed.

The CBSPs can also be prepared by a novel procedure termed bulk modification, which involves a co-hydrolysis and co-condensation of tetraethoxysilane or poly(ethoxysiloxane) and a corresponding organotriethoxysilane. In this way, the organic functional groups become a constituent of the bulk phase as well as of the surface.

The retention behaviour of solutes on columns packed with both types of packings was examined in normal and reversed-phase liquid–solid chromatography. Corresponding to the high surface concentration of functional groups, the capacity factors of solutes normalized to unit surface area of the adsorbent, k'/S_{BET}, were found to be relatively larger on bulk- than on surface-modified silica packings for a given bonded group. For *n*-alkyl bonded phases in reversed-phase chromatography, the plot of log k'/S_{BET} *versus* the number of carbon atoms in the *n*-alkyl chain was linear for each solute.

Further, it is shown that the selectivity of solutes on polar modified packings is largely controlled by the type of polar functional groups substituting the silanol groups.

INTRODUCTION

In the past 5 years, chemically bonded silica packings (CBSPs) have gained increasing importance in high-performance liquid chromatography (HPLC)[1–5]. Bonded phases offer some practical advantages in use compared with liquid stationary phases. The most substantial feature, however, is their potential for high separation selectivities.

CBSPs are usually prepared by means of surface reactions between a given silica support and an appropriate modifier, yielding a phase that is covalently bonded to the siloxane matrix. As the selectivity of bonded phases is largely controlled by their chemical and geometrical structure, our primary objective was to attain bonded species with a defined and reproducible composition.

This paper deals with the principles of surface modification of silica and with the conditions for obtaining a dense monolayer of functional groups bonded via an Si–O–Si–C linkage. In addition to surface modification, a procedure termed bulk modification is presented, by which CBSPs can be prepared without any post-treatment. The characteristics of surface- and bulk-modified silica packings are discussed and the retention behaviour of solutes on both types of packings has been investigated in different modes of liquid–solid chromatography (LSC).

EXPERIMENTAL

Synthesis of bonded packings

Surface modification. Surface modification involves a reaction between completely hydroxylated porous silica and an appropriate modifier such as organochlorosilanes or organoethoxysilanes.

Silica microspheres in narrow sieve fractions prepared by means of the poly(ethoxysiloxane) procedure were used as supports[6–8]. Most of the silane reagents were commercial products (Dynamit-Nobel, Troisdorf, G.F.R.; Wacker-Chemie, Burghausen, G.F.R.; E. Merck, Darmstadt, G.F.R.), while some of them were prepared by the appropriate Grignard reactions. All reagents were purified by fractional distillation under vacuum and stored in closed glass ampoules. Purity was controlled by means of gas chromatography.

Ten-gram charges of silica and an ampoule containing a molar excess of the modifier were placed in a specially designed glass apparatus[9]. After prolonged heating of the silica at 523 °K under high vacuum, the ampoule was broken and the silane was condensed on the silica sample. The wetted silica then was heated at 20 °K below the boiling point of the corresponding silane, mostly in the range between 473–523 °K, for 8 h in dry nitrogen atmosphere. Finally, the product was washed with diethyl ether and again subjected to a vacuum treatment at 473 °K.

Bulk modification. Bulk modification consists in a co-hydrolysis and co-condensation of tetraethoxysilane (TES) and poly(ethoxysiloxane) (PES), respectively, with an organotriethoxysilane as modifying agent. Two procedures were applied as follows, depending on whether TES or PES was used as the starting material.

In Procedure 1, a given portion of organotriethoxysilane and TES were dissolved in ethanol. Addition of dilute hydrochloric acid started the partial hydrolysis and co-condensation, forming a poly(organoethoxysiloxane) (POES). By evaporation of ethanol, a viscous liquid of POES was obtained. The POES was then emulsified in a water–ethanol mixture. While stirring vigorously, a basic catalyst of known concentration was added, which completed the hydrolysis and polycondensation, yielding beads of organosilica hydrogel. After washing and dehydration, the beads became porous, containing covalently bonded organic groups in the bulk as well as at the surface.

As an example, consider bulk-modified silica with benzyl groups:

(1) Preparation of poly(benzylethoxysiloxane):

Charge: 312 ml (≡ 1.4 mole) of tetraethoxysilane, 155 ml (≡ 0.6 mole) of benzyltriethoxysilane, 250 ml (≡ 4.3 mole) of ethanol and 35 ml of 0.01 *N* hydrochloric acid (≡ 1.94 mole of H_2O).

Yield: 240 ml of poly(benzylethoxysiloxane), $MW_n = 700$.

(2) Preparation of benzyl silica beads:

Charge: 120 ml of poly(benzylethoxysiloxane) (≡ 1 mole of SiO_2), 60 ml (≡ 0.51 mole) of cyclohexane, 90 ml (≡ 1.55 mole) of ethanol, 270 ml (≡ 15.0 mole) of water and 50 ml of concentrated ammonia (≡ 0.67 mole).

