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New insights into silica-based NMR "chromatography"

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ABSTRACT

Silica is used as an important component for NMR "chromatography". In this study the effect of the binding strength to silica of a variety of compounds on their diffusion rate is measured for the first time. Over two orders of magnitude of diffusion difference enhancement was obtained in the presence of silica for some compounds. An explanation of the enhancement is given that also allows one to predict the "chromatographic" behavior of new compounds or mixtures. The binding strength is divided into categories of weakly bound, singly bound and multiply bound. Carboxylates, sulfonates, and diols are found to be particularly strongly bound and to diffuse up to 2½ orders of magnitude more slowly in the presence of silica.

1. Introduction

NMR spectroscopy is an excellent method and therefore usually a tool of choice for precise structural characterization of organic and bio-molecules. NMR is most suited to the analysis of pure compounds. However, NMR spectroscopy is limited and almost useless when several compounds of various structures are present in the solution mixture. Most organic syntheses initially yield a mixture of compounds that require a separation process prior to structural characterization by NMR, if indeed separation is possible. Hence, there have been efforts to achieve hyphenated techniques where the mixture is separated by chromatography and NMR is then used to analyze each component [1].

To a certain extent, 2D and multidimensional NMR can separate simple mixtures but a more suitable method is to separate the components according to their diffusion coefficients [2]. This is achieved with pulsed magnetic gradients using self-diffusion (SD) NMR techniques, also known, when presented as a 2D contour map, as diffusion ordered spectroscopy (DOSY) [3–5]. The chemical shift of the spectrum is plotted on one (usually the horizontal) axis while the diffusion rate is plotted on a perpendicular (usually the vertical) axis. Each component yields a separate regular 1D spectrum corresponding to its diffusion constant. This application of DOSY has been dubbed NMR "chromatography" [6]. In later reports NMR chromatography has been used to refer to DOSY spectra where structured media such as silica gel was used to enhance the separation in the diffusion dimension [7–10,11]. The NMR "chromatography" technique is not true classical chromatography because there is no phase possessing mean movement. Although the term "NMR chromatography" has been used in several papers, it may be better to term this technique pseudochromatographic NMR.

The main disadvantage with the conventional DOSY method is that in most cases there is insufficient separation in the diffusion axis to fully separate the components of the mixture. The free induction decay (FID) of a DOSY experiment is a sum of decaying sinusoids in the acquisition dimension and a sum of Gaussian decays in the diffusion dimension. The acquisition dimension is easily analyzed by a Fourier transform yielding high resolution in the frequency domain. However, analysis of the diffusion dimension involves an inversion of the Laplace transform (ILT) [12–14]. While this is quite accurate, as little as 2% for a single decay [15], it has very low resolution when separating two or more overlapping signals with little chance of resolving diffusions of signals that have overlapping frequencies that differ by less than 30–50% [16,17].

In this work ILT was not implemented analytically but the Levenberg–Marquardt fitting algorithm was applied [18,19] as supplied with the spectrometer, because it is more robust than the analytical method in the presence of noise. We did not use other processing methods such as CONTIN [20] or MEM [21] as they were found to yield inferior and less reliable results with the data produced in this work.

An attempt to enhance the separation in the diffusion dimension was made by adding a solid chromatographic medium such as silica gel [7–11]. This separation is termed the diffusion

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difference enhancement (symbol, Δlg(D)) and is defined by the change in the difference in lg(D) upon addition of the chromatographic medium, where D is the self-diffusion coefficient and lg refers to $\log_{10}$. In conventional chromatography, the silica gel differentially binds each compound, giving each compound a different translational velocity in the column. Likewise, there is a differentiation in the compounds' effective diffusion rates even though there is no flow. However, using conventional NMR techniques solid silica gel broadens the signals arising from the liquid to hundreds or thousands of Hertz due to inhomogeneous magnetic susceptibility. Signals arising from solids that tumble slowly if at all are broadened to tens of kilohertz due to chemical shift anisotropy. In this work we deal only with signals arising from molecules that tumble freely, although some are bound to silica only to the extent that they are held in position while free to tumble. DOSY requires narrow signals in 1D NMR in order to yield signals in a diffusion spectrum. This is because broad signals relax almost completely during the diffusion pulse sequence prior to acquisition. Therefore, solid-state NMR techniques such as high resolution magic angle spinning (HR-MAS) have been used to observe the spectrum. The disadvantages of this technique are:

- It requires an NMR spectrometer with an HR-MAS probe and MAS spinning capability.
- When using reversed phase silica, the proton signals of the coating of the silica may interfere [9].
- The measured diffusion rate while spinning may be inaccurate due to vortexing in low-viscosity liquids for sample volumes of 55 μL or more, although the use of 12 μL samples seems to resolve the problem [22].

The mechanism by which the molecules are bound to the silica is investigated here. The aim of this study is to relate the binding mechanism to the diffusion difference enhancement. This knowledge would make it possible to estimate the strength of the binding, the diffusion rate, and hence the diffusion difference enhancement of molecules. As a result, prediction of the likely enhancement and usefulness for a particular combination of compounds could be made, allowing easier application of the NMR "chromatography" method.

