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NMR Wool Tube: a novel method for NMR solution analysis of derivatized glass surfaces

Olivia Maria Cholewa*

School of Pharmacy, University of Wisconsin–Madison, 777 Highland Avenue, Madison, WI 53705-2222, USA

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Abstract

Glass wool was placed within an NMR tube as a solid support for the covalent attachment of a molecule to allow for a simple one-dimensional ^{1}H FT NMR solution analysis. This novel procedure avoids the use of expensive sample tubes or platforms, as required for magic angle or fast spinning, exotic pulse sequences, isotopic labeling or the use of a large number of scans to provide the ability to analyze the structure, mobility, ligand binding, and solvent interactions of the surface bound molecule.

Keywords: Glass wool; Nuclear magnetic resonance spectroscopy; Derivatized glass surface

1. Introduction

The derivatized surfaces commonly used in HPLC resins are dynamic surfaces whose properties and characteristics are dependent upon their interaction with the solvent(s) used in the mobile phase. Current methods in the examination of modified glass surfaces involve the use of solid-state NMR with cross-polarization-magic angle spinning (CP-MAS), scanning electron microscopy (SEM), atomic force microscopy (AFM) or other methods [1–8]. The main disadvantage of the solid state NMR, SEM and other studies is that they must be carried out in the absence of solvent and therefore do not elucidate the actual bonded phase characteristics that exist under the conditions used in liquid chromatography [9], and AFM is limited in resolution.

The concepts of the hydrophobic bonded phase configurations have evolved from the early static models [13,21] to more recent dynamic models where bonded chains can undergo changes in orientation and mobility dependent on various parameters such as temperature, solvent type and composition [26] or aggregate under defined conditions [27,28]. The dynamic models were further bolstered by studies [29] that showed that alkyl chains (i) possess a flexibility related to the mobile phase used and carbon content of the bonded phase, and (ii) that they exist in various conformations with the range extrema going from rigid brushes to a convoluted form.

These basic models provided a physical description to explain how the hydrophobic bonded phase interacts with the mobile phase to form a solvation layer, an interphase [16], and how the bonded phase retains solute molecules. These NMR experiments presented a theoretical foundation to help explain the behavior and problems associated with derivatized materials commonly used in HPLC that cannot be emulated by any other experimental design. The design and development of chromatographic materials were a direct result from these early structural and dynamic NMR studies.

The bonded phase has been physically characterized using a variety of methods with solid-state and, to a limited extent, with solution NMR [10–47]. Solution NMR studies of chromatographic packings were performed using either ^{2}H or ^{13}C spectra (selectively isotopically labeled ^{2}H, ^{13}C or natural ^{13}C) with ^{2}H or ^{13}C FT NMR. The direct examination of dry materials, as required for example, SEM or solid

* Present address: Molecular Probes, Inc., 29851 Willow Creek Road, Eugene, OR 97402, USA. Tel.: +1 541 335 0314; fax: +1 541 335 0504.
E-mail address: ocholewa@students.wisc.edu (O.M. Cholewa).

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state NMR cannot fully address solvent interactions with the bonded phase. The solution NMR experiments performed had considerable experimental challenges and provided only a rudimentary theoretical foundation.

The ability to perform more assays on the same sample allows for a more rigorous analysis. In standard solution NMR, if multiple experimental conditions are required, preparations of milligram quantities of a molecule of interest must be available in more than one NMR tube or the molecule used in one experiment must again be separated/purified from the components of the previous experiment with potential losses to that sample.

This study outlines the use of glass wool placed within an NMR tube as a solid support for the covalent attachment of a solute. With a covalently attached molecule, (i) the removal of excess unbound or smaller ligands (separation) and the change of solvents, buffers or salts (desalting) can be accomplished with little or no loss; (ii) non-specific binding can be limited to nearest neighbor molecule or possibly no non-specific binding dependant upon the size of the molecule; (iii) potential ligands may be subjected to a more rigorous binding assays and variations in experimental conditions are easier to prepare. This method allows for a simple solution NMR method to probe the proton and carbon structure of a molecule bound to the surface of glass without the use of expensive sample tubes or platforms, as required for magic angle or fast spinning.

2. Experimental

2.1. Chemicals

All chemicals were ACS or HPLC-grade; HPLC-grade water with conductivity in the 14–17 MΩ cm was used for all aqueous reactions. Dimethylphenylchlorosilane ("Phe"), dimethyloctylchlorosilane ("C_8") and were purchased from Fluka (Buchs, Switzerland); 3-aminopropyltriethoxysilane ("APTES") was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All deuterated solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

Organic solvents used in the silanization reactions were chemically dried prior to mixing with the silane using either sodium metal or anhydrous magnesium sulfate; final moisture content of reaction solutions was not determined.

2.2. Materials

NMR tubes (5 mm × 185 mm) were purchased from Wilmad (Wilmad Glass Co., Inc., USA) and filled with FisherBrand® Glass Wool, Spun Soft Glass using a 2 mm diameter glass rod. FisherBrand® Disposable Borosilicate Glass Pasteur Pipettes with Constriction (16 or 23 cm) (Fisher Scientific, Pittsburgh, PA) were used to create an NMR tube cleaning apparatus. Half-gallon, wide-mouth Ball® Mason jars (Alltrista Corp., Muncie, IN) were used to dry and store derivatized NMR Wool Tubes. Other assorted labware included 50 mL polypropylene screw cap culture tubes, rubber septa, two-hole black rubber stoppers, 500 mL flask with three vertical necks with S.T.-joints, S.T.-side-arm connector tubes, and vacuum pump with dry ice trap for pump.

All reaction solutions were mixed, measured and dispensed in oven-dried glassware (95–100 °C in excess of 1 week). The polypropylene containers and pipette tips used were stored in a 65 °C dry incubator until use. The alkyl chain chlorosilane (C_8) and APTES were dispensed with polypropylene pipette tips; Phe was dispensed with glass pipettes; APTES and water were mixed in 50 mL polypropylene screw cap culture tubes.

2.3. Equipment

The NMR experiments were performed at room temperature (293–299 °K) in one of three instruments: a Bruker Aspect-3000 console with either a Bruker 250 MHz, a 300 MHz magnets, or a Varian UNITY/INOVA 500 MHz magnet. For proton experiments, the 250 MHz magnet required, at most experiments, 64 scans to provide a good baseline; the 300 and 500 MHz required only 16 scans for excellent, near noise-free or noise-free baselines. Sixty-four scans were used occasionally with the 300 and 500 MHz instruments to compare previous 250 MHz experiments with these instruments. Experiments for natural ^{13}C required from 256 to 12,000 scans, dependent upon the concentration of the samples examined and the magnet used.

