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SPECTROSCOPIC INVESTIGATION OF THE HYDROLYSIS OF γ-GLYCIDOXYPROPYLSILANE BOUND TO SILICA SURFACES

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SUMMARY

Chemically modified silica gels were prepared under various conditions with γ-glycidoxypropyltrimethoxysilane in order to investigate the effect of various methods of hydrolyzing the terminal epoxide group. The silylated substrates were then examined by ^{13}C solid-state NMR with cross-polarization and magic angle spinning. NMR results were correlated with infrared results obtained by diffuse reflectance Fourier transform infrared spectroscopy. By reaction with a primary alkyl amine the percentage of unopened epoxide may be determined by Fourier transform infrared spectroscopy. Infrared data indicate that as little as 10% epoxide remaining after attempted hydrolysis can influence chromatographic capacity factors.

INTRODUCTION

High-grade porous silica gels with a narrow distribution of particle diameters, well defined pore volume and various surface areas are now widely available. Uses of such materials are strongly dependent on the chemical properties of the support surface. Chemical modification of support materials by bonding an organosilane to the surface is common. There exists a wide variety of functional groups from which to choose. An important example of a surface modification useful for protein liquid chromatography is the attachment of a diol group[1]. Diol phases are prepared by attaching γ-glycidoxypropyltrimethoxysilane to silica followed by hydrolysis of the epoxide group under acidic conditions[2]. The diol phase may be used directly for exclusion chromatography of hydrophilic polymers and proteins, or after further modification for the attachment of ligands for affinity chromatography. Few investigations have provided a detailed description of the chemical species which exists on surfaces prepared in this way. Therefore, reproducible preparations of modified silica are difficult to obtain and variations of retention times and selectivity between

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batches is common[3]. Methods used for the characterization of chemically modified silica gels such as thermogravimetry in combination with other techniques, or hydrolytic cleavage of Si–O–Si bonds have the disadvantage of destroying the chemically bonded material. Solid state ^{13}C NMR with cross-polarization and magic angle spinning (^{13}C CP-MAS-NMR) is an excellent technique for surface characterization[4]. However, it is relatively time consuming and expensive compared to other spectroscopic techniques, and restricted availability limits the extent of detailed and systematic investigations which can be performed. An inexpensive and readily available technique such as infrared spectroscopy is a desirable approach to obtain information about the bonded species for characterization of modified silica gels. The information obtained by infrared spectroscopy can be augmented by the use of simple chemical treatments. Leyden and co-workers[5,6] have shown diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) to be an important qualitative and quantitative tool for the investigation of modified silica surfaces. In addition to the ease of sample preparation, DRIFTS is superior to transmission techniques because methods involving sample pellet preparation can lead to erroneous results[7]. This report describes the use of chemical probes and ^{13}C CP-MAS-NMR and Fourier transform infrared (FT-IR) spectroscopy for the characterization of silica gel modified with γ-glycidoxypropyltrimethoxysilane. The extent of the hydrolysis reaction used to produce the diol as determined spectroscopically is correlated with the retention properties of liquid chromatographic columns prepared with these materials.

EXPERIMENTAL

Preparation of modified silica gel

Materials and equipment. The γ-glycidoxypropyltrimethoxysilane was purchased from Petrarch Systems (Bristol, PA, U.S.A.). The entire amount was transferred into smaller vials and sealed to protect against hydrolysis and polymerization. The vials were stored in a refrigerator. Periodic acid and perchloric acid were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Potassium dichromate and sodium thiosulfate were obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). The silica gels used in this study were 30-μm Nucleosil 100-30 (reported surface area of 300 m^2/g and pore size of 100 Å) from Machery-Nagel, F.R.G. Titrations of the diols were done with a Radiometer Auto-buret (Model ABU-11, Copenhagen, Denmark) equipped with a 2.5-ml buret assembly.