In our previous work it has been shown that enhanced separation of molecules such as hexanol, propylene glycol, and DSS (3-(trimethylsilyl)-1,2-propanesulfonic acid sodium salt) can be achieved in the DOSY spectrum by adding a solid chromatographic medium in a regular high-resolution NMR spectrometer [23,24]. Compounds can be slowed by up to two orders of magnitude while retaining reasonable line-widths between 2 and 15 Hz even though the sample contains solid silica. This is achieved by susceptibility matching the solvent to the silica by choosing a mixture of solvents that possess the same magnetic susceptibility as that of the silica gel.

In this work the application of this technique was tested on a wide variety of compounds to demonstrate its generality and to clarify the mode and mechanism of the binding to the silica. The compounds that were separated include diols, amines, organic acids, and salts. Compounds with differing binding properties to silica were chosen; among them some that do not bind significantly (weakly bound) such as naphthalene and some that are hypothesized to be singly bound such as 1,2-octanediol and some that form multiple bonds to silica such as dodecylbenzenesulfonic acid sodium salt.

2. Results and discussion

A method for measuring DOSY of suspensions of silica gel is presented here that produces very little (about 10 Hz) line broadening and does not require a magic angle spinning (MAS) probe. The sample must be a magnetically homogeneous spheroid or a long prism aligned with the magnetic field in order to obtain narrow signals [25]. Normal suspensions of silica do not possess these properties and yield very broad signals. Conventional cylindrical NMR samples fall under the category of a long prism. In order to make the sample magnetically homogeneous, thereby yielding narrow signals, the susceptibility of the solvent must be matched to the silica gel. Silica is much more diamagnetic than most regular NMR solvents, so less common solvents, usually brominated or iodated solvents, are required. This offers the prospect of a routine way of simultaneously carrying out structural analysis of the components of a mixture by NMR [23]. A mixture of $CDCl_3$ and diiodomethane was used as the magnetically susceptibility matched solvent. This solvent yielded signals that were narrow by comparison with a silica suspension in $CDCl_3$.

An investigation of the diffusion properties of a large number of compounds indicates that the amount by which the diffusion is slowed by the addition of silica is related to the number and strength of the molecular bonds to the silica surface. Another factor affecting binding strength is the considerable variation in the hydrogen-bonding strength. As a result, the number of bonds to silica is a major, but not overriding, factor in determining the binding strength to silica.

Diffusion rates (D) (Table 1) for small entities (usually molecules but sometimes referring to supramolecular aggregates) were observed to be either fast ($>3\times10^{-10}\,m^2\,s^{-1}$) or slow ($<3\times10^{-10}\,m^2\,s^{-1}$). The borderline between fast and slow is in the center of an almost empty region of the diffusion dimension between the unbound and bond molecules. One notable supramolecular system is formic acid, which is a small molecule that forms aggregates in non-polar solutions [26] and therefore diffuses slowly. The small entities (Table 1), which diffuse quickly with similar diffusion constants to those in chloroform solution, were found to be unbound or very weakly bound to the silica. By contrast the slow diffusers (Table 2) were found to be loosely bound to the silica. Being loosely bound means that they remain on the surface of the silica but can still tumble freely and isotropically, thereby yielding an NMR signal that is narrow enough to make useful high-resolution NMR measurements. If the molecules were not tumbling rapidly and isotropically their NMR signals would be very broad [27].

As mentioned above, some compounds yield signals in the fast diffusion range (Table 1) while others yield signals in the slow diffusion range (Tables 2 and 3). However, there are some compounds, particularly the carboxylic acids that yield two sets of peaks corresponding to two diffusion rates, one very similar (within 0.1 orders of magnitude) to that for the solution without silica and one considerably slower. These rates for a series of carboxylic acids were measured. Two diffusion rates were observed for each, one for the free acid and one for the bound acid. In the case of acetic acid, the ratio of unbound to bound acid increased as its concentration increased from 1 to $10\,g\,L^{-1}$ and with changes in the concentration of silica. Their bound diffusion rate was found to be slow, in the range between 3×10^{-11} and $1.1\times10^{-10}\,m^2\,s^{-1}$. There was some indication of a slowing of diffusion with increasing chain length, an effect that is known to be due to increasing molecular size [28]. The unbound diffusion rates of carboxylic acids were found to be relatively fast, in the range between 4×10^{-10} and $1.1\times10^{-9}\,m^2\,s^{-1}$, with the exception of formic acid that had a slower diffusion in both states. The difference was attributed to the tendency of formic acid to form polymers in non-polar solvents [26] as mentioned above.

While the diffusion measurements clearly showed binding to the silica, relaxation measurements were made in order to provide independent confirmation of the existence of binding. The

Table 1
Diffusion constants and their ratios for compounds that do not interact significantly with silica, with and without 4 wt.% H60 silica in susceptibility matched $CDCl_3$ and CH_2I_2.

