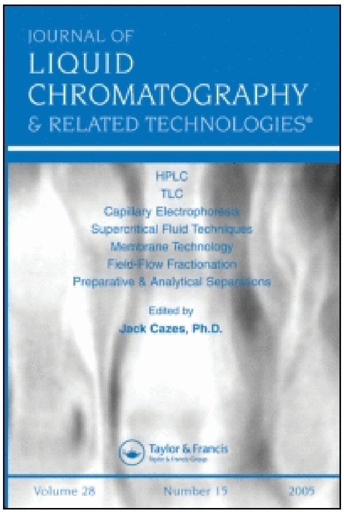

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Engelhardt, Heinz and Orth, Peter(1987) 'Alkoxy Silanes for the Preparation of Silica Based Stationary Phases with Bonded Polar Functional Groups', Journal of Liquid Chromatography & Related Technologies, 10: 8, 1999 — 2022

To link to this Article: DOI: 10.1080/01483918708066809
URL: http://dx.doi.org/10.1080/01483918708066809

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(8&9), 1999-2022 (1987)

ALKOXY SILANES FOR THE PREPARATION OF SILICA BASED STATIONARY PHASES WITH BONDED POLAR FUNCTIONAL GROUPS

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ABSTRACT

For preparation of polar bonded phases with alkoxy silanes an activator and a catalyst are required to achieve surface coverages comparable to those obtained with chlorosilanes. For activation a monolayer of water on the silica surface is sufficient. The most active catalyst in many cases has been p-toluene sulfonic acid, however, for silanes with basic groups triethylamine gives better coverages. Silanes with polar groups tend to adsorb also with this group onto the surface thus preventing chemical binding via alkoxy groups. Long time experiences in the preparation of amino phases, anion and cation exchangers and hydrophilic bonded phases for protein analysis are summarized.

INTRODUCTION

The rise of HPLC is closely connected to the increasing use of chemically modified silicas as stationary phases. Consequently, most of the papers dealing with the preparation and characterization of chemically bonded phases have been published in the early seventies ((1-24)) and reviewed in several books ((11,13)). The surface silanol groups have been modified by esterification ((2,14)), modification of surface silicic acid chlorides with amines ((15)) and by the reaction of these chlorides with

Grignard ((16-18)) or lithium organic compounds ((19)). However, from all these different reactions only that of reacting surface silanols with chlorosilanes prevailed. Commercially available stationary phases are exclusively prepared by this reaction nowadays.

Primarily only trichloro silanes have been technically available. But these silanes can polymerize to organo silicones in the presence of water, which can also be bonded to the silica surface. Polymeric phases should give higher retention due to the larger "volume" of the bonded stationary phase, but should have the disadvantage ((12)) of a slower speed of mass transfer in the thick polymeric layer compared to that achieved with "monomeric" bonded phases. With these phases only a monomolecular layer of organic groups is attached to the surface (sometimes these types are referred to as "bristle" type stationary phases ((2))). To reduce the danger of polymerization, resulting in stationary phases with excess peak broadening, it has been recommended to exclude water systematically ((20-22)) during phase preparation, and to use monochlorosilanes exclusively ((23)).

To adjust the selectivity of alkyl bonded phases (reversed phases, RP) functional groups have been introduced, especially to prepare silica based ion exchangers, e.g. by reacting aryl or naphthyl modified silicas with chlorosulfonic acid to prepare cation exchangers or by introducing chloromethyl groups which have been reacted in a second step with amines to form anion exchangers. All these secondary reactions on surface modified silicas do not proceed quantitatively. Organic reactions with almost quantitative yields in bulk phase chemistry give only 20-50 % yields, if carried out with reactants already surface bonded ((25)). These reactions are always accompanied by a loss of carbon, indicating the cleavage of surface bonds. Consequently, the selectivity of stationary phases prepared by consecutive reactions is governed by that of the introduced polar group, by that of the initial bonded aryl silane and by the presence of surface silanol groups generated during reactions.

To prepare stationary phases with polar functional groups with an unequivocal retention mechanism one has to go the tedious way to prepare first the appropriate silane and bond this to the surface ((26-28)). Because of the high reactivity of chlorosilanes the introduction of functional groups into a silane seems only possible by using the less reactive alkoxy silanes ((26,29)). Alkoxy silanes are relatively stable in the absence of a catalyst, even in aqueous solutions ((30)). On the other hand, this low reactivity of alkoxy silanes and the presence of polar functional groups requires a different type of bonding reaction to attach these silanes to the silica surface. In this paper our studies in the preparation of surface modified silicas with bonded polar fuctional groups with optimum surface coverage will be summarized. The stationary phases prepared with alkoxy silanes are suitable for the analysis of sugars ((31)), for ion exchange chromatography ((44)) or size exclusion chromatography as well as hydrophobic interaction chromatography ((26,29,32,33) of proteins.

EXPERIMENTAL CONDITIONS

The chromatographic measurements were performed with a modular system built of standard components (Waters pump M 6000, Injection system Water U6K, Waters refractometer R 401 (all from Waters Assoc., Milford, Mass.), and a Perkin Elmer LC 85 B (Bodenseewerk, Überlingen) variable wavelength detector.

Columns (150 or 300 mm in length, 4.1 mm inner diameter) were packed as described ((34)).