Silanization schemes. The following procedure is based on Watson's[8] modification of the method of Regnier and Noel[2]. A 5.0-g sample of 30-μm silica was weighed and placed in a 500-ml erlenmeyer flask. A 5.0-ml sample of γ-glycidoxypropyltrimethoxysilane was added to 100.0 ml, 10 m*M* acetate buffer solution of pH 5.5. The mixture was added to the silica and the slurry was degassed by sonication under vacuum for 10 min. After breaking the vacuum, the slurry was swirled and degassed for an additional 10 min. The flask was then tightly stoppered with an aluminum foil wrapped neoprene stopper and placed in a 90 $\pm$ 3°C oil bath such that the liquid levels were equal. The flask was swirled every 15 min for the first 2 h and then every 0.5 h thereafter for a total of 6 h. Approximately 10 min after the start of the reaction the solution became quite turbid. After 1 h the solution became clear and colorless. The flask was removed from the bath and allowed to cool to room temperature. We call this sample "A".

pH 3.0 Ring opening (preparation of sample B). The silica silanized as above was filtered in a 30-ml, fine-grade sintered glass funnel and washed with pH 3.0 acetic acid (10 m*M*), and degassed as above. The flask was then placed in a 90 ± 3°C oil bath for one additional hour. The flask was swirled every 15 min. The solution above the silica remained clear and colorless throughout the process. After the flask was removed from the bath, deionized water was added to cool the reaction. The support was filtered as before and washed with 25–30 bed volumes of water, acetone, and 2–3 bed volumes of diethyl ether. The support was not endcapped. This sample was labeled sample "B".

pH 3.0 Ring opening (preparation of sample C). A 2.0-g aliquot of sample B was placed in a 250-ml erlenmeyer flask with 50.0 ml of pH 3.0 (10 m*M*) acetic acid buffer. The slurry was degassed as stated earlier. The flask was stoppered and the reaction vessel placed in a 90 ± 3°C oil bath for approximately 10 h. The phase was filtered and washed as in the previous procedure. This sample was labeled "C".

pH 2.0 Ring opening (preparation of sample D). A 1.0-g aliquot of sample A was placed in a 125-ml erlenmeyer flask with 20.0 ml of pH 2.0 sulfuric acid solution. The slurry was outgassed, the flask stoppered, and the reaction vessel heated for 1 h. The flask was swirled every 15 min. The resulting phase was filtered and washed as in the previous procedure. This sample was labeled "D".

Perchloric acid ring opening (preparation of sample E). A 1.0-g aliquot of sample A was placed in a 125-ml erlenmeyer flask with 25.0 ml of 35% perchloric acid solution. The slurry was outgassed and the flask was stoppered. The flask was swirled every 15 min. The phase was filtered and washed as in the previous procedure. This sample was labeled "E".

Pre-hydrolysis (preparation of sample F). A 2.5-ml sample of γ-glycidoxypropyltrimethoxysilane was dissolved in 50 ml of pH 2.0 sulfuric acid solution. The stoppered solution was heated in a 90 ± 3°C oil bath for 1 h. The solution was removed from the bath and the pH was raised to pH 5.0 by the addition of 2.0 ml of 0.5 *M* sodium acetate. A 1.0-g sample of silica was added to the solution and the slurry was outgassed. The solution was heated for an additional 6 h. The reaction was swirled every 15 min for the first 2 h, and then every 0.5 h for the remaining reaction time. At no time during the reaction was any turbidity observed in the solution above the silica. The phase was filtered and washed as in the previous procedure. This sample was labeled "F".

All phases were left overnight (approximately 12 h) in an oven at 90°C. Before being analyzed or packed, the phases were sieved through a set of 200–400-mesh sieves to remove polymer. All of the phases prepared above were evaluated for their hydrophobicity by studying the retention of a select group of solutes. These solutes were chosen because they had the highest retention on the diol phase of all the solutes tested.

Titration procedure

The following procedures were followed in preparing the solutions for the diol titrations. The thiosulfate solution was prepared from freshly boiled distilled deionized water. Approximately 25.0 g of thiosulfate and 0.1 g of sodium carbonate were dissolved in 1.0 l of water. Chloroform (3–4 drops) was added to inhibit bacterial growth. The solution must be standardized to four significant figures. The potassium iodide solution was prepared daily and was made by dissolving 1.0 g of potassium iodide in 50.0 ml of distilled deionized water. The flask must be stoppered when not in

use. The periodic acid solution was prepared by dissolving 0.25 g of acid in 10 ml of distilled deionized water. After the solid completely dissolved, 40 ml of glacial acetic acid were added and the solution thoroughly mixed. This solution was stored in a brown glass bottle and kept in the dark until used. The 35% perchloric acid solution was prepared by diluting a 70% acid solution. The 0.2% (w/v) starch solution was prepared by boiling 250 ml distilled deionized water and slowly adding 0.5 g of soluble starch. When the solution had cooled, 3–4 drops of chloroform were added.

