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ALLYL-BONDED STATIONARY PHASE AS POSSIBLE INTERMEDIATE IN THE SYNTHESIS OF NOVEL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PHASES

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SUMMARY

A procedure has been developed for the synthesis of allyl to silica gel-bonded stationary phase using surface chlorination followed by a Grignard reaction. The phase is evaluated by electron spectrometry for chemical analysis, Fourier transform infrared spectroscopy, ^{13}C magic angle spinning Fourier transform nuclear magnetic resonance, and elemental analysis as well as chromatographically. In addition, the pH stability of the material was investigated. The procedure developed produces an high-performance liquid chromatographic phase which is very stable and free of salt deposits. The presence of the double bond could be exploited in subsequent reactions to produce additional bonded phases that may be difficult or impossible to synthesize by current procedures.

INTRODUCTION

In 1969, Halasz and Sebastian¹, described an esterification reaction between an alcohol and the free silyl hydroxyl groups at the surface of the silica gel particle. The major disadvantage with this phase was the relative ease of hydrolysis. This precluded the use of water in the mobile phase and had the potential of exchange at the surface with alcohols in either the mobile phase or the sample.

Shortly thereafter, commercial phases utilizing organosilanes became available. The major advantage of this type of bonded phase was its contribution of greater stability both to hydrolysis and to pH. The exclusive use of organosilanes to create these phases is notable. Today this is still the reaction used to make commercially available columns. Also notable is the fact that the C_{18} phase is the most commonly used column in liquid chromatography (LC). It has been estimated that 60-80% of all separations currently done by LC are accomplished by reversed-phase separations².

A number of reactions that bond modifying groups to the surface of solid supports have not resulted in commercially available column packings. One of these reactions, the chlorination of the silica surface followed by a reaction of the chlorinated silica with a Grignard or organolithium reagent, produces a highly stable Si-C bond.

It is interesting that all of the aforementioned reactions were described much earlier in the literature in papers strictly unrelated to chromatography. Work describing the physical properties of organosilanes bonded to glass surfaces was published in 1947³. This later became a cornerstone for the column packings that are currently commercially available. In 1959, Deuel and co-workers^{4,5} described surface modifications of silica gel particles using both the esterification reaction and the chlorination-Grignard reactions.

The purpose of this investigation is the production of a phase utilizing the chlorination-Grignard method that could serve as an intermediate for further modification by numerous reagents resulting in a variety of useful column packings. This report focuses on preparing and characterizing an intermediate allyl phase bonded to the surface of 10- μ m silica gel particles.

EXPERIMENTAL

Preparation of the allyl phase

Chlorination of the silica gel. An amount of 20 g of silica was transferred to a 500-ml round-bottomed flask. Then 50 ml of dry thionyl chloride and 450 ml of dry toluene were added. Gentle mechanical stirring was used to mix the contents of the flask. The stirring bar was kept in the flask throughout the chlorination step for use later in the procedure. The flask was fitted with a 60-cm West condenser and the contents were allowed to reflux for 16-18 h. Then the heating mantle was removed and the contents were allowed to cool before a septum was wired onto the flask. Using a cannula technique⁶ the chlorinated silica was pumped into 50-ml septum-capped centrifuge tubes. The tubes were counterbalanced with centrifuge tubes containing water and spun at 3000 rpm (*ca.* 1000 g). To remove the excess thionyl chloride, toluene was pumped into a waste container, and 45 ml of dry toluene were pumped into the tubes. The tubes were again counterbalanced and centrifuged as described. This process was repeated three times, with dry ether being substituted for toluene in the final wash. The ether supernatant was kept over the chlorinated silica gel until the Grignard reagent was available.

Preparation of allyl magnesium bromide. One neck of a three-necked round-bottomed flask was equipped with a septum, another neck with a 60-cm West condenser, and the third neck with a 500-ml separatory funnel. To this flask were added 17 g of magnesium and a stirring bar. Before initiating the Grignard reaction, the flask was flamed with a fischer burner. During the flaming the flask was purged with nitrogen through the septum port via a syringe needle connected to the end of the nitrogen line. The nitrogen purge was continued throughout the reaction.

A mixture of 55 ml of distilled allyl bromide in 350 ml of distilled ether was then added to the separatory funnel. This mixture was added to the flask slowly with adequate stirring. The reaction proceeded smoothly and could be controlled easily with an ice bath. After the complete addition of the allyl bromide-ether mixture, the contents of the reaction vessel were allowed to mix for an additional 30 min. The condenser and the funnel were removed and quickly replaced with septa. When the last septum was in place, the nitrogen line was removed.