	Without silica		With silica		Ratio	Log_{10} (ratio)
	Diffusion[a]	log_{10} (diffusion)[b]	Diffusion[a]	log_{10} (diffusion)[b]		
N,N-Diisopropylamine	81	−9.09	150	−8.82	0.54	−0.27
1,2-Diaminopropane			153	−8.82		
Hexane	170	−8.77	275	−8.56	0.62	−0.19
Naphthalene	150	−8.82	100	−9.00	0.67	−0.18
NNNN-tetramethyl-1,4-phenylenediamine	86	−9.06	127	−8.90	0.67	−0.16
Trihexylamine	78	−9.11	84	−9.08	0.93	−0.03
1,2-Phenylenediamine	147	−8.83	135	−8.87	1.1	0.04
N-Methylethylenediamine			124	−8.91		
Cyclohexylamine			120	−8.92		
Triethylamine	135	−8.87	117	−8.93	1.2	0.06
Ethanol	214	−8.67	148	−8.83	1.4	0.16
β-Naphthalamine	162	−8.79	123	−8.91	1.3	0.12
4-Nitroaniline	134	−8.87	87	−9.06	1.5	0.19
TMS	186	−8.73	112	−8.95	1.7	0.22
Trimethylphosphate			98	−9.01		
Diethylene glycol monomethyl ether	128	−8.89	79	−9.10	1.6	0.21
β-Citronellol	112	−8.95	77	−9.11	1.5	0.16
Hexamethyldisiloxane	153	−8.82	75	−9.13	2.0	0.31
N-Methyldioctylamine			67	−9.17		
2-Methoxy-1,4-phenylendiamine sulfate hydrate			62	−9.21		
2,5-Dimethylene-2,5-Hexanediol			61	−9.21		
trans-1,2-Cyclohexanediol	142	−8.85	58	−9.24	2.4	0.39

[a] 10^{-11} m^2 s^{-1}.
[b] log_{10} (m^2 s^{-1}).

Table 2
Diffusion constants and their ratios for compounds that show medium-strength binding to silica with and without 4 wt.% H60 silica in susceptibility matched $CDCl_3$ and CH_2I_2.

	Without silica		With silica		Ratio	Log_{10} (ratio)
	Diffusion[a]	log_{10} (diffusion) [b]	Diffusion[a]	log_{10} (diffusion)[b]		
Formic acid	19	−9.73	1.9	−10.73	10	1.00
Methylamine	303	−8.52	25	−9.60	12	1.08
Valeric acid	115	−8.94	7.5	−10.13	15	1.13
1,2-Pentanediol			7.0	−10.15		
Isobutylamine			6.9	−10.16		
1,2-Octanediol	99	−9.01	6.1	−10.21	16	1.21
Linoleylamine	85	−9.07	4.4	−10.36	19	1.29
Lauric acid	94	−9.02	4.8	−10.32	20	1.30
Stearic acid	92	−9.04	4.2	−10.37	22	1.33
Butyric acid	136	−8.87	5.8	−10.24	23	1.37
Citric acid	50	−9.30	2.1	−10.67	23	1.37
Trimethylamine	194	−8.71	7.8	−10.11	25	1.40
1,3-Propanediol	166	−8.78	6.0	−10.22	28	1.44
Hexanoic acid	113	−8.95	3.8	−10.42	30	1.47
Acetic acid	349	−8.46	11	−9.97	32	1.51
Propionic acid	193	−8.72	6.0	−10.22	32	1.51
(±)-1-Phenyl-ethanediol	128	−8.89	3.8	−10.42	34	1.53

[a] 10^{-11} m^2 s^{-1}.
[b] log_{10} (m^2 s^{-1}).

compounds that are bound to the silica yielded short relaxation times. The longitudinal relaxation time (T_1) varies with tumbling rate. T_1 is long for fast (as in liquids) and very long for slow (as in solids) tumbling rates, and short for medium tumbling rates [29]. As the molecular motion is constrained by being bound to silica, its tumbling rate is reduced from being fast towards being medium, leading to a reduction in T_1. This reduction in T_1 further supports the correlation of diffusion rate with the strength of silica binding. Additionally, there is a weak correlation ($R = 0.7$) between the logarithms of T_1 and the diffusion rate and a clear separation into bound and unbound regions in the correlation plot (Fig. 1 and Table 4).

The picture that emerged from the relaxation measurements (Fig. 1) is that molecules are either free in solution (diffusing faster than 4×10^{-10} m^2 s^{-1}) or bound to silica (diffusing slower than 8×10^{-11} m^2 s^{-1}). The only two compounds that yielded diffusion rates in the intermediate range (between 8×10^{-11} and 4×10^{-10} m^2 s^{-1}), while expected to be in free solution, are formic acid and methylamine because they aggregate in solution and, according to our explanation, form multiple bonds with the silica [26]. The range of diffusion rates for the bound compounds (2×10^{-12}–8×10^{-11} m^2 s^{-1}) is very large and does not correlate with the molecular size as measured by their van der Waal's radius. These molecules move a distance of several micrometers, less than the size of a silica particle that is typically tens of micrometers in diameter, during the evolution time (typically 300 ms) of the diffusion measurement. Therefore, the experiment measures the rate of diffusion in the vicinity of the silica surface for bound entities against the rate of diffusion in solution for unbound entities. Our explanation is an empirical observation of the average of more complex system that includes an average of diffusion in many sites including compartmentalized diffusion in pores, and specific

Table 3
Diffusion constants and their ratios for compounds that show strong binding to silica with and without 4 wt.% H60 silica in susceptibility matched $CDCl_3$ and CH_2I_2.