A blank titration was performed on the same day as the diol titration. The 2.5-ml auto-buret was thoroughly rinsed and filled with standardized thiosulfate. A 1.0-ml sample of the periodic acid solution was pipetted into a 10-ml erlenmeyer flask. A 2-ml volume of the potassium iodide solution was then added. The PTFE tube from the buret was inserted into the flask and the solution was stirred. Titrant was added until the solution changed from a dark brown to a light brownish yellow. A 2-ml volume of starch solution was then added and the titration continued until the solution color changed from blue to colorless.

For particles of high surface area, much smaller amounts of sample were needed for the titration. Three samples whose weights ranged from 10 to 20 mg were weighed out and placed in clean, dry 10-ml erlenmeyer flasks with stir bars. A 1-ml volume of 35% perchloric acid was added to the first sample. After stirring for 1 h at room temperature, 1.0 ml of periodic solution was again added and stirred for another 0.5 h. The rest of the titration procedure is identical to the blank titration.

The surface coverage was calculated in moles of diol/m^2 by the following equation:

$$\text{Surface coverage} = \frac{(V_b - V_s)\, M}{2000\, S_A\, S_W}$$

where V_b and V_s are the blank and sample titration volumes in ml, respectively, M is the molarity of the thiosulfate solution, S_A is the surface area of the silica and S_W is the weight of the sample in g.

Modified silica–amine reactions

A 0.5-g aliquot of γ-glycidoxypropylsilane-modified silica gel was treated with 25 ml of 1.0 M reagent-grade n-butylamine in toluene previously dried over molecular sieve. Samples were allowed to react while standing for 24 h at room temperature. After vacuum filtration, samples were washed three times with 15-ml aliquots of dry toluene and dried under 0.1 mmHg vacuum at 100°C for 4 h.

INSTRUMENTAL

A Nicolet 60SX FT-IR spectrometer purged with dry air and equipped with a liquid nitrogen-cooled wide-band mercury cadmium telluride detector was used to obtain infrared spectra. All spectra were acquired by signal averaging 100 scans at a nominal resolution of 4 cm^{-1}. DRIFTS samples were 5% (w/w) dispersions of modified silica gel in potassium chloride (< 5 μm particle size, dried at 120°C for 8 h) and were prepared by mixing in a Wig-L-Bug (Crescent Dental Manufacturing) without the grinding ball for 30 s. A DRA-2CN diffuse reflectance accessory (Harrick

Scientific) was used to obtain the DRIFT spectra. The ^{13}C NMR spectra were obtained on a Nicolet NT-150 spectrometer at a carbon frequency of 37.735 MHz with a home-built CP-MAS unit, including the probe. The decoupling field was 13 G (55 kHz). The spinning system is a modified version of that reported by Wind *et al.*[9] with a sample volume of 0.3 cm^3. The samples were rotated at 3800 rps. The CP contact time was 2 ms, and the repetition time was 2 s. Acquisition conditions involved collection of 2024 data points with a spectrum width of 20 kHz and an acquisition time of 52 ms. Chemical shifts were measured relative to external tetramethylsilane, with hexamethylbenzene as a secondary standard (methyl signal at 17.35 ppm).

RESULTS AND DISCUSSION

In the first four synthetic procedures presented in the experimental section, the same method for immobilizing the silane on the silica was used. During this step, it has been reported[10] that polymer does form in the first hour. The solution appeared to have a light precipitate dispersed throughout the supernate during this time period. After 1 h the precipitate settled out of the solution.