Lichrosorb materials from Merck AG, Darmstadt, obtained in different batches, specifications (Si 60, Si 100, Si 200, Si 500, Si 1000) and consequently differing in surface areas have been used. Surface areas were determined by inverse size exclusion chromatography ((35)). Silanes were purchased from Dynamit Nobel, Troisdorf (n-octyltriethoxysilane, 3-aminopropyltriethoxysilane), from Petrarch Systems, Levittown PA (N,N,N-trimethyl-propyl (trimethoxy) silyl ammonium chloride, N-triethoxysilylpropyl urea) or synthesized in our own laboratory (4-chlorosulfon-phenyl-propyl-triethoxysilane).

For binding procedures standard laboratory equipment (3 necked flasks, glass stirrer, reflux condenser) was used. Silica was cleaned as described below, and dried for 24 hours at 150°C before use.

RESULTS AND DISCUSSION

Standardization of silica surface

The achievable surface coverage in the preparation of chemically bonded stationary phases is a function of the silanol concentration on the silica surface, the silanization reagent and the reaction conditions. Because it has been difficult to obtain identical surface coverages (measured via carbon content of the bonded phase) with the same silane and different batches of silica with comparable physical properties (surface area, pore diameter, pore volume), a standard procedure has been developed for the preparation of a chemically homogeneous silica surface. In these studies a very active silanization reagent, bis(trimethyl-silyl) acet amide (BSA) was used. It can be seen in Fig. 1, that with standard conditions (12 m Mol BSA dissolved in 200 ml

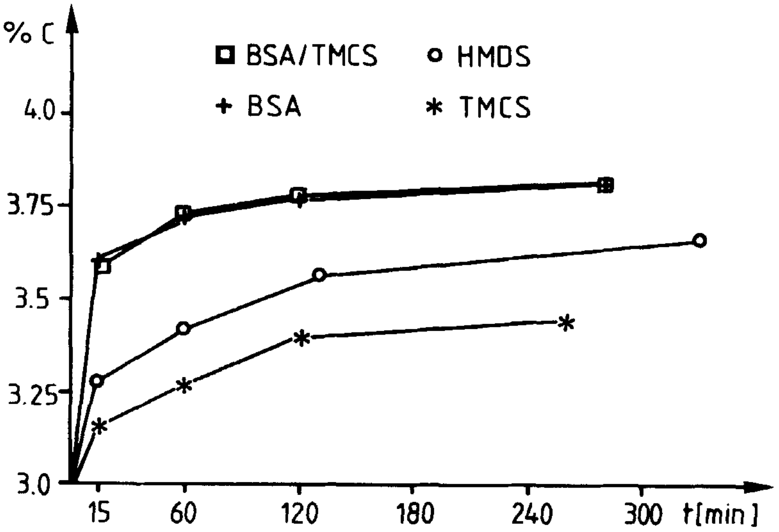

Figure 1. Reactivity of various trimethylsilylation reagents. Reaction conditions see text. BSA = bis-trimethylsilyl acetamide; HMDS = hexamethyldisilazane; TMCS = trimethylchlorosilane.

dry toluene (stored over molecular sieve 3 A) added to 2 g silica and reflux) only BSA gave the highest coverage and the reaction was completed within 2 hours. The other commonly used trimethyl silanization reagents did not give a constant value in carbon content within five hours of reaction. The surface coverage obtained with BSA could not be achieved with the other reagents.

When silicas differing in age were used in this standard silanization procedure, great differences in the surface coverage were obtained. However, if the silica has been "cleaned" before silanization, almost identical coverages are achieved. These values are summarized in Table I. The "cleaning" was achieved by heating the silica (10 g) in 100 ml of a 9:1 mixture of sulfuric acid (conc) and nitric acid until the development of nitrosic gases ceased. The material was then washed with water to neutrality. Water was removed with methanol and after washing with dichloromethane the silica was dried at 120°C for 24 hours. This cleaning procedure not only removed organic and metallic impurities but also resulted in a more homogeneous fully hydroxylated surface. Consequently, all silica samples were cleaned by this procedure before use. The effect of this "cleaning" procedure was the more noticeable the older the initial silica had been (batch EH 12). Here a 20 % increase in carbon content was achieved by silica cleaning. The carbon content of all cleaned silicas, achieved with BSA, showed a linear correlation with the specific surface area.

The average surface coverage with trimethyl silyl (TMS) groups thus achieved has been 4.68 ± 0.25 μmol/m² if one neclects the values obtained with silicas with a surface area below 100 m²/g. In these cases the contribution of the error of CH analysis is too great. If one assumes a van der Waals radius of a TMS group of 0.4 nm ((36)), one can calculate a maximum achievable surface coverage of 4.4 μmol/m². Consequently, with BSA optimum, i.e. maximum surface coverage, can be obtained. Similar surface coverages have been published by Kovats ((37)) with 4.7 μmol/m² and Unger ((20)) with 4.2 μmol/m² for TMS groups. On binding alkyl silanes with longer alkyl groups to silica only lower surface coverages can be achieved. With octadecylsilanes values between 2.5 and 3.2 μmol/m² have been reported ((8)).

