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Communication

High-resolution NMR "chromatography" using a liquids spectrometer

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ABSTRACT

NMR spectroscopy is an excellent tool for the structural analysis of pure compounds. However, for mixtures it performs poorly because of overlapping signals. Diffusion can be used to separate compounds of widely differing molecular weight but the amount of separation is usually insufficient.
Addition of a solid medium, analogous to the stationary phase in chromatography, can preferentially slow the diffusion of some components of a mixture permitting separation in the diffusion dimension. However, this would usually require a solid-state NMR spectrometer otherwise the signals would be too broad.
Susceptibility matching the solvent to the solid medium yields a spectrum with narrow signals allowing the measurement of a DOSY spectrum with enhanced separation in the diffusion dimension.

1. Introduction

NMR spectroscopy is usually the tool of choice for precise structural characterization of organic and bio-molecules. NMR is best suited to the analysis of pure compounds. However, most organic syntheses initially yield a mixture of compounds that require separation 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 used as the detector of each component [1].

In principle, NMR can be used on its own to separate the mixture by exploiting different diffusion characteristics of each component [2]. This is achieved with pulsed magnetic gradients using self-diffusion (SD) NMR techniques, also known as diffusion ordered spectroscopy (DOSY) [3]. The chemical shift of the spectrum is on one (usually the horizontal) axis while the diffusion rate is on a perpendicular (usually vertical) axis. Each molecule yields a separate spectrum corresponding to its diffusion constant.

The problem lies in separating overlapping signals. 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 [4–6]. While this is quite accurate at up to 2% for a single decay [7], 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% [8,9].

The separation can be enhanced by adding a solid chromatographic medium such as silica gel [10–12]. This separation is termed the diffusion difference enhancement [symbol, $\Delta\lg(D)$] 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 where there is no flow. However, solid silica gel broadens the signals to hundreds or thousands of Hertz using conventional NMR techniques. DOSY requires narrow signals in order to work. Therefore solid-state NMR techniques such as HR-MAS have been used to observe the spectrum. The disadvantages of this technique are:

1. It requires an NMR spectrometer with solid-state capability.
2. When using reversed phase silica, the proton signals of the silica may interfere [12].
3. The diffusion rate measured while spinning is not the true diffusion rate.

DOSY has been used to separate ligands in different discrete binding states using a technique called affinity NMR [13,14]. DOSY has similarly been used to study the slowing of diffusion in many cases of binding [15,16] but not with the specific purpose of separating different molecules. The diffusion separation can be enhanced by adding a binding molecule such as cyclodextrin but the enhancement is only about a factor of two as compared with two orders of magnitude with silica gel [17].

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We present here a method for measuring DOSY of suspensions of silica gel with very little line broadening and without the need for spinning or the use of a solid-state NMR spectrometer. Suspensions of silica are not magnetically homogeneous. In order to obtain narrow signals the sample must be a magnetically homogeneous spheroid or a long prism aligned with the magnetic field [18]. The conventional cylindrical NMR samples fall under the category of a long prism. In order to make the sample magnetically homogeneous, the susceptibility of the solvent is matched to the silica gel, yielding narrow signals. This offers the prospect of a routine way of simultaneously carrying out structural analysis of the components of a mixture by NMR [17].

2. Results and discussion

2.1. Selection of solvent magnetic susceptibility

Solvent systems were contrived from selected components and susceptibility matched with silica gels.

The line-widths of the solvent and other signals in the spectrum yield a minimum width when plotted against the magnetic susceptibility of the solution (Fig. 1). The magnetic susceptibility of the silica gel was measured by adjusting the magnetic susceptibility of the solvent mixture to yield the narrowest NMR signal. The susceptibility of a solvent mixture is given by the following equation:

$$\kappa_{\text{mix}} = x\kappa_1 + (1-x)\kappa_2 \tag{1}$$

where κ_1 and κ_2 are the volume magnetic susceptibilities of the pure solvents (Table 1), κ_{mix} is the volume magnetic susceptibility of the solvent mixture and x is the volume fraction of the first solvent in the mixture. Note that the susceptibilities are given here using SI system while many literature values [20–22] are given using the cgs system and need to be multiplied by 4π to convert them to the SI system.

