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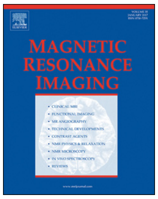

Original contribution

Evaluation of benchtop NMR Diffusion Ordered Spectroscopy for small molecule mixture analysis

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ABSTRACT

Diffusion Ordered Spectroscopy (DOSY) is an attractive method for analyzing chemical mixtures in the liquid state because it separates spectra by the molecular weight of the associated molecule. It has been compared with hyphenated chromatographic and analytical methods such LC-MS and has broad potential in servicing those same applications including forensics, reaction analysis, quality control, and fraud detection. Benchtop NMR can collect quality spectra on small molecules, however, lacks the chemical shift dispersion of high field instruments, can suffer from spectral overlap common in mixtures, and the diminished sensitivity of the lower field compounds these problems. In this work, we show that existing high field pulse sequences and processing methods perform well at 43 MHz. Spectra from molecular mixtures where the constituents had 20% differences in diffusion coefficients and significant overlap were able to be matched to a bespoke spectral library and identified correctly. In addition, spectra from mixtures with constituents that have severe overlap in the spectrum and differ by 50% in diffusion coefficients were also able to be match and identified correctly. The combination of benchtop NMR and easy implementation of modern pulse sequences and processing show promise of bringing these useful methods to chemistry laboratories in research and industrial environments.

1. Introduction

NMR is one of the main analytical methods for chemical structure analysis and therefore well situated for chemical identification [1]. A major problem is access to high resolution NMR instruments for everyday chemical analysis. While high field NMR instruments are abundant, they are expensive and require special facilities that are most often situated away from the laboratories where wet chemistry is practiced. Benchtop NMR provides inexpensive and direct access within the chemistry laboratory, however, there is a significant compromise on the field strength. Benchtop NMR spectrometers are typically in the range of 40–80 MHz proton resonance frequency compared to basic high field spectroscopy systems that start at 300 MHz. This field reduction results in decreased chemical shift dispersion proportional to the field strength without any decrease in J-coupling frequency because it is independent of the Larmor frequency. The spectra from benchtop NMR, therefore, suffer from peak overlap when J-coupling and line width are on the order of the chemical shift difference between nuclei. These shortcomings could be overcome by sufficient signal to noise and gradient steps; however, sensitivity and therefore signal-to-noise is much lower on benchtop NMR instruments because it decreases with Larmor frequency. High resolution NMR spectroscopy uses the benefits of the higher fields or multidimensional NMR experiments to overcome these spectral resolution problems [2]. Because benchtop NMR takes a step back in field strength it must go forward in another area to compensate. Multidimensional NMR experiments have already been used to increase the resolution on benchtop NMR instruments [3,4] and show great promise for further development.

Pulse-field gradient Fourier transform NMR spectroscopy (PFG-FT-NMR spectroscopy), commonly called Diffusion Ordered Spectroscopy (DOSY) after the most common analysis method, has great potential for decreasing the complexity of spectra of mixtures [5,6]. In a diffusion NMR experiment, where a series of FIDs are collected with increasing gradient strength, the signal observed (S) is derived from by the Stejskal-Tanner equation [7].

$$S = S_0 e^{-D\gamma^2\delta^2 g^2 \Delta'} \tag{1}$$

where S_0 is the signal observed in the absence of a gradient, D is the diffusion coefficient of the molecule the signal originates from, γ is the magnetogyric ratio of the observed nucleus, δ is the gradient duration, g

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is the gradient amplitude, and Δ' is the diffusion time in the limit of a small gradient duration as compared to the separation of the gradient pulses. A typical experiment steps through a range of gradient amplitudes and an FID is collected at each step. In DOSY processing, the FID is Fourier transformed to the frequency domain and each peak is fitted to the Stejskal-Tanner equation. In the ideal situation, considering liquid-state NMR, where all peaks are fully resolved and their signal attenuation is solely derived from diffusion, then each peak originating from a similar molecule will show the same diffusion coefficient. This process breaks down at lower fields where peak overlap is expected, resulting in multi-exponential behavior in all but the simplest mixtures of the simplest molecules and where data sets have an inadequate signal to noise to fit the data to these more complex models.