Table 1

SILANIZATION OF SILICAS WITH BSA BEFORE AND AFTER ACID TREATMENT

Silica	Surface area* m² /g	Carbon content % w/w	Surface coverage µmol/m²
Lichrosorb Si 60			
before	336	3.80	3.4
after	389	5.81	4.7
Lichrosorb Si 100			
EH 12 before	292	3.68	3.8
after	322	4.60	4.4
Lichrosorb Si 100			
F 1468 before	379	5.02	4.1
after	317	5.18	5.0
Lichrosorb Si 200			
before	182	2.70	4.3
after	177	2.78	4.6
Lichrosorb Si 500			
before	66	0.81	3.5
after	65	1.29	5.7
Lichrosorb Si 1000			
before	20	0.30	4.2
after	20	0.36	5.0
LS 500 before	85	0.96	3.2
	75	1.35	5.1

* determined by inverse size exclusion chromatography ((35))

Preparation of polar bonded phases

a) Influence of reaction solvent

The primary requirements for a good solvent for the bonding reaction should be: chemical inertness, solubility for the silanes, relatively high boiling point and it should not itself be adsorbed to the surface too strongly. One can imagine, that the first step for reaction would certainly be the adsorption of the silane onto the surface, and then subsequently the reaction under removal of alcohol, water or hydrochloric acid should take place. When ethers, esters or chloroform were used, bad reproducibility (± 25 % deviation in surface coverage) has been noticed. A possible explanation may be that during their purification the stabilizers were removed and degradation occurred during storage and reaction. Reproducible results could only be obtained by using aromatic hydrocarbons as reaction solvent. In Fig. 2 the nitrogen content is plotted for different reaction temperatures, achieved with different aromatic hydrocarbons. In this and in the following studies the silane was added always in a concentration sufficient for all present silanol groups (7-8 μmol/m²) to react with one molecule of the silane. As can be seen, the maximum of the nitrogen content, i.e. maximum reaction of silica with triethoxypropylaminosilane, has been achieved around the boiling point of toluene (111°C). On the other hand, at these intermediate temperatures the C/N ratio of the bonded phases approaches the theoretical value of 3. The average C/N ratio with all three aromatic hydrocarbons determined here is 3.14 ± 0.05. At lower and higher temperatures larger C/N ratios have been obtained. The average nitrogen content is 1.85 % corresponding to a surface coverage of 4.7 μmol/m². From this it may be concluded that at intermediate temperatures either all three alkoxy groups are able to react with the surface - or that alkohol is removed by a reaction of two adjacent bonded silanes. The possibility of this reaction has been proved recently by NMR measurements ((39,40)) and experimentally ((41)). The larger C/N ratios measured at lower and at higher temperatures may be explained by the presence of alkoxy groups or decomposition of the aminosilane. The latter is likely to occur at higher temperatures.

In Fig. 3 the kinetics of the bonding reaction in three different aromatic hydrocarbons at their boiling points is shown.

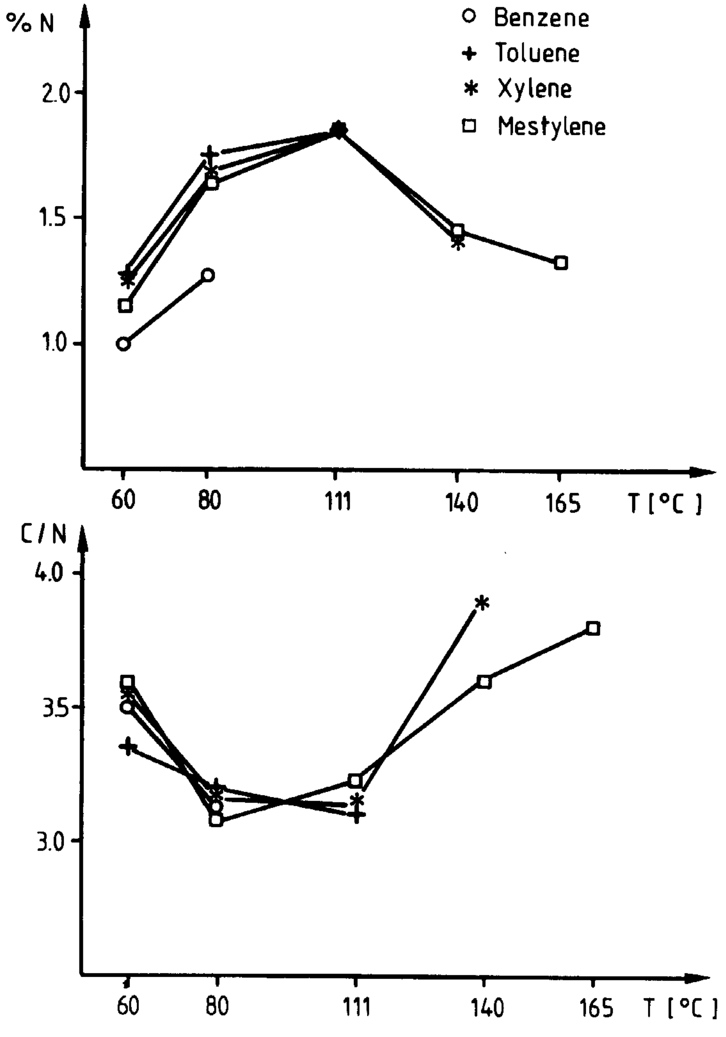

Figure 2. Preparation of amino bonded phases.
Silane: 3-aminopropyl(trialkoxy)silane. Reaction conditions see text.
Upper diagram: nitrogen content as function of reaction temperature.
Lower diagram: carbon-nitrogen ratio of amino phases as function of reaction temperature.