	Without silica		With silica		Ratio	Log_{10} (ratio)
	Diffusion[a]	log_{10} (diffusion)[b]	Diffusion[a]	log_{10} (diffusion)[b]		
Ethylene glycol	41	−9.39	1.0	−10.99	40	1.60
Benzylamine	148	−8.83	3.5	−10.45	42	1.62
1,2-Propanediol (PG)	117	−8.93	2.5	−10.60	47	1.67
1,10-Decanediol	120	−8.92	2.4	−10.62	50	1.70
n-Octadecylamine	76	−9.12	1.4	−10.84	54	1.72
1,4-Butanediol	151	−8.82	2.5	−10.60	60	1.78
Dodecylbenzene sulfonic acid sodium salt	17	−9.77	0.25	−11.60	68	1.83
n-Hexylamine	138	−8.86	2.5	−10.60	55	1.84
n-Pentylamine	151	−8.82	1.9	−10.71	80	1.89
3-Phenyl-1-propylamine	128	−8.89	1.1	−10.96	117	2.07
n-Butylamine	163	−8.79	1.3	−10.89	126	2.10
Sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 (TSP)	182	−8.74	1.2	−10.93	155	2.20
Tartaric acid	295	−8.53	1.2	−10.93	251	2.40
Tri-^{t}butyl-phosphonium tetra-fluoroborate			0.45	−11.35		
3-(trimethylsilyl)-1,2-propanesulfonic acid sodium salt (DSS)	94	−9.03	0.26	−11.59	362	2.56
Sodium oleate	437	−8.36	0.87	−11.06	502	2.70

[a] 10^{-11} m^2 s^{-1}.
[b] log_{10} (m^2 s^{-1}).

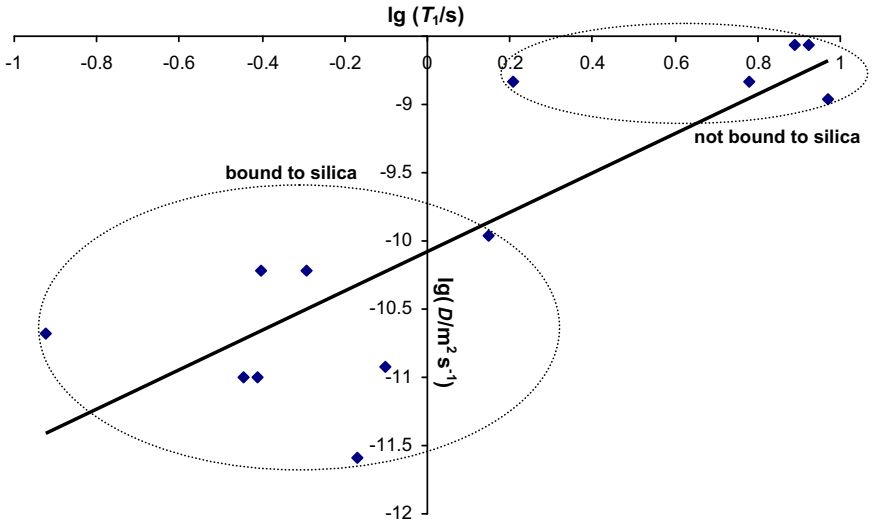

Fig. 1. Correlation between the logarithms of T_1 relaxation and diffusion rate for nine compounds: 1,3-propanediol, ethylene glycol, ethanol, hexane, citric acid, acetic acid, TMS, TSP, and DSS.

Table 4
Data for correlations between T_1 relaxation and diffusion rate.

Compound	Proton	log (T_1)	log (D)
1,3-Propanediol	1	−0.40	−10.22
1,3-Propanediol	2	−0.29	−10.22
Ethylene glycol	CH_2	−0.41	−11.00
Ethylene glycol	OH	−0.44	−11.00
Ethanol	1	0.78	−8.83
Ethanol	2	0.21	−8.83
Hexane	2	0.93	−8.56
Hexane	1	0.89	−8.56
Citric acid		−0.92	−10.68
Acetic acid		0.15	−9.96
TSP		−0.10	−10.92
DSS		−0.17	−11.59
TMS		0.97	−8.96

surface interactions. Their diffusion rates' lack of correlation with molecular size indicates that the freedom to move on the surface of the silica is more restricted in some cases than in others. The experimental evidence provided by DOSY indicates that the mechanism by which this occurs is due to differences in binding strength to the silica. Previous studies of surfactants on silica used capillary diffusion measurements to investigate their binding and the binding of organic materials in the presence of surfactants to silica [30] and showed differential binding to silica.

Of the compounds tested, we found that at least some of certain classes showed binding to silica. The classes of compounds that we consider here are sulfonates, amines, diols, and polycarboxylic acids.

