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THE ALLYL-BONDED STATIONARY PHASE

III. *IN SITU* CONVERSION TO A CATION-EXCHANGE MATERIAL

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SUMMARY

An allyl-bonded stationary phase, produced by a chlorination–Grignard reaction scheme, is used as an intermediate in the synthesis of a cation-exchange material. The double bond of the allyl phase serves as a site for a free radical addition of bisulfite in the presence of azobisisobutyronitrile as a catalyst. The conversion is carried out by both a bulk reaction and an *in situ* procedure. Both methods result in about 90% conversion to the sulfonated material. The stationary phases are characterized by retention indices of alkyl aryl ketones and by Fourier transform infrared spectra. Both types of converted column materials give satisfactory results for typical separations of amino acids and catecholamines. It appears that the allyl phase can serve as a useful intermediate in the synthesis of new bonded phases.

INTRODUCTION

The bonding of organic materials to silica via organosilane reagents has led to significant advances in chromatographic separations in recent years. These stationary phases offer high temperature stability in gas chromatography and high solvent stability (particularly hydrolytic stability) in high-performance liquid chromatography (HPLC). The organosilane reaction is used almost exclusively in the production of commercial chemically bonded stationary phases[1]. The durability of these phases appears to be governed only by the nature of the chemical bonds and the stability of the silica surface itself[2]. Their versatility is determined by the types of organosilane reagents that can be synthesized.

Another approach to bonding organic moieties to silica involves first the chlorination of the silica surface:

$$\overset{|}{\underset{|}{-Si}}-OH + SOCl_2 \rightarrow \overset{|}{\underset{|}{-Si}}-Cl + SO_2 + HCl$$
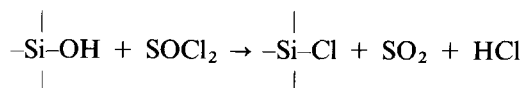
This is then followed by reaction of the chlorinated surface with a Grignard reagent:

$$-Si-Cl + RMgBr \rightarrow -Si-R + MgBrCl$$
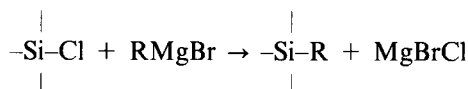
Such a reaction scheme was first reported for phases used in gas chromatography[3,4] and has been shown to be applicable to carbon as well as silica[5]. Both the organo-silane and the chlorination–Grignard reaction schemes were described for modifying glass surfaces much before their use for producing chemically bonded stationary phases[6-8].

In an earlier report[9] we described the synthesis of an allyl-bonded stationary phase for HPLC by the chlorination–Grignard method. Preliminary results[9] which are being investigated further indicated pH stability over a wide range. The presence of the double bond offers the possibilities of subsequent modifications to form new stationary phases with completely different properties. One such possibility would be conversion to a cation-exchange material. It should be possible to add bisulfite to a double bond by use of a free-radical initiator, such as azobisisobutyronitrile (AIBN)[10]:

$$R-CH=CH_2 + HSO_3^- \rightarrow RCH_2CH_2SO_2^-$$
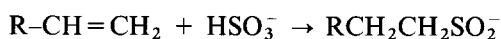
This reaction proceeds under moderate conditions (ambient or slightly elevated temperatures in aqueous methanol as solvent). Thus it should be possible to carry out the conversion *in situ* in an already packed column of allyl-bonded stationary phase. Because the allyl phase has already been shown to exhibit reversed-phase properties[9], the transformation to a cation-exchanger would produce a column with much different separation capabilities.