Conditions: room temperature; stirring speed, 2000 r.p.m.

In Procedure 2, poly(ethoxysiloxane) (PES) was prepared by means of partial hydrolytic polycondensation of TES in a dilute acidic medium[6,7]. In a subsequent step, a certain amount of organotriethoxysilane was dissolved in PES and the solution was emulsified in a water–ethanol mixture. By adding a basic catalyst while stirring, the liquid droplets were converted into gelatinous beads. The reactions involved consisted in the total hydrolysis of the ethoxy groups of PES and of the modifier followed by a co-condensation of silanol groups. The organosilica hydrogel beads were washed and dried, yielding porous microparticles containing covalently bonded organic groups.

As an example, consider bulk-modified silica with 1,2-diol-3-propoxypropyl groups:

(1) Preparation of poly(ethoxysiloxane):

Charge: 445 ml (≡ 2.0 mole) of tetraethoxysilane, 233 ml (≡ 4.0 mole) of ethanol and 40 ml of 0.01 *N* hydrochloric acid (≡ 2.2 mole of H_2O).

Yield: 240 ml of poly(ethoxysiloxane), $MW_n = 800$.

(2) Preparation of bulk-modified organosilica:

Charge: 120 ml of poly(ethoxysiloxane) (≡ 1 mole of SiO_2), 120 ml (≡ 0.43 mole) of 1,2-epoxy-3-propoxypropyltriethoxysilane, 20–90 ml (≡ 0.17–0.8 mole) of cyclohexane, 160 ml (≡ 2.74 mole) of ethanol, 480 ml (≡ 26.6 mole) of water and 10–50 ml of 1.0 *N* sodium hydroxide (≡ 0.01–0.05 mole) or 6–150 ml of concentrated ammonia (≡ 0.08–2.0 mole).

Characterization of packings

The particle size was characterized by the quantity dp_{50}, which corresponds to the dp value at 50% of the cumulative undersize distribution. The specific surface areas, S_{BET}, of the packings were obtained from nitrogen sorption measurements at 77 °K according to the BET method using $A_m (N_2) = 0.162$ nm^2 per molecule[10]. Pore structure parameters such as the specific pore volume, V_p, and the pore volume distribution, *PVD*, were obtained from the nitrogen desorption isotherm applying the method of Brunauer *et al.*[11]. The mean pore diameter, D, corresponds to the most frequent D value of the differential *PVD*.

The types of bonded functional groups were identified by means of infrared spectroscopy using transmission as well as the attenuated total reflection technique[12]. The surface concentration, α, of surface-bonded species was calculated from carbon analysis and the specific surface area:

$$\alpha_{exp.} \,(\text{mole/m}^2) = \frac{w}{M \cdot S_{BET}} \tag{1}$$

where w = weight of functional group (grams per gram of adsorbent), M = molar weight of the bonded functional group (g/mole) and S_{BET} = specific surface area of the starting support corrected by the weight increase due to modification (m²/g).

The surface concentration of hydroxyl groups on the original and modified silicas was estimated by means of isotopic exchange with tritium-labelled water[13].

Column packing techniques

Columns were packed using the slurry technique. The slurry made by ultrasonic mixing of silica and tetrachloroethane was pumped rapidly at a constant pressure of 400 bar through the column, which was closed at the end by stainless-steel screens.

Chromatographic measurements

The liquid chromatograph was a DuPont Model 830 instrument with a fixed-wavelength UV detector (254 nm, 8-μl cell volume). The columns used were of length 25 cm and I.D. 4.2 mm, made of precision-bore Type 304 stainless-steel tubing (Nennen Chemicals, Dreieichenhain, G.F.R.). Empty columns were carefully cleaned before use. Porous stainless-steel plugs (Type PSSH) of 1/10-in. thickness (Pall, Sprendlingen, G.F.R.) were fitted in the column outlet. Connections were made with 1/4-in. Swagelok fittings.

Chromatographic solvents and samples were of analytical-reagent grade (E. Merck). Samples were injected with a Type HP 305 5-μl syringe (Schmidt, Hamburg, G.F.R.).

RESULTS AND DISCUSSION

Modes in CBSP formation

The CBSPs should fulfill the following requirements:

(1) in order to prevent a mixed retention mechanism, the bonded phases should completely cover the original silica surface;

(2) they should exhibit a defined and reproducible composition;

(3) they should be available with graduated polarity in order to perform separations in normal and reversed-phase LSC;

(4) the bonded phases should have high chemical and thermal stability.

It has been found that phases with an Si–O–Si–C linkage possess the highest degree of variation with respect to polarity of functional groups. They are also the most stable compared with those containing Si–N and Si–O–C linkages. The Si–O–Si–C linkage can be obtained by either surface or bulk modification.