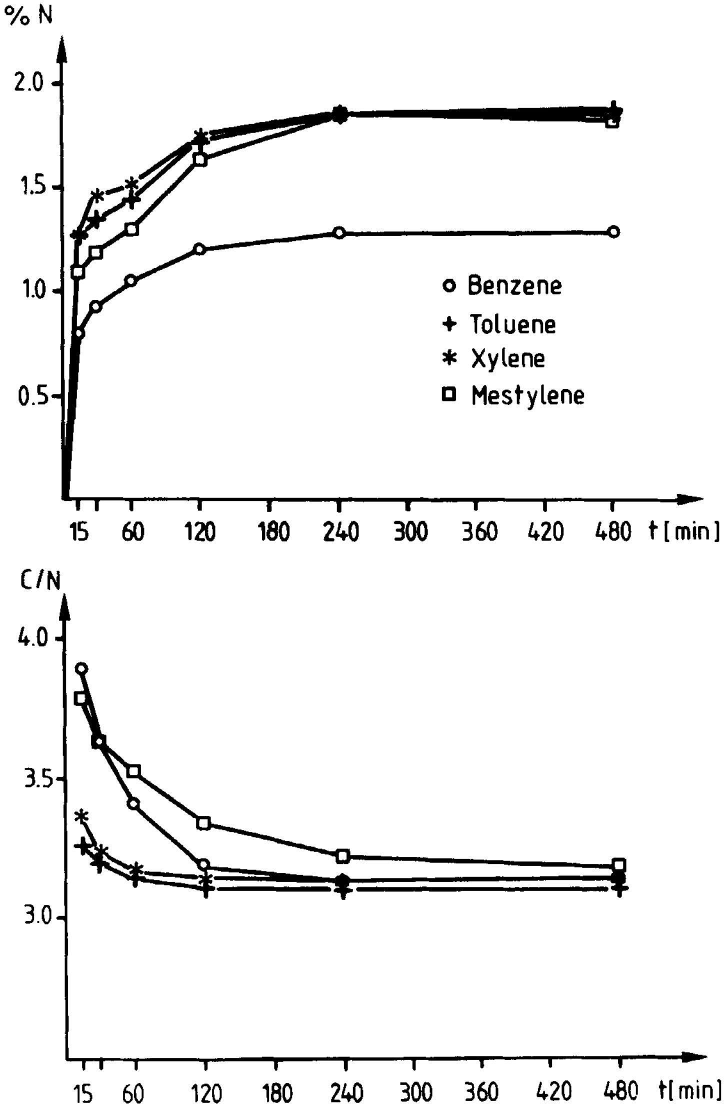

Figure 3. Reaction kinetics for amino phases.
Conditions as in Fig. 2
Upper diagram: nitrogen content and reaction time.
Lower diagram: carbon-nitrogen ratio and reaction time.

After 4 hours constant nitrogen content is achieved with all three solvents. Only with benzene as solvent, due to the lower reaction temperature at boiling point, a smaller surface coverage is obtained. It is interesting to look at the changes of the C/N ratio as a function of reaction time, also shown in this figure. After 4 hours reaction time a constant C/N ratio of approximately 3.1 is approached. Only with mesitylene at the higher reaction temperature a slightly larger C/N ratio is obtained. The decrease of the C/N ratio in the initial stage of reaction may indicate that the silane reacts primarily with the surface with one ethoxy group and the removal of remaining ethoxy groups and subsequent additional binding occurs in a secondary reaction. Both solvents, toluene and xylene, gave identical results, therefore, in the following toluene has been used exclusively.

Similar behaviour as discussed here for amino silane has been observed with all other silanes with polar functional groups, but the surface coverage was always lower than that obtained with the amino propyl silane. With the quarternary ammonium silane a maximum coverage of only 2.2 $\mu mol/m^2$ could be achieved. With the octylsilane reacted under the conditions discussed above, a maximum surface coverage of only 0.3 $\mu mol/m^2$ was calculated from a carbon content of 1.4 % w/w. This indicates that the reaction mechanism of alkoxysilanes with silica is different from that of chlorosilanes, where with octylchlorosilanes carbon contents around 10 % w/w have been achieved under otherwise identical reaction conditions.

b) Influence of water

In about 95 % of all papers dealing with surface modification of silica with chlorosilanes extreme caution has been taken to prevent the contact with moisture during reaction. Only Majors et al. ((42)) described the addition of water to the silica in the case of reaction with alkoxy silanes, but here also a warning has been given in order to prevent polymerization. Only very recently the presence of water in the preparation of polar bonded phases with trialkoxy silanes has been recommended ((43)). In extensive studies on the stability of alkoxy silanes in aqueous medium it has been found ((30)), that the hydrolysis of alkoxysilanes is fast, however, the polycondensation of the generated silanols to siloxanes is a relatively slow process. Consequently, with alkoxy silanes the influence of water added to the silica in the range of monolayer coverage for possible activation of binding was studied. In Fig. 4 the reaction of amino propyl silane with silica in toluene with different water contents is shown. With a water concentration of 200 ppm and more in the toluene not only a slightly higher nitrogen content (better surface coverage) is achieved, but also the reaction proceeds much faster and is already completed after two hours compared to 4 hours in the absence of water. At a water concentration of 500 ppm the toluene (saturated at room temperature) contained so much water that under the reaction conditions (ratio silica to volume of toluene) a monomolecular surface coverage of water on the silica could be calculated.

For the reaction of alkoxyoctylsilane with silica the influence of water on the surface reaction is much more striking, as can be seen in Fig. 5. However, the maximum coverage achievable with chlorosilanes could not be reached under this conditions.

c) Catalytic acceleration of surface modification

The reaction of surface silanols with alkoxysilanes corresponds to an anhydride formation from an acid (silica) and an ester (alkoxysilanes). These reactions can be catalyzed either by acids or bases. The fast reaction of aminopropyltriethoxysilane with silica and the high achievable surface coverage suggests that this reaction is autocatalyzed by the amino groups present.