A number of solutions were prepared with a range of magnetic susceptibilities, 4 wt% of silica gel added and the line-width measured of the CH_2I_2 resonance in the resulting suspension. The line-width as a fraction of the resonant frequency (conventionally expressed in ppm) is given by Eq. (2), where $\bar{\alpha}$ is the effective shape factor, f is an empirical value indicating the fraction of the solution affected by the silica and w_0 is the line-width without silica.

$$w = (1/3 - \bar{\alpha})f|\kappa_{\text{solvent}} - \kappa_{\text{silica}}| + w_0 \tag{2}$$

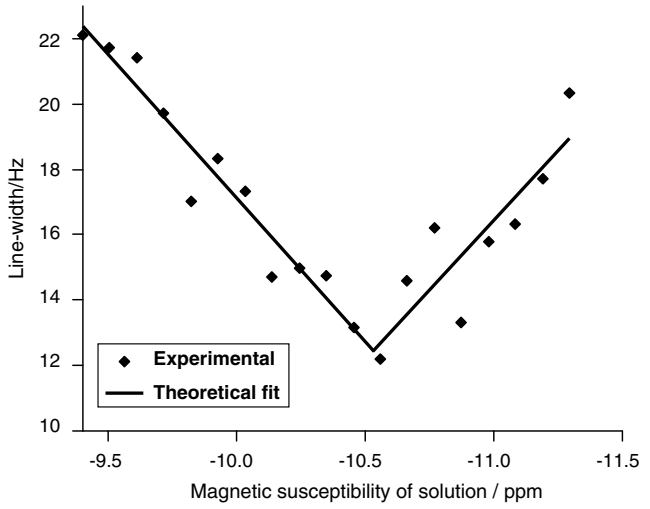

Fig. 1. Variation of line-width with solvent susceptibility in the presence of silica gel 60H.

Table 1
Magnetic susceptibilities of various solvents

Solvent	Volume susceptibility (ppm)	Molar susceptibility/ $\times 10^{-6}$ cm^3 mol^{-1}
Acetone-d_6	−5.838 [19]	−429.3
DMSO-d_6	−7.868 [19]	−556.5
D_2O	−8.974 [20–22]	−162.8
$CDCl_3$	−9.291 [19]	−745.6
$CDBr_3$	−11.76 [23]	−1023.8
CBr_3COOH (in D_2O)[a]		−1880.6
CH_2I_2	−14.35 [22,24]	−1156.1

[a] We expect that applications for this technique will be found in aqueous media that will require aqueous solutions with a greater magnetic susceptibility than water in order to match that of silica. We therefore measured the susceptibility of a solution of CBr_3COOH in D_2O as it was considered likely that this water soluble compound may have a high enough magnetic susceptibility. (Being a solid, the volume susceptibility of CBr_3COOH is not important to this work.) The density of a 3.433 M solution was 1.3727 g cm^{-3} and had a volume susceptibility of −9.658 ppm. Assuming a linear relationship (which is expected to be a reasonable approximation), the susceptibility of a solution of CBr_3COOH in D_2O is −8.974–0.200[CBr_3COOH] ppm.

For a typical NMR sample $\bar{\alpha}$ is 0.007 [18] so the equation becomes the following equation:

$$w = 0.326f|\kappa_{\text{solvent}} - \kappa_{\text{silica}}| + w_0 \tag{3}$$

The magnetic susceptibility of the silica gel corresponds to the minimum line-width. Once the magnetic susceptibility of the silica gel was established then the value of x was calculated using the following equation:

$$x = \frac{\kappa_{\text{mix}} - \kappa_1}{\kappa_2 - \kappa_1} \tag{4}$$

A variety of silicas were tested. Those with granule sizes greater than 100 μm precipitated before a spectrum could be measured. On the other hand, fumed silica that is composed of aggregated nanoparticles, precipitated over many hours to days. Three types of silica that were tested are listed in Table 2.

The reversed phase R202 silica has a susceptibility low enough to be accessible to aqueous solutions of CBr_3COOH (~3.5 M). However, we have yet to see a significant diffusion difference enhancement in aqueous solutions (need to try different eluents). We still have to see if it can be used with a neutral salt such as CBr_3COONa or CBr_3COOCs.