Data was processed using WinNMR or WinNUTS software. All data was baseline corrected, zero filled, Fourier transformed and phase corrected. All NMR spectra are in chemical shift (δ, ppm). Tetramethylsilane was not added to any of the samples used in these experiments to avoid complicating data interpretation. All spectra were calibrated and peaks assigned using landmark protonated solvent peaks for the solvents used in the respective experiments. ^{13}C spectra were referenced to solvent carbon peaks where applicable.

2.4. Sample preparation

NMR tubes were weighed individually and the weights recorded. Using a 2 mm-thick glass rod, small tufts of glass wool were inserted consecutively into each pre-weighed NMR tube to yield under 10 mg/cm of glass wool within the bottom 2.5–3 cm of the tube. Care was taken to avoid forming compact clumps of glass wool, uneven packing, empty spaces within the glass wool packing or accidental inclusion of extraneous materials (dust particles, non-glass fibers, etc.).

An NMR Wool Tube could not be considered usable until a ^1H NMR analysis with either D_2O or another deuterated solvent was done to evaluate the quality of the solvent peaks. This pre-derivatization analysis of NMR Wool Tubes to determine suitability for further processing is termed validation. With a validated NMR Wool Tube proton peaks should resemble the control, the same deuterated solvent in an NMR

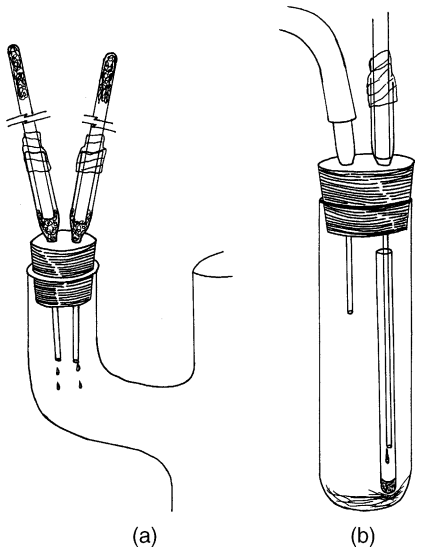

Fig. 1. Assembly for vacuum aspiration of solvent from NMR Wool Tubes. (a) Flask/side arm adaptor setup for liquid removal from NMR Wool Tubes; (b) 150 mL culture tube for collecting liquid from NMR Wool Tubes for analysis.

tube with no wool, as closely as possible. NMR Wool Tubes that exhibit difficulty in shimming, show an uneven baseline, skewed peaks or excessive broadening of the solvent peak, as compared to solvent control, were not suitable for further use and were repacked.

To perform the vacuum aspiration of NMR Wool Tubes to remove excess solvent, the uppermost portion of a 14 cm Pasteur pipettes were cut to remove the tube constriction to create cleaning pipettes. A small amount of glass wool was packed into the cleaning pipette opening to act as a cushioning support for the NMR Wool Tube to prevent breakage. The NMR Wool Tube inserts into the cleaning pipette to rest upon the cushioning glass wool support wad at the neck of the pipette. Approximately 20 cm of Teflon tape was wrapped around the pipette and NMR Wool Tube to seal against a vacuum.

NMR Wool Tubes were cleaned by attaching the sidearm adaptors to two openings of a 500 mL three-hole flask (Fig. 1, item a). The center hole of the three-hole flask was plugged with a one-hole rubber stopper connected to tubing leading to a vacuum pump with a dry ice vacuum trap.

To collect free liquid from the NMR Wool Tube for analysis, Fig. 1, item b depicts a setup for collecting into a clean NMR tube. A 150 mm culture tube is capped with a two-hole rubber stopper. A tube connected to a vacuum pump is inserted into one hole to create a vacuum within the culture tube. The NMR Wool Tube is inserted into a cleaning pipette with the longer pipette tip placed into the other stopper hole leading into a clean NMR tube for collection.

Modified half-gallon, wide-mouthed Mason jars were used as storage containers and as vacuum or chemical drying chambers for NMR Wool Tubes. A hole was drilled into the Dome Lid® of the Mason jar to allow insertion of small rubber septa as a portal for the insertion of a needle connected to either a vacuum system for evacuation or for the entry of inert gas. Mason jars and culture tubes used as vacuum drying chambers have packaging tape wrapped around the exterior as a precaution against implosion.

To keep the chamber dry, one 50 mL polypropylene culture tube was filled with KOH pellets and capped with a culture tube screw cap with small holes drilled into the top.

Only tubes containing the same solvent were stored together, i.e., tubes filled with D_2O were not stored with tubes containing benzene-d_6. Tubes containing organic solvents were not stored with the plastic NMR tube caps on.

The Mason jars under vacuum were never opened to the surrounding air to avoid picking up dust and to avoid absorbing atmospheric moisture or other protonated vapors that may be present in a lab. To purge the vacuum in the jars, a syringe needle connected to tubing connected to a gas line was inserted into the Dome Lid® septa and filled with a small amount of dry, filtered inert gas; once the Dome Lid® visibly swells with gas, the addition of gas was halted.

Vacuum drying within the Mason jars is usually complete within 8–24 h, dependent upon: (i) type of solvent, (ii) type of derivatized surface, (iii) number of tubes in the jar, and (iv) number of jars in the vacuum setup.

All silanization reactions were performed with the glass wool packed within the NMR tube. The silanizing reagents were mixed with solvent (‘silane and reaction solvent’, Table 1) and then immediately, 1 mL of the mixture was added to validated NMR Wool Tubes. The tubes were capped and each tube was subjected to short centrifugal force from a quick hand-whip motion to force all solution into the glass wool.

The NMR Wool Tubes filled with silane/solvent were placed on their sides, uncapped, into the hood air stream for a slow hydrolysis (conversion of chlorosilane or ethoxysilane to reactive silanol) from exposure to atmospheric moisture (‘reaction time/temperature’, Table 1) until most of the ex-

Table 1
Silanizing reaction and post-reaction drying and washes

Silane and reaction solvent (v/v)	Reaction time/ temperature	Initial drying time (RT)	Incubator drying time (42 °C)	Post-reaction washes	Vacuum drying time (RT)
Phe, 20% in heptane	48 h/RT	19.5 h	9.5 h	3× heptane; 2× methanol	7.5 h
C_8, 20% in heptane or C_8, 33% in heptane	46 h/RT	21.5 h	5 days 7 h	4× heptane; soak in heptane 23 h; soak in heptane 3.5 h	19 h
APTES, 5% in ddH_2O	30 min/37 °C	1 h	None	4× H_2O; 3× acetone	18.5 h

“Phe”, dimethylphenylchlorosilane; “C_8”, dimethyloctylchlorosilane; “APTES”, 3-aminopropyltriethoxysilane.

cess liquid above the glass wool appeared to have evaporated. Excess silanizing solution in the glass wool was removed by vacuum aspiration and again, the tubes were laid flat in the hood for another drying time ('initial drying time', Table 1). This was the time required for the appearance of all liquid to have evaporated and the glass wool to visually exhibit a dry appearance.