Reaction of allyl magnesium bromide with chlorinated silica. The ether remaining in the centrifuge bottles after the wash steps previously described was pumped

off and Grignard reagent was pumped in to the predetermined 50-ml mark. The septa were removed from the centrifuge bottles and fitted with ground-glass stoppers. The contents were mixed thoroughly for a minimum of 1 h.

Clean-up step. The bottles were centrifuged as previously described and the excess Grignard reagent was decanted into a waste container. A 0.01 *M* sodium hydroxide solution was added to the mark. The contents of the flasks were thoroughly mixed and then centrifuged. The supernatant was decanted, a solution of 0.10 *M* hydrochloric acid was added to the mark, and the process was repeated. This acid-base cycling was repeated three more times followed by a last wash with deionized water. The contents of the centrifuge bottles were then transferred to petri dishes and dried overnight at 110°C.

Spectroscopic evaluation

The spectroscopic experiments were carried out as follows: small-spot electron spectrometry for chemical analysis (ESCA) was done at Surface Science Laboratories (Mountain View, CA, U.S.A.). Fourier transform infrared spectroscopy (FT-IR) was carried out at the IBM Research Laboratory (San Jose, CA, U.S.A.). Elemental analyses were run primarily by the University of California's Chemical Analytical Services (Berkeley, CA, U.S.A.). Elemental analyses of some samples were done at Merck (Rahway, NJ, U.S.A.). Finally, the magic angle spinning Fourier transform nuclear magnetic resonance (MAS FT-NMR) spectra were run at the University of Guelph (Guelph, Canada).

pH stability studies

A 150-mg quantity of allyl-bonded phase was added to each of six 16 × 100 mm screw-capped test tubes. One tube was prepared for each of six pH conditions: 1, 2, 3, 8, 9 and 10. Of each solution 10 ml were added to the appropriate test tubes. Then, to ensure that the particles were uniformly suspended throughout the duration of the experiment, the tubes were agitated gently using a labquake rocker. After 36 h, the samples were spun at 3000 rpm (*ca.* 1000 *g*) for 5 min. The particles were then washed three times with deionized water and one last time with methanol. Finally, the samples were dried overnight at 110°C.

Materials

The silica support used was distributed by Supelco under the trade name Chromosorb LC6. This material has an average pore size of 100–200 Å and a surface area greater than 400 m²/g. Prior to use, all reagents were distilled over molecular sieves to remove any water that might interfere in the preparation of the bonded phase.

All materials used to prepare the alkylarylketone standards were reagent grade. In most cases, the standards were used from the container without further handling. Any material showing more than one peak was distilled before further use. The alkylarylketone series included acetophenone (Columbia Organic Chemical Co), propiophenone (Aldrich), butyrophenone (Aldrich), valerophenone (Eastman Organic), hexanophenone (Aldrich), and octanophenone (Eastman Organic). The solvents used for high-performance liquid chromatography (HPLC) were reagent-grade methanol (Fisher Scientific) and reversed-osmosis deionized water that was prepared immediately before use on a Millipore apparatus.

Equipment

Each 150 × 4.6 mm I.D. column (Alltech) was packed with 2.75 g of the allyl-bonded phase via a Haskel pump with a head pressure of 8000–10 000 p.s.i.g. Methanol was used as the solvent. The equipment used included a Spectra Physics Model 740 pump, a Spectra Physics Model 8200 UV detector, and a Spectra Physics Model 4100 computing integrator. The injector was a Rheodyne fixed-loop injector (10 μ l).

RESULTS AND DISCUSSION

Although the preparation of alkyl phases by Grignard reactions for GC has been previously described^{7,8}, it was necessary to address three major considerations when preparing an allyl phase for LC. (i) Since the particle sizes for LC are a great deal smaller than those for GC and cause large increases in settling time, the procedure for washing the chlorinated silica described for gas chromatography^{7,8} had to be modified to reduce the time required to wash the 10- μ m particles. (ii) Because the chlorinated surface is extremely susceptible to hydrolysis and must be protected from atmospheric moisture, the chlorinated silica particles had to be protected from atmospheric conditions during any transfer from the original reaction vessel for the purpose of washing. (iii) For LC, the magnesium salts that occur both as a by-product of the reaction with surface chlorinated sites and as a result of the post-reaction hydrolysis step are undesirable. These salts had to be effectively removed from the silica surface.