In Table 2 the influence of different catalysts on the reaction of octyltriethoxysilane with silica in toluene at two different water concentrations is summarized. The catalysts were added at a concentration of 4 % relative to the amount of silane (5 mol/g Silica) used. Without catalyst the maximum surface coverage achieved in wet toluene has been 1.3 µmol/m² as discussed above. With various catalysts surface coverages between 2.2 and 2.6 µmol/m² in wet toluene have been achieved. However, toluene sulfonic acid was found to be the most effective catalyst, resulting in a surface coverage of 4.1 µmol/cm² and a carbon content of 11.6 % w/w. These values are comparable to those achieved with the corresponding chlorosilanes. It should also be noted that with this catalyst already in dry toluene a reasonable surface coverage of 2.6 µmol/cm² could be obtained.

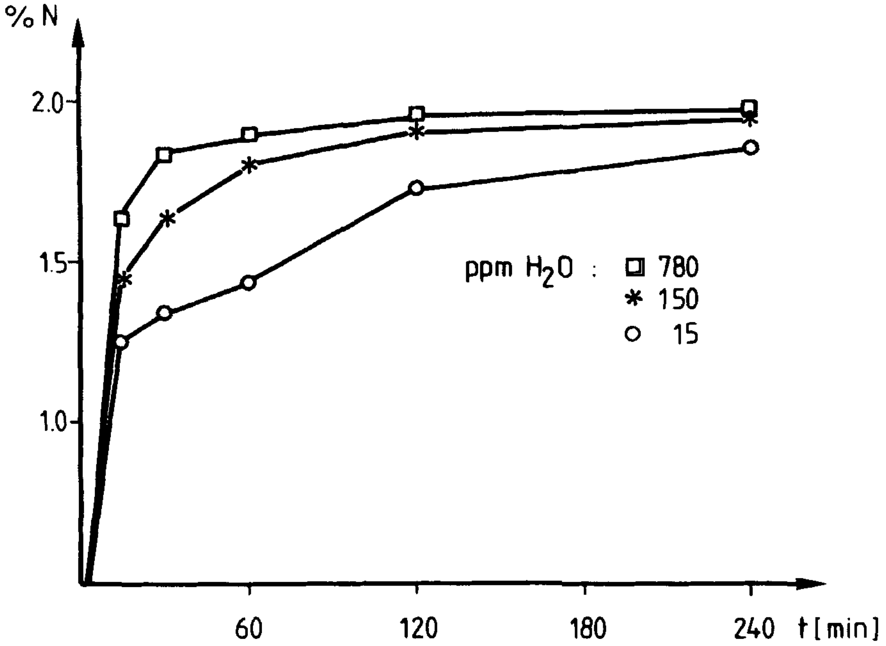

Figure 4. Influence of water in the preparation of amino phases. Conditions as in Fig. 2 except that water concentration in the toluene was varied between 15 ppm and 780 ppm.

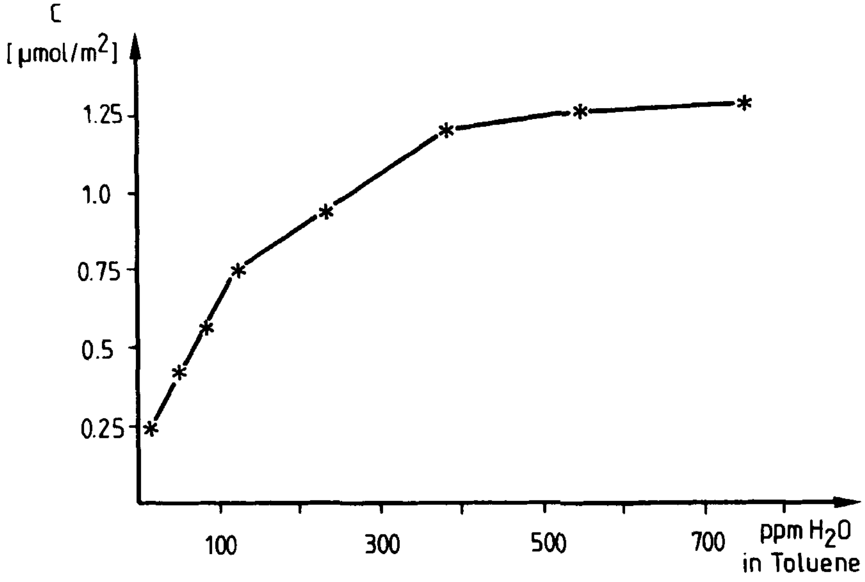

Figure 5. Achievable surface coverage as a function of water added.
Silane: trialkoxy-n-octylsilane.

Table 2

SURFACE COVERAGE WITH ALKOXYOCTYLSILANE.
INFLUENCE OF CATALYST IN DRY AND WET TOLUENE
LICHROSORB Si 100 (F 1468)

Catalyst	Reaction in Toluene			
	15 ppm H_2O		750 ppm H_2O	
	%C	coverage μMol/m^2	%C	coverage μMol/m^2
Pyridine	3.13	1.07	6.62	2.18
Triethylamine	3.59	1.23	7.11	2.36
Ammonia (32% in H_2O)	3.82	1.32	6.78	2.24
Formamide	6.19	2.21	7.02	2.33
Benzoylperoxide (+ 20% H_2O)	7.09	2.57	7.78	2.61
pToluene sulfonic acid	7.07	2.57	11.6	4.15
+ BSA	7.87	2.89	11.8	4.25

From these results it can be deduced that the reaction of alkoxy silanes with the silica surface requires catalysts (amino silanes are autocatalysts) for acceleration and that the reaction has to be additionally activated by using enough water to form a monolayer at the surface.