Surface modification. As a result of surface modification, a chemisorbed monolayer, multilayer or polymer layer can be formed. For simplicity, all of these forms are termed phases, although this terminology is not correct from the physico-chemical point of view. Polymer layers are obtained, for instance, by adding a polymerizing agent to the silica support[1,14]. Multilayers can be built up by repeated reactions between a modifier and a previously chemisorbed layer that has reactive groups[1]. In the preparation of both types, difficulties arise in controlling the composition, the extent of cross-linking and the homogeneity of the layers. The simplest situation is expected for monolayer formation, which occurs according to the following equation:

$$\equiv \text{Si–OH} + \text{X–Si–R} \rightarrow \equiv \text{Si–O–Si–R} + \text{HX} \qquad (2)$$

where X = Cl, OCH_3 or OC_2H_5 and R = organic radical. Before such a reaction is carried out, two conditions should be satisfied with respect to the surface of the silica support:

(1) the support should have the maximum number of hydroxyl groups per unit surface area, *i.e.*, a completely hydroxylated surface;

(2) physisorbed water has to be quantitatively removed from the surface, otherwise the modifier may be hydrolyzed and condense, yielding a physisorbed organosilicon polymer.

These conditions are fulfilled after prolonged treatment of a hydrated silica support at 473 °K under vacuum[15]. Then, the amount of physisorbed water will be negligibly small and the surface concentration of hydroxyl groups amounts to about 8.0 μmole/m^2 or 4.8 hydroxyl groups/nm^2 (refs. 12 and 20).

In order to achieve maximum conversion, a molar excess of the modifier has to be employed. As the reaction rate is controlled by diffusion of the reactant to the active surface sites within the porous particles, the reaction temperature has to be as high as possible and the reaction time should be long enough for maximum conversion to be attained. However, the temperature should not exceed about 523 °K because otherwise dehydroxylation of hydroxyl groups takes place, with the formation of unreactive siloxane bonds. One must also bear in mind that, depending on the type of modifier, its thermal decomposition becomes appreciable at high temperatures.

The introduction of a certain organic functional group may be possible by using one of the three homologues R_nSiX_{4-n} $(1 \leqslant n \leqslant 3)$. According to the number of reactive Si–X groups, they are termed monofunctional (R_3SiX) bifunctional (R_2SiX_2) and trifunctional ($RSiX_3$) modifiers. The stoichiometry of the surface reaction (eqn. 2), expressed by the factor F, will be strongly determined by the functionality of the modifier; F is the ratio of the number of moles of hydroxyl groups reacted to the number of moles of modifier reacted.

The stoichiometry of surface reactions with various modifiers has been studied extensively using different techniques[12,16,17], and the results are summarized in Fig. 1. Except in the trivial case of the monofunctional modifier with only one reactive Si–X group per molecule, the factor F has been found to be $1 \leqslant F \leqslant 2$ for the bi- and trifunctional modifiers; $F = 3$ could never be established experimentally and seems highly improbable for steric reasons. In conclusion, using bi- and trifunctional modifiers such as R_2SiX_2 and $RSiX_3$ in surface modification, one or two Si–X groups per bonded functional group remain unreacted. After treatment with water, hydrolysis takes place and additional hydroxyl groups will be formed, having about the same concentration as that of the organic functional groups. The presence of organohydroxyl silyl instead of organosilyl groups may affect the retention behaviour of solutes and also influence the stability of the packing in aqueous solutions at $pH > 7$.

Bulk modification. The basic idea of this procedure consists in the introduction of an organic functional group, R, to the silica surface by means of co-hydrolysis and co-condensation between tetraethoxysilane and poly(ethoxysiloxane) as starting material and a corresponding organotriethoxysilane as modifier. The gel obtained consists of a siloxane network, in which silicon atoms are attached to R by a stable Si–C bond. Thus, the organic functional groups are a part of the framework and also grafted at the surface.

It is worth noting that the total hydrolysis and condensation of organotri-

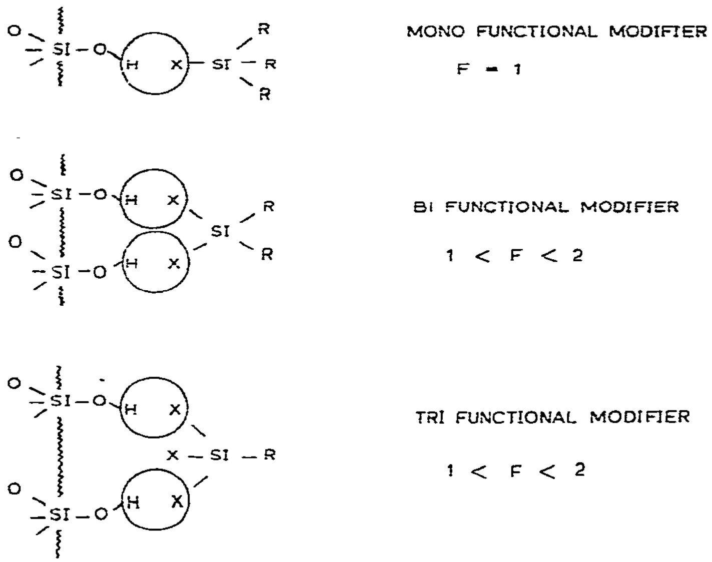


Fig. 1. Stoichiometry of surface reactions between hydroxyl groups at the silica surface and modifiers R_nSiX_{4-n} ($1 \leqslant n \leqslant 3$), which differ in the number of reactive Si–X groups.