The spectroscopic techniques previously mentioned were used to assess the extent of the reaction and the effective removal of undesired by-products of reaction.

Elemental analysis

The average carbon loading from four syntheses of the allyl phase was 2.05%.

FT-IR

Scans for two batches of allyl-bonded phase are shown in Fig. 1. Because sample Al-LC-A had the highest reported carbon loading and sample Al-LC-B had the lowest, these two scans represent the range of responses anticipated for all four batches. The spectra were taken using the same instrument settings with constant sample size and represent 2000 repeated scans. Thus, comparisons of peak intensities are valid. In accordance with the respective percentage carbon loading, the peak intensity at 1620 cm^{-1} is notably greater for the first batch than for the second.

The information provided by FT-IR can indicate much more than just whether the surface has been modified with the alkene functional group. If a subsequent reaction is carried out on the allyl phase, the band at 1620 cm^{-1} should not be apparent when the intermediate group is successfully reacted to form a new stationary phase. The FT-IR information is therefore important both at the initial bonding step and at further steps when modifications are made to the intermediate brush.

MAS FT-NMR

An example of an MAS FT-NMR spectrum for the allyl-bonded phase is shown in Fig. 2. The spectrum represents 11 000 repeated scans. Evident at 178 ppm,

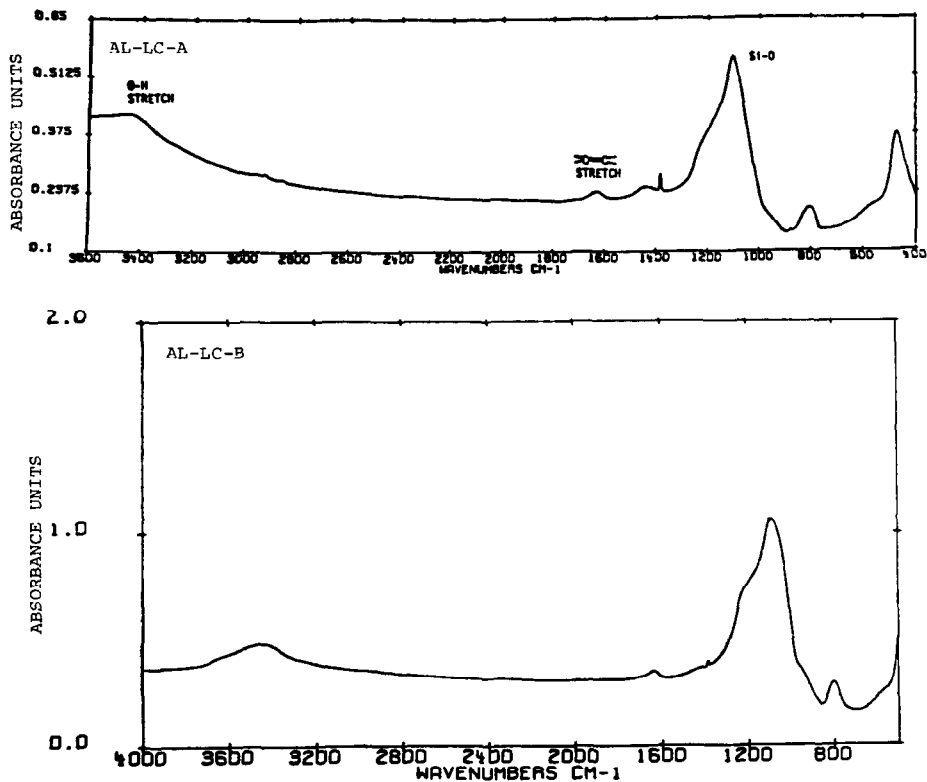


Fig. 1. FT-IR spectra of allyl-bonded stationary phase.