The surface coverage thus obtained seems to be almost complete, because an additional treatment of the octylsilica with BSA does not significantly increase the carbon content (0.2 % w/w). On the other hand, the octyl phase prepared in dry toluene seems still to contain accessible and reactive silanols, because here with BSA the carbon content increased by 0.8 % w/w in this end-capping reaction. The corresponding values have also been included in Table 2. Fig. 6 demonstrates chromatographically the

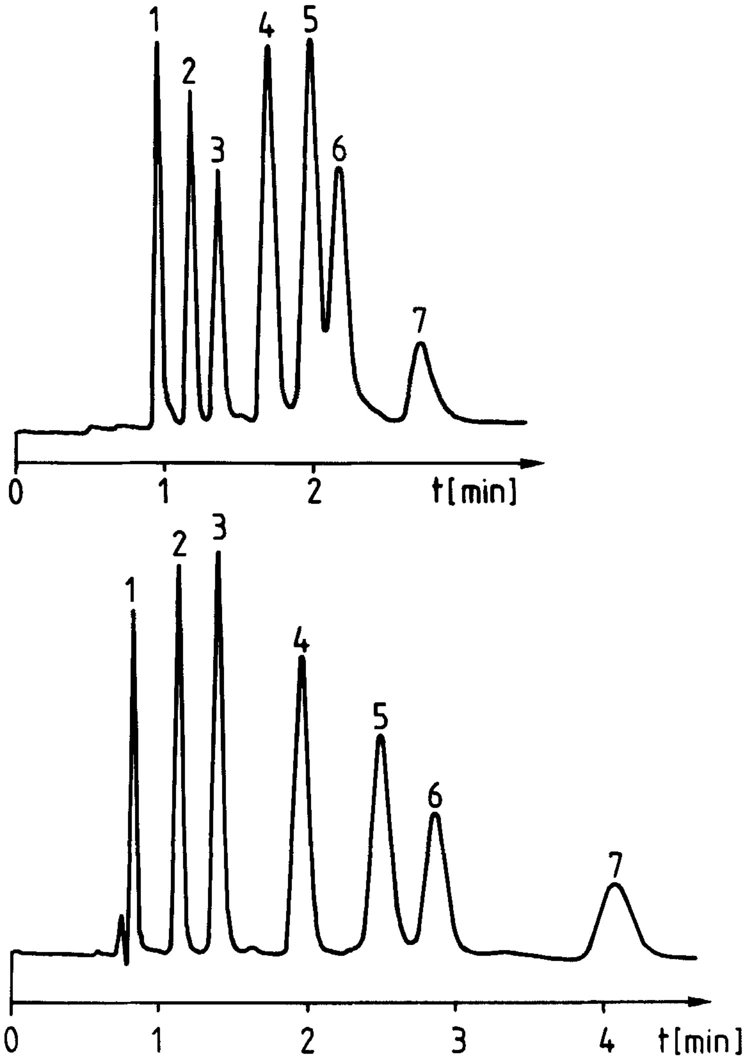

Figure 6. Chromatographic differences of octyl phases prepared in dry and wet toluene. Separation of aromatic hydrocarbons.
Columns: 300 x 4 mm; Eluent: methanol-water, 7-3, w-w; Solutes: 1 = nitromethane (k' = 0.02; 0.08), 2 = benzene (0.26; 0.48), 3 = naphthalene (0.46; 0.83), 4 = anthracene (0.80; 1.57), 5 = pyrene (1.10; 2.2), 6 = chrysene (1.31; 2.7), 7 = perylene (1.91; 4.3).

differences between the two octyl phases prepared with catalyst in dry and wet toluene.

The very active catalyst toluene sulfonic acid is, of course, desactivating the reaction of amino silanes with silica. In Table 3 the nitrogen content of bonded phases achieved with triethylamine as catalyst is compared with that obtained with toluene sulfonic acid catalyst. Approximately 20 % less groups have been bonded to the surface with this acidic catalyst. This amount is even smaller than that obtained with dry toluene and without any catalyst. Consequently, for basic silanes an acidic catalyst should not be used.

Removal of adsorbed silanes

During the post reaction cleaning procedures it has been found that by washing with dilute hydrochloric acid around 1.8 % carbon and 0.25 % nitrogen were lost. The C/N ratio of the material removed by washing was 8.5. This indicates that physically adsorbed silane with a theoretical C/N ratio of about 9 has been desorbed. In repeating this treatment several times, the carbon and nitrogen content as well as the C/N ratio remained constant after this initial loss. This is proof of the stability of the bonded phase against dilute acids. By treatment of this washed stationary phase (after drying) with BSA the carbon content increased by 0.85 %. The silanols blocked by adsorbed amine could thus be covered by TMS groups. From the increase in carbon content a concentration of 0.96 $\mu mol/m^2$ could be calculated. This physical adsorption indicates what might be expected from chromatography: in the nonpolar toluene the silanes are either adsorbed via the ester group, where surface reaction is possible or via the amino group where only physical adsorption takes place. From this orientation reaction to bonded phases is impossible. Similar observations have been made with the ammonium silane, where about 10 % of the silane was physically adsorbed.