General methods for solving the overlap problem include multi-exponential high resolution-DOSY (HR-DOSY) and inverse Laplace transforms, however they address an ill-posed problem with essentially infinite solutions for spectra with excessive peak overlap and will be highly dependent on their regularization. Many other methods exist, such as DECRA [8,9], MRC-ALS [10], and Maximum Entropy [11] that incorporate *a priori* knowledge of the number of species in the sample to in order to constrain and simplify the data analysis. This ties down the number of solutions and removes the dependence on regularization. The CORE (component resolved NMR) method by Stilbs et al. [12] also requires *a priori* knowledge of the number of components within the mixture and forces this particular number of unique spectra and diffusion decays. While HR-DOSY produces a 2-dimensional map of peaks and diffusion coefficients comparable to other 2D correlation methods, CORE produces a single spectrum and diffusion coefficient for each molecular component in the sample. CORE relies on a constrained alternate least squares method that can be slow for large data sets typical of DOSY NMR. More recently, this method was speeded up by using matrix algebra within the algorithm and rebranded as SCORE by Nilsson et al. [13]. SCORE retains the same underlying method, while at the same time improves the robustness and performance. CORE and SCORE acknowledge that the gradient dependent signal decays and spectra produced should be highly correlated in both the diffusion attenuation and spectral dimensions such that the observed signal as a function of frequency and gradient amplitude, $I(f,g)$, can be represented as the product of the spectra, $S(f)$, and the diffusive attenuation decays, $C(g)$ summed over all the components, i, of the mixture [13].

$$I(f,g) = \sum_{i=1}^{M} S_0^i(f) C^i(g) \tag{2}$$

SCORE uses the equivalent matrix algebra to speed up the minimization process by optimizing matrices of spectra, S, decays, C, and residuals E, where X is a matrix of spectra as a function of gradient strength and $^{\mathrm{T}}$ is the transpose of a matrix [13].

$$\mathrm{X} = \mathrm{CS}^{\mathrm{T}} + \mathrm{E} \tag{3}$$

It is fair to say that extremely successful initial PFG-FT-NMR experiments were conducted on 100 MHz NMR instruments [14] nearly four decades ago. Stilbs and Moseley's [15] original work published in 1980 on Fourier transform pulse field gradient spin echo was intended to measure diffusion coefficients of multiple components but Stilbs also suggested its use for analytical chemistry [5] and demonstrated ^{13}C PGSE to address J-modulation as well as difference spectroscopy in proton NMR to address signal overlap. These measurements were conducted at 100 MHz and 25 MHz and diffusion coefficients were determined for individual peaks similar to HR-DOSY and presented in tables as opposed to 2D plots. While these measurements were possible, they were not easy. In his 1987 review [14] Stilbs stated that PFG hardware was still being developed for the complex gradient pulses required for the work presented here and that implementation of even relatively simple sequences was challenging with these hardware limitations. Today these hardware limitations have been addressed and implementing these sequences *is* trivial, even on benchtop NMR instruments. Around the time of that review, Stilbs's group, and the author presumes most others, migrated to 300 MHz superconducting systems, which seems to have also ended high resolution low-field FT-PFG NMR method development due to the superior performance of these systems.

Even though the development went to high field, the problems such as signal overlap remained due to the increased complexity of the mixtures and spectra, lower concentrations, and more components within the mixtures. Further developments in pulse sequences [16–19] and data analysis [12,13,20] eventually addressed spectrum quality and overlapping signals, but these were not carried back to low-field until there was adequate reason to so, *i.e.* the advent of high resolution benchtop NMR spectroscopy [4].

Benchtop NMR instrumentation differs from high field superconducting instruments in the static magnetic field strength, geometry, and to some extent homogeneity, yet the spectrometer hardware that controls specialized pulse sequences remains the same. This means that all the experimental development in pulse sequences to produce high-quality data can be transferred directly to benchtop NMR spectrometers. Specifically, the optimized pulse sequences that implement bipolar gradient pulses, and shaped RF and gradient pulses can be directly adopted. The differences in the magnet field strength and homogeneity can sometimes diminish the effectiveness of high field methods, for example, pre-saturation solvent suppression. Here we apply these advanced diffusion methods to a 43 MHz benchtop NMR system using an alcohol mixture system to evaluate their effectiveness. Furthermore, we apply the optimized SCORE method to obtain spectra of the component and compare it to the most general method HR-DOSY, which was recently demonstrated on a benchtop NMR instrument [21,22], as well as the simple a one dimensional (1D) proton spectrum of the mixture.

2. Materials and methods

2.1. Samples

Alcohols from two to ten carbons long originally obtained from Sigma-Aldrich were acquired from the departmental chemical stores and no attempt was made to dry or purify them. The DMSO-d_6 had significant water contamination, however, this did not adversely affect the experiments. Samples were prepared by diluting 40 μl of the specified alcohol into 560 μl of DMSO-d_6 and spiked with an additional 40 μl of benzene for a line shape and chemical shift reference at 7.37 ppm.

2.2. Instrument

Data were collected on a 43 MHz Spinsolve benchtop NMR instrument (Magritek Ltd., Wellington, New Zealand) with a custom diffusion gradient coil and amplifier providing a maximum gradient amplitude of 1.0 T/m. Gradient orientation was perpendicular to the axis of the magnet and parallel to the main field. This gradient orientation limits the effect of signal attenuation due to convection [23]. Samples were measured at the magnet bore temperature of approximately 27 °C. No significant sample heating or convection was observed during the experiments.