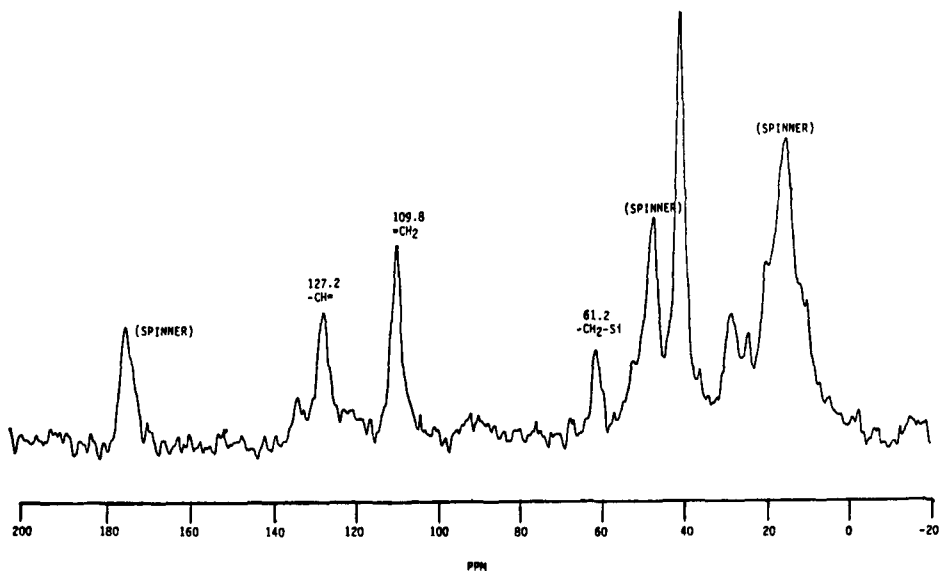


Fig. 2. ¹³C MAS FT-NMR spectrum of allyl-bonded stationary phase.

45 ppm, and 18 ppm are peaks corresponding to background contributed by the spinner. All peak assignments are in reference to trimethylsilyl. After eliminating peaks corresponding to the spinner, assignment of the three remaining peaks to the carbon species in the allyl-bonded phase can be readily done.

ESCA

Fig. 3 represents a typical ESCA spectrum of the allyl-bonded phase. ESCA analyses were done on three of the four batches. Of particular interest in the ESCA analyses is information concerning the presence or absence of magnesium. This determination can be used to evaluate the clean-up procedure for the removal of residual salts from the Grignard reaction. The presence of the anticipated hydrocarbon groups gives further support to the information yielded by other instrumental methods.

Of five scans taken on one batch, two showed traces of magnesium and three did not. Of two scans taken on another batch, neither showed any trace of magnesium. None of the ESCA spectra showed by any traces of halide. This information suggests the following. (i) Trace amounts of magnesium, if present, can be found easily via ESCA. Two magnesium Auger peaks are apparent at 300 and 350 eV. (ii) One batch had small amounts of magnesium inhomogenously distributed throughout the material. Since repeated samples from other batches did not appear to have magnesium present, it can be assumed that the clean-up step is almost completely effective in removing magnesium. The fact that one batch showed some samples that contained magnesium suggests that the clean-up step is critical. Care must be taken at this step to ensure complete removal of magnesium. It is possible that more than

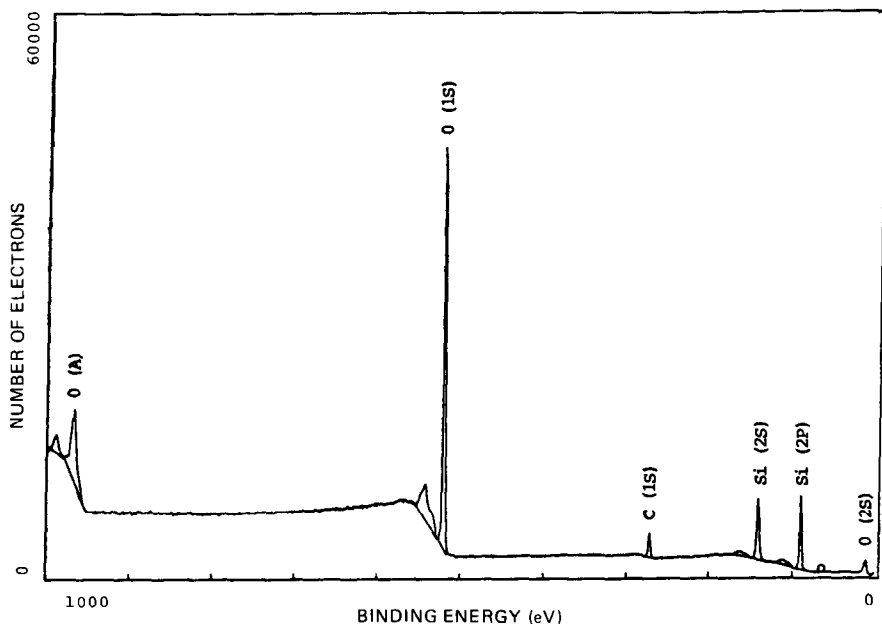


Fig. 3. ESCA spectrum of allyl-bonded stationary phase.

three acid-base cycles may be necessary to consistently obtain magnesium-free batches.

