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Resolving DOSY spectra of isomers by methanol-d_4 solvent effects

Introduction

A wide variety of NMR experiments have been devised to elucidate the structures and concentrations of analytes as well as the composition of multicomponent samples. Among these, diffusion methods hold promise to resolve mixture components according to their physical properties: molecular shape, size and mass.[1] If presented in the form of 2D diffusion-ordered spectroscopy plots (DOSY),[2] then diffusion NMR provides an intuitive way of correlating isolated spin systems and estimating the relative size and the number of analytes in a mixture.[3]

Diffusion-ordered spectroscopy complements the spectral resolution from 1D NMR with separation by diffusion in the second dimension. It can easily provide meaningful information if two prerequisites are met: (i) There is minimal spectral overlap among compounds of interest and (ii) analytes have different rates of thermal diffusion. While a multitude of advanced NMR techniques have been developed to address the first prerequisite, options to increase discrimination of analytes with similar diffusion properties are limited. This has been a hindrance in application of DOSY in the analysis of mixtures of isomers or otherwise highly similar compounds. Herein, we show how solvent effects in methanol-d_4 substantially enhance the diffusion resolution of isomeric compounds. We will demonstrate clear discrimination among eight analytes in a molecular weight (MW) range of three units, allowing to unambiguously correlate all signals.

Diffusion-ordered spectroscopy has been used for the analysis of drug formulations,[4] biological extracts,[2,5] protein aggregation,[6] intramolecular interactions in supramolecular chemistry,[7] structures of organometallic complexes[8] and evaluation of polymer MWs.[9] Spectral overlap issues in complex samples have been addressed by combining the diffusion experiment with a 2D NMR experiment (creating pseudo-3D experiments, such as DOSY-COSY, DOSY-TOCSY and DOSY-HSQC),[7] with the pure shift experiment,[10] maximum quantum experiments[11] or detection via nuclei other than proton (i.e. fluorine,[12] carbon[13] and silicon[14]). Unfortunately, all these come with noticeable penalties in sensitivity and experiment time or, in the case of fluorine, require the presence of the specific nucleus.

Alternatively, advanced data processing techniques have emerged to address overlap: Multiexponential fitting,[15] multivariate techniques,[16] covariance analysis,[17] etc. have been proposed. Yet, none of these methods work reliably in all situations and usually require some prior assumptions to be made – a drawback compared with traditional FT-NMR spectroscopy.

A further solution to resolving spectral overlap is experimenting with solvents. Anisotropic solvents like benzene are known to resolve otherwise overlapping signals[18] and may help DOSY in problematic situations. Combining all these approaches suggests that sufficient spectral resolution can be found often even in moderately complex samples. Still, for DOSY to help identify mixture components, resolution in the diffusion dimension is required.

Results and Discussion

The diffusion coefficients D of analytes are related to their molecular size, as described by the Stokes–Einstein equation[1]:

$$D = \frac{k_b T}{6\pi\eta r_S} \quad (1)$$

where k_b is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solution, and r_S is the hydrodynamic radius of the analyte. Although only correct for infinitely diluted spherical particles, Eqn (1) is a good approximation to real solutions as far as analytes with similar shapes are concerned.

Because the resolution in the second dimension in DOSY is derived from differences in D, one would expect it to perform poorly in a sample of compounds with similar MWs, shapes and hydrodynamic radii. Unsurprisingly, this is what was observed in the mixture herein when analyzed in the two most common solvents in NMR: D_2O and chloroform-d. The mixture of eight isomeric substituted pyridines and pyrazines (Fig. 1) contains compounds **1**–**5** with equal atomic composition, a further pair of isomeric alkylpyridines **6**–**7** and a related methoxypyridine **8**. While all analytes can be recognized on the DOSY plot in D_2O (Fig. 2A), only compounds **1** and **3** are somewhat resolved and do not suffer from overlap in neither dimension. Thus, the overall diffusion dispersion is clearly not good enough in D_2O for definitive signal correlation.

Surprisingly, compound **8** is well resolved in the diffusion dimension in chloroform-d (Fig. 2B), but two of its signals spectrally overlap with other components (dotted circles in Fig. 2B). The slowest diffusing analyte also appears to be resolved in Fig. 2B, but we argue that such minute differences in D in both solvents cannot be used as reliable proof considering the uncertainties often encountered in DOSY. We conclude that neither solvent is a suitable medium for DOSY analysis of the mixture herein.

The analysis of mixtures of compounds **1**–**8** and their numerous analogues would, however, be highly desired due to their importance in the food, fragrance and pharmaceutical industries.[22] Alkylpyrazines and alyklpyridines are also important flavour constituents of coffee, but the analysis of such highly isomeric compound mixtures is a challenge for the industry's standard techniques.[23] Diffusion NMR would be a convenient tool for the analysis of such isomer mixtures if sufficient spectral and diffusion resolution could be obtained.

Additional diffusion dispersion has been achieved previously by complementing the sample with further slow diffusing

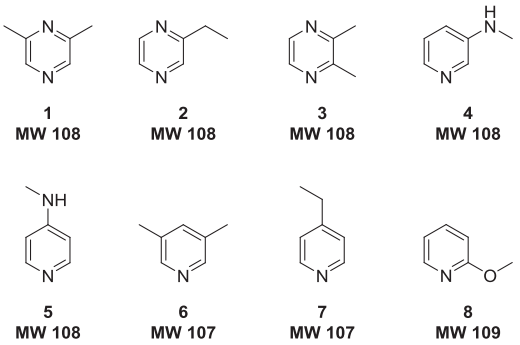

Figure 1. Structures of compounds **1**–**8**.

components that have varying degrees of interaction with different analytes. The approach is called chromatographic NMR[24] or more often matrix-assisted DOSY and involves the addition of chromatographic medium-like macrocyclic compounds (i.e. cyclodextrins),[24] metal complexes,[25] micelles,[26] (soluble) polymers[27] or silica gel[28] to the sample. General guidelines for the choice of matrix have been developed,[29] and the technology has found applications, for instance, in the analysis of a flavonoids mixture.[30] Nevertheless, addition of chromatographic medium may cause line shape distortions and additional spectral crowding and complicate the recovery of sample.

A more elegant approach was developed by Codling *et al.* who manipulated the solvent environment to modify diffusion coefficients.[31] Similarly to achieving spectral resolution with benzene, they improved diffusion resolution for three dihydroxybenzene regioisomers by optimizing the solvent composition in a mixture of D_2O and nondeuterated monohydridic alcohols, achieving sufficient changes in internal solution structure and hydrogen bonding interactions with analytes. Although methanol was not used in the report,[31] we reasoned that it should also provide additional diffusion resolution. Its deuterated counterpart, methanol-d_4, also has hydrogen bonding solution structure[32,33] and is commonly found in NMR laboratories. Considering that hydrogen bonds in deuterated solvents may be stronger[34] and that the hydrogen bonding patterns of pyridine in methanol and methanol-d_