ethoxysilanes alone yield organogels in the form of finely divided powders that are not suitable as packings for LC. Thus, a sufficiently large siloxane content in the final product is necessary for obtaining mechanically stable porous organosilica microparticles. On the other hand, the content of organic groups should not be too low, in order to ensure a significant variation of the bulk and surface properties of the packing. In practice, the maximum amount of organotriethoxysilane in tetraethoxysilane is limited to about 30 mole-%. At higher contents, appreciable swelling of the product occurs. As shown under Experimental, two procedures were developed for the bulk modification, which differ only in the means of introducing the modifier. According to Procedure 1, a bulk-modified silica with bonded benzyl groups was prepared and was tested as a reversed-phase packing. The content of benzyl groups in the final support could be established simply by the ratio of TES to benzyltriethoxysilane. Reactions at the benzyl organosilica, such as sulphonation, revealed that more than 50% of the total amount of benzyl groups could be substituted and hence are accessible at the surface.

A hydrophilic material was obtained by means of Procedure 2. In the hydrolysis and condensation during the second step, using sodium hydroxide as a catalyst, the epoxy groups of the modifier were cleaved, yielding a 1,2-dihydroxy-3-propoxypropylsilyl group. When ammonia was employed as a basic catalyst, instead of a 1,2-diol a hydroxylamine structure was obtained. The ratio of amine to hydroxyl groups was found to be a function of the concentration of ammonia.

The two procedures simultaneously permit the control of the pore structure parameters of the microbeads. The specific surface area, mainly determined by the type and the concentration of the basic catalyst, could be varied reproducibly between 100 and 500 m^2/g. As shown previously[7], the specific pore volume and the mean pore diameter are a function of the mean molecular weight of the intermediate product (PES or POES). In order to increase the porosity of the beads up to about 85%, cyclohexane was added to PES and POES as a volume modifier. As the main interest in these packings is their application in LC, detailed information about the variation of the chemical composition and that of the pore structure will be given in a separate paper.

Characteristics of packings

Identification of surface species. The primary objective relates to the type of surface species. Identification is preferably made by means of infrared spectroscopy using transmission and the attenuated total reflection technique[12,18] or by means of mass spectrometry[19]. Chemical bonding can easily be proved by gas chromatographic analysis of a suitable solvent previously used as an extractive. When the surface functional groups contain atoms other than carbon and hydrogen, *e.g.*, nitrogen or halogens, the molar ratio of C:N from elemental analysis can be estimated and compared with that of the expected or predicted ratio.

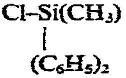
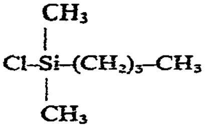
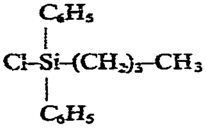
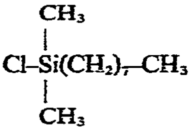
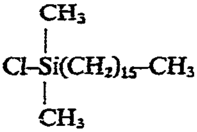
Surface coverage. Most of the commercial CBSPs are only characterized by their carbon content, which gives poor and incomplete information. The most valuable parameter is the surface concentration of bonded groups, $\alpha_{exp.}$, calculated according to eqn. 1. This approach has the advantage of comparing products of different surface concentrations, irrespective of their origins and their specific surface areas. Furthermore, $\alpha_{exp.}$ is also a measure of the completeness of surface coverage. It could be shown by careful experiments that on increasing the reaction temperature and duration, $\alpha_{exp.}$ will reach a certain limit that may indicate a dense surface coverage[20]. These values are listed for different reversed-phase packings in Table I.