After air-drying at room temperature ('initial drying time', Table 1), the silanized NMR Wool Tubes were stored in a Mason jar, the jar covered but not sealed, and placed in a 42 °C incubator, tubes open, to continue the condensation and curing process ('incubator drying time', Table 1).

Each silanized NMR Wool Tube was washed of excess, unreacted silane and reaction solvent by adding 1–2 mL of solvent, hand-whipping the solvent into the wool and then removing excess liquid by vacuum aspiration ('post-reaction washes', Table 1). After the final wash and removal of liquid by vacuum aspiration, the tubes were then placed into a Mason jar with KOH pellets and vacuum dried. The silanized NMR Wool Tubes were stored in a sealed Mason jar, with vacuum intact, until NMR analysis.

The method of Nisnevitch et al. [48] outlines the immobilization of antibodies onto glass wool utilizing a 3-aminopropyltriethoxysilane ("APTES") derivatized glass surface. In regards to the derivatization with APTES, their protocol was followed with minor modification.

At the time of NMR analysis, solvent was added to the NMR Wool Tubes (0.5–0.75 mL total volume of solvent), individually capped with NMR tube caps and the solvent was hand-whipped into the tube glass wool until no further hand-whipping changed the solvent level within the wool. This is usually one or two strong applications of manual force.

For NMR Wool Tubes with air bubbles within the solvent/wool, their individual tube caps were removed and the tubes placed inside a Mason jar. The Mason jar was sealed tightly and vacuum briefly applied, usually under 5 min, until the solvent appeared to percolate up each NMR Wool Tube but not yet ejecting out of the tubes. The vacuum pump was halted but vacuum maintained by not dislodging the septa. The Mason jar was flooded with dry, filtered inert gas and the solvent that was percolating up the NMR tube retracted into the glass wool. The NMR Wool Tubes were visually examined to verify that all air bubbles had been removed.

3. Results and discussion

The complete preparation of silanized NMR Wool Tubes for NMR analysis requires (i) rinsing the glass wool repeatedly in a suitable solvent, (ii) removing the previous solvent or reaction solution from the glass wool by repeated vacuum aspiration, (iii) vacuum drying, (iv) storing the tubes in a vacuum, with or without KOH pellets to keep the chamber dry, (v) flooding the chamber with dry, filtered inert gas prior to opening the jar, (vi) adding 0.5–0.75 mL of deuterated solvent to each tube, (vii) capping each tube and hand-whipping solvent into the wool, (viii) degassing to remove air bubbles, if necessary, and (ix) recapping each tube and proceed to NMR experiments.

In this work, 40 C_8-derivatized NMR Wool Tubes were manufactured per reaction; 24 tubes were reacted with 20% C_8-silanization solution and 16 tubes with a 33% C_8-silanization reaction solution. A higher concentration of dimethyloctylchlorosilane did not improve the signal compared to signal derived from tubes reacted with a lower concentration of silane. The solvent soak time and number of washes were not determined empirically, but were monitored in this initial exploratory test.

Silanization could not be done with the glass wool outside the NMR tube and then the tube stuffed with silanized glass wool because the physical handling of the glass wool shears the glass wool, creating unsilanized surfaces. The free silanols formed alter the surface characteristics. Also, silanized glass wool clumps readily and is more difficult to pack, and the introduction of particulate contaminants is more probable. Endcapping was not performed to avoid complicating the spectra.

Surprisingly, all manipulations of the glass wool within the NMR tubes (solvent changes, hand-whipping, vacuum aspiration and degassing) did not deform the wool from its original packing.

Throughout the spectra, "P" refers to an standard solution NMR analysis of sample in solvent in a "plain" NMR tube with no glass wool present; "PGW" refers to "plain glass wool" for solution NMR analysis of sample in solvent in an NMR tube filled with glass wool that has not undergone any derivatization reaction; "DW" refers to "derivatized wool" for solution NMR analysis of sample covalently attached to the glass surface on glass wool within an NMR tube, henceforth referred to as an "NMR Wool Tube".

3.1. Dimethylphenylsiloxane on glass in derivatized NMR Wool Tubes

Fig. 2 provides a comparison of ^{1}H spectra of dimethylphenylsiloxane on glass in three derivatized NMR Wool Tubes ("DW-1", "DW-2", and "DW-3") submerged in D_2O. The lower left inset shows proton peak assignments per the structure of dimethylphenylsiloxane on glass. The methylsilyl protons are designated as "α" at the broad peak at 0.2–0.3 ppm with minor peaks or peak splitting for the same protons designated "α′", "α″". The phenyl protons are designated "b", with again minor peaks or split peaks designated "b′" and "b″". Solvent peaks, for both the solvent used in these experiments and the presence of residual solvent from post-reaction washes are designated "s(#)"; "s(1)" is residual acetonitrile-d_3 (∼1.6 ppm) and "s(2)" is acetone/water (∼2.9 ppm) from previous NMR analysis (data not shown) and "s(3)" is residual methanol (∼3.3 ppm) from washing; "s(4)" at 4.8 ppm is from the protonated population in the D_2O. The spectra are referenced to the protonated water peak at 4.8 ppm.

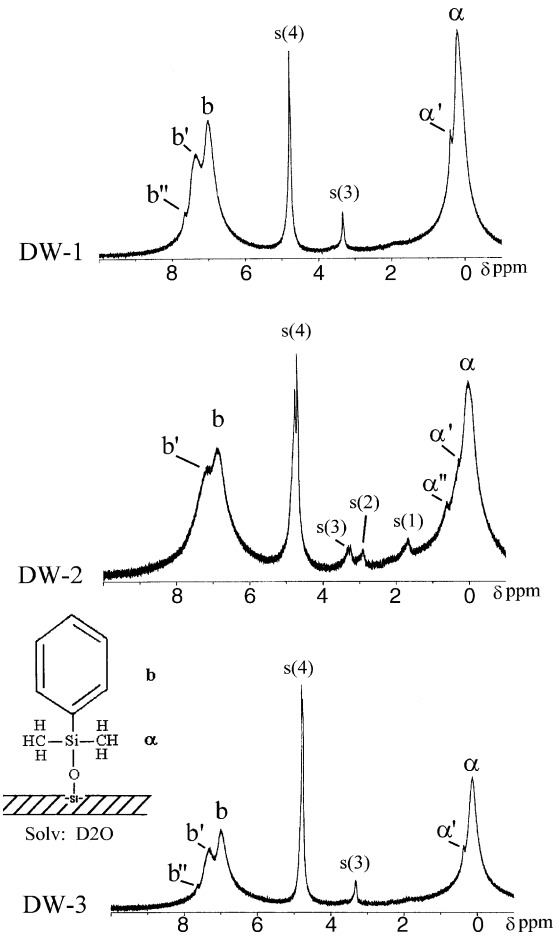

Fig. 2. Proton spectra of three NMR Wool Tubes ("DW-1", "DW-2", and "DW-3") derivatized with dimethylphenylchlorosilane to yield dimethylphenylsiloxane/glass wool (structure and proton assignment on bottom inset). "s(#)" denotes solvent peaks.