2.2. Effect of silica concentration

As expected from Eq. (3), the line-width varies linearly with the wt% concentration of silica gel (Fig. 2).

In conventional liquid chromatography, TLC, HPLC, etc., the eluent solution flows over the adsorbent medium for a period of typically several minutes over a distance of tens of centimeters. During this time the substrates are repeatedly adsorbed and desorbed a very large number of times. In the NMR experiment, the solution diffuses for a period of between 50 ms and 1 s and typically moves several micrometers, a smaller distance than the size

Table 2
Susceptibilities and matching solvents for three types of silica

Silica type	Susceptibility (ppm)	Solvent mixture		
Silica 60H	−10.4	$CDCl_3$:$CDBr_3$ 49:51	DMSO:$CDBr_3$ 31:69	$CDCl_3$:CH_2I_2 76:24
Fumed silica FK700	−10.4	$CDCl_3$:$CDBr_3$ 49:51	DMSO:$CDBr_3$ 31:69	$CDCl_3$:CH_2I_2 76:24
Reversed phase R202	−9.7	$CDCl_3$:$CDBr_3$ 87:17	DMSO:$CDBr_3$ 56:44	$CDCl_3$:CH_2I_2 94:6

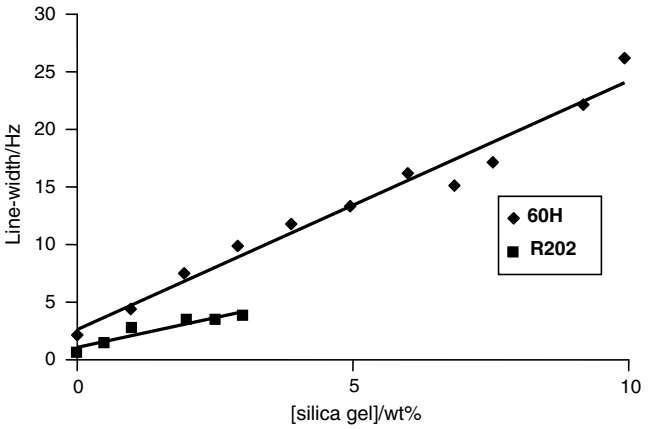

Fig. 2. Dependence of line-width on silica gel concentration for two types of silica: 60H and the reversed phase R202. The gel became too solid to prepare at concentrations greater than 3.5 wt% of R202.

of a silica particle. For example, a molecule of a solvent with a self-diffusion coefficient of 10^{-9} m^2 s^{-1} and an inter-pulse delay of 300 ms would have a mean displacement magnitude of 17 μm while a molecule that is bound to the silica with a diffusion coefficient of 10^{-11} m^2 s^{-1} would have a mean displacement magnitude of 1.7 μm. Nonetheless, even when the diffusion is slowed 100-fold, the signal strength is not significantly reduced indicating that the molecule tumbles rapidly and isotropically while loosely bound to the silica. If the molecule was strongly bound to the silica its NMR signal would be like that of a solid and too broad to be observed using a high-resolution spectrometer. If this were not the case then dipolar coupling would broaden the signal to tens of kHz in a similar manner to solid-state NMR, making it unobservable. In addition, the diffusion coefficient is independent of the inter-pulse delay, in the region of 37.5 ms to 1.2 s, showing that the diffusion is unrestricted on this timescale.

The change in diffusion difference enhancement is not linear but asymptotically approaches a maximum (Fig. 3), giving an optimum silica concentration at 3–4% for 60H and 1–1½% for R202. This indicates the amount of silica to bind the substrate.

In most cases, the silica either floats or sinks after a period of minutes to hours. A regular diffusion experiment will be affected by such linear motion. In regular samples, if there is linear motion on the timescale of the diffusion detection (~300 ms), this is caused by convection. To compensate for this, the convection compensated diffusion experiment was developed [25]. This special diffusion experiment was applied here in order to remove any effects of settling out (sinking or floating) of the silica and the effect was found to be undetectably negligible.