There have been a number of reports dealing with the synthesis of silica-based cation exchangers. For example, Weigand *et al.*[11] used an amonium sulfite substitution reaction on bromoalkylsilanes or oxidation of alkylmercaptans to produce relatively low-capacity materials. Wheals[12] also oxidized mercaptans but used potassium permanganate. While higher exchange capacities were produced, the acidic condition of the oxidation could lead to cleavage of the bonded phase. Fazio *et al.*[13] has shown that thiols can be oxidized with organic peroxyacids at room temperature without significant bond cleavage. It also has been shown that cation exchangers can be synthesized by sulfonating a phenyl phase[14]. A similar sulfonation was performed on a phase which was synthesized by first chlorinating the surface and then reacting the chlorinated surface with benzyllithium[15]. Apfel *et al.*[16] have demonstrated that addition to double bonds on polysiloxanes can be carried out in high yield. Our purpose was the production of a cation-exchange material by a method applicable to *in situ* conversions.

EXPERIMENTAL

Materials

All of the columns used were 15.0 cm × 4.6 mm I.D. and contained packings

with 10-μm Partisil (Whatman, Clifton, NJ, U.S.A.) having a surface area of 350 m^2/g. The allyl phase was prepared according to a previously described procedure[6].

Amino acid samples and alkyl aryl ketones (Touzart-Matignon, Vitry-sur-Seine, France) were used as received. The catecholamine samples were a gift of the French National Army Hospital. Chromatographic-grade solvents (Touzart-Matignon) also were used as received. Water was filtered through a Milli-Q (Millipore, Bedford, MA, U.S.A.) apparatus. All other chemicals used were of reagent grade.

Conversion to the cation exchanger was accomplished by using a saturated solution of AIBN and sodium bisulfite in methanol–water (1:1, v/v). The conversion was carried out in bulk by heating 50 ml of the mixture at 70°C with 5 g of allyl-bonded silica for 14 h. The reacted material was filtered off and washed with 50 ml of water and then 50 ml of methanol. The washing was repeated two more times and then the material was dried at 110°C for 12 h. About 3 g of this material was used for packing into a column by standard slurry techniques. The *in situ* conversion was carried out by circulating an AIBN–bisulfite solution identical to the one used for bulk conversion through a packed allyl column with a chromatographic pump for 16 h. After the *in situ* conversion, 100 ml of methanol, followed by 100 ml of water, were passed through the column at a flow-rate of 2 ml/min.

The procedure for measuring the ion-exchange capacity involved placing the material in 0.01 M hydrochloric acid for 5 h to ensure that all of the sites were fully protonated. After filtration, the bonded phase was placed in 0.1 M sodium chloride for 4 h and the liberated hydrogen ion was titrated with standard sodium hydroxide, using a pH meter for end-point detection. The results of a blank titration of the allyl starting material were subtracted from the results of the titration of the cation exchanger when determining the exchange capacity. The *in situ* converted material was tested by the same titration procedure after it was removed from one of the columns.

Carbon and sulfur elemental analyses were performed by Chemical Analytical Services (Berkeley, CA, U.S.A.). The results for the allyl phase and the two types of cation exchangers are shown in Table I along with the calculated ligand densities and exchange capacities.

Infrared spectra were recorded on a Model 1800 (Perkin-Elmer, Norwalk, CT, U.S.A.) spectrometer operated in the diffuse-reflectance (DRIFT) mode. The samples were prepared by making a mixture of 15% bonded phase in dry potassium bromide. Difference spectra were obtained by subtracting the spectrum of a 15% mixture of bare silica in dry potassium bromide from the sample spectrum.

TABLE I

BONDED PHASE PROPERTIES

Phase	%C	%S	Ligand density ($\mu mol/m^2$)	Exchange capacity (mequiv./g)
Allyl	0.63	–	0.50	–
Cation (bulk)	0.60	0.54	0.48	0.17
Cation (*in situ*)	0.61	0.55	0.48	0.17

Chromatography

The retention index determinations were made according to the procedure of Smith[17], using methanol–water (10:90) as the mobile phase. Amino acid and catecholamine separations were carried out with 0.1 M disodium hydrogen phosphate at pH 4.3 as the eluent. Void volumes were determined by injection of ^2H_2O.