The fourth procedure (sample F) essentially reversed the first and second steps that are used in the other procedures. The ring was opened in pH 2.0 buffer and then the silane was attached to the silica. It is important to note that at no time during the reaction was there any indication of polymer formation. The resulting phase also passed through the sieves more quickly than any other phase.

There were two reasons why the pre-hydrolysis procedure was investigated. When a glycidoxy group is attached to silica, the reaction is assumed to occur between the silanol groups on the silica surface and the methoxy groups on the silane. However, it is possible that the silanols on the surface may react with the epoxide ring rather than the methoxy group. This type of reaction has been demonstrated by the reaction of 1,4-dioxane and silica silanols[11]. If the ring is opened before the silane is exposed to the surface, then this reaction cannot occur. Secondly, after the silane has been immobilized on the surface of the silica, there may be interactions involving the epoxide group and the surface that interfere with ring opening.

The surface coverage of sample B, as determined by the diol titration procedure before treatment with perchloric acid, was determined to be 2.5 (±0.1) $\mu mol/m^2$. After treatment with perchloric acid, the surface coverage increased to 3.1 (±0.2)

TABLE I

CAPACITY FACTORS FOR IODOBENZENE ON THE MODIFIED SILICA SAMPLES AND MOBILE PHASES EMPLOYED

Mobile phase	*Stationary phase*	*k'*	*Mobile phase*	*Stationary phase*	*k'*
Water–methanol (90:10)	A	5.59	Water–methanol (70:30)	A	2.84
	B	4.93		B	2.15
	C	4.62		C	1.91
	D	4.14		D	1.74
	F	1.04		F	0.43

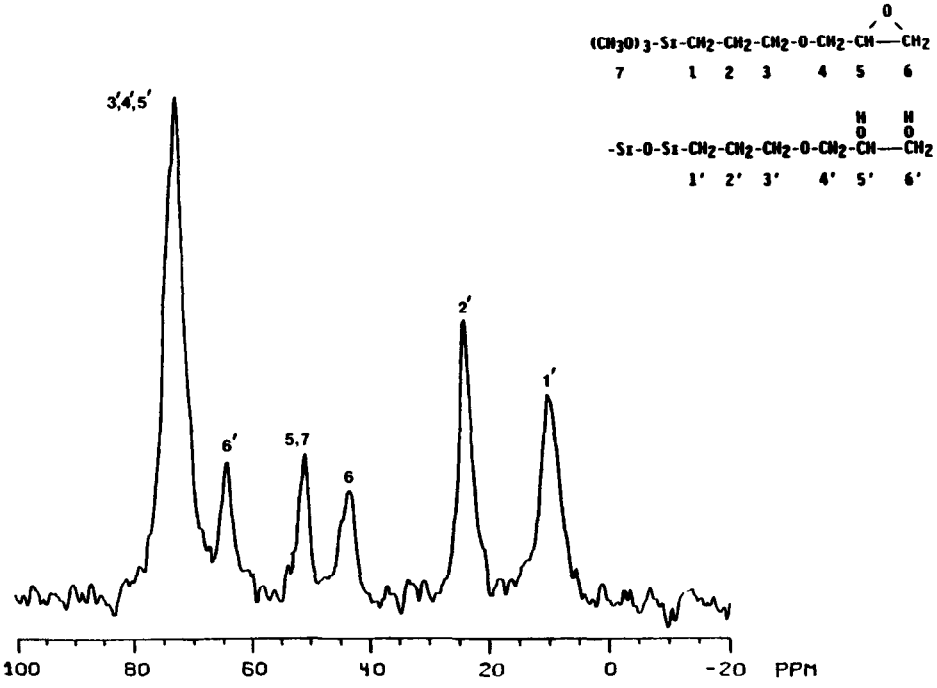

Fig. 1. Solid-state ^{13}C CP-MAS-NMR for sample A (3936 scans).