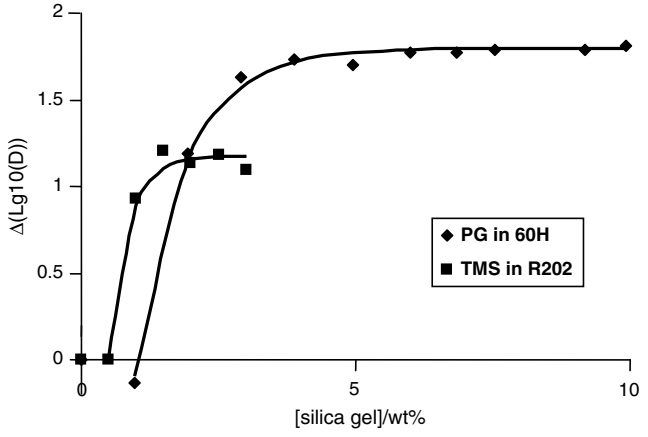

Fig. 3. Enhancement in diffusion separation as a function of silica gel concentration.

There was a marked difference between the diffusion difference enhancements for different silicas (Table 3). The underivatized 60H silica bound strongly with the polar molecules 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS), ethylene glycol and propylene glycol (Fig. 4) and, to a lesser extent, hexanol and Tween 80. On the other hand, the reverse phase R202 silica showed a strong affinity to less polar molecules such as cyclohexane and TMS. The diffusion rate of the eluent solvent mixture, $CDCl_3$ and CH_2I_2 is barely affected. The solvents were chosen because of their magnetic susceptibilities. $CDCl_3$ is the most diamagnetic of the common NMR solvents and CH_2I_2 has a very high magnetic susceptibility. $CDBr_3$ was also used as a more diamagnetic analog of $CDCl_3$. This allows susceptibility matching with only a little CH_2I_2 or a larger amount of $CDBr_3$. The selection of less polar solvents that are better suited to chromatography is expected to be the subject of future research. Reversed phase C_8–C_{18} do not show enhancements with non-polar solvents and new, polar solvent mixtures probably need to be developed in order to achieve such results.

2.3. Factors affecting 'chromatographic' separation

This NMR method shows separation effects that parallel those in regular chromatography. The stationary phase, mobile phase and substrate each affect the separation efficiency [26]. Our stationary phase was chosen to be silica and its derivatives because its diamagnetism is low enough to be matchable with solvents that are not too esoteric. However, silica is less adsorbent than MgO, charcoal or alumina that may be the subject of future study. Nonetheless, silica and its derivatives are capable of yielding good separation when used with the correct combination of substrate and eluting solvent.

Underivatized silica absorbs low-polarity substrates better than high-polarity ones while the opposite is true for reverse phase silica. R202 is fumed silica after treated with polydimethylsiloxane (PDMS). This gives it some properties of reverse phase silica while remaining useful in less polar solvents than those that are suited to other reverse phase silicas that are bound to long chain derivatives of dimethylsiloxane.

The eluting power of the solvent depends on its polarity with petroleum ether being non-polar and of low eluting power while water and ionic acids are polar and of high eluting power. In general, low-polarity eluents such as cyclohexane give good separations for untreated silica while high-polarity solvents such as water give good separation with non-polar reverse phase silica. For our initial studies, we used mixtures based on the common NMR solvent $CDCl_3$. While this solvent is not ideal in that it usually

Table 3
Diffusion difference enhancements for a variety of compounds in regular and reversed phase silica[a]

	$\lg D_{none}$	$\lg D_{60H}$	Enhancement_{H60}	$\lg D_{R202}$	$\text{Enhancement}_{R202}$
DSS	−9.48	−11.45	1.97	−9.90	0.42
Ethylene glycol	−8.92	−10.15	1.23	−8.98	0.06
Propylene glycol	−8.98	−9.84	0.86	−8.98	0.00
Hexanol	−8.80	−9.35	0.55	−10.10	1.30
Tween 80	−9.40	−9.90	0.50	−10.74	1.34
Cyclohexane	−8.60	−8.82	0.22	−9.90	1.30
Hexamethyldisiloxane	−8.93	−8.94	0.01	−9.39	0.46
TMS	−8.72	−8.85	0.13	−10.00	1.28
CH_2I_2	−8.83	−8.84	0.01	−8.86	0.03
$CHCl_3$	−8.73	−8.75	0.02	−8.77	0.04

[a] $\lg D$ is $\log_{10}$ (diffusion constant/m^2 s^{-1}).