Evaluation of the allyl-bonded phase by LC

The method of Smith⁹ was used to evaluate the phase. The use of a homologous series of alkylaryketones similar to the Kovats index used in GC was suggested by Smith as a retention index scale for reversed-phase partition phases. Under reversed-phase partition conditions, this series would be separated by increasing molecular weight. The use of the series was thought to be ideal for obtaining the information sought. For a summary and representation of the results obtained using the allyl-bonded phase to separate the homologous series of alkylaryketones, see Fig. 4.

The anticipated relationship between log capacity factor, k' , and carbon number was obtained using the allyl-bonded phase. Although the k' values obtained were

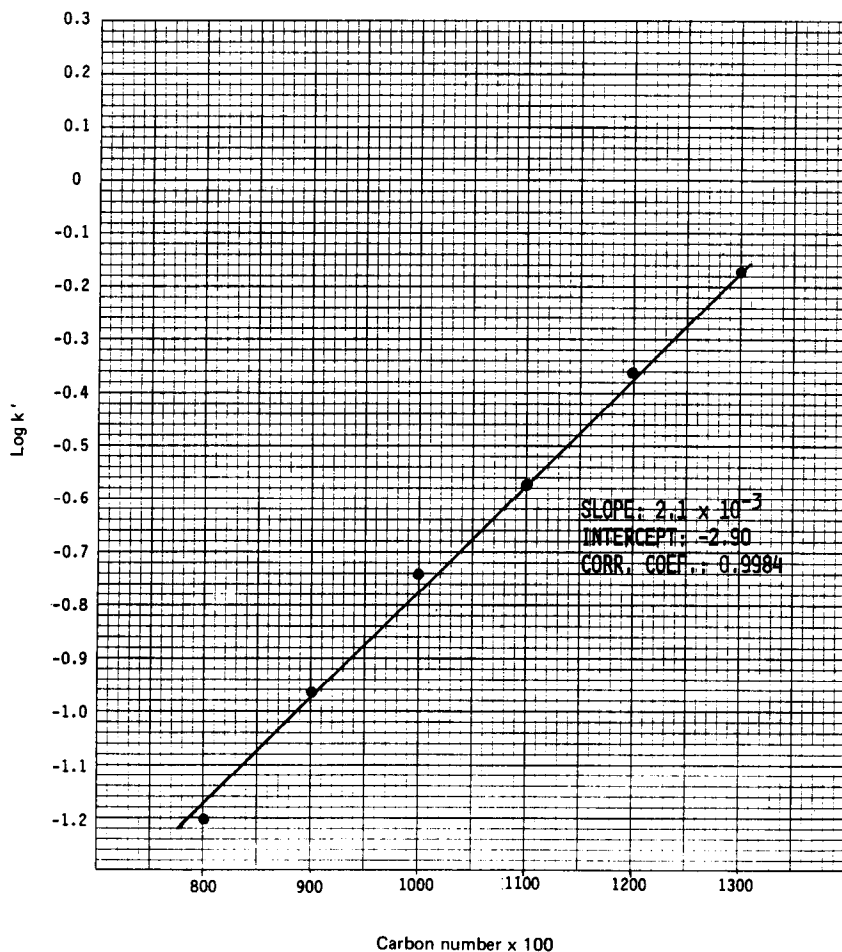


Fig. 4. Plot of log k' for various alkylaryketones vs. carbon number $\times 100$ for allyl-bonded stationary phase.

TABLE I
EFFECT OF pH ON CARBON LOADING

<i>pH condition</i>	<i>Carbon loading (%)</i>
Control (Samples 2-6)	2.21
pH 1	2.62
pH 2	2.29
pH 3	2.12
pH 8	2.00
pH 9	1.89
Control (Sample 8)	2.16
pH 10	2.14

low compared to those required for optimal separation, the requirements of this experiment did not call for an optimized system. Little or no tailing was observed in all of the alkylarylketone peaks. Clearly, the peak symmetry and elution order were indicative of a hydrocarbon phase with good coverage.