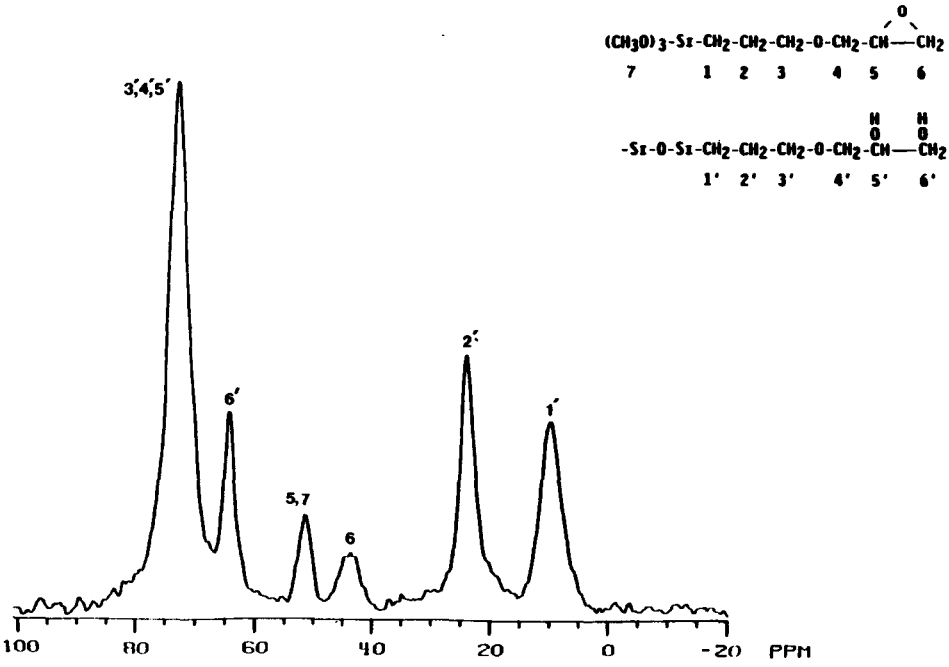

Fig. 2. Solid-state ^{13}C CP-MAS-NMR for sample B (30 000 scans).

μmol/m^2. Sample F did not show a similar increase in coverage. The coverage before treatment was determined to be 2.4 ($\pm$0.2) μmol/m^2, while after treatment the coverage was 2.5 ($\pm$0.3) μmol/m^2.

DRIFT spectroscopy of the modified silica does not allow the direct observation of whether the terminal epoxide group on the silane has been completely hydrolyzed. This results primarily from the overlap of the epoxide ring vibrations in the 750–950 cm^{-1} region with absorption from the silica gel matrix. Absorption by residual silanol groups on the silica substrate interferes with the direct determination of hydroxyl groups on the diol moiety. These large interferences also impede spectral subtraction techniques because spectral artifacts in the low wavenumber region prevent accurate interpretation of the difference spectra. Reaction of the unhydrolyzed epoxide group with a primary alkylamine provides a very convenient chemical probe of the amount of residual oxirane. Absorption by the alkyl group of the reacted amine is free of matrix interference problems. The presence of water is required for the reaction to proceed[12], and may limit the amount of reaction if an inadequate quantity of water is present. Silica samples equilibrated for 48 h in a 50% relative humidity atmosphere showed no additional reaction with the amine than those dried overnight at 110°C. Therefore, it is assumed for the surface coverage involved that there is sufficient surface adsorbed water present to allow for the reaction. Chromatographic data for the modified silica samples (A, B, C, D and F) and mobile phases employed are shown in Table I. Sample E, which was treated for 1 h at 90°C with a 35% perchloric acid solution, shows virtually complete removal of bound silane, as determined by DRIFTS and is not considered further in this work.