2.1. Sulfonates

Sulfonated species are known to bind bidentately with silica [31]. By contrast, carboxylates are singly charged and therefore would be expected to make a single bond. As a result, sulfonates such as dodecylbenzenesulfonic acid sodium salt ($D = 2.5 \times 10^{-12}$ m^2 s^{-1}) and DSS ($D = 2.6 \times 10^{-12}$ m^2 s^{-1}) were found to be more strongly bound and less able to move over the silica surface than carboxylates, thereby explaining their slower diffusion rates. Carboxylic acids were generally found to have diffusion rates that suggest a single

hydrogen-bond to silica. Most of their diffusion rates are in the range 3.7×10^{-11}–1.2×10^{-10} m^2 s^{-1}.

2.2. Amines

Secondary, tertiary, and aromatic primary amines, with the exception of trimethylamine, do not bind strongly to silica and have diffusion rates greater than 10^{-10} m^2 s^{-1}. Aliphatic primary amines with the exception of methylamine were found to bind with what appears to be a single bond with diffusion rates usually significantly less than 10^{-10} m^2 s^{-1}. Trimethylamine is very polar by comparison with other tertiary amines, which may explain its unusual diffusion rate of 7.8×10^{-11} m^2 s^{-1}. Methylamine polymerizes in solution *via* hydrogen-bonding, reducing its capability to hydrogen-bond with the silica and giving it a relatively fast diffusion rate of 2.5×10^{-10} m^2 s^{-1}.

2.3. Diols

Diols show varying diffusion rates. According to our explanation this indicates that some are not substantially bonded and some are singly bonded to the silica. All the 1,2-diols except *trans*-1,2-cyclohexanediol were found to diffuse at a rate indicative of bonding. This is a very strong indication of the role of intramolecular hydrogen-bonding that is absent in all these non-bonded compounds. This is particularly telling for *trans*-1,2-cyclohexanediol where intramolecular hydrogen-bonding is sterically unfeasible. Intramolecular hydrogen-bonding causes the oxygen to become slightly negatively charged, encouraging further hydrogen-bonding with the silica (Fig. 2). 1,4-butanediol and 1,10-decanediol bonded very strongly to the silica due to the possibility of two binding sites arising from long range intramolecular hydrogen-bonding. On the other hand, 1,3-propanediol was less strongly bonded because intramolecular hydrogen-bonding is sterically hindered, precluding efficient direct interaction. This is illustrated in Fig. 3 for a mixture of ethanol (not bound), 1,3-propanediol (weakly bound), and ethylene glycol (strongly bound).

2.4. Polycarboxylic acids

As stated above, carboxylic acids generally have diffusion rates that indicate a single hydrogen-bond with diffusion rates in the range 3.7×10^{-11}–1.2×10^{-10} m^2 s^{-1}. If our explanation is workable, then one can expect the polycarboxylic acids to have multiple binding sites (Fig. 4) and therefore diffuse slower. To this end, the diffusion rates of citric acid and tartaric acid were measured. Their diffusion constants were found to be considerably less than the monocarboxylic acids, being 2.1×10^{-11} m^2 s^{-1} for citric acid and 1.2×10^{-11} m^2 s^{-1} for tartaric acid.

A mixture of acetic acid (a monocarboxylic acid expected to be singly bound), citric acid (a polycarboxylic acid expected to be doubly bound), and hexane (not expected to show significant binding) were used to illustrate the diffusion difference enhancement (Fig. 5).

Three trimethylsilyl compounds (TMS, TSP – sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4, and DSS) were chosen to illustrate this effect because their chemical shift is close to zero, well away from the diiodomethane resonance (3.86 ppm). TMS is not strongly bonded, with a diffusion coefficient of 1.1×10^{-9} m^2 s^{-1}. TSP makes a single hydrogen-bond with the silica and diffuses more slowly, 1.2×10^{-11} m^2 s^{-1}. DSS diffuses the slowest, 2.5×10^{-12} m^2 s^{-1}, because it makes two hydrogen-bonds (Fig. 6). The diffusion spectrum without silica is poorly resolved while with silica the signals are well separated (Fig. 7).