It seems to be a general problem in the preparation of polar bonded phases, that surface silanols are blocked by physically adsorbed silanes. After desorption during cleaning or column use additional silanols are present and may affect chromatographic selectivity. Therefore, it is recommended to remove physically

Table 3

TYPE OF CATALYST FOR BASIC SILANES

Catalyst	Triethylamine		Toluene sulfonic acid
Toluene	dry	wet	wet
Nitrogen content with			
Aminopropylsilane	1.9	2.2	1.8
Ammonium silane	1.1	1.2	0.85

adsorbed material and react the now freed silanols either with BSA or in a second step again with the corresponding silane. Of course, BSA will also react with free amino groups. However, the thus formed Si-N bond is hydrolysed in aqueous media even around neutral pH values. By this method it has been possible to prepare good and stable amino phases for sugar analysis ((31)), anion exchangers ((44)), and amide phases for protein separation ((33)).

Polymeric or monomeric phases?

The presence of water during reaction may cause the formation of polysiloxanes and their subsequent fixation onto the surface. Such stationary phases should show bad mass transfer resulting in larger C terms of the van Deemter plot or at least result in less efficient columns ((12)). No significant differences in column efficiency could be observed between stationary phases prepared in the presence of a water monolayer with trialkoxyoctylsilane and octyl phases prepared with a monofunctional dimethyl octyl silane. Certainly, the water was adsorbed first to the surface of the dry silica, therefore, polysiloxane formation in solution has been less likely. It should be mentioned that this way of water addition for bonded phase preparation is different from that proposed in literature ((5,45-47)), where the water is added in large excess and polycondensation is more likely. On the other hand, it was tried to prepare a "polymeric" phase starting with a silica reacted with trialkoxyoctylsilane. After hydrolysis of alkoxy groups the bonded phase was treated again with alkoxyoctylsilane in a subsequent reaction in the way of Merrifield peptide synthesis. Even after three subsequent steps no increase in carbon content was noticeable within the error of measurement of the CH analysis. Therefore, it seems not possible to form a polymeric layer on the surface in this way. It should be mentioned, however, if polymeric alkoxy siloxanes with molecular weights between 1000 and 5000 are bonded to silica the mass transfer is already restricted and reduced plate heights around 10 could be achieved only ((48)). Also if silica is coated with polyamide, column efficiency is worse than with monomeric bonded phases and plate numbers decreased with increasing polymeric layer thickness ((49)).

Applications

With the bonding procedure described in this paper silica based bonded stationary phases with polar functional groups have been prepared and used in various chromatographic applications. Stationary phases with aminopropyl groups have been used in the analysis of sugars ((31)). The phases derived from this silane by acetylation, which has to take place before bonding reaction, have been used extensively in size exclusion chromatography of proteins ((33)) and other water soluble polymers ((29)). These acetamide bonded phases are also well suited for hydrophobic interaction chromatography of proteins ((32)).

By reacting silica with N,N,N-trimethyl-propyl-ammoniumsilane an anion exchanger with an effective and stable exchange capacity of 0.9 mVal/g could be prepared. This anion exchanger could be used for the separation of nucleotides. An example is shown in Fig. 7. With this short chain anion exchanger no hydrophobic interaction could be noticed. The plot of k' vs reciprocal ionic strength leads to the origin. When silica was reacted with substoichiometric amounts of the ammonium silane, followed by a reaction with acetaminopropylsilanes to remove remaining silanols, dilute ion exchangers could be prepared, which have also been successfully applied in nucleotide analysis ((44)).

Starting with 3-phenylpropylalkoxysilane a silane with sulfonic acid groups could be prepared by chlorosulfonation in chloroform. The excess sulfuric acid was removed with anhydrous barium carbonate. By this no water was introduced which would have caused polycondensation. This silane also has been bonded to silica by the procedures described in this paper. A stable cation exchanger with phenylsulfonic acid groups and an exchange capacity of 0.85 mVal/g could be prepared. It should be mentioned that the packing density of silica based cation exchangers is more than double that of resinous ion exchangers. Therefore, the capacity achieved with silica is comparable to that of classical resinous exchangers. Fig. 8 shows the application of such a cation exchanger for the separation of nucleobases. They have also be used for amino acid analysis in combination with post-column derivatization ((50)). It should be mentioned, that with this ion exchanger hydrophobic interactions of solutes with the relatively large organic group are not negligible. Phenylalanine is retarded more strongly than expected from its pK value and coelutes with the basic amino acids in the usual gradient elution program.

CONCLUSIONS

The reproducible preparation of chemically modified stationary phases by using alkoxy silanes is possible if the following important points are considered: The silica should have a clean and fully hydroxylized surface. This has recently been reconsidered ((51)). Medium polar solvents like aromatic hydrocarbons

