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STABILIZATION OF REVERSED PHASES FOR LIQUID CHROMATOGRAPHY

APPLICATION OF INFRARED SPECTROSCOPY FOR THE STUDY OF BONDED-PHASE STABILITY

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SUMMARY

This paper examines the spectroscopic characterization of chromatographic silica gels at three stages: before bonding of an *n*-butyl group to the silica surface, after bonding, and after exposure to hydrolytic conditions expected to lead to some loss of the alkyl groups from the surface. Hydrolysis was achieved by circulating an aqueous acetonitrile solution containing 0.5% (v/v) trifluoroacetic acid at 27°C through a loosely packed bed of the *n*-butyl-bonded silica. This hydrolytic treatment results in a rapid partial loss of bonded phase over about 1 day, followed by a much more gradual loss over the next six days. The extent of hydrolysis depends upon the silica used and the initial degree of coverage of the bonded phase. The results show that a portion of the bonded sites of the bonded phase are hydrolyzed more rapidly under acidic conditions, and that higher initial bonded-phase coverages lead to shielding of the more reactive sites and thus less overall hydrolytic loss. Diffuse-reflectance infrared Fourier transform (DRIFT) spectroscopy was used to study three different silicas before and after hydrolysis of the bonded phase. On one of these silicas, rapid hydrolysis of the *n*-butyl bonded phase was associated with reemergence of isolated silanol groups on the silica surface. On the other two silicas, the same hydrolytic conditions led to much slower loss of coverage, and spectroscopy did not detect a reemergence of lone silanol groups. Although all three unbonded silicas apparently had similar physical and chemical properties, one of them was clearly different from the others in stability of a subsequently attached alkyl phase. This difference could only be detected by bonding, followed by hydrolysis. One possible explanation of these results is that different manufacturing conditions lead to a different distribution of silanol groups on the silica surface, which is not detectable by routine physical and chemical characterization methods, yet nevertheless affects bonded-phase stability.

INTRODUCTION

Interest in the design, synthesis and use of chemically bonded silica gels in high-performance reversed-phase liquid chromatography remains high. A sizable body of data has been developed on the synthesis of organosilane coatings on the surface of silica gel[1-3]. Recent studies have focused on the role of the silica gel used to prepare the bonded phase as well as the type of organosilane and reaction conditions employed[1,4-7]. A limited number of studies is available on the characterization of hydrolyzed bonded phases[8]. Advances in these areas will lead to better characterized stationary phases with extended lifetime in chromatographic use.

Improved stability of alkyl-bonded silicas in reversed-phase chromatography may be achieved in a variety of ways, including: use of a fully hydroxylated silica[9], use of high-coverage synthetic conditions[6], use of bulky alkylsilanes (*e.g.*, those containing ethyl[10] or isopropyl[7] side groups), multi-reactive silanes (*e.g.*, dichloro- or triethoxysilanes[11]) or siloxane polymer coatings[12,13]. Although all of these approaches can lead to improved stability in individual experiments, they should also be evaluated for their ability to be incorporated into a reproducible manufacturing process.

The silica gel used for bonding plays an important role in the preparation of improved alkyl-bonded phases. Several studies have characterized chromatographic behavior associated with "good" or "bad" silica gel precursors and attempted to relate silica surface chemistry to observations in chromatographic peak shape of basic compounds and column stability[6,14,15].

The early literature[16,17] suggests that differences exist in adsorptive behavior and chemical reactivity of the various silica surface species, including siloxanes ($\equiv$Si–O–Si$\equiv$), isolated or one silanols ($\equiv$SiOH), vicinal silanols ($=$Si(OH)–O–(OH)Si$=$) and geminal silanols ($=$Si(OH)$_2$)[18]. Geminal groups have usually been classified as more like isolated silanols in their IR and chemical behavior[16,19]. "Associated silanols" is a general term for those silanol groups capable of interacting with neighbors through hydrogen bonding[5,6]. The distinction between isolated and associated silanols is one of proximity: neighboring silanols separated by 2.6 to 3.1 Å are close enough to interact, while silanols separated by more than 3.1 Å are isolated on the surface[20]. Spectroscopic methods, such as IR, NMR and fluorescence provide complementary qualitative data on these surface species; identification of specific spectroscopic peaks can be provided by a variety of thermal[20-23], adsorptive[17,24,25], and isotopic exchange[26,27] techniques. Unfortunately, quantitative analysis of surface species is still difficult, although progress has been reported by several groups[28,29].

More recent studies, which often employ spectroscopic techniques, show that a fraction of the total silica surface silanols is more reactive in the typical silanization reaction used for alkyl-bonded phase preparation. Interestingly, published reports disagree on the relative reactivity of isolated, geminal, and vicinal silanols toward a typical monochloro-organosilane[4-6,20,23,27]. Many studies have shown the presence of a more reactive subset of silanols; reports are divided on the identity of this subset. Finally, it must be noted that in these studies silica gels containing variable impurity levels, produced by variable manufacturing processes, were used.

The goal of this research is the generation of a reproducible silica surface chemistry for the preparation of high-coverage, acid-stable alkyl-bonded phases. As a critical use of reversed phases today is in the area of biopolymer separations, which

commonly require the use of acidic conditions, we felt that reversed-phase stability under acid conditions is an appropriate starting point for the general study of stationary phase stability. In particular, we examined the role of the silica gel used in the reaction and the role of initial coverage of the bonded phase on its stability under acidic conditions. We assessed stability directly by measuring the loss of bonded phase upon hydrolysis, using a hydrofluoric acid dissolution/gas chromatography sampling technique. Fourier transform (FT) IR spectroscopy of the unbonded silica and the bonded silica before and after hydrolysis suggests pathways by which the reaction proceeds. Taken together, the results provide greater insight into the variable surface reactivity that underlies stationary-phase instability.

EXPERIMENTAL

Chemicals and materials

n-Butyldimethylchlorosilane was obtained from Petrarch Systems (Bristol, PA, U.S.A.) and was used without further purification. Trifluoroacetic acid (TFA), orthophosphoric acid and acetonitrile were "Baker Analyzed"-reagent grades (J. T. Baker, Phillipsburg, NJ, U.S.A.). HPLC-grade water was prepared in-house. Three experimental silica gel batches were supplied by PQ Corp. (Valley Forge, PA, U.S.A.) and used as received. Their physical and chemical characteristics are listed in Table I.

TABLE I

CHARACTERISTICS OF SILICA GELS STUDIED

	Batch 1	Batch 2	Batch 3
Date of manufacture	March, 1984	February, 1986	May, 1986
Pore volume (ml/g)*	1.3	1.21	1.39
Surface area (m^2/g)*	261	238	254
Median pore diameter (Å)*	186	197	188
Particle size properties			
Dv, 50 (μm)**	19.2	17.0	16.4
Dv, 50/Dp, 50**	1.09	1.16	1.18
Dv, 10/Dv, 90**	1.55	1.76	1.72
Metals, anions (ppm)***			
Al	45	50	88
Ca	38	7	<25
Fe	53	33	50
Na	10	31	41
SO$_4^{2-}$	25	<25	31
Cl$^-$	27	<25	17
pH§	4.0	5.2	4.7
Loss on drying§§	<5	4.2	4.4

   * Porisimetry determined by nitrogen sorption (BET method).
  ** Particle-size analysis by Coulter counter.
 *** Cation analysis was by hydrofluoric acid digestion of silica followed by flame atomic absorption spectroscopy for Na and inductively coupled plasma spectroscopy for Al, Ca, Fe and Mg determination. Anion analysis was by ion chromatography of water extracts.
   § pH measured of a 10% slurry of silica in water.
  §§ Determined from the weight change of the silica after heating at 105°C for 2 h.

The silica gels were bonded by the procedure of Kinkel and Unger[1]. Before bonding, the silica gel was dried for about 12 h under vacuum at 120°C.

Hydrolysis procedure

In our hydrolysis procedure, which was discussed in a previous paper[10], the bonded phase was exposed to a recirculating hydrolysis solvent, composed of 32% aq. acetonitrile, the aqueous component being 10 mM orthophosphoric acid, and, in the final solution, the TFA concentration being 0.5% (v/v). The aqueous component was adjusted to a pH of 1.6, using potassium hydroxide prior to addition of the acetonitrile. The hydrolytic mobile phase was prepared freshly at frequent intervals to prevent any equilibration with the bonded phase during the hydrolysis experiment. Aliquots of the bonded silica gel (*ca.* 500 mg) were sampled at various intervals and washed with 50 ml each of water, methanol and acetone. Bonded-phase coverage was determined by a previously developed HF digestion/gas chromatographic method[30].

DRIFT Studies

Spectra were obtained on a Model 20SXB spectrometer (Nicolet, Madison, WI, U.S.A.) operating at 2 cm^{-1} spectral resolution with full aperture opening. A Happ-Genzel apodization function was used and 100 scans were collected on the typical sample. Approximately 30 mg of the stationary phase was added with minimal packing as a 10% (w/w) mixture in dry potassium chloride to a sample cell, connected to a vacuum pump. Typically, several hours of pumping at room temperature were needed to remove physically adsorbed water to enhance observation of the isolated and vicinal bands. Peak position was assigned by comparison of the characteristic spectra obtained with literature data[31].

RESULTS AND DISCUSSION

The goal of this paper is to relate the spectroscopic properties of silica-based bonded phases to chromatographic stability of the stationary phase. The hydrolysis technique developed previously[10] serves as a testing procedure for the assessment of stability and as a method for preparing various hydrolyzed surfaces. The acid achieves hydrolysis of the bonded ligands in a reasonable time and mimics typical mobile phases used in the reversed-phase HPLC of proteins. Determination of alkyl group coverage is achieved via hydrofluoric acid digestion/headspace gas chromatography[30]. The technique directly measures surface group coverages with the advantage of ligand chain speciation by use of the gas chromatography separation. Thus, a quantitative measure of bonded-phase stability may be generated by appropriate use of the hydrolysis and hydrofluoric acid/gas chromatographic procedures. We next turn to a discussion of the roles of the silica gel precursor used as well as alkyl group coverage level on the stability of the resultant bonded phase.

Effect of base silica gel

Three prototype silicas (batches 1, 2 and 3) were allowed to react with *n*-butyldimethylchlorosilane to the maximum coverage achievable by our procedure, and the bonded phases were subjected to the hydrolysis evaluation described previously. Fig. 1 presents the loss in bonded phase coverage as a function of hydrolysis time.
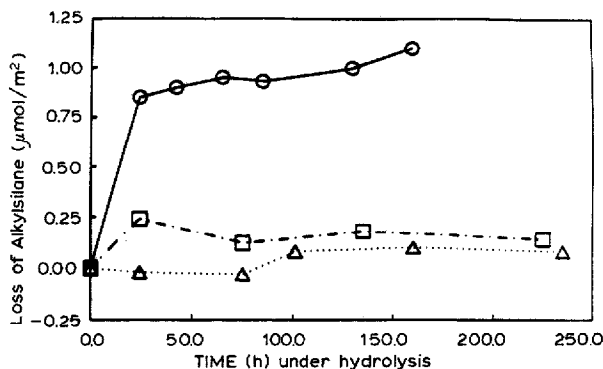
Fig. 1. Hydrolysis response of batch 1, 2 and 3 silicas, bonded with *n*-butyldimethylchlorosilane. Initial coverages are as noted in the figure. ○ = Silica batch 1 (initial concentration, 3.67 μmol/m^2); △ = batch 2 (2.83 μmol/m^2); □ = batch 3 (4.04 μmol/m^2). For conditions, see text.

Note that initial coverage levels varied between silicas, which may reflect type and amount of surface silanols available for reaction. C$_4$-bonded batch 1 exhibited a rapid loss of coverage from 3.67 to about 2.8 μmol/m^2 (about 25% over 25 h), followed by a much more gradual loss of coverage. Interestingly, C$_4$-bonded batches 2 and 3 exhibit losses of only about 0.1 μmol/m^2 in the course of the experiment. While different manufacturing conditions were used in the production of these silica gels, Table I shows no great differences in the characteristics typically measured on these 200-Å pore diameter, 20-μm particles. Analysis of metals and anions in the gels revealed low levels of these impurities with some variability in the Al, Ca, Fe and Na content. Results obtained by other investigators[6] prompted us to examine silica surface phenomena as a possible source of variability. We next turned to the examination of the influence of coverage levels on the stability of the C$_4$-bonded batch 1 silica.

Effect of alkyl group surface concentration
        The influence of bonded-phase coverage on stability of the material was studied by synthesizing a series of bonded phases on several quantities of batch 1 silica to levels of 1.95, 3.00, 3.17, and 3.67 μmol/m^2 of *n*-butyldimethylsilyl groups. Fig. 2 presents the loss in bonded-phase coverage of these packings as a function of hydrolysis time. For initial coverages on the same silica of 1.95, 3.00, and 3.17 μmol/m^2, a similar rapid loss in coverage of ca. 1.6 μmol/m^2 over 30 h is observed, followed by a much more gradual loss. For the bonded phase initially bonded to highest coverage (3.67 μmol/m^2), a rapid loss of coverage of only 0.8 μmol/m^2 occurs over the same 30 h period, followed again by a much more gradual loss. Fig. 3 provides selected data of Fig. 2 including a plot of initial coverage, the coverage loss at 137 h of hydrolysis, and the remaining surface coverage as a function of initial bonding coverage. First, each of the three partially bonded phases showed about the same 1.6 μmol/m^2 "average" loss of coverage over the course of the hydrolysis experiment, suggesting the presence of a subset population of more easily hydrolyzed alkylsilylsiloxanes on the surface. Note that the magnitude of this population remains constant, even though the initially bonded coverage increases from 1.95 to 3.17 μmol/m^2. The residual coverage levels
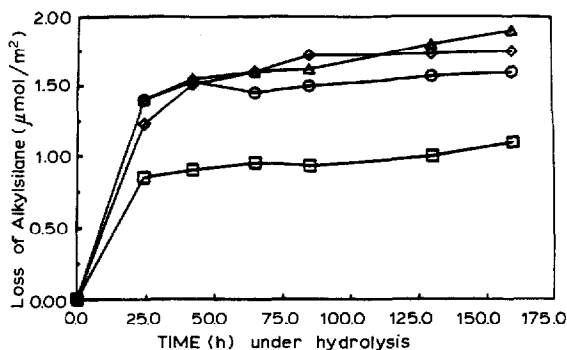
Fig. 2. Hydrolysis response of batch 1 silica, bonded with *n*-butyldimethylchlorosilane to different coverages. Bonded phases of 1.95, 3.00, 3.17 and 3.67 μmol/m^2 initial coverage were evaluated. Initial ligand concentrations: $\bigcirc$ = 1.95 μmol/m^2; $\triangle$ = 3.00 μmol/m^2; $\diamond$ = 3.17 μmol/m^2; $\square$ = 3.67 μmol/m^2. For conditions, see text.

after hydrolysis of *ca.* 0.35, 1.4 and 1.6 μmol/m^2, corresponding to the partial initial coverages of 1.95, 3.00 and 3.17 μmol/m^2, respectively, represent a less reactive population of sites, which is not hydrolyzed as easily as the first set of sites. The observation that hydrolysis behavior does not proceed more readily after about 30 h suggests that a shielding effect of initially higher but not maximum coverage is not significant. However, evidence of such a shielding effect is noted for the maximum coverage of 3.67 μmol/m^2, where coverage loss has been decreased to about 1 μmol/m^2 over 137 h. For this phase, a relatively greater proportion of the less reactive sites has been bonded so that steric hindrance reduces the easily hydrolyzed population from about 1.6 to about 1 μmol/m^2.

The results indicate that the most densely covered phases will exhibit better stability, but that coverage increases to less than the maximum level will not necessarily result in better stability. Furthermore, even at maximum coverage, some silicas give better stability than others that appear to be similar in typically measured properties. Thus, while improved bonding methods can serve to increase stability[10], it is
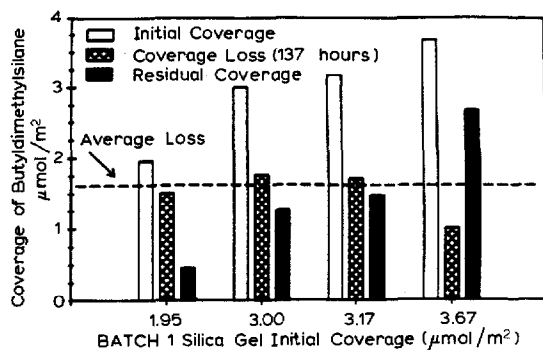

Fig. 3. Comparison of initial coverage level, coverage loss after 137 h of hydrolysis, and residual coverage for batch 1 silicas, bonded with *n*-butyldimethylchlorosilane to variable coverage levels. See text for further discussion.

obviously an advantage to use a silica (*e.g.*, batches 2 or 3) that provides alkylated surfaces of greater stability.

We decided to explore the capability of modern spectroscopic methods for the further identification of differences observed in the stability of bonded phases based on batches 1, 2 and 3. Note that for the remainder of this paper, batches 2 and 3 are grouped as silicas that are "good" or "stable", while batch 1 silica is "bad" or "unstable". The subsequent spectroscopic results therefore use batches 2 and 3 interchangeably as examples of desirable silica gel precursors.

Fourier transform infrared spectroscopic studies

An evaluation of the bare silicas, butyl-bonded phases, and hydrolyzed stationary phases was undertaken by the FT-IR method. Previous literature studies have shown how this method can be used to identify different types of silanol groups on the silica surface. The paper of McDonald[31] provides a useful summary of peak assignments in the IR region. Recent work has shown the value of the FT-IR technique in characterizing the *n*-alkyl-bonded phase, especially as it relates to various pre-treatment procedures of the silica gel precursor[28,32]. FT-IR provides a rapid qualitative analysis of silica surfaces, particularly in the identification of isolated silanol groups compared to other associated or hydrogen-bonded silanol sites. Fig. 4 provides DRIFT spectra of the three unbonded silica surfaces used in this study.

Three bands of interest are evident in each of the three spectra. The sharp band centered at 3735 cm^{-1} is assigned to the isolated silanol sites, while the more diffuse band centered at 3655 cm^{-1} has been tentatively identified as weakly hydrogen-bonded silanols or vicinal silanols[31]. The broad peak at around 3500 cm^{-1} is assigned to physically adsorbed water.

The assigment of IR band intensity to geminal silanols has generated some contradictions in the literature[4,18]. It appears that the predominant view is that the position of the geminal silanol band in the IR is closer to that of the isolated silanol
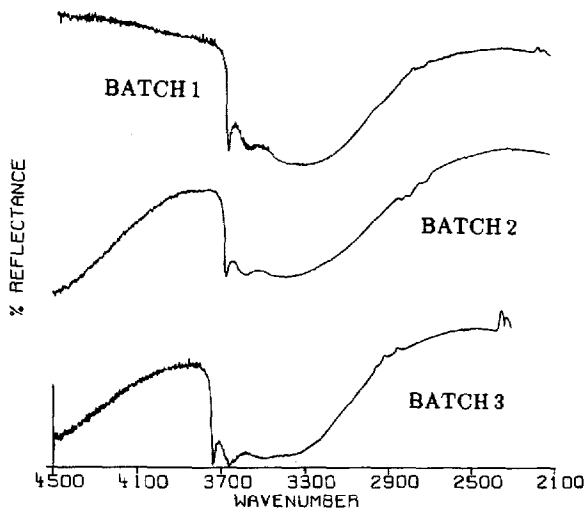

Fig. 4. DRIFT spectra of batch 1, 2 and 3 silicas. See text for discussion.
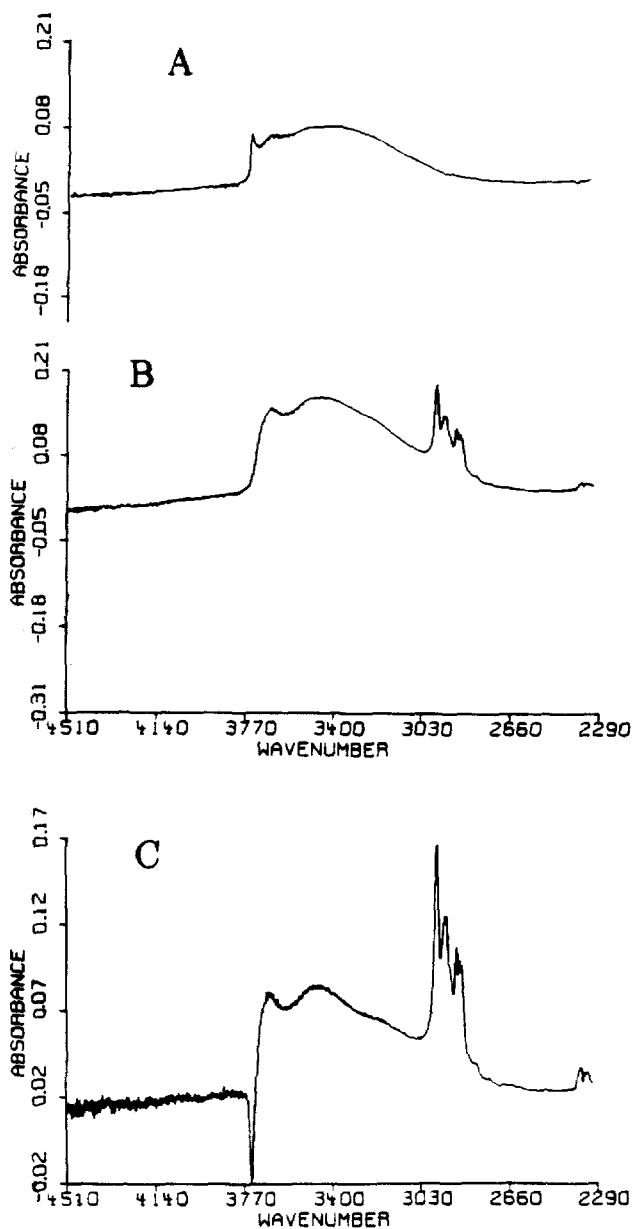
Fig. 5. FT-IR spectra of unbonded and bonded batch 1 silica. (A) Silica batch 1; (B) batch 1 silica, bonded to a coverage of 3.67 μmol/m^2 butyldimethylsilyl groups; (C) difference spectrum, B − A.

rather than the associated silanols[6,16,19]. The IR spectra presented here do not allow any conclusions to be made with respect to the presence of geminal silanols on these silicas.

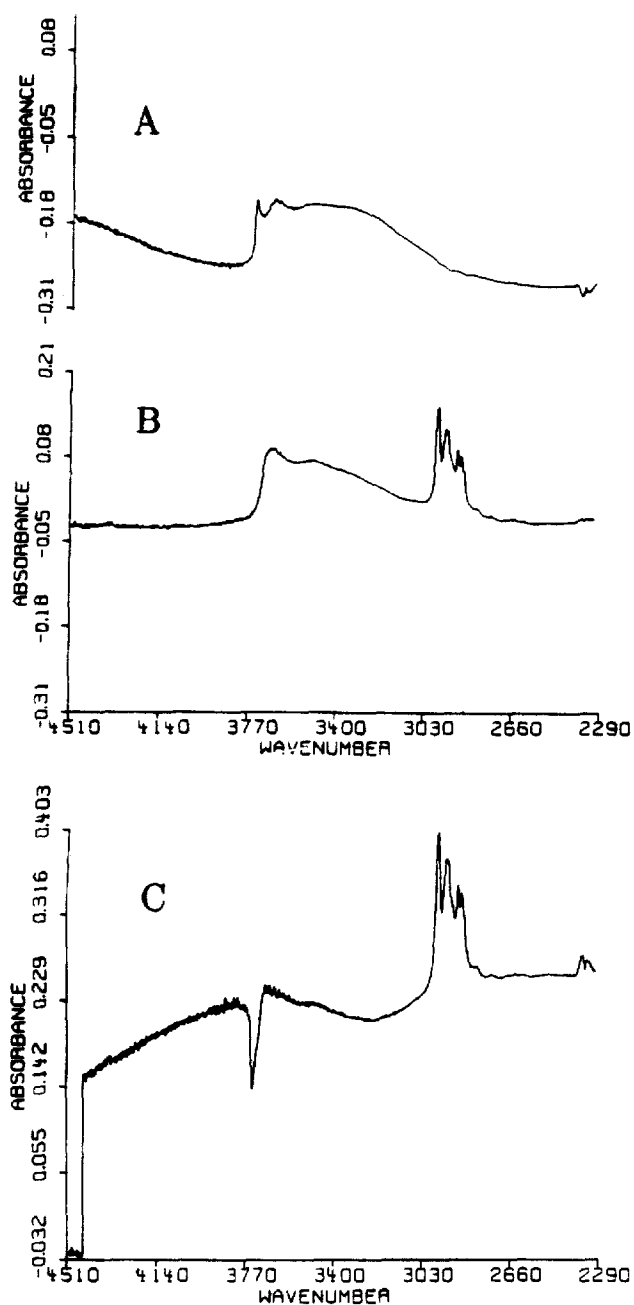While we did not attempt to quantitate the observed bands in the IR spectra, a

Fig. 6. FT-IR spectra of unbonded and bonded batch 3 silica. (A) Silica batch 3; (B) batch 3 silica, bonded to a coverage of 4.0 μmol/m^2 with butyldimethylsilyl groups; (C) difference spectrum, B − A.

qualitative comparison indicates that there are minimal changes from spectrum to spectrum for these three silicas. Interestingly, silica batches 2 and 3 appear to have a more pronounced band at 3655 cm^{-1} than batch 1. This observation may indicate a greater proportion of vicinal silanols.

Figs. 5 and 6 present IR spectra of unbonded and *n*-butyl-bonded phases, based on batch 1 and batch 3 silicas, respectively. As may be readily observed in both spectra, isolated silanol band intensity is decreased upon bonding the silica with *n*-butyldimethylchlorosilane, with the resultant appearance of CH group stretches at about 2900 cm^{-1}. Interestingly, the associated silanol band intensity does not seem to change significantly. Spectral subtraction of the unbonded silica spectrum from the bonded phase spectrum confirms that the major observable change between the spectra is the loss of isolated silanol intensity and gain in hydrocarbon intensity as indicated by FT-IR. Several other researchers have made this observation in the bonding of monochloroalkylsilanes to silica gel[5,6,20,23].

We next examined the FT-IR of *n*-butyl-bonded phases as a function of hydrolysis time. Figs. 7 and 8 provide spectra of hydrolyzed bonded phases, corresponding to points for batches 1 and 2, respectively, in Fig. 1. Fig. 7 shows that the hydrolysis of *n*-butyl-bonded batch 1 silica leads to a reemergence of isolated silanol intensity at the expense of the hydrocarbon peak area. As short a hydrolysis time as 18 h produces isolated silanols with a corresponding 17% decrease in alkyl group coverage. The isolated silanol peak appears to grow as the hydrolysis of the bonded phase is extended to 137 h.

Interestingly, Fig. 8 shows that a much longer hydrolysis time of 35 days on the *n*-butyl-bonded batch 2 silica for a similar loss of coverage of *ca.* 24% does not lead to a large increase in isolated silanol intensity (although the inflection of the lower spectrum of Fig. 8 suggests that some isolated silanols are present). Figs. 9 and 10 clarify these observations by providing the difference spectrum of the hydrolyzed stationary phases, subtracted from the freshly prepared bonded phase for batches 1 and 2, respectively. After 35 days, batch 2 shows about 37% of the isolated silanol intensity observed for batch 1 after only 137 h (about 6 times less) of hydrolysis. Chromatography of basic amine solutes on both packings indicates that the bonded phase has deteriorated to the extent that broad and tailed peak shapes were observed (data not shown). Thus, while the bonded phases on both batches 1 and 2 have deteriorated, only batch 1 shows a rapid instability associated with the reappearance of isolated silanols. Slow hydrolysis (< 6 times longer than for batch 1) of the batch 2 material under the same hydrolytic solution conditions does not lead to a significant reappearance of isolated silanols and suggests that hydrolysis occurs by a different mechanism or occurs on different sites than in batch 1.

One may speculate that the isolated (and/or geminal) silanol population may represent a more reactive set of sites on the surface and are among the first to bond in the typical silanization reaction. This hypothesis is supported by the FT-IR data (Figs. 5 and 6). It is possible that these sites are more reactive even as the bonded siloxane species and so are among the first groups to be hydrolyzed. Hence, isolated and/or geminal silanol intensity reemerges in the IR (Fig. 7) upon hydrolysis of the bonded phase. The lengthy time scale of hydrolysis for bonded phases on batch 2 suggests that a different set of siloxane sites undergoes reaction, and therefore implies the absence of isolated siloxane sites on this silica. Fig. 3 suggests that the population
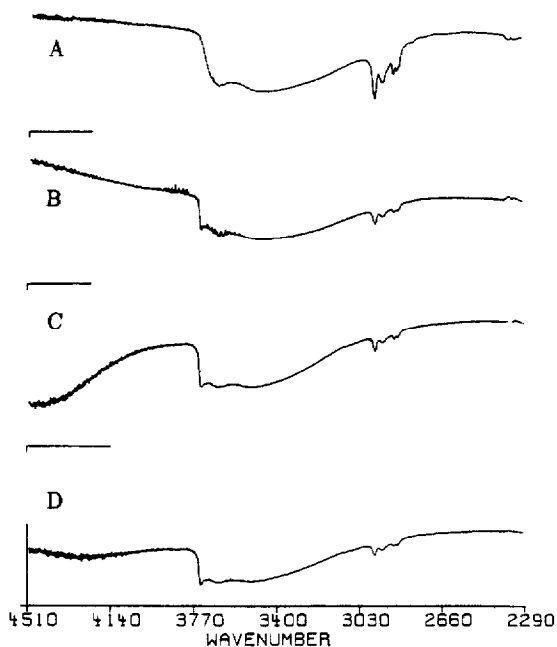
Fig. 7. DRIFT spectra of bonded and hydrolyzed batch 1 stationary phases. (A) *n*-Butyldimethylsilyl-bonded batch 1 silica, 3.67 μmol/m^2; (B) same phase as in A after 18 h of hydrolysis, 3.03 μmol/m^2; (C) same stationary phase as in A after 42 h of hydrolysis, 2.77 μmol/m^2; (D) same stationary phase as in A after 137 h of hydrolysis, 2.64 μmol/m^2. Hydrolysis conditions are described in the Experimental section.

of easily hydrolyzed bonded sites on batch 1 is *ca.* 1.6 μmol/m^2, reflecting a population of *ca.* 1.6 μmol/m^2 of isolated and/or geminal silanols on the surface.

Fig. 11 presents a set of IR spectra, corresponding to a series of *n*-butyl-bonded batch 1 silicas synthesized to coverage levels of 0, 0.83, 2.0 and 3.67 μmol/m^2. The major loss in intensity from spectrum A to spectrum B is at the isolated silanol

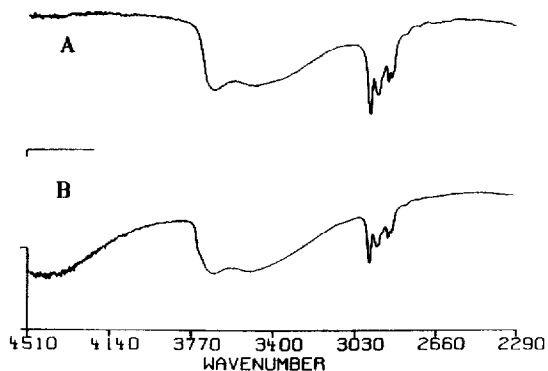Fig. 8. DRIFT spectra of bonded and hydrolyzed batch 2 stationary phases. (A) *n*-Butyldimethylsilyl-bonded batch 2 silica, 2.83 μmol/m^2; (B) same stationary phase as in A after 35 days of hydrolysis, 2.15 μmol/m^2. Hydrolysis conditions are as described in the Experimental section.
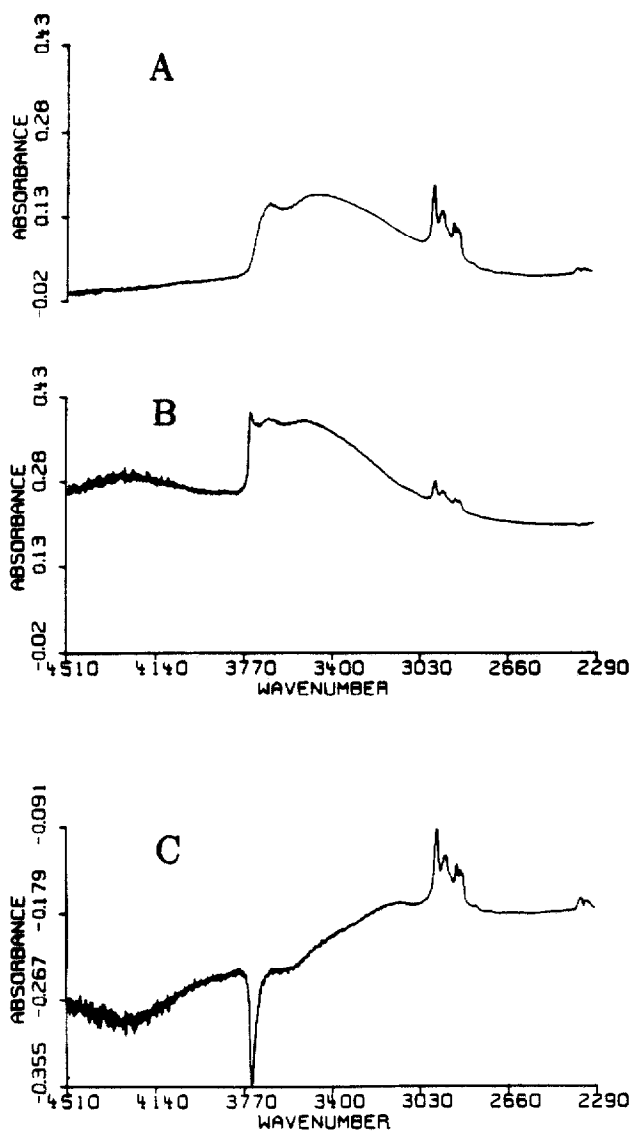
Fig. 9. FT-IR spectra of bonded and hydrolyzed batch 1 stationary phases. (A) *n*-Butyldimethylsilyl-bonded batch 1 silica, 3.67 μmol/m^2; (B) same stationary phase as in A after 137 h of hydrolysis, 2.64 μmol/m^2; (C) difference spectrum, A − B.

position, which reflects the preferential bonding of these silanol sites on the silica surface. It is evident that the lone silanol intensity is lost after silanization of the surface to between 0.83 (spectrum B) and 2.0 (spectrum C) μmol/m^2 coverage, in rough agreement with the 1.6 μmol/m^2 of hydrolyzable sites. These data suggest that the lone or geminal silanol sites react first in bonding and are also hydrolyzed first; consequently, stable stationary phases should contain a minimum of these sites. This
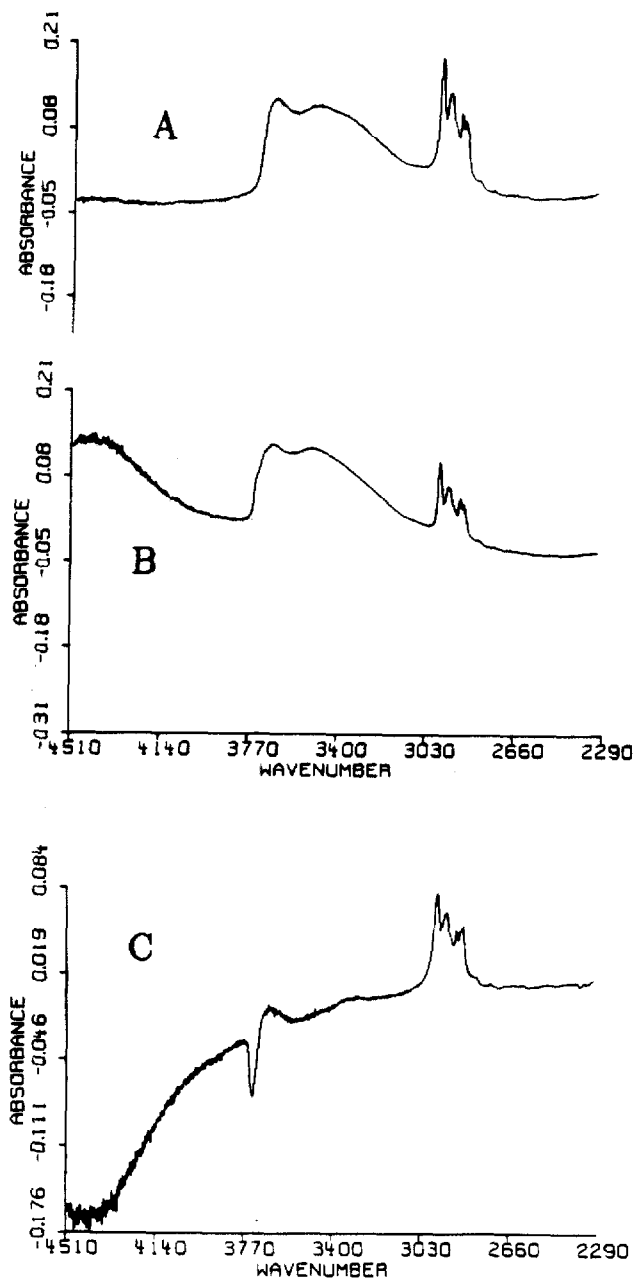
Fig. 10. FT-IR spectra of bonded and hydrolyzed batch 2 stationary phases. (A) *n*-Butyldimethylsilyl-bonded batch 2 silica, 2.83 μmol/m^2; (B) same stationary phase as in A after 35 days of hydrolysis, 2.15 μmol/m^2; (C) difference spectrum, A $-$ B.
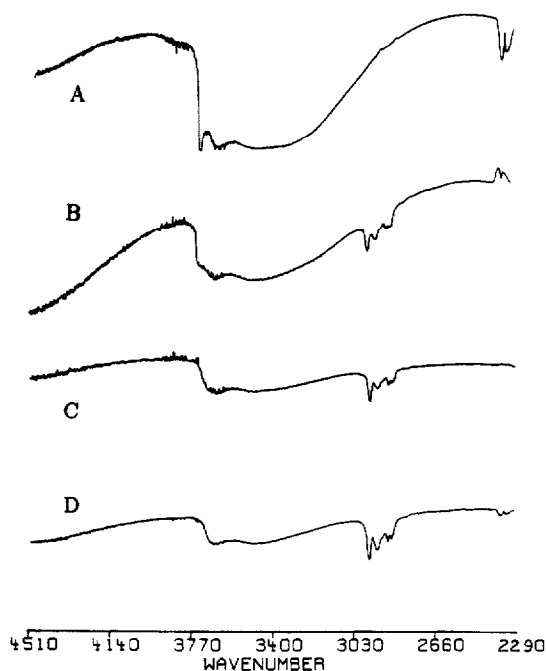
Fig. 11. DRIFT spectra of unbonded and partially *n*-butyldimethylsilyl-bonded batch 1 silicas. (A) Batch 1 silica; (B) C_4-bonded batch 1 silica, 0.83 $\mu mol/m^2$; (C) C_4-bonded batch 1 silica, 2.0 $\mu mol/m^2$; (D) C_4-bonded batch 1 silica, 3.67 $\mu mol/m^2$. See Experimental section for synthesis conditions.

conclusion is in agreement with the literature[6]. Further work to confirm this hypothesis including the use of ^{29}Si NMR spectroscopy is under way in our laboratories.

The main difference between the manufacturing processes used to produce batch 1 silica and batches 2 and 3 silica is in the presence or absence of salts (*e.g.*, sodium sulfate) during drying of the hydrogel. Interestingly, only batch 1 silica possessed about 1000 ppm sodium sulfate as the silica was being dried, and subsequent washing gave the values listed in Table I. One hypothesis now under study in our laboratory is that the presence of salts during drying gives rise to a disadvantageous silanol population on the silica gel surface, perhaps by a "template effect" in which ionic species from the salts that are adsorbed on the silica surface interact with the silanol groups that are forming during the drying process, preventing those silanol groups from interacting with each other. Thus, a higher proportion of isolated groups may form and remain even after the salts are later washed away.

CONCLUSIONS

This paper examined the DRIFT spectroscopic characterization of three batches of *n*-butyl-bonded silica gels before bonding, after bonding, and after hydrolytic removal of the alkyl chains from the surface. Batch 1 silica, when bonded with C_4 chains, yields a characteristic and rapid 25% loss of alkyl groups upon hydrolysis

over a period of *ca.* 25 h, followed by a much more gradual loss. DRIFT results reveal that the rapid hydrolysis of the bonded phase is associated with the reemergence of the isolated silanol groups on the silica gel surface. Initial bonded-phase coverage levels on this silica affect the magnitude of the bonded group loss but not the time scale. A shielding effect is evident at high coverage, which serves to diminish alkyl group loss by hydrolysis. Bonded phases, based on silica batches 2 and 3, manufactured under different conditions, exhibited much slower losses of coverage and did not show reemergence of isolated silanol intensity in spectroscopy. The results indicate that improved coverage serves to decrease coverage losses by hydrolysis and that the silica gel precursor selected has a major role to play in the resultant bonded-phase stability. The data suggest that direct characterization of silica gel products is necessary but not sufficient to quantitate the stability of the bonded phase. Differences in the inherent stability of bonded phases on these two types of silica were only evident after bonding and hydrolysis studies.

ACKNOWLEDGEMENTS

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