These experiments were performed to compare the consistency and extent of the silanization reaction in various tubes by comparing the consistency of the signal of the bonded phase, the attached dimethylphenylsiloxane on glass. These spectra display discrepancies in peak quality that may be due to the amount and quality of packing and amount of dimethylphenylsiloxane coating the glass wool (height of peaks). These experiments were also done to examine the utility of derivatized NMR Wool Tubes to resolve spectral characteristics of solutes that have insoluble or immiscible solvents added.

The broad peaks seen in Fig. 2 are due to loss of mobility. In a completely miscible solvent, the dimethylphenyl groups are free to rotate about the axis of attachment with glass silanols and to exhibit side-to-side writhing, most noticeably the "α" peak is narrower and the ring protons provide sharper dual peaks at ∼7–7.5 ppm (data not shown). The smaller size of the dimethylphenyl group may still allow for solute–solute aggregation with a nearest neighbor dimethylphenyl to provide for hydrophobic exclusion of water. The spectra are considered that of a molecule attached to glass because the same wash/vacuum aspiration/drying sequence performed on a PGW tube filled with 1 mL toluene provided no NMR signal.

3.2. 3-Amino-1-propanol and 3-aminopropylsiloxane on glass in D_2O

Fig. 3 provides a comparison of ^{1}H spectra of 3-amino-1-propanol (0.4 mL) in D_2O (0.3 mL) in a plain NMR tube ("P"), in an NMR tube filled with plain glass wool ("PGW") and an NMR Wool Tube derivatized with APTES to yield 3-aminopropylsiloxane on glass ("DW") submerged in D_2O. These experiments were done to determine what affect the presence of glass wool within an NMR tube has upon peak quality for the P, PGW, and DW samples.

For the solute 3-amino-1-propanol in P and PGW tubes, the exchangeable hydroxyl proton (–OH) is designated "a" with the two exchangeable amino protons ($-NH_2$), designated "e", provide a minor contribution within the protonated water for the peak assigned "s(1)" at 4.8 ppm. The two methylene protons ($-CH_2-O$) adjacent to the oxygen, "b", comprise the peak adjacent and upfield (∼3.6–3.7 ppm) from the water peak. The four methylene protons ($-CH_2-$), designated "c" and "d", are the two peaks upfield from "b", with "c" at ∼1.7 ppm and "d" at ∼2.7 ppm. For the solute, 3-aminopropylsiloxane on glass; the two methylenesilyl protons ($-CH_2-Si-$) are assigned the letter "b" at ∼0.6 ppm; methylene protons ($-CH_2-$) designated "c" at ∼1.7 ppm and "d" at ∼2.9 ppm. Amino protons ($-NH_2$) are designated "e" and are furthest downfield to provide a minor contribution within the protonated water peak "s(1)" at 4.8 ppm.

In comparing the DW with the P and PGW samples, the assignment of peaks are unrelated due to the presence of the silicon molecule, but "c" and "d" protons retain relatively similar chemical shifts. The presence of glass wool contributes to peak broadening and overlap in the PGW and DW sample. Aminopropyltrisilanol ($NH_2(CH_2)_3Si(OH)_3$) or aminopropylsilane ($NH_2(CH_2)_3SiH_3$) in P and PGW would have been a more appropriate control to compare with the derivatized surface, the DW NMR Wool Tube.

In the absence of a comparison with a better free solute control in P and PGW, the spectra for DW may be considered the spectra of an immobilized solute to the surface of glass wool from the observation from the repeated washes in various solvents and subsequent solvent removal with vacuum aspiration. After repeated washes and soaks with various solvents and removal by vacuum aspiration, the signal of the 3-aminopropylsiloxane on glass persists. The same wash regimens completely removed 1 mL of 3-amino-1-propanol (neat) added to PGW tubes to provide no NMR signal. Other materials (1 mL of either silicone pump oil, olive oil, octane, dodecane, or toluene) added undiluted to individual PGW tubes subjected to the same wash regimens applied to DW tubes were completely removed to provide no NMR signal, whereas the derivatized NMR Wool Tubes consistently provided signal, even after more washes, with no change

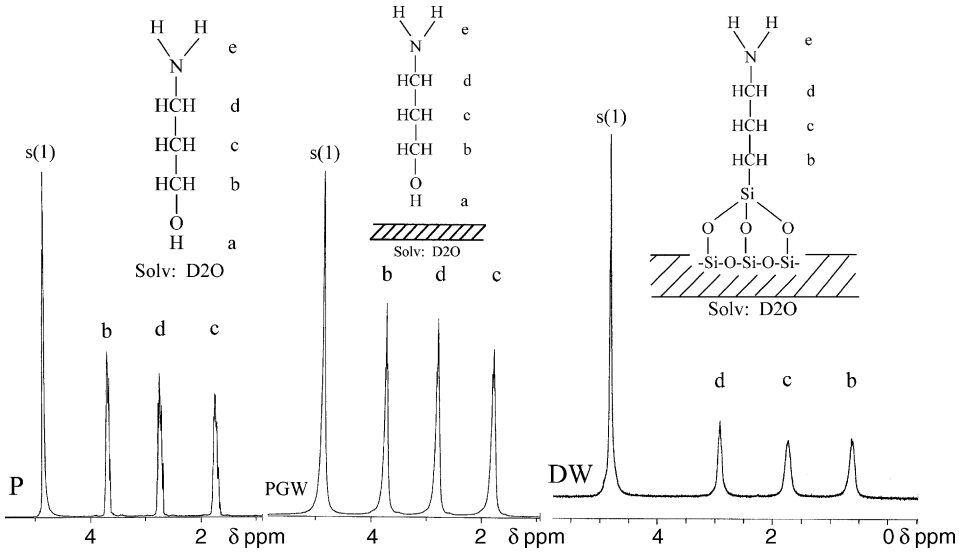

Fig. 3. Proton spectra of a solution of 3-aminopropanol ("P"), 3-aminopropanol in an NMR Wool Tube ("PGW") and NMR Wool Tube derivatized with APTES to yield 3-aminopropylsiloxane/glass wool ("DW"), all in D_2O (structures and proton assignments on insets). "s(1)" denotes protonated water peak at 4.8 ppm.

in signal peak quality, after examination with the same deuterated solvents.