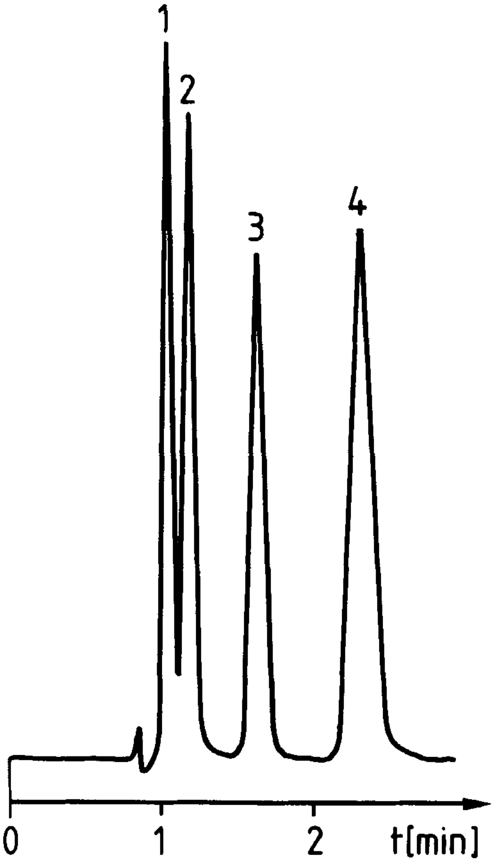

Figure 7. Anion exchange chromatography of nucleotide monophosphates.
Stationary phase: Ammoniumsilane, capacity 0.9 mVal/g; Eluent: 0.1 m phosphate buffer pH 3.5, F = 2 ml/min; Solutes: 1 = CMP (k' = 0.2), 2 = AMP (0.34), 3 = UMP (0.88), 4 = GMP (1.67)

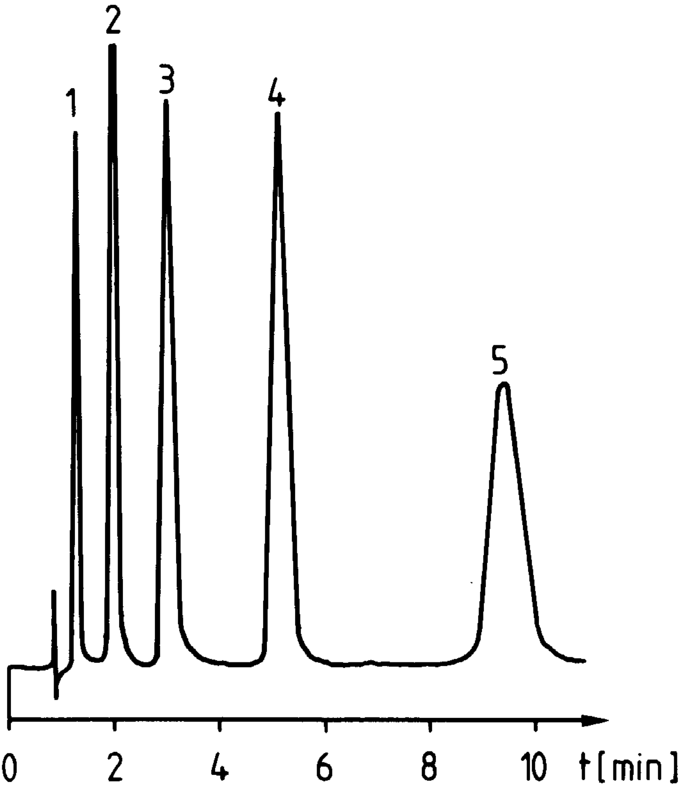

Figure 8. Cation exchange chromatography of nucleobases. Stationary phase: propyl phenyl sulfonic acid, capacity 0.85 mVal/g. Eluent: 0.1 m phosphate buffer pH 6.0, F = 2 ml/min. Solutes: 1 = uracil (k' = 0.4), 2 = thymine (1.22), 3 = guanine (2.41), 4 = cytosine (4.9), 5 = adenine (10).

are optimally suited as reaction media, because they are able to dissolve even polar silanes and they have boiling points high enough to be able to work at the optimum reaction temperature of 80 to 120°C. At least a 1:1 ratio of silanol groups and silane should be used. With these solvents the alkoxy silanes are adsorbed onto the surface. Too polar solvents prevent the silane from being adsorbed, which is necessary for binding.

Acidic or basic groups in the silane act autocatalytically for the reaction with the surface. For the reaction of non polar silanes water is required as accelerator for the silanization reaction. In the case of the autocatalyzed reaction the addition of water is advantageous.

Through the addition of catalysts (optimal are either triethyl amine or toluene sulfonic acid) the reaction can be accelerated and the yield (surface coverage) can be improved.

An additional treatment of bonded phases with dilute hydrochloric acid is required to hydrolyze alkoxy groups and to desorb silane with basic functional groups from the silica surface. The silanol groups freed by this treatment can be removed by reaction with BSA in toluene (endcapping). Silanization of basic amino groups may occur. However, these bonds are not very stable in aqueous medium and are removed during washing procedures.

The reaction starts with the adsorption of the silane. The water is required for the hydrolysis of the alkoxy groups. The still adsorbed silane reacts consecutively with the surface silanol under removal of water. Therefore, polymerization - or oligomerization - is unlikely. The achieveable surface coverages and the C/N ratios of bonded phases confirm the statements ((20,52)), that on an average 1.5 alkoxy groups of the silane are able to react with the surface. In other words, 50% of the silane are bonded with one bond to the surface and 50% are bonded with two siloxane bonds onto the surface. Because of sterical reasons the third alkoxy group seems not to be able to react with the surface directly. Evidence has been reported that these silanol groups may react with similar ones of adjacent silanes during an additional heat treatment, described as phase curing ((40)).

ACKNOWLEDGEMENT

This paper is part of the Ph. D. thesis of P. Orth, Saarbrücken, 1979. The thesis was financed by grants of the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg and the Fonds der Chemischen Industrie, Frankfurt. Part of this paper has been reported at the 13th International Symposium on Chromatography, Cannes, 1980.

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