It has been shown that the $\alpha_{exp.}$ value for a given functional group varies within 10% for different silica supports. The surface concentration of the smallest modifier molecule available, trimethylchlorosilane, is about 4.2 μmole/m^2. This means that only 50% of the total hydroxyl groups have reacted. Nevertheless, the surface can be considered as a dense layer of trimethylsilyl groups shielding the remaining hydroxyl groups. The value of 4.2 μmole/m^2 corresponds fairly well to the theoretical value, $\alpha_{theor.}$, calculated from the molecular cross-sectional area of a trimethylsilyl group[21]. It is interesting to study the influence of the bulk volume and the chain length of the modifier on its maximum surface concentration. For bonded C_n *n*-alkyl groups ($n > 4$), $\alpha_{exp.}$ changes only slightly with increasing chain length; the average value for $4 \leqslant n \leqslant 16$ is $\alpha_{exp.} = 3.5\,\mu$mole/m^2 ± 15%. In contrast, the substitution of methyl groups by bulky phenyl groups in *n*-alkyl groups drastically reduces the $\alpha_{exp.}$ values, as shown in Table I. Special problems arise in the evaluation of $\alpha_{exp.}$ of bulk modified CBSPs, because the carbon content accounts for both bulk and surface functional groups. For two reasons, substitution reactions of surface functional groups are not an effective means in the determination of $\alpha_{exp.}$: (1) the accessibility of surface functional groups may be restricted by hindered diffusion of the reactant and (2) the chemical stability of the Si–C bond must be taken into account. An estimation seems to be possible by means of isotopic exchange.

In LC, the conversion has sometimes been expressed as the ratio of the hydroxyl concentration present before reaction to that after the reaction, assuming a certain stoichiometry. However, this quantity was not measured neither before or

TABLE I

DATA ON A SERIES OF SURFACE-MODIFIED REVERSED-PHASE SILICA PACKINGS

Modifier	*Bonded organic functional group*	*Surface concentration, $\alpha_{exp.}$ (μmole/m^2)*	
		A^*	B^{**}
$\mathrm{Cl{-}Si(CH_3)_3}$	Trimethylsilyl	4.5	4.1
 $\mathrm{Cl{-}Si(CH_3)_2(C_6H_5)}$	Dimethylphenylsilyl	2.6	—
 $\mathrm{Cl{-}Si(CH_3)(C_6H_5)_2}$	Diphenylmethylsilyl	—	—
$\mathrm{Cl{-}Si(C_6H_5)_3}$	Triphenylsilyl	1.5	—
 $\mathrm{Cl{-}Si(CH_3)_2{-}(CH_2)_3{-}CH_3}$	*n*-Butyldimethylsilyl	3.6	3.7
 $\mathrm{Cl{-}Si(C_6H_5)_2{-}(CH_2)_3{-}CH_3}$	*n*-Butyldiphenylsilyl	1.8	1.7
 $\mathrm{Cl{-}Si(CH_3)_2(CH_2)_7{-}CH_3}$	*n*-Octyldimethylsilyl	3.8	3.4
 $\mathrm{Cl{-}Si(CH_3)_2(CH_2)_{15}{-}CH_3}$	*n*-Hexadecyldimethylsilyl	3.4	3.0

* Silica support, $S_{BET} = 211$ m^2/g.
** Silica support, $S_{BET} = 301$ m^2/g.

after the reaction. It should be noted that in all evaluations the dense coverage of organic functional groups can be estimated by simple chromatographic measurements. For instance, reversed-phase packings can be tested as follows:

(1) Use the CBSP as a polar adsorbent with dry *n*-heptane as a mobile phase. As a result of the dense coverage, the capacity factors of different solutes should be $k' \approx 0$. Methanol, diethyl ether and acetone should show completely symmetrical peaks and also have $k' \approx 0$.

(2) An additional treatment of the modifier with trimethylchlorosilane should not increase the carbon content and change the chromatographic properties according to point (1).

TABLE II

VARIATION OF PORE STRUCTURE PARAMETERS OF SILICA BY MEANS OF SURFACE MODIFICATION

Type of packing	*Specific surface area*		*Specific pore volume*		*Most frequent pore diameter, D (nm)*
	S_{BET} (m^2/g)	$-\Delta S_{BET}$ (%)	V_p (ml/g)	$-\Delta V_p$ (%)	
Original SiO_2	300	—	1.32	—	12.6
Octylsilyl groups bonded	201	33	0.82	38	11.4
Octadecylsilyl groups bonded	139	54	0.52	61	7.0; 11.4

Pore structure. It is sometimes neglected that surface modification also changes the pore structure of the support. Theoretically, the attachment of a chemisorbed layer to a mesoporous silica leads to a diminution of the mean pore diameter by twice the thickness of the layer. As a result, the specific surface area, S_{BET}, and the specific pore volume, V_p, also decrease[22]. An example is given in Table II for two reversed-phase packings, showing that the decrease in S_{BET} and the more pronounced decrease in V_p increase with the chain length of the modifier. One would expect that with decreasing S_{BET} of the packing, the capacity factors of solutes would also decrease. However, the capacity factors of solutes under given conditions are larger on C_{18}-bonded than on C_8-bonded phases. This means that the first effect is over-compensated by doubling the chain length of the *n*-alkyl group (see *Non-polar CBSPs*).