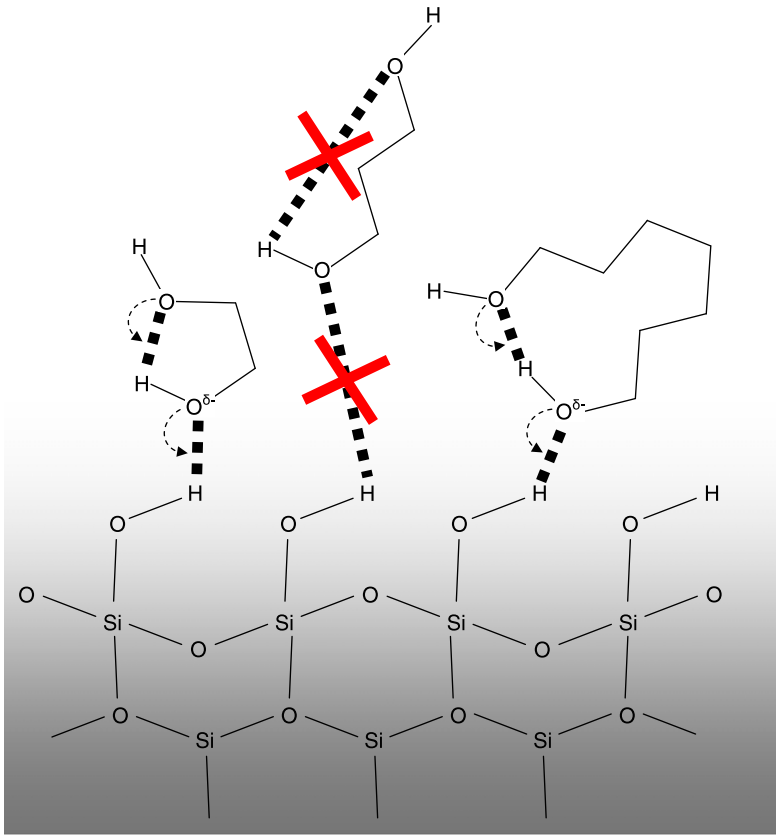

Fig. 2. Schematic illustration of attachment of diols to silica.

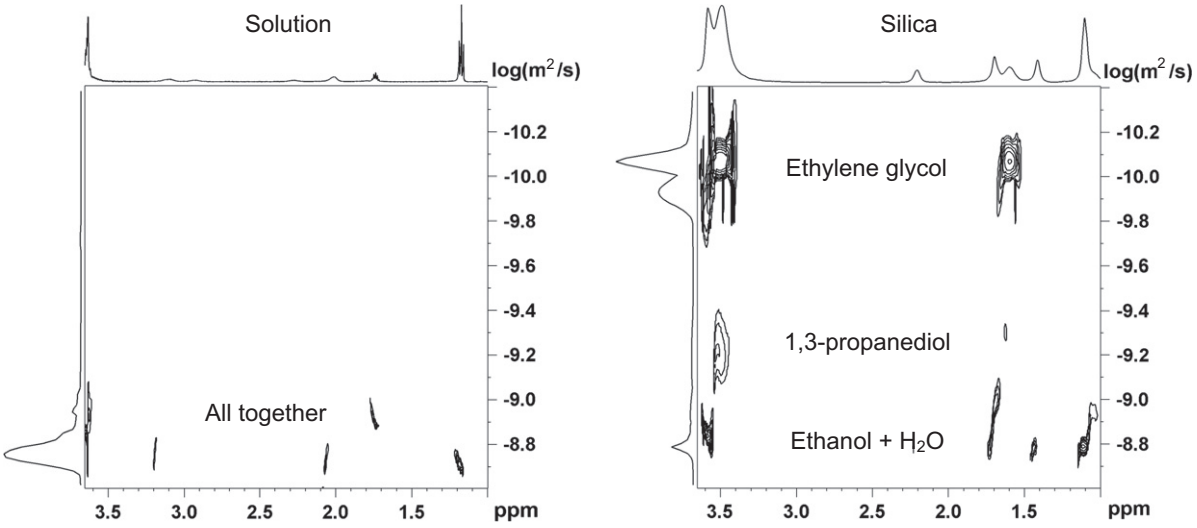

Fig. 3. The DOSY spectrum of a mixture of ethanol, 1,3-propanediol, and ethylene glycol solution without and with silica.

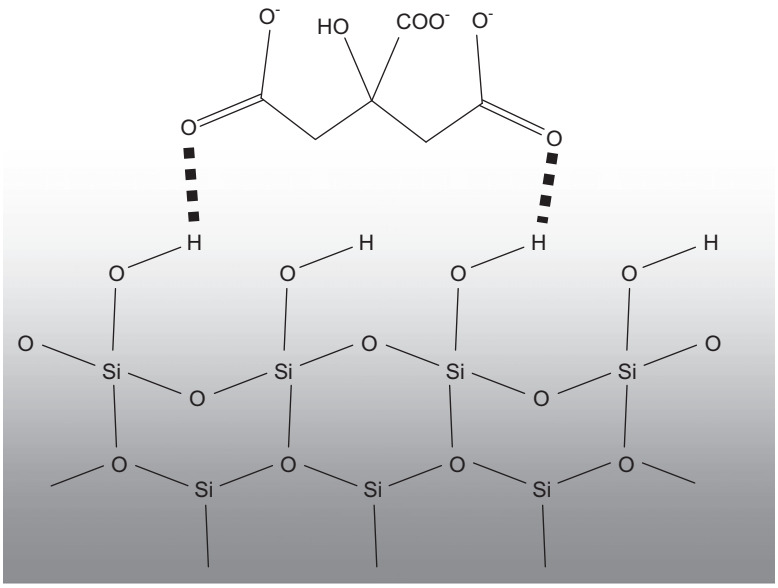

Fig. 4. Schematic illustration of the binding of citric acid, a polycarboxylic acid, to silica.