2.3. FT-PFG NMR pulse sequence

Data were acquired using the Oneshot45 pulse sequence [16], with 1.7 ms gradient pulses, a 200 ms diffusion delay, an *alpha* value of 0.2, and a 0.3 T/m maximum gradient amplitude. For mixtures, a repetition time of 7.5 s, 32 scans, and 32 gradient steps was used for a total experiment time of 2 h 8 min. For pure alcohols, 8 scans and 10 gradient steps were used for a total acquisition time of 10 min. The gradient steps were spaced as a function of gradient amplitude squared to meet the linearity requirement of multivariate methods [13].

2.4. Data processing

The data presented here were processed using DOSY Toolbox [20] in MatLab (MathWorks). The spectrum of the first or second gradient step was used to manually phase and baseline correct the spectrum. Reference deconvolution was performed using FIDDLE [24] and benzene as the reference line shape with a target of 1 Hz Gaussian line shape.

HR-DOSY and SCORE were performed over the range of 0 to 5 ppm, excluding the benzene peak that was used only as a chemical shift and line shape reference. For SCORE this removed one component from the fitting that was of little interest and speeded up the computation. HR-DOSY fits were performed using threshold peak picking, pure monoexponential fitting (as opposed to non-uniform gradient corrections). Dual exponential fitting was tested, but took much longer and rarely converged on more than one component per peak. It may have been possible to converge on two components with optimized initial guesses, but manual starting parameters are not possible in the software used. In addition, higher signal to noise and therefore smaller residuals could possibly provide a better solution for two-exponentials. SCORE fits were performed with three components, exponential fits, and non-negativity constraints on decays and spectra. The initial guesses to the diffusion coefficients were generated from fits to the bulk data as described in the DOSY Toolbox documentation [20].

Monte Carlo-like simulations, similar to Stilbs and Moseley [25], were performed to estimate the error of the SCORE method as well as provide a data set that could be transformed into an HR-DOSY representation of the SCORE data. Briefly, an initial SCORE fit is used to create a seed set of residuals. The residuals were used to estimate the root mean squared (RMS) error of the fit. A normally distributed noise matrix with the same RMS error as the residuals was created and added back into the residuals and then scaled to the original RMS noise value. The new residuals were added to the initial fit to simulate a new data set with a new random noise profile yet retaining the systematic deviations of the original data from the fit. This was repeated 100 times to get statistics on the diffusion coefficients and spectra for each component.

These simulations take a long time when using the same non-negativity constraints that were used with the first fitting. In contrast, the unconstrained simulation produces the same mean spectrum as the constrained fit and takes the same time as a single non-negativity constrained fit. The error in the unconstrained version has a higher error and has the possibility of producing outliers.

The Monte Carlo-like simulations were then used to convert the SCORE simulation data into an HR-DOSY representation to further compare the two methods. The simulations produced 100 spectra matrices, S, and 100 decay matrices, C. The diffusion coefficients were determined from the decays and binned into a histogram over the range of the diffusion coefficients for each component of the sample. A simulated HR-DOSY data set was created by taking the outer product of the spectrum and the histogram and mapping them onto the frequency and diffusion coefficient ranges.

3. Results

Fig. 1 shows the FT-PFG NMR data set for the 2-butanol and 1-octanol mixture and is representative of all the other data sets with the exception that only 10 gradient steps were acquired for pure alcohol samples. The diffusion coefficients and spectra of the pure alcohols were determined using the SCORE algorithm with two components to remove the water contamination peak and a non-negativity constraint. These spectra and diffusion coefficients were later used to evaluate the fidelity of spectral components and diffusion coefficients extracted from the mixture samples.

3.1. Mixture analysis with HR-DOSY

The mixtures were initially analyzed using the HR-DOSY method that fits each spectral peak to a single or double exponential in the diffusion dimension [26]. The spectral overlap of the peaks caused the diffusion coefficient for these peaks to be a compromise between the true diffusion coefficients of the molecules associated with them. The HR-DOSY map of 2-butanol and 1-octanol in Fig. 2 demonstrates this with the octanol peaks shifted towards faster than expected diffusion, while the butanol and water peaks are shifted to slower diffusion. In general, it is difficult to assign peaks in this 2D plot to a specific component and even more difficult to identify the components.