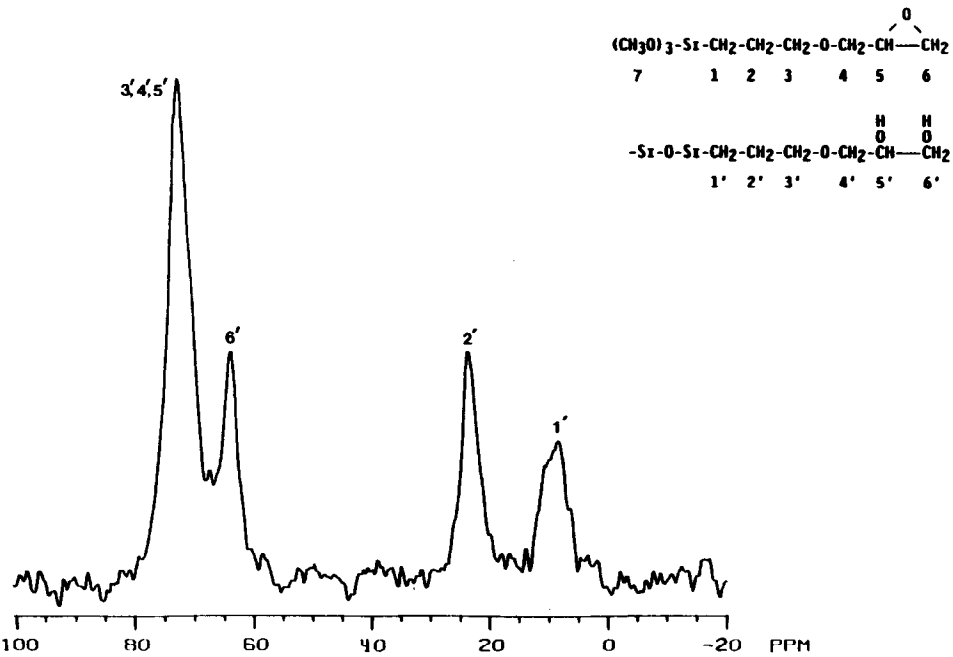

Fig. 3. Solid-state ^{13}C CP-MAS-NMR for sample F (6000 scans).

For most of the above samples, chromatographic capacity factors were larger than expected for the very polar diol stationary phases. A possible explanation of the high retention is that for all samples other than F (the pre-opened epoxide), some unhydrolyzed epoxide remains on the surface imparting a much less polar environment than a diol group.

The existence of unhydrolyzed epoxide groups on the above materials was confirmed by solid-state ^{13}C NMR. Fig. 1 shows a solid-state ^{13}C CP-MAS-NMR spectrum of sample A. Chemical shifts are assigned according to Bayer *et al.*[4]. Assignments are as indicated on the spectrum. The peaks at 43.4 and 50.1 ppm indicate the presesence of carbons 6 and 5, respectively, in the epoxide, and the resonance at 63.1 ppm indicates the presence of carbon 6′ in the diol. Some of the signal at 50.1 ppm may result from unhydrolyzed methoxy group carbons on the silane and, therefore, this peak is given less importance in the interpretation than that at 43.4 ppm. Fig. 1 clearly shows that some diol formation has occurred under the mild hydrolysis conditions, but hydrolysis of the epoxide is incomplete. Fig. 2 shows that when this sample is heated to 90°C at pH 3.0 for 1 h (sample B), the resonance at 43 ppm remains indicating that hydrolysis of the epoxide is still incomplete. Fig. 3 is the solid-state ^{13}C NMR spectrum of sample F. The resonances at 43.4 and 50.1 ppm are not present indicating complete hydrolysis of the epoxide group and the methoxy groups.