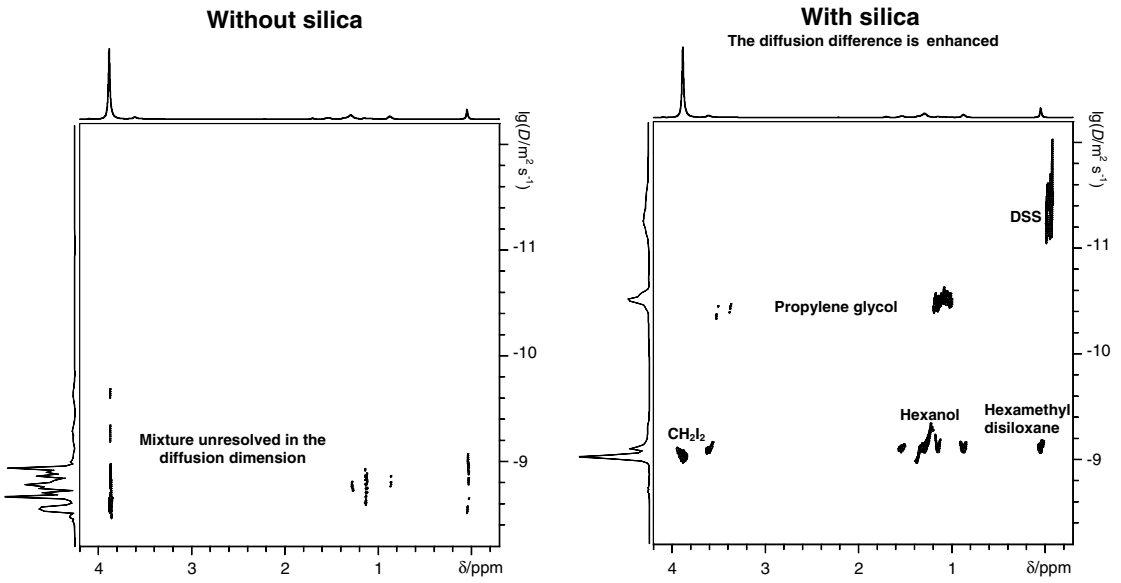

Fig. 4. Comparison of regular and enhanced DOSY for a mixture of DSS, hexamethyldisiloxane, propylene glycol and hexanol in a susceptibility matched mixture of $CDCl_3$ and CH_2I_2. The spectrum shows enhancement for DSS and propylene glycol but not for the other components.

contains nearly 1% water and traces of HCl, making it a mixture of low- and high-polarity, it does provide some separation in certain circumstances (Fig. 4).

3. Conclusions

We have shown that enhanced separation of molecules such as hexanol, propylene glycol and DSS can be achieved in the DOSY spectrum by adding a solid chromatographic medium in a regular high-resolution NMR spectrometer. Our results show diffusion of compounds slowing 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. The behavior of the various solutes in the NMR technique closely parallels their behavior in liquid chromatography.

Future work is aimed at understanding the relationship of this new NMR "chromatography" to other forms of chromatography, improving the separation enhancement and extending this method to a wide range of chemical systems.

4. Experimental

All NMR experiments were performed with a Bruker DRX 400 spectrometer equipped with BGU II gradients and a 5 mm BBI probe with a *z*-gradient coil with a maximum gradient strength of 0.534 T m^{-1}. Diffusion was measured using an asymmetric bipolar LED [27,28] experiment with an asymmetry factor of 20% ramping the strongest gradient from 2% to 95% of maximum strength in 32 steps. Gradient pulses of 1–4 ms and intergradient delays between 0.1 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 [29,30] 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 and added to a weighed amount of silica gel Sigma-Aldrich 60H (an example of underivatized regular silica) or Degussa Aerosil R202 (an example of a reversed phase silica). Where necessary to aid dissolution of the substrates, the sample was sonicated for up to 10 min.

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