3.2. Mixture analysis with SCORE

The SCORE algorithm was used here to extract the spectra, S, and diffusion dependent decays, C, from the pulsed field gradient data sets, X, by minimizing the residuals, E, as described in Eq. (3). The raw data, as shown in Fig. 1, was represented by an $ng \times nf$ matrix, X, where ng was the number of gradient steps in the first dimension and nf was the number of frequency data points in the spectrum in the second dimension. The pulsed field gradient data, X, was factored into an $ng \times nc$ decays matrix, C, where ng was the number of gradient steps and nc was the number of components in the mixture and an $nc \times nf$ spectra matrix S, where nc was again the number of components and nf was the number of frequency points in the 1D spectrum. The decays, C, were constrained to Eq. (1), so that the final result of SCORE processing was a spectrum and diffusion coefficient derived from the decay of each component (Fig. 3). The number of points in the spectrum and number of gradient steps were defined by the data, while the user chose the number of components.

Individual pure alcohol samples were analyzed with two components, $nc = 2$, the alcohol and the solvent contaminant. Three components, $nc = 3$, representing each alcohol and the solvent were used for the mixtures. The spectral range was limited to 0 to 5 ppm to remove the benzene reference peak and simplify the analysis.

The diffusion coefficients and spectra of the pure alcohols (Table 1) were compared to the spectra extracted from the mixtures using SCORE. The diffusion coefficients were directly compared while the spectra were compared using cosine similarity ($cos\theta$)

$$cos\theta = \frac{AB'}{\|A\|\,\|B\|}$$

where A was the spectral component from the mixture and B was the spectral component from the pure alcohol.

Prior to comparison, the spectra were aligned using the icoshift algorithm [27]. This created optimistic correlations for spectra that were not a match, however, poor matches would reap little benefit compared to true molecular matches. Icoshift allows a maximum shift, or selection of reference regions, which may be useful in the future. However, this was not used in this study because the greatest shift observed was two points. The cosine similarity results are shown in Table 2.

3.2.1. 2-Butanol and 1-octanol mixture

The 1D spectrum, or more accurately the first gradient step of the mixture, was compared to individual alcohol spectra to show the necessity of a more advanced analysis method than just a simple 1D spectrum of the mixture. The cosine similarity gave the best match of 0.78 with 1-pentanol. This demonstrated the averaging effect of a bulk spectrum, where the best match was neither component, but rather a compromise between the components. This match was not much better than that for 1-butanol (0.71), 1-hexanol (0.75), or 1-octanol (0.73). A bulk range of diffusion coefficients was estimated by taking the amplitude at the beginning of each FID at each gradient step and performing an inverse Laplace transform to yield a distribution of diffusion

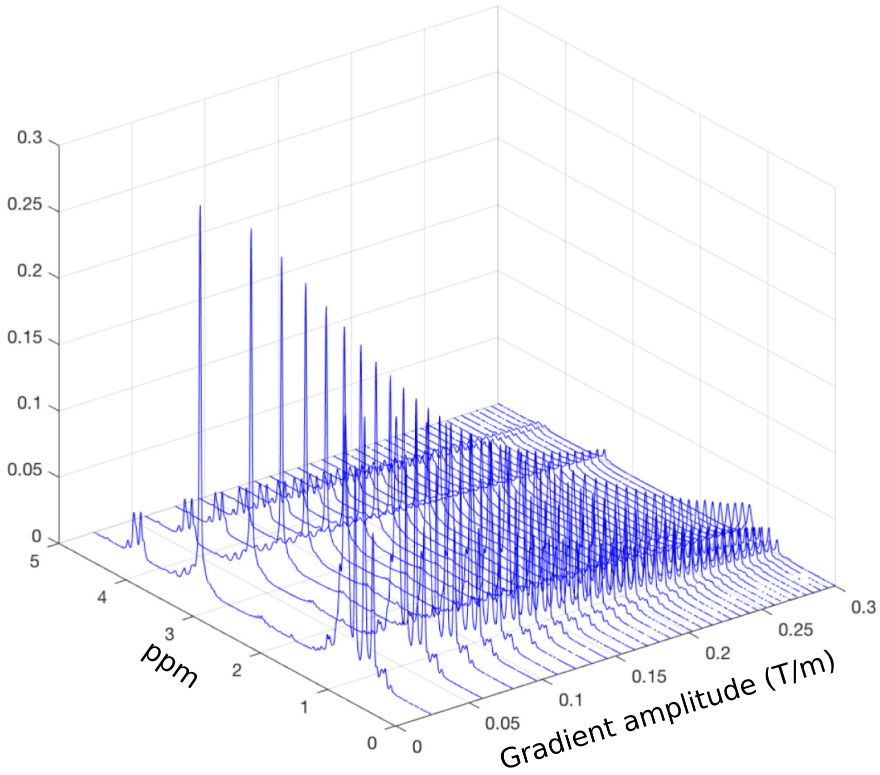

Fig. 1. Typical pulsed field gradient NMR data set of the 2-butanol and 1-octanol mixture acquired using OneShot45 pulse sequence. The data have been Fourier transformed along the read dimension and cropped to the 0–5 ppm range. The spectra have *nf* points and *ng* gradient steps defining the size of the two-dimensional data matrix.