3.3. *Octane in acetonitrile-d_3 and dimethyloctylsiloxane on glass submerged in acetonitrile-d_3*

Fig. 4 provides a comparison of ^1H spectra of octane in acetonitrile-d_3 as either a P or PGW sample and an NMR Wool Tube derivatized with dimethyloctylchlorosilane to yield a surface modified with dimethyloctylsiloxane on glass, designated as DW, in acetonitrile-d_3.

Either dimethyloctylsilanol ($C_{10}H_{23}SiOH$) or dimethyloctylsilane ($C_{10}H_{23}SiH$) would have been a more useful solute choice than octane for a comparison with dimethyloctylsiloxane on glass, but the main focus in this study is to observe the quality of peaks.

The peak assignments per structure on the insets, the proton designation of dimethyloctylsiloxane on glass follows the order of proton assignments for octane but with the two methylsilyls designated as "α". The methyl protons of octane designated as "a" are the first chain methylene protons in the dimethyloctylsiloxane structure and retain the designation of "a". The methylene protons retain the letter designation of "b", "c", "d", "e", "f", and "g"; in the DW spectrum there is separation into three peaks, "g" (two protons), "b, f" (four protons) and "c, d, e" (six protons). The peak assigned as

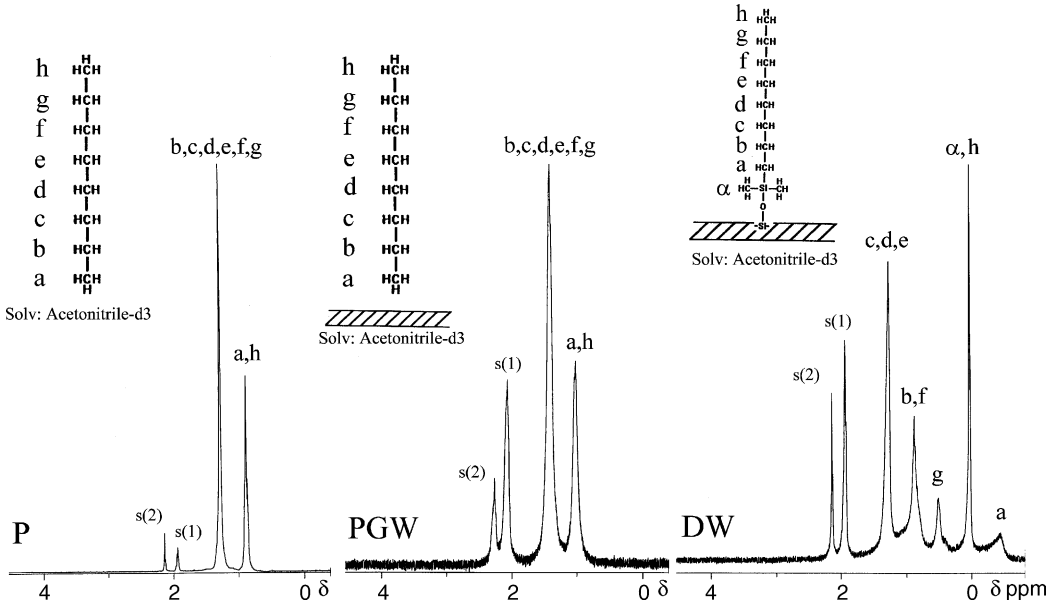

Fig. 4. Proton spectra of octane in acetonitrile-d_3 in a plain NMR tube ("P"), a plain NMR Wool Tube ("PGW") and of dimethyloctylsiloxane on glass submerged in acetonitrile-d_3 in a derivatized NMR Wool Tube ("DW"). Structures and proton assignments on insets; DW assignments are speculative.

"α,h" includes the six methylsilyl protons, "α", plus the three protons from the terminal methyl group "h".

"s(1)" and "s(2)" designates the first and second solvent peaks for acetonitrile-d_3 (1.94 ppm) and residual acetone-d_6 (2.05 ppm), from the post-reaction analysis using first acetone-d_6 (spectra not shown) and then acetonitrile-d_3.

The protons of DW are separated into five peaks as compared to three peaks for the published spectrum of dimethyloctylchlorosilane (spectrum not shown) [49] and the two peaks for octane. The two methylene protons attached to silicon, "a", show as the peak furthest upfield at −0.444 ppm as referenced the peaks to the acetonitrile-d_3 peak at 1.94 ppm. The six methylsilyl protons, "α" plus the three terminal methyl protons, "h", show up as a single peak at 0.031 ppm. The two methylene protons, "g", proximal to the terminal methyl protons "h", show up as a discrete peak at 0.517 ppm. The two methylene protons "b", proximal to "a", and the two methylene protons "f", proximal to "g", are at opposite ends of the chain, but are similar in magnetic field strength and create the peak designated "b, f" at 0.883 ppm. The six innermost chain methylene protons, "c", "d", and "e" create the single peak "c, d, e" and are furthest downfield, at 1.278 ppm. Pending further work with selective isotopic labeling, these peak assignments are speculative.

Using the same solute, octane, in the same solvent, acetonitrile-d_3, the spectrum of PGW and P in these experiments provides a comparison in the quality of peaks due to the presence of glass wool. The spectrum of PGW shows broader peaks and a noisier baseline compared to P, but the peaks are distinctive.

Using the solute octane in the solvent acetonitrile-d_3 in the presence of glass wool for the PGW experiment to compare the quality of peaks of dimethyloctylsiloxane on glass, the DW experiment, it is evident that the DW peaks are of comparable quality to the PGW peaks. Both spectra exhibit a similar extent of peak broadening, not only for the solute peaks but also for the solvent peaks. The DW experiment shows less baseline noise than the PGW, and this may be related to the quality of the packing of glass wool in each tube.

3.4. Octane in D_2O and dimethyloctylsiloxane on glass submerged in D_2O

Fig. 5 provides a comparison of ^{13}C spectra of octane in D_2O in a plain NMR tube ("P"), a plain NMR Wool Tube ("PGW") and dimethyloctylsiloxane on glass in a derivatized NMR Wool Tube ("DW"). The ^{13}C spectra shows sharper peaks for both P and PGW samples compared to the DW sample.

Per the insets, the structure of octane with ^{13}C assigned by letters; methyl carbons ($^{13}CH_3$–) are designated "a" and "h"; methylene carbons (–$^{13}CH_2$–) are designated with the letters "b", "c", "d", "e", "f", and "g". The structure of dimethyloctylsiloxane on glass with carbons assigned by letters; methylsilyl carbons ($^{13}CH_3$–Si–$^{13}CH_3$) are designated "α". The former methyl carbon ($^{13}CH_3$–) designated "a" on the octane structure is now methylene carbon (–$^{13}CH_2$–). The terminal methyl carbon ($^{13}CH_3$–) is designated "h"; methylene carbons (–$^{13}CH_2$–) retain the designated letters "b", "c", "d", "e", "f", and "g" as assigned on octane.