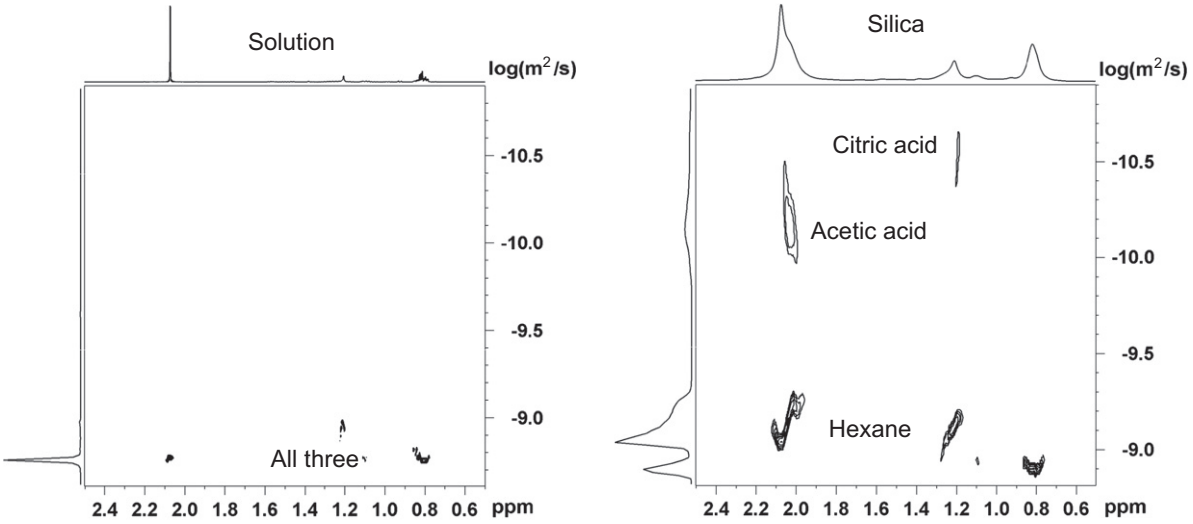

Fig. 5. The DOSY spectrum of a mixture of hexane, acetic acid, and citric acid solution without and with silica.

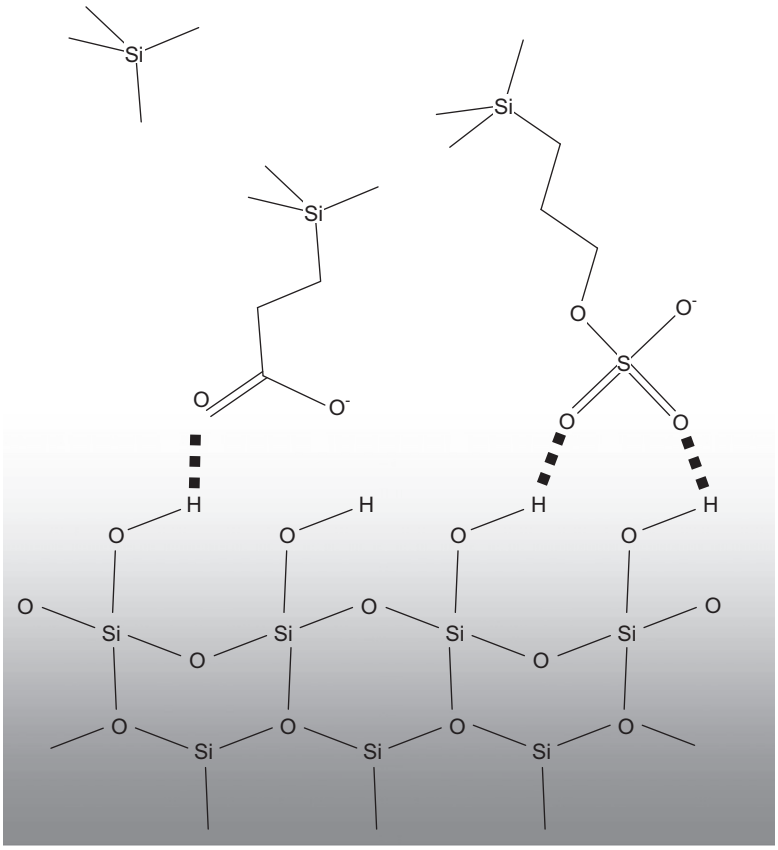

Fig. 6. Schematic illustration according of different types of trimethylsilyl compounds used as examples of different types of interaction with silica.

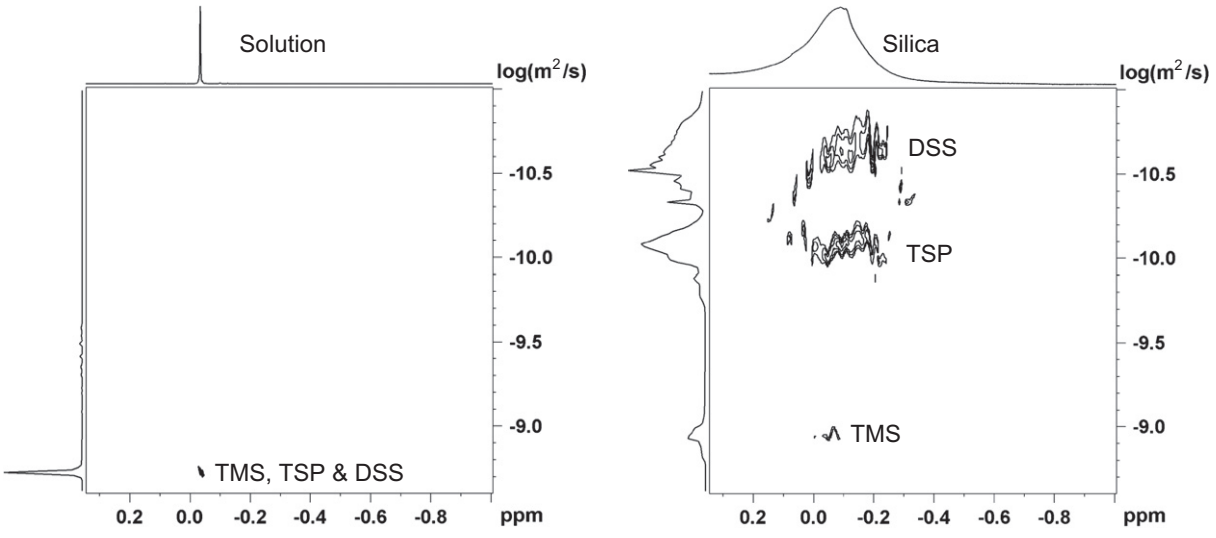

Fig. 7. The DOSY spectrum of a mixture of TMS, TSP, and DSS solution without and with silica.

3. Experimental

All NMR experiments were performed with a Bruker AVII 500 spectrometer equipped with GREAT 1/10 gradients and a 5 mm BBI probe with a *z*-gradient coil with a maximum gradient strength of 0.536 T m^{-1}. Diffusion was measured using an asymmetric bipolar LED [32,33] experiment with an asymmetry factor of 20% ramping the strongest gradient from 2% to 95% of maximum strength in 32 steps. Gradient pulses of 1 to 4 ms and intergradient delays between 0.07 and 1 s were used in order to achieve a decay curve that decayed most of the way but not completely to zero in order to optimize the accuracy of the diffusion measurement. The spectrum was processed by a Fourier transform in the acquisition (t_2) dimension and by a Levenberg–Marquardt [18,19] fit to decaying Gaussians, supplied with the Bruker TOPSPIN software, in the gradient ramp evolution (g) dimension. NMR spectra were recorded at 298 ± 0.5 K. Samples were prepared with measured amounts of solvents from a micropipette. In this work the solvents used were

$CDCl_3$ and CH_2I_2 in a volume ratio of 3.58:1 in order to achieve a magnetic susceptibility match to silica gel (−10.4 ppm in SI units). Silica suspensions were prepared by mixing the solution to 4% by weight of silica gel 60H with a particle size of approximately 60 μm that was not specially pretreated or dried. This method of preparation has been previously shown to yield maximum or near maximum diffusion enhancement [23]. Where necessary to aid dissolution of the substrates, the sample was sonicated for up to 10 min. The viscosity of the solution is similar to that of water, sufficient to prevent convection effects from disturbing diffusion measurements.

In the absence of silica, some compounds, such as DSS, were not sufficiently soluble in $CDCl_3/CH_2I_2$ to be observed by ^1H NMR. In these cases a small (<1% v/v) amount of water was added in order to solubilize a trace of the compound under study.

The CH_2I_2 signal is very strong in the spectrum, not being deuterated and comprising a large portion of the solvent. Presaturation was applied to the proton and diffusion spectrum when the strength of the signal interfered with observation of the compound studied. The presaturation pulse was centered on the CH_2I_2 signal and applied for a period of 5 s with an effective field width of 150 Hz.

Relaxation measurements were made for deoxygenated suspensions under argon using a J-Young tube. This was to ensure that the relaxation was not affected by the paramagnetism of dissolved oxygen.

4. Conclusions

Silica suspensions can provide enhancement of diffusion difference in NMR spectroscopy that can be used to separate the spectra of mixtures and/or to study their binding behavior to silica.

The diffusion separation is enhanced by differences in the binding order of the molecules to the silica surface, separating freely dissolved species from bound species. Among the bound species, diffusion separation is enhanced by differences in bond order with singly bonded moieties diffusing faster than doubly bonded moieties.

Using this explanation, it is possible for the first time to estimate the strength of the binding, the diffusion rate, and hence the diffusion difference enhancement of molecules. As a result, prediction of the likely enhancement and usefulness for a particular combination of compounds can be made. The examples show that silica-based NMR "chromatography" yields useful separation in cases where conventional DOSY is insufficient to distinguish between components of a mixture.

The silica-based NMR "chromatography" method separates compounds based on their hydrogen-bonding affinity to silica. While conventional DOSY separates compounds based on their molecular weight, this type of "chromatography" can separate compounds of similar molecular weight if they have different functionalities. For example, this method can separate alcohols from carboxylates and in turn from sulfonates. Often the difference in functionality can be more subtle; for example, 1,2-diols from non-adjacent diols, primary from secondary amines, or carboxylates from polycarboxylates. Even steric factors that affect intramolecular (and as a consequence intermolecular) hydrogen-bonding can yield a significant diffusion difference enhancement.

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