Whereas in the preparation of reversed-phase packings S_{BET} decreases considerably, bulk-modified reversed-phase materials will be obtained with relatively high surface areas. Again, comparing the bulk- and surface-modified packings containing benzyl groups, the large difference in the capacity factors is mainly due to the higher surface concentration of benzyl groups in the bulk-modified material.

Another point of view that has not been investigated up to now is related to the question in which way the drastic variation in pore structure on surface modification changes the mass transfer of solutes in the stationary phase.

Stability. The chemical stability of CBSPs is primarily determined by that of the silica matrix. Hence, these packings can be employed in aqueous solutions between pH 0 and 8.5. In this range, the Si–C bond is fairly stable, depending slightly on the type of organic functional group attached to silicon. The stability of a CBSP, particularly in salt solutions at $pH > 7$, is very sensitive to the presence of silanol groups, which act as weakly acidic ion-exchange groups. For this reason, we prefer a dense monolayer of functional groups instead of a partial coverage. In most instances, thermal stability is adequate up to a temperature of 473 °K. The stability can easily be determined by means of thermogravimetry.

Retention behaviour of solutes on surface- and bulk-modified silica packings

Polar CBSPs. A series of polar bonded silica packings made by surface modification are now commercially available[23]. In this study, only one type was investigated as a representative, having 1,2-dihydroxy-3-propoxypropylsilyl functional groups. Chemical bonding was done by both surface and bulk modification. Regarding the surface composition of these phases, the protons of silanol groups are replaced by propoxypropyl-carrying dihydroxy groups at the end. Compared with silanol groups, the carbinol group is still polar but less acidic than Si–OH. However, the carbinol groups constitute dihydroxy groups, and this may increase the strength of the interaction between the adsorbent and the solutes. Indeed, it could be established that the capacity factors of solutes normalized to unit surface area of the adsorbent are generally higher on polar modified packings than on the original silica (Table IV).

As the silanol concentration on modified packings is vanishingly small, the solute capacity factors are expected to be less sensitive to a variation of the water content of the mobile phase, which could be established experimentally[24]. A slight reversed-phase effect on the modified packings was observed, which may be due to the influence of the long alkyl chain separating the diol group from the surface. This is also evidenced by the reversed elution order of carboxylic acids, differing in pK_a values (Table III).

TABLE III

CAPACITY FACTORS OF CARBOXYLIC ACIDS ON A COLUMN PACKED WITH A SURFACE-MODIFIED SILICA PACKING CONTAINING 1,2-DIHYDROXY-3-PROPOXYPROPYL GROUPS

Column length, 20 cm; I.D., 4 mm.

Sample	pK_a	*Acetonitrile–methanol eluent composition (v/v)*			
		95:5, $\varepsilon = 0.56$[25]	*90:10,* $\varepsilon = 0.59$[25]	*80:20,* $\varepsilon = 0.64$[25]	*70:30,* $\varepsilon = 0.66$[25]
Pyridine-2-carboxylic acid	5.52	∞	Large	Large	0.75
Pyridine-3-carboxylic acid	4.85	9.40	1.90	0.40	0.15
Benzoic acid	4.19	1.20	0.60	0.30	0.10
2-Phthalic acid	2.89	0.30	0	0	0

With bulk-modified derivatives, hydroxyl groups could easily be substituted by amine groups, yielding a hydroxylamine structure. As shown in Table IV, on increasing the amine relative to the hydroxyl content, the solute capacity factors also increase. In the same direction, the selectivity improves considerably for both acidic and basic solutes.

Non-polar CBSPs. A large number of surface-modified reversed-phase packings were prepared for further studies (Table I)[26]. In addition, a bulk- and a surface-modified packing having the same functional group (benzyl) were prepared. In this section, only a brief discussion will be given on the packing properties that influence the retention behaviour of solutes in reversed-phase chromatography.

As generally valid in adsorption chromatography, the capacity factors are proportional to the surface area of the adsorbent and to the concentration of functional groups attached to the surface. In particular, in reversed-phase chromatography, the chain length of the bonded *n*-alkyl group becomes important. In Table V, the capacity factors normalized to unit surface area of some aromatic hydrocarbons on columns packed with different reversed-phase packings are listed. It is shown that for a given *n*-alkyl group, the quantity k'/S_{BET} for the solute is independent of the specific surface area of the adsorbent. Further, a plot of log k'/S_{BET} against the number of carbon atoms in the *n*-alkyl group gives a straight line for

TABLE IV

CAPACITY AND SELECTIVITY FACTORS OF POLAR SOLUTES ON DIFFERENT BULK-MODIFIED SILICA PACKINGS AT CONSTANT ELUENT COMPOSITION (DICHLOROMETHANE–ACETONITRILE, 95:5, V/V)

Column length, 20 cm. Column 1: packing $S_{BET} = 400$ m^2/g; original silica. Column 2: packing $S_{BET} = 394$ m^2/g; functional group $Si(CH_2)_3$–O–CH_2–CH(OH)–CH_2OH. Column 3: packing $S_{BET} = 401$ m^2/g; functional group $Si(CH_3)_3$–O–CH_2–CH(OH)–$CH_2OH(NH_2)$ (molar ratio OH:NH_2 = 6). Column 4: packing $S_{BET} = 430$ m^2/g; functional group $Si(CH_3)_3$–O–CH_2–CH(OH)–CH_2NH_2 (molar ratio OH:NH_2 = 1).