Fig. 4 shows DRIFT spectra of sample B before and after reaction with

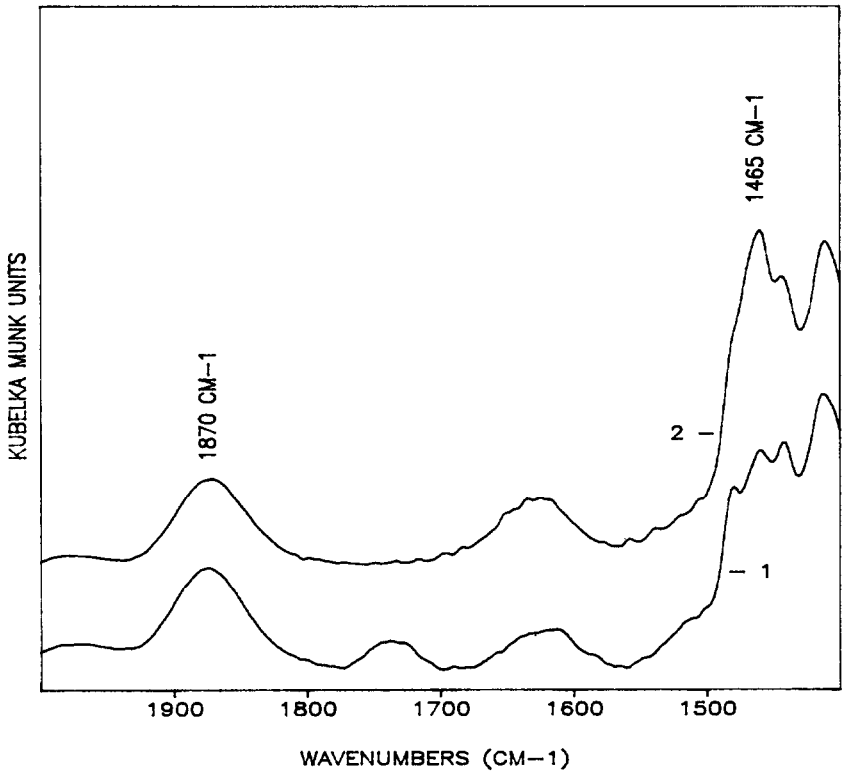

Fig. 4. DRIFT spectra of sample B before amine treatment (1) and after amine treatment (2).

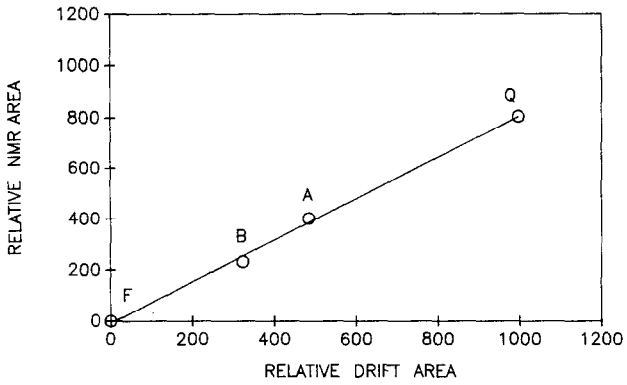

Fig. 5. Plot of relative epoxide NMR band area *versus* FT-IR amine band area.

n-butylamine. The spectra show an increase in absorption at approximately 1465 cm^{-1}. This is attributed to a combination of the asymmetrical deformation band of the terminal methyl group and the methylene scissoring band from reacted amine[13]. Integration of this band and taking the ratio of the result to the area of the Si–O–Si combination band at 1870 cm^{-1}, provides a relative band area for each sample. This band area ratio is an indication of the amount of epoxide on the surface. One can similarly ratio the area of the solid-state ^{13}C CP-MAS-NMR resonance at 43.4 ppm to the area of the resonance of carbon 1′ at 9.6 ppm. Fig. 5 shows a plot of the relative NMR band area *versus* the relative FTIR band area. Point Q was established by making the assumption that the resonance for each carbon in the solid-state ^{13}C CP-MAS-NMR spectrum yields the same area. Therefore, the sum of the areas for the peaks at 43.4 ppm (carbon 6) and 63.1 ppm (carbon 6′), each ratioed to the peak at 9.6 ppm, represents an estimate of the relative area of each peak for a surface containing pure expoxide or diol, respectively. This estimate is accurate to perhaps 10–15%. Point Q was located by plotting the total NMR relative area on the fitted line. Fig. 5 shows

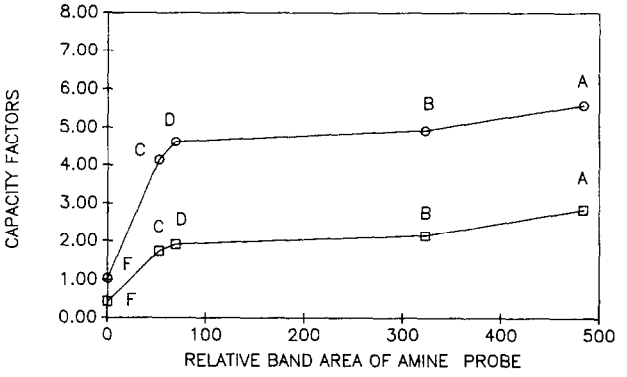

Fig. 6. Plot of capacity factors of iodobenzene *versus* the relative band area of the FT-IR peak for *n*-butylamine reacted with the γ-glycidoxypropylsilane-modified silica. Mobile phase: water–methanol (90:10) (○) or (70:30) (□).