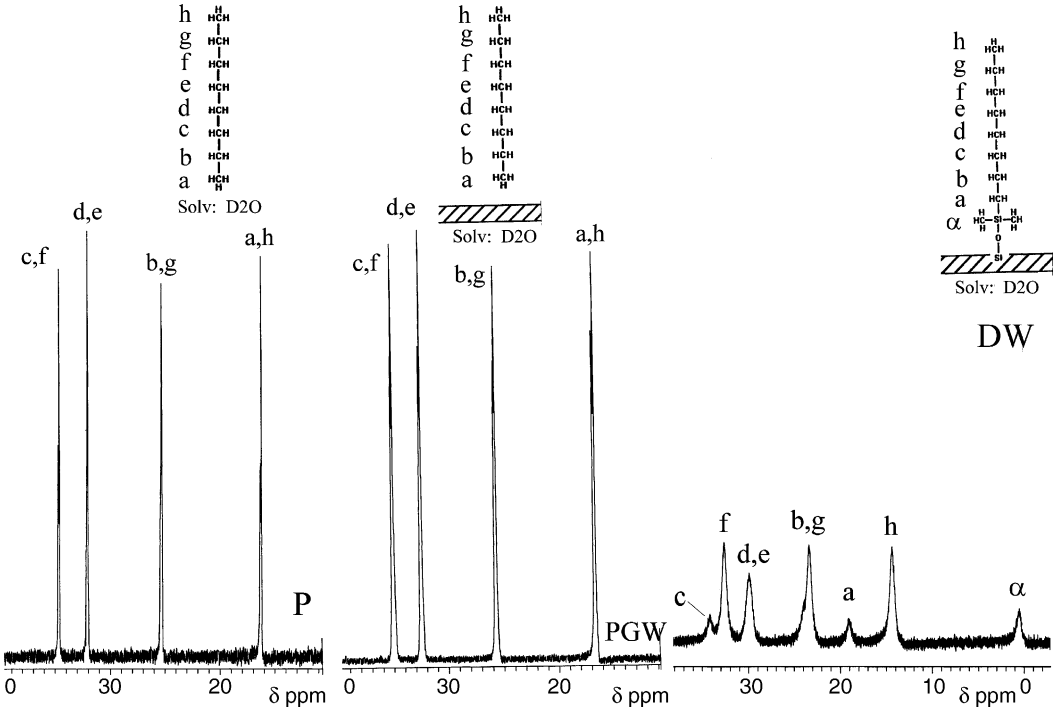

Fig. 5. ^{13}C spectra of octane in D_2O in a plain NMR tube ("P"), a plain NMR Wool Tube ("PGW") and of dimethyloctylsiloxane on glass submerged in D_2O derivatized NMR Wool Tube ("DW"). Structures and proton assignments on insets.

For octane, the terminal methyl carbons designated “a” and “h” are assigned to the most upfield peak (∼17 ppm) as “a, h”. The 2,7-methylene carbons designated “b” and “g” are assigned to the peak downfield from the methyl carbons, as peak “b, g” (∼26 ppm). The 4,5-methylene carbons designated “d” and “e” are assigned to the peak at ∼32–33 ppm, as “d, e”. The 3,6-methylene carbons are designated “c” and “f” and are assigned the peak at ∼35 ppm as “c, f”.

The peak assignments for carbons on the dimethyloctylsiloxane on glass for the DW the methylsilyl carbon “α” is assigned to the peak at ∼0.8 ppm, the terminal methyl carbons “h” at 14.5 ppm, the methylsilyl carbon “a” at 19.2 ppm, the methylene carbons “b” and “g” at 23.5 ppm, the methylene carbons “d” and “e” at ∼30.2 ppm, and methylene carbons “f” and “c” at 33 and 34.5 ppm, respectively, following the assignments of Bayer et al. [50].

3.5. Comparison of different NMR Wool Tubes with the same derivatization—consistency of spectra

Fig. 6 provides a comparison of the ^{1}H spectra of six of a group of ten DW NMR Wool Tubes all silanized, cleaned, dried, stored, submerged in benzene-d_6 and analyzed under the same conditions as a group (four spectra are not shown).

Peak assignments per the structure of dimethyloctylsiloxane on glass are shown on the inset; the two methylsilyls, “α”, at ∼0.002–0.05 ppm; the methylenesilyl protons, “a”, at ∼−0.3 to −0.4 ppm). The area under the peak at −0.3 to 0.4 ppm, assigned as “a”, is commensurate for two protons. The methylene protons “b”, “c”, “d”, “e”, “f”, and “g”, separate into three peaks, “g” (two protons, ∼0.5 ppm), “b, f” (four protons, 0.8 ppm) and “c, d, e” (six protons, 1.2 ppm). The peak assigned as “α,h ” includes the six methylsilyl protons, “α” and the three methyl protons, “h”. Again, pending selective isotopic labeling, these peak assignments are speculative.

“s(1)” and “s(2)” designates the first and second solvent peaks for acetonitrile-d_3 (1.94 ppm) and residual acetone (2.05 ppm) from the post-analysis cleaning regimen, respectively.

Spectral variations may be directly related to discrepancies in wool packing and amount of bonded phase attached to the surface of the glass wool. The amount of overlap may be related to the lessened mobility of the octyl chain relative to the amount of dimethyloctylsiloxane bound to the glass surface. With a more dense surface coating, the neighboring octyl chains have a greater affinity to aggregate with each other than mixing with the solvent, benzene.