Sample	*Column 1*		*Column 2*		*Column 3*		*Column 4*	
	k'	*α*	*k'*	*α*	*k'*	*α*	*k'*	*α*
2-Chlorophenol	0.21		0.25		0.46		0.72	
		3.75		3.4		2.3		1.6
Phenol	0.80		0.85		1.06		1.14	
		0.86		0.94		1.16		1.54
4-Chlorophenol	0.69		0.80		1.23		1.75	
		1.23		1.48		1.83		5.18
4-Nitrophenol	0.85		1.18		2.26		9.08	
Aniline	1.49		1.00		2.26		4.08	
		—		1.3		2.0		2.25
α-Picoline	large		1.30		4.6		10.8	

TABLE V

CAPACITY FACTORS OF AROMATIC HYDROCARBONS NORMALIZED TO UNIT SURFACE AREA OF THE ADSORBENT ON COLUMNS PACKED WITH DIFFERENT REVERSED-PHASE MATERIALS

Eluent, acetonitrile–water (80:20, v/v); temperature, 20 °C.

Sample	*$k'/S_{BET} \cdot 10^3$*							
	Column 1: bonded trimethylsilyl groups		*Column 2: bonded n-butyldimethylsilyl groups*		*Column 3: bonded n-octyldimethylsilyl groups*		*Column 4: bonded n-hexadecyldimethylsilyl groups*	
	S_{BET} = 168 m^2/g	*S_{BET} = 244 m^2/g*	*S_{BET} = 115 m^2/g*	*S_{BET} = 225 m^2/g*	*S_{BET} = 163 m^2/g*	*S_{BET} = 212 m^2/g*	*S_{BET} = 97 m^2/g*	*S_{BET} = 175 m^2/g*
Naphthalene	0.6	0.4	0.9	0.8	1.7	2.1	4.2	4.5
Phenanthrene	1.1	1.3	2.6	2.3	4.4	4.6	13.8	12.6
Anthracene	1.5	1.8	3.0	3.1	5.1	5.7	15.7	14.9
Fluoranthene	1.4	2.4	3.3	4.3	6.8	7.5	21.3	19.7
Pyrene	2.0	—	3.4	—	7.1	—	24.3	—
Chrysene	2.7	—	5.2	—	9.3	—	30.9	—
3,4-Benzpyrene	3.3	—	5.7	—	13.6	—	62.5	—
Perylene	4.8	—	6.1	—	14.1	—	55.7	—
Indenopyrene	6.9	—	8.5	—	25.1	—	114.4	—

each solute, indicating that the total area of the alkyl chain will be accessible for hydrophobic interactions. On the other hand, the selectivity factors do not change on varying the chain length, all other conditions being kept constant (Table VI). Comparison between bulk- and surface-modified benzyl phase reveals that the high

TABLE VI

CAPACITY AND SELECTIVITY FACTORS OF AROMATIC HYDROCARBONS ON SURFACE-MODIFIED *n*-ALKYL REVERSED-PHASE PACKINGS

Eluent: methanol–water (80:20, v/v); temperature, 50° C. Column 1, functional group = trimethylsilyl; column 2, functional group = *n*-butyldimethylsilyl; column 3, functional group = *n*-octyldimethylsilyl; column 4, functional group = *n*-hexadecyldimethylsilyl.

Sample	*Column 1*		*Column 2*		*Column 3*		*Column 4*	
	k'	*α*	*k'*	*α*	*k'*	*α*	*k'*	*α*
Naphthalene	0.10		0.18		0.46		0.80	
		3.30		2.94		2.13		2.78
Phenanthrene	0.33		0.53		0.98		2.22	
		1.39		1.09		1.10		0.95
Pyrene	0.40		0.58		1.08		2.13	
		1.15		1.22		1.10		1.18
Anthracene	0.46		0.71		1.19		2.53	
		1.30		1.36		1.34		1.36
Fluoranthene	0.80		0.97		1.6		3.46	

TABLE VII

CAPACITY AND SELECTIVITY FACTORS OF SOLUTES ON COLUMNS PACKED WITH SURFACE- AND BULK-MODIFIED SILICA

Column 1: surface-modified silica with benzyl groups; $S_{BET} = 225$ m²/g; $\alpha_{benzyl} = 4.3$ μmole/m².
Column 2: bulk-modified silica with benzyl groups; $S_{BET} = 460$ m²/g.
Column length = 25.0 cm; I.D. = 4 mm. Eluent,: methanol–water (80:20, v/v); temperature, 50.0 °C.