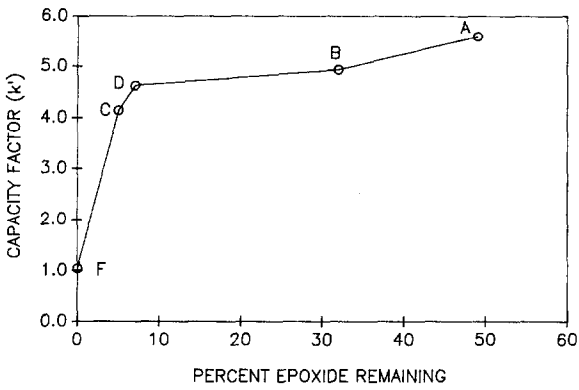

Fig. 7. Plot of capacity factors for iodobenzene *versus* percent unhydrolyzed epoxide as determined by FT-IR. Mobile phase: water–methanol (90:10, v/v).

that the FT-IR-amine probe method can be used as a rapid and simple estimate of the fraction of epoxide on the surface.

Fig. 6 is a plot of the capacity factors for iodobenzene *versus* relative amine band area determined by the infrared method using two different mobile phase compositions. The plot indicates that the surface coverage of epoxy groups must be below a critical level before a significant decrease in retention occurs on this type of modified silica.

The data shown in Fig. 5 may be used to estimate the percent epoxide remaining on the surface using the *n*-butylamine probe and FT-IR. Fig. 7 shows a plot of the chromatographic capacity factor (k') for iodobenzene *versus* percent of total surface γ-glycidoxypropylsilane present in the unhydrolyzed epoxide form. The conclusion is that the retention is dramatically affected by the presence of as little as 10% unhydrolyzed surface epoxide group.

CONCLUSIONS

All samples of γ-glycidoxypropylsilane modified silica contained some residual epoxide on the surface. Only the sample in which the γ-glycidoxypropylsilane was pre-hydrolyzed (F) contains little or no unhydrolyzed epoxide. Solid-state NMR results allow estimation of the extent of hydrolysis of the epoxide as a function of chemical treatment. NMR spectra also support the results obtained using DRIFT spectroscopy. By simple chemical treatment of the modified silica, changes can be monitored by using infrared techniques. As a result, infrared spectroscopy can be used to further describe surface characteristics and determine relative amounts of epoxide left unhydrolyzed.

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REFERENCES

1 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.
2 F. E. Regnier and R. Noel, *J. Chromatogr. Sci.*, 14 (1976) 316.
3 J. N. Kinkel and K. K. Unger, *J. Chromatogr.*, 316 (1984) 193.
4 E. Bayer, K. Albert, J. Reiners and M. Nieder, *J. Chromatogr.*, 264 (1983) 197.
5 J. P. Blitz, R. S. S. Murthy and D. E. Leyden, *Appl. Spectrosc.*, 40 (1986) 829.
6 R. S. S. Murthy and D. E. Leyden, *Anal. Chem.*, 58 (1986) 1228.
7 J. P. Blitz, *Ph.D. Dissertation*, Colorado State University, Fort Collins, CO, 1987.
8 M. Watson, *Ph.D. Dissertation*, University of Minnesota, Minneapolis, MN, 1981.
9 R. A. Wind, F. E. Anthonio, M. J. Duijvestijn, J. Smidt, J. Trommel and G. M. C. de Verre, *J. Magn. Reson.*, 52 (1983) 424–434.
10 S. H. Chang, K. M. Gooding and F. E. Regnier, *J. Chromatogr.*, 120 (1976) 321.
11 F. Schmitz, D. Leyendecker and D. Leyendecker, *J. Chromatogr.*, 389 (1987) 245.
12 S. P. McManus, C. A. Larson and R. A. Hearn, *Synth. Commun.*, 3 (1973) 177.
13 A. L. Smith, *Applied Infrared Spectroscopy*, Wiley, New York, 1979.