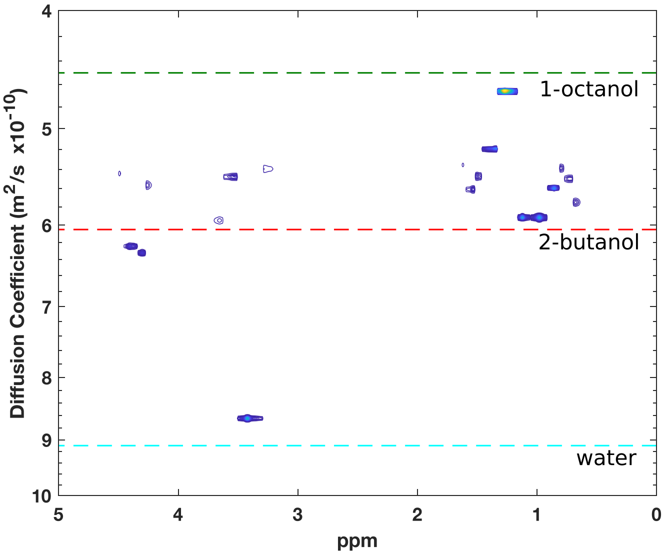

Fig. 2. The HR-DOSY 2D spectrum of 2-butanol and 1-octanol mixture in DMSO-d_6. The blue, red, and green lines show the expected diffusion coefficients for 1-octanol, 2-butanol, and water respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

coefficients. This range spanned 5–7 × 10^{-10} m^2/s, which included a match to propanol, butanol, pentanol, and hexanol. It excluded 1-octanol that was a true component in the mixture. This result is similar to the visual interpretation of the 2D HR-DOSY spectrum shown in Fig. 2.

Three spectra and decays were extracted from the NMR data for the 1-octanol and 2-butanol mixture using the SCORE method. The diffusion coefficients, 4.4 ± 0.3 × 10^{-10} and 5.9 ± 0.3 × 10^{-10} m^2/s, obtained from fitting the extracted decays to Eq. (1) best matched octanol and butanol and a third matched water with a diffusion coefficient of 9 × 10^{-10}. The best cosine similarity matches were 0.99 to 1-octanol and 0.99 to 2-butanol in separate comparisons. The water spectrum was not tested for similarity to any of the alcohol spectra. This showed that the presented method was able to accurately match the spectral components of this model mixture sample to a library of likely spectra.

3.2.2. 2-Butanol and 1-hexanol

The same process was repeated with 2-butanol and 1-hexanol to examine molecules with similar diffusion coefficients. Butanol had a predicted and measured diffusion coefficient of 6.05 ± 0.06 × 10^{-10} m^2/s compared to hexanol which was 5.0 ± 0.03 × 10^{-10} m^2/s. The 1D proton spectrum acquired from the first gradient step that represents the 1D spectrum of the mixture was compared to the individual alcohols and matched best to 2-butanol with a similarity of 0.81. Three other alcohols had similarities greater than 0.7 including 1-butanol, 1-pentanol, and 1-hexanol. This was not surprising considering the matches resemble the closest alcohols in size and structure.

The alcohol spectra extracted using SCORE had diffusion coefficients of 5.12 ± 0.03 × 10^{-10} and 6.34 ± 0.04 × 10^{-10} m^2/s, which matched closest to the butanol and hexanol diffusion coefficients measured from the individual alcohol samples, but there was a systematic error towards faster diffusion. Looking at the similarity of the extracted spectra to the individual alcohols the best matches correlated to the correct alcohols with 0.95 to 1-hexanol and 0.98 to 2-butanol. While there were discrepancies in the diffusion coefficients measured, the analysis method identified the most likely components correctly.

3.2.3. 1-Butanol and 1-octanol

A third mixture of 1-butanol and 1-octanol was measured to look at the impact of spectral similarity. The overlay of the spectra in Fig. 4 shows that all the peaks are fully overlapping with only minor differences in shape and amplitude.

The diffusion coefficients of the two alcohol components within the mixture were measured to be 4.8 ± 0.3 × 10^{-10} and 6.7 ± 0.2 × 10^{-10} m^2/s. This was significantly lower for hexanol and

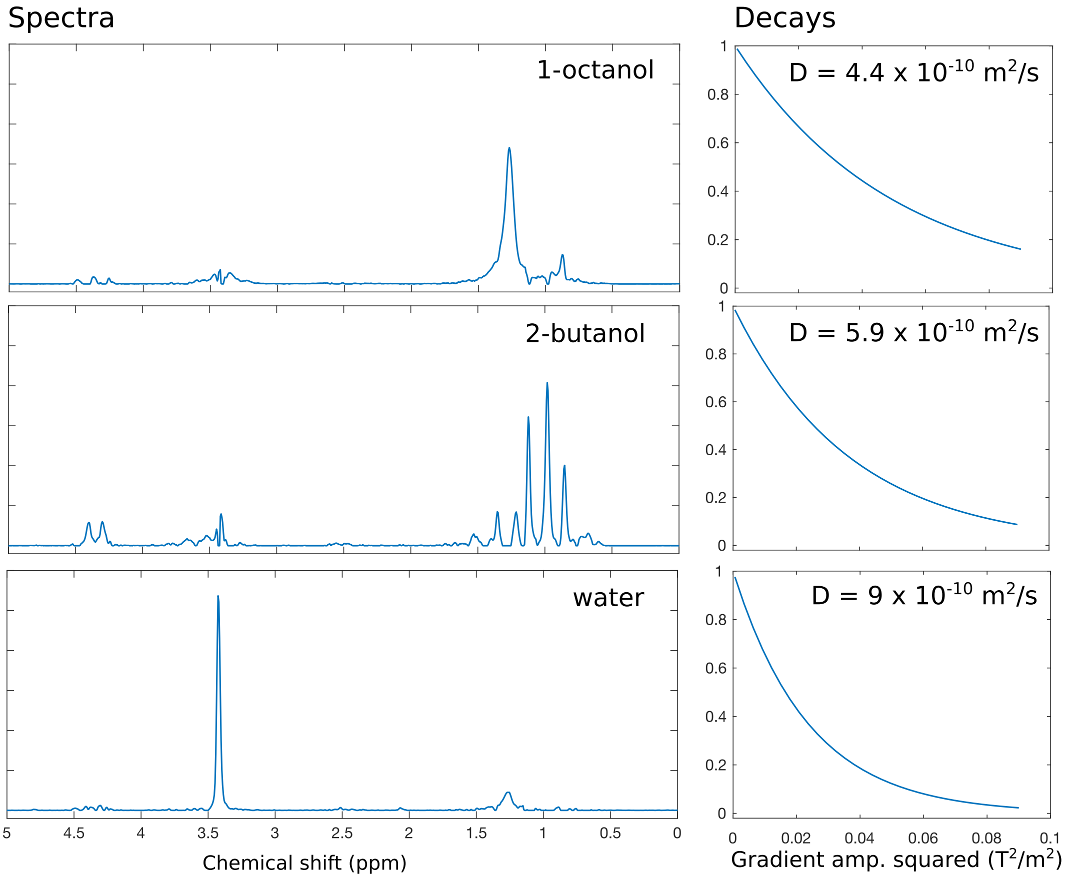

Fig. 3. The SCORE algorithm factors the NMR data, shown in Fig. 1, into spectral and amplitude decay components as shown here for the 2-butanol and 1-octanol mixture.

Table 1
Properties of the alcohols used in this study.

Alcohol	Carbons	Molecular weight (g/mol)	Diffusion coefficient at 27°C in DMSO-d_6 ($\times 10^{-10}$ m^2/s)	
			Predicted [29]	Measured
Ethanol	2	46	7.8	7.78 (0.03)
2-Propanol	3	60	6.7	6.49 (0.01)
1-Butanol	4	74	6.0	6.01 (0.03)
2-Butanol	4	74	6.0	6.09 (0.03)
1-Pentanol	5	88	5.5	5.70 (0.05)
1-Hexanol	6	102	5.0	5.04 (0.05)
1-Octanol	8	130	4.4	4.49 (0.01)
1-Decyl alcohol	10	158	4.0	4.02 (0.01)

higher for butanol than expected for the individual components and matched closest to hexanol (or heptanol if it was included in this study) and propanol. While this was disappointing, the spectra matched closest to 1-octanol with 0.99 and 1-butanol with 0.90 cosine similarity.

3.3. SCORE simulations

The unconstrained SCORE simulation was used to recreate the HR-DOSY-like spectrum shown in Fig. 5. An unconstrained SCORE method was used in the simulation because it was much faster and the non-negativity was frequently pushed up against a negative boundary at many points decreasing the freedom of the fit. None of the error bars pushed the diffusion coefficient into negative values and the mean spectrum was the same spectrum obtained from the initial constrained fit. There were a few outlying spectra and diffusion coefficients, but they were still included in the statistics.

Table 2
Cosine similar of the mixture spectrum and each SCORE component spectrum compared to the alcohol spectral library for the mixtures.

Alcohol	2-Butanol and 1-octanol			2-Butanol and 1-hexanol			1-Butanol and 1-octanol		
	1D	Comp 1	Comp2	1D	Comp 1	Comp2	1D	Comp 1	Comp2
Ethanol	0.14	0.12	0.08	0.14	0.17	0.10	0.17	0.15	0.20
2-Propanol	0.48	0.56	0.87	0.65	0.25	0.85	0.16	0.13	0.21
1-Butanol	0.71	0.64	0.46	0.73	0.82	0.53	0.86	0.74	**0.90**
2-Butanol	0.62	0.24	**0.99**	0.81	0.43	**0.98**	0.34	0.30	0.39
1-Pentanol	0.79	0.78	0.46	0.76	0.94	0.52	0.88	0.86	0.74
1-Hexanol	0.75	0.89	0.36	0.73	**0.95**	0.46	0.90	0.92	0.54
1-Octanol	0.73	**0.99**	0.26	0.64	0.93	0.36	0.90	**0.99**	0.41
1-Decyl alcohol	0.68	0.98	0.22	0.56	0.86	0.32	0.84	0.97	0.30

The best spectral matches are displayed in bold.

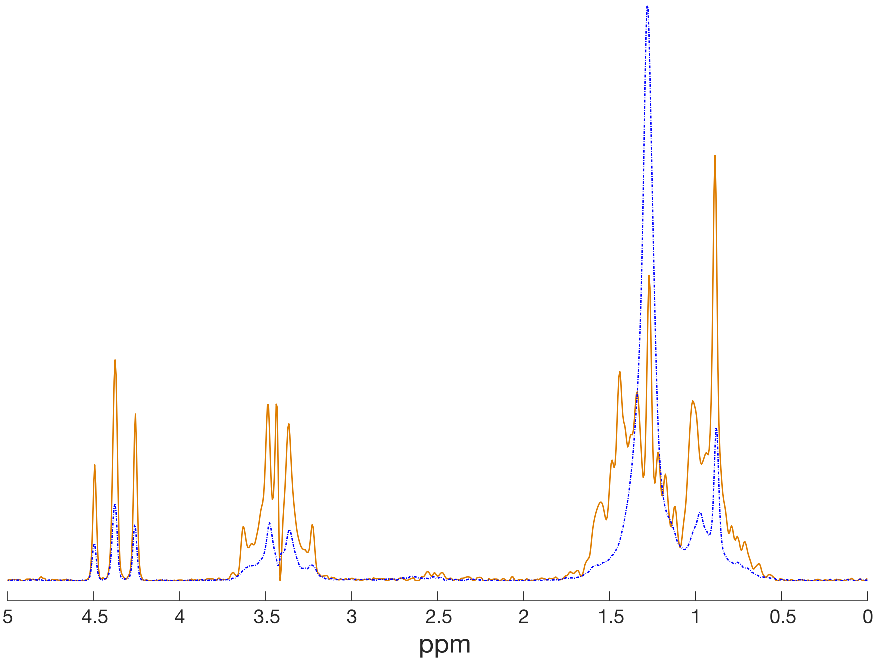

Fig. 4. 1D spectral overlay of 1-butanol (solid) and 1-octanol (dashed) acquired from SCORE processing of individual alcohols to remove solvents peaks.

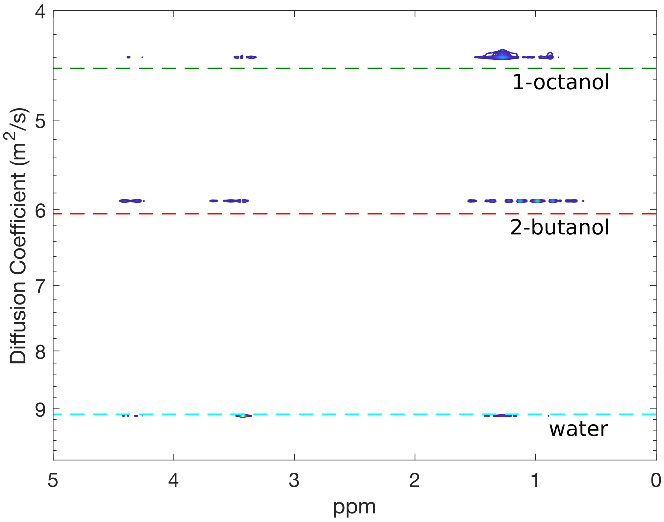

Fig. 5. An HR-DOSY representation of 2-butanol and 1-octanol reconstructed from the diffusion coefficients and average spectrum obtained from a SCORE simulation.

4. Discussion

In most cases, the HR-DOSY and SCORE processing methods will be complementary, such as when studying a new system or an unknown mixture. These studies would most likely be carried out at high field on the best performing NMR instrument available to the researcher. However, if these analyses are implemented as a routine analysis on a benchtop instrument, where most outcomes will be expected, one simple analysis method would be preferable. In these cases, it is valid to compare these two methods for suitability to specific applications. The alcohols tested in this study represent such an application with expected outcomes.

The 2D HR-DOSY representation shown in Fig. 5 allows for a more direct comparison between HR-DOSY (Fig. 2) than the standard SCORE output of spectra and decay components (Fig. 3). The nature of SCORE processing confines the spectra to a narrow range of diffusion coefficients in the diffusion dimension defined by the variance in the simulations. There is also some crosstalk between spectra that is particularly evident between the water alcohol protons that will add some uncertainty to the analysis. Despite these artifacts, this representation and the high correlation between the expected spectra show the clear advantage that SCORE processing has over the more traditional HR-DOSY processing in this low field NMR mixture analysis problem where there are severe overlap and only moderate differences in molecular size. The alcohols are easily matched to the correct spectrum and diffusion coefficient in the data library.

The overall method demonstrated here can be extended to other similar mixtures, where only a few components make up the sample, the spectra have different characteristics, the components differ in molecular size, and there is a significant concentration of each component in overlapping peaks. The degree to which compromise can be made on each criterion depends on how well the others are satisfied. This sample space was not exhaustively explored here, but the three examples demonstrate resolving similarly sized molecules with significant overlap, but differently shaped peaks in the 2-butanol and 1-hexanol, while the 1-butanol and 1-octanol mixture shows that spectra can be severely overlapped if there is a two-fold size difference between the molecules and the spectral intensity patterns still differ significantly.

Collection of good raw data and processing methods that clean up and analyze the data are critical to the separation and identification process. The Oneshot45 pulse sequence [16] produces clean data from the three key characteristics of bipolar gradients, unbalanced gradient pairs, and 45-degree purge pulse. Bipolar pair gradients minimize the impact of eddy currents created by the pulsed gradients [19]. By unbalancing the pairs, unwanted coherences are dephased. This could allow for a decreased phase cycling scheme, but 16 transients were necessary to accumulate sufficient signal to noise. At low-field significant signal averaging will be necessary for most samples making the shortened phase cycle unnecessary. The only detriment of this method is a decrease in the maximum gradient amplitude by the factor of *alpha* required for mismatched bipolar gradient pulses. In this study, the *alpha* value of 0.2 results in a 20% decrease in the maximum gradient pulse as compared to a method using matched bipolar gradient pulses or a standard Hahn echo or stimulated echo pulse sequence. Finally, the 45-

degrees purge pulse removes antiphase magnetization that distorts the line shape. This is critical in producing pure absorption lines in a very crowded spectrum.

Reference deconvolution is useful for cleaning up the remaining line shape artifacts [28]. In this study we used a benzene singlet peak as a reference, which resulted in a spectrum with reproducible line shapes and widths. This allowed for high reproducibility and in turn comparable spectra. While benzene was an appropriate line shape reference for this project, there are many other line shape references that could be used in other mixtures.

The spectrum and diffusion coefficient are two independent parameters for identifying molecular components of the mixture. This combination of characteristics allows for the identification of molecules of similar structure or similar size within a mixture as demonstrated here. The 1-hexanol and 2-butanol mixture has shown that different chemical functionality that results in different spectra allows for separation even when diffusion coefficients differ by only 20%. Substitution reactions are common in organic chemistry and this method could be used to evaluate such a reaction. This method also has promise in evaluating reaction mixtures with minimal purification where a synthetic chemist would not bother acquiring a high field spectrum due to time, availability, or convenience constraints. The ease of removing the signal from contaminants could also be of interest for forensics such as excluding cutting agents from spectra for more confident identification or supplying information about possible contaminants underlying quantitation peaks.

Due to its much lower magnetic field strength, benchtop NMR, as compared to high field NMR, suffers limitations such as a lack of chemical shift dispersion and lower signal to noise. Many methods have been developed to compensate for signal overlap due to lack of sufficient chemical shift dispersion, however, sufficient signal to noise is often required to apply them. Benchtop NMR systems have sufficient signal to noise for many applications, but the crosstalk in the simulated DOSY spectrum is an example of a limitation. The correlations of the SCORE spectra with known sample spectra and their fitted diffusion coefficients provide compelling evidence of identification in the known system presented here, however, it is prudent to deploy this method in well-known systems with expected outcomes as opposed to open outcome studies due to the performance limitations of benchtop NMR.

5. Conclusions

This work shows that molecular components of a mixture can be distinguished at low field from similar molecules. The combination of pulsed field gradient methods, preprocessing, and spectral analysis compensates for the lack of chemical shift dispersion that high field NMR measurements benefit from. The pulsed field gradients separate out residual solvent and components that vary in size. Processing, such as reference deconvolution, creates reproducible spectra that can be used to query databases of known spectra. Analysis methods such as SCORE can be used to successfully pull out reproducible spectra. This procedure is easy to carry out and could be automated to improve the accessibility of analytical NMR to those not accustomed to working with it. Accessibility of these methods and the modest footprint of the hardware could extend NMR analysis to chemical laboratories where it would be most useful on a day to day basis as opposed high field facilities that are only used when structural confirmation is necessary.

Declarations of interest

None.

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