pH stability of the allyl-bonded-phase

The single greatest disadvantage of currently available bonded phases is that they are unable to withstand prolonged exposure to pH-range extremes (pH less than 3 or greater than 8). The allyl-bonded phase was subjected these pH extremes for a 36-h period. Elemental analysis was performed on the samples after they were subjected to these extremes (See Table I). The pH 10 sample was compared with a control sample by FT-IR. The intensity of the peak at 1620 cm^{-1} was used as the basis for comparison with the control sample. Reports in the literature state that the surface

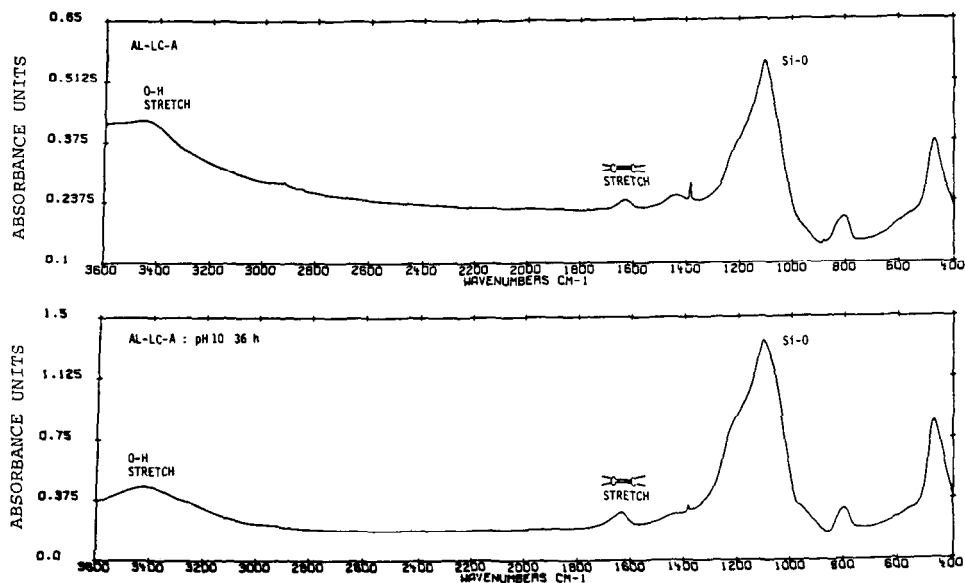


Fig. 5. FT-IR spectra for allyl-bonded phase. Top, unused; bottom, exposed to pH 10 solution for 36 h.

of the silica begins to dissolve at a pH above 9 (ref. 10). Therefore, it was felt that additional information was required in order to evaluate the pH stability of the allyl-bonded phase.

When the data are analyzed as two distinct groups, high pH group and a low pH group, the statistical parameters for the two groups are as follows: for low pH: average carbon, 2.34%; S.D., 0.25; $n = 3$; and for high pH: average carbon loading, 2.01%, S.D. 0.13; $n = 3$.

An hypothesis test comparing the two means shows that the means are not distinctly different ($P < 0.05$). The average of the high pH group is well within ± 2 S.D. of the mean of the low pH group.

When all this is taken into consideration, the meaning drawn from the experiment is that while there may be some lessening of carbon loading as pH increases, the loss at high pH is not significant. Further, no trend has been established. Based on the known chemistry of silica, no trend was anticipated in the variation seen in the low pH data. This leads one to believe that within-batch inhomogeneity may exist. Further confirmation of the high pH result is obtained by FT-IR analysis (Fig. 5). This data which shows no decrease in the 1620 cm^{-1} peak qualitatively reinforces the results of elemental analysis leading to the conclusion that this phase is not greatly affected at high pH. Further pH studies comparing this and similarly produced phases to other available phases are in progress.

CONCLUSION

The object of this investigation was to produce an allyl phase bonded to the surface of silica gel suitable as an intermediate packing for HPLC. To be suitable for use in HPLC, the bonded phase had to show good surface coverage and an absence of magnesium salts. This type of phase was successfully produced. Both spectroscopic analysis of the phase and HPLC results obtained using the material as a column packing support that conclusion. An additional feature of a brush anchored to the solid support via a Si-C bond is its tendency to resist degradation, notably hydrolysis.

With the intermediate phase successfully produced, future investigations of this column packing can focus on the ultimate goal: the conversion of the intermediate phase to a variety of column packing materials. These bonded-phase packing materials may be of many types, including ion-exchange, reversed-phase, chiral, or affinity column packings. This type of intermediate column, one with the capability of becoming any of a variety of columns upon demand, has a great potential for HPLC.

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