Sample	*Column 1*			*Column 2*		
	k'	*k'/S*	*α*	*k'*	*k'/S*	*α*
Benzene	0.12	0.53		1.27	2.76	
			1.42			2.26
Naphthalene	0.17	0.75		2.88	6.26	
			1.87			2.12
Phenanthrene	0.31	1.38		6.09	13.24	
			1.31			1.13
Anthracene	0.41	1.82		6.90	15.00	
			1.07			0.94
Pyrene	0.44	1.95		6.50	14.13	
			1.05			1.26
Fluoranthene	0.46	2.04		8.20	17.82	
			1.64			1.12
Chrysene	0.75	2.50		9.17	19.93	
Phthalic acid	0.07	0.31		0.72	1.56	
			1.51			1.00
Terephthalic acid	0.11	0.49		0.72	1.56	
			1.09			1.43
Benzoic acid	0.12	0.53		1.03	2.24	
			1.25			1.33
Naphthol-1	0.15	0.66		1.37	2.98	
			1.19			1.03
Naphthol-2	0.17	0.75		1.41	3.06	
			1.88			2.56
1-Nitronaphthalene	0.31	1.38		3.56	7.74	

capacity factors, k'/S_{BET}, on the bulk-modified packing may be due to the high concentration of benzyl groups. Again, selectivity factors show no significant difference between bulk- and surface-modified material (Table VII). The high concentration of benzyl groups in the bulk-modified material makes it possible to decrease the water content of the mobile phase for optimization of solute capacity factors. These packings in particular offer a high capacity for trace organic compounds.

REFERENCES

1 E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1974.
2 D. C. Locke, *J. Chromatogr. Sci.*, 11 (1973) 120.
3 M. Novotný, S. L. Bektesh and K. Grohmann, *J. Chromatogr.*, 83 (1973) 25.
4 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.
5 R. E. Majors, *Analusis*, 3 (1975) 549.
6 K. Unger, J. Schick-Kalb and K. F. Krebs, *J. Chromatogr.*, 83 (1973) 5.
7 K. Unger, J. Schick-Kalb, B. Straube and B. Scharf, *Colloid Polym. Sci.*, 253 (1975) 658.
8 K. Unger and B. Scharf, *J. Colloid Interface Sci.*, 55 (1975) 377.
9 K. Unger, K. Berg and E. Gallei, *Kolloid-Z. Z. Polym.*, 234 (1969) 1108.
10 S. J. Gregg and K. S. W. Sing, *Adsorption, Surface Area and Porosity*, Academic Press, New York, 1967.
11 S. Brunauer, S. Skalny and J. Odler, in S. Modry and M. Svata (Editors), *Proceedings of the International Symposium, Rilem/IUPAC, on Pore Structure and Properties of Materials*, Volume I, C-3, Academia, Prague, 1974.
12 A. V. Kiselev and V. I. Lygin, *Infrared Spectra of Surface Compounds*, Wiley-Interscience, New York, 1975.
13 K. Unger and E. Gallei, *Kolloid-Z. Z. Polym.*, 237 (1970) 358.
14 J. J. Kirkland and P. C. Yates, *Ger. Pat.*, 2,125,428 (Dec. 2, 1971).
15 L. T. Zhuravlev and A. V. Kiselev, in D. H. Everett and R. H. Ottewill (Editors), *Proceedings of the International Symposium on Surface Area Determination*, Butterworths, London, 1970, pp. 155–160.
16 L. H. Little, *Infrared Spectra of Adsorbed Species*, Academic Press, New York, 1966.
17 M. L. Hair, *Infrared Spectroscopy in Surface Chemistry*, Marcel Dekker, New York, 1967.
18 A. Ahmed, E. Gallei and K. Unger, *Ber. Bunsenges. Phys. Chem.*, 79 (1975) 66.
19 L. T. Zhuravlev, A. V. Kiselev and V. P. Naidina, *Russ. J. Phys. Chem.*, 42 (1968) 1200.
20 K. Unger, *Angew. Chem., Int. Ed.*, 11 (1972) 267.
21 K. Unger, K. Berg, E. Gallei and G. Erdel, *Fortschr. Kolloide Polym.*, 55 (1971) 34.
22 G. Erdel, K. Unger, H. Fischer and B. Straube, in S. Modry and M. Svata (Editors), *Proceedings of the International Symposium, Rilem/IUPAC, on Pore Structure and Properties of Materials*, Volume III, B-127, Academia, Prague, 1974.
23 V. Rehak and E. Smolkova, *Chromatographia*, 9 (1976) 219.
24 K. Unger, N. Becker and E. Krämer, *Chromatographia*, 8 (1975) 283.
25 D. L. Saunders, *Anal. Chem.*, 46 (1974) 470.
26 K. Unger and P. Roumeliotis, to be published.