The chromatographic system consisted of a Model 110A pump (Beckman, Fullerton, CA, U.S.A.) and a Model 7130 (Rheodyne, Cotati, CA, U.S.A.) 10-μl injection system. ^2H_2O for void volume measurements and amino acid samples were detected by a Model R401 (Waters, Milford, MA, U.S.A.) refractive index detector. Catecholamine samples and alkyl aryl ketone standards were detected by a UVIDEC 100 IV (Varex, Rockville, MD, U.S.A.) variable-wavelength detector.

RESULTS AND DISCUSSION

Fig. 1 shows a plot of log k' *vs.* carbon number $\times$ 100 for the alkyl aryl ketone homologous series. The eluent was methanol–water (10:90). Line I represents the unmodified allyl bonded stationary phase. The result is similar to those previously obtained[9] for allyl columns prepared by the chlorination–Grignard method. Line II represents an allyl phase that had been treated with chlorotrimethylsilane before packing. The allyl phase used in this study was synthesized to have a relatively low capacity (see Table I). Higher-capacity resins have been shown to have poor mass transfer[18]; the exchange capacity choosen (*ca.* 0.17 mequiv./g) in this study is close to the optimum values reported elsewhere[13,18]. As might be expected, the log k'
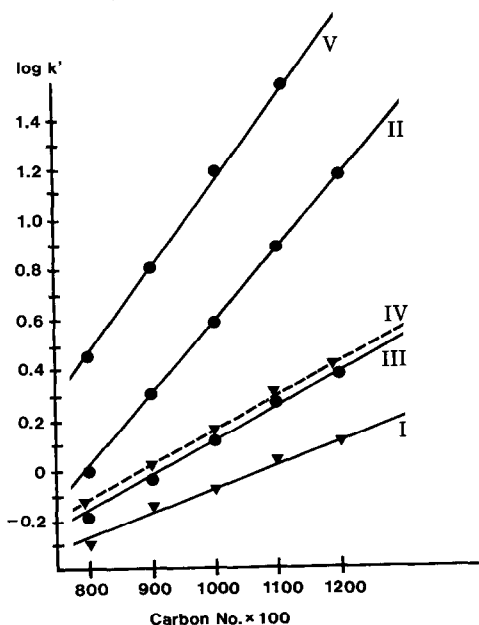

Fig. 1. Log k' *vs.* carbon number $\times$ 100 for alkyl aryl ketones. Line I = allyl phase, line II = endcapped allyl phase, line III = allyl phase after bulk addition of bisulfite, line IV = allyl phase after *in situ* addition of bisulfite, and line V = allyl phase, endcapped after bulk addition of bisulfite. Mobile phase, methanol–water (10:90).

values increase due to the greater carbon loading, but a straight-line relationship is still maintained. The change in the slope is probably due to the masking of the residual surface hydroxyls, which have been endcapped.

Line III represents the cation-exchange column which was produced by the bulk conversion of the allyl-bonded phase with bisulfite in the presence of AIBN. Again, both a change in k' values and a change in slope is observed. The increase in k' values is probably due to interactions (hydrogen bonding,, dipole, etc.) between the sulfonic acid group and the carbonyl of the alkyl aryl ketones which do not exist for the allyl phase. Titration of the reacted material indicated that $90 \pm 2\%$ of the allyl phase had been converted by bisulfite addition to a cation-exchange phase. The titration data is also supported by the results of the sulfur elemental analysis on this phase (Table I). Line IV represents a column in which the allyl phase was converted by the *in situ* method to a cation-exchange phase. The retention behavior of both the allyl phase that was converted in bulk and the allyl phase that was converted *in situ* is identical. Removal of the cation-exchange phase from the column where it was formed and subsequent titration indicated $91 \pm 2\%$ conversion of the allyl phase. This result is also supported by the results of the sulfur elemental analysis (Table I). Line V represents a column that was converted by bisulfite addition and then treated
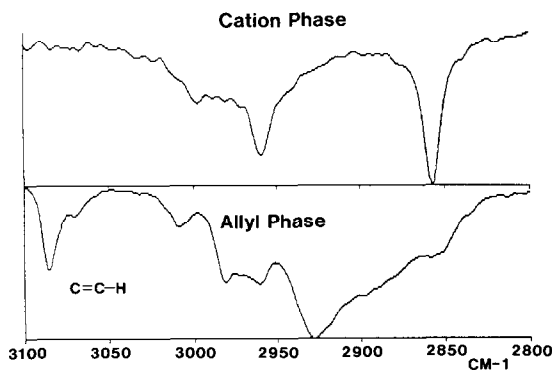

Fig. 2. DRIFT difference spectra of the cation-exchange phase (top) produced by the bulk conversion process and the allyl stationary phase (bottom).
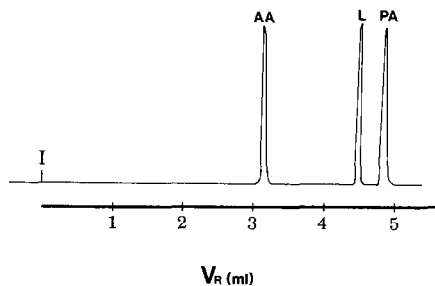

Fig. 3. Separation of some amino acids on bulk-converted allyl-to-sulfonate phase. Mobile phase, 0.1 M disodium hydrogen phosphate (pH 4.3). AA = aspartic acid, L = leucine, and PA = phenylalanine. I = injection.
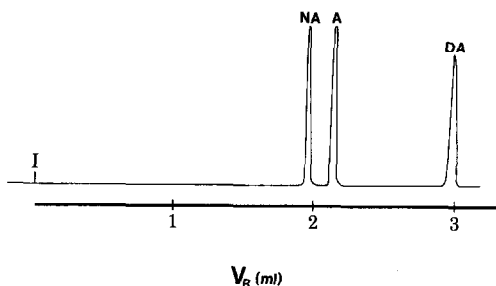
Fig. 4. Separation of some catecholamines on *in situ* converted allyl-to-sulfonate phase. Mobile phase, 0.1 *M* disodium hydrogen phosphate (pH 4.3). NA = noradrenaline, A = adrenaline, and DA = dopamine. I = injection.

with chlorotrimethylsilane. The retention behavior indicates a mixture of retention mechanisms, produced by the effects of the conversion to a cation-exchange phase and by removal of the residual silanols by endcapping.

Fig. 2 shows a comparison of the DRIFT difference spectra of the starting allyl phase and the converted cation-exchange phase. The carbon–hydrogen stretching region is particularly suitable for monitoring any modifications of the allyl phase that involve addition to the double bond. For example, the olefinic carbon–hydrogen stretch at 3080 cm^{-1} is readily apparent in the spectrum of the allyl phase. When the bisulfite reaction is successful, the peak disappears, as shown in the spectrum of the cation-exchange phase. In addition, the overall carbon–hydrogen stretching region between 3000 and 2800 cm^{-1} is markedly different in the two spectra. The spectra obtained for the cation-exchange material, produced by the bulk conversion process and the *in situ* conversion process, are identical.

In order to characterize the converted material further, some separations typically carried out on cation exchangers were tried. Fig. 3 shows a separation of some amino acids on column III, the bulk-converted allyl-to-sulfonate phase. A similar separation was achieved with the *in situ*-produced column (IV). This separation is similar to the one reported by Fazio *et al.*[13] for the components in this mixture obtained with a chemically bonded cation-exchange phase that was synthesized from sulfonated chlorodimethyl(3-phenylpropyl)silane.

Fig. 4 shows a separation of some catecholamines on the *in situ* converted column. A similar separation was achieved with a column made from the bulk-converted allyl-to-sulfonate phase. Again, the separation is comparable to the one reported[13] for a cation-exchange column prepared with a sulfonated silane.

CONCLUSIONS

Evidently, the allyl-bonded stationary phase can serve as an intermediate in the synthesis of new bonded phases. This could lead to the development of bonded phases that might be difficult or impossible to produce with silane reagents. In addition, the possibility of *in situ* conversions also has been demonstrated. Therefore, the allyl phase might serve as a basic column that could be changed on demand to one with much different properties.

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