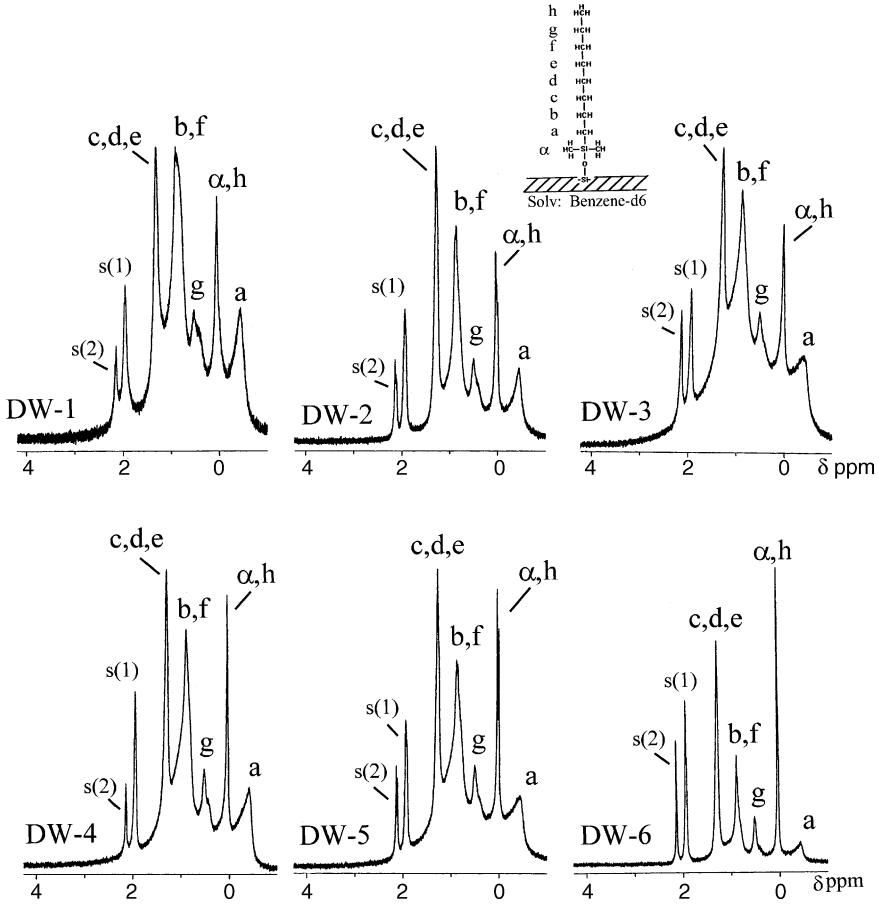

Fig. 6. Proton spectra of six NMR Wool Tubes (“DW-1” to “DW-6”) derivatized with dimethyloctylchlorosilane to yield dimethyloctylsiloxane/glass wool in benzene-d_6 (structure and speculated proton assignments on top inset).

Table 2
Statistical analysis of peaks from Fig. 6 (columns DW-1 through DW-6; not shown columns DW-7 through DW-10)

^{1}H	δ (ppm)									
	DW-1	DW-2	DW-3	DW-4	DW-5	DW-6	DW-7	DW-8	DW-9	DW-10
α	0.024	0.029	0.030	0.026	0.050	0.031	0.016	−0.002	−0.002	0.032
a	−0.460	−0.428	−0.388	−0.422	−0.421	−0.444	−0.449	−0.471	−0.471	−0.379
b	0.876	0.880	0.892	0.886	0.874	0.883	0.869	0.851	0.851	0.883
c	1.288	1.279	1.290	1.286	1.299	1.278	1.275	1.247	1.247	1.282
d	1.288	1.279	1.290	1.286	1.299	1.278	1.275	1.247	1.247	1.282
e	1.288	1.279	1.290	1.286	1.299	1.278	1.275	1.247	1.247	1.282
f	0.876	0.880	0.892	0.886	0.874	0.883	0.869	0.851	0.851	0.883
g	0.514	0.525	0.516	0.517	0.531	0.517	0.511	0.476	0.476	0.517
h	0.024	0.029	0.030	0.026	0.050	0.031	0.016	−0.002	−0.002	0.032
s(1)	1.940	1.940	1.940	1.940	1.940	1.940	1.940	1.940	1.940	1.940
s(2)	2.133	2.134	2.142	2.141	2.159	2.138	2.124	2.100	2.100	2.134

^{1}H	Maximum	Minimum	Average	Variance	S.D.
α	0.050	−0.002	0.023	0.0002	0.016
a	−0.379	−0.471	−0.433	0.0009	0.032
b	0.892	0.851	0.875	0.0002	0.014
c	1.299	1.247	1.277	0.0003	0.017
d	1.299	1.247	1.277	0.0003	0.017
e	1.299	1.247	1.277	0.0003	0.017
f	0.892	0.851	0.875	0.0002	0.014
g	0.531	0.476	0.510	0.0003	0.019
h	0.050	−0.002	0.023	0.0002	0.016
s(1)	1.940	1.940	1.940	0.0000	0.000
s(2)	2.159	2.100	2.131	0.0003	0.018

Table 2 provides a statistical analysis of the peaks from all 10 spectra. The low variance and standard deviation values show that the derivatized NMR Wool Tubes can provide consistent sample data.

One spectrum, DW-6, shows little peak overlap, peak heights commensurate with number of protons and a clean baseline. This one spectrum establishes the utility of derivatized NMR Wool Tubes as a relevant method for solution NMR analysis. While the others highlight the limitations created by hand-packing, with proper packing to eliminate field inhomogeneities, the one exceptional spectrum, DW-6, proves that good packing can provide excellent data.

3.6. The spectral characteristics of the bonded phase in the presence of varying solvent composition

Fig. 7a shows the ^{1}H spectra and speculated peak assignments of six tubes of dimethyloctylsiloxane on glass as a derivatized NMR Wool Tube ("DW") in either acetonitrile-d_3 only, or a mixture of D_2O and acetonitrile-d_3. The D_2O/acetonitrile-d_3 mixture follows a gradient with 0.5 mL total volume in every tube: 0.25 mL D_2O with 0.25 mL acetonitrile-d_3; 0.2 mL D_2O with 0.3 mL acetonitrile-d_3; 0.15 mL D_2O with 0.35 mL acetonitrile-d_3; 0.1 mL D_2O with 0.4 mL acetonitrile-d_3; 0.05 mL D_2O with 0.45 mL acetonitrile-d_3 and 0.5 mL acetonitrile-d_3 only.

The experiments were performed locked onto D_2O solvent values provided on the solvent settings for data acquisition. The acetonitrile-d_3 solvent values were also used in a second round of NMR experiments performed within the same sitting. There we no difference in quality of the spectra between the two solvent lock choices (spectra from the acetonitrile-d_3 lock settings not shown).

The object of this experiment was to determine what affect the hydrophilic/hydrophobic character of the solvent has upon the dimethyloctylsiloxane on glass in NMR Wool Tubes. Using a solvent gradient that approximates what is commonly used in HPLC separations, these spectra show the affect the solvent composition of the "mobile phase", the solvent added to the tubes, has upon the behavior of the "bonded phase", the dimethyloctylsiloxane on glass. As can be clearly seen, the more hydrophilic the solvent, the less distinct the separation of the peaks of the "bonded phase", compared to the same samples exposed to solvents of a greater hydrophobic character, i.e., a greater percentage of acetonitrile-d_3.

The greater the percentage of D_2O relative to acetonitrile-d_3, the less the resolution of the protons (Fig. 7a, the four left-most spectra) directly related to the loss of mobility of the octyl chain at the surface of the glass. The chains are aggregating, individually or with other octyl chains, to exclude water and the mobility of the chain is likewise reduced, as may be graphically represented in Fig. 7b, items a and b. The greater the percentage of acetonitrile-d_3 relative to D_2O, the better the separation of protons as seen with the presence of more peaks (Fig. 7a, the two right-most spectra). With increase in the hydrophobic character of the mobile phase, as in increasing the acetonitrile-d_3 concentration relative to water, the octyl chains on the surface of glass have increased mobility, as depicted going from items b to c to d in Fig. 7b. These spectra (Fig. 7a) and their

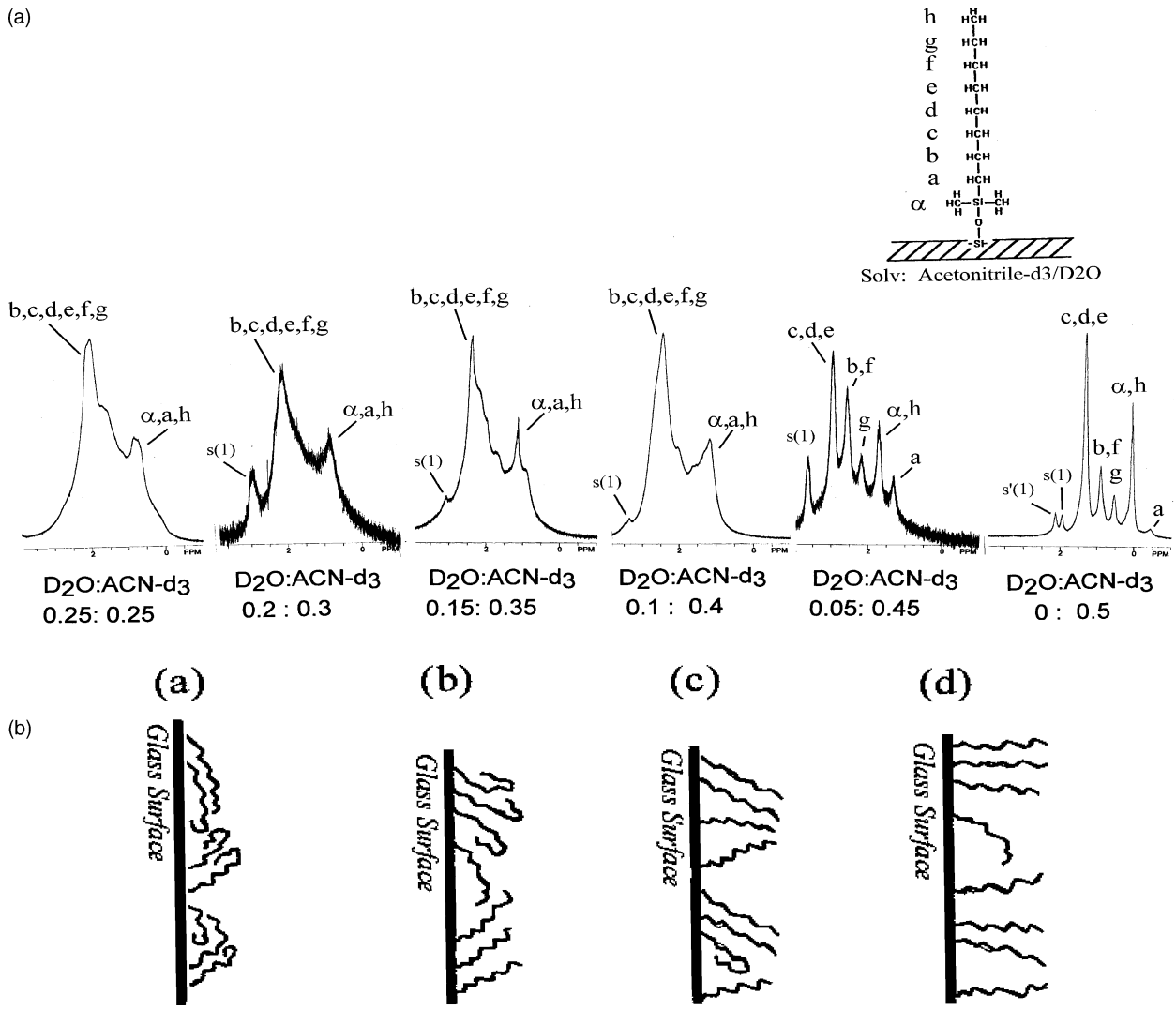

Fig. 7. (a) The proton spectra of dimethyloctylsiloxane on glass in various ratios of D_2O/acetonitrile-d_3 ("ACN-d_3") for a total volume of 0.5 mL solvent added to the derivatized NMR Wool Tubes, from left to right 0.25 mL D_2O with 0.25 mL acetonitrile-d_3, 0.2 mL D_2O with 0.3 mL acetonitrile-d_3; 0.15 mL D_2O with 0.35 mL acetonitrile-d_3; 0.1 mL D_2O with 0.4 mL acetonitrile-d_3; 0.05 mL D_2O with 0.45 mL acetonitrile-d_3 and 0.5 mL acetonitrile-d_3 only (structure and speculated proton assignment on top inset). (b) Simplified drawings of the possible conformations that surface bound dimethyloctylsiloxane chains may assume in the presence of an immiscible solvent (a) progressing to a more miscible solvents (b and c) to a fully miscible solvent (d).

respective idealized drawings (Fig. 7b) follows the "bristle brush", "haystack", "ink bottle", and other models presented by Halasz and Sebestian [21], Hemetsberger et al. [22], Lochmuller and Wilder [27] and others [23–25]. These experiments reiterate the solution NMR experiments done on alkyl-chain derivatized HPLC silica particles packed within an NMR tube [9,12,15–18,26,28,29,31,32], albeit, with the NMR Wool Tube experiments, more solvent mixtures were examined.

4. Conclusion

It is the premise of this work that glass wool: (i) provides enough surface area for an adequate quantity of small molecules to attach upon to generate signal; (ii) provides a solid support that allows easy diffusion of solvent in situ without the need for particle settling or packing and resettling/repacking (as done in past HPLC silica particle solution NMR studies); (iii) packed suitably within an NMR tube, does not interfere with nuclear or electronic phenomena of the attached surface molecule; (iv) in the absence of direct physical handling, does not deform during manipulation with solvent addition, removal of solvent under vacuum, sonication, low centrifugal force or the application of heat under 125 °C, the maximum temperature allowed for NMR tubes [51].

From the spectra presented with hand-packed glass wool, the physical and experimental criteria listed above have been met. With better packing of the glass wool with an even, consistent spooling to eliminate field inhomogeneities, the use of glass wool packed within an NMR tube has the potential to be a viable experimental tool to study surface bound molecules

in a solution environ. Also, future work with NMR Wool Tubes will require selective isotope labeling to define peaks and elemental analysis necessary to determine the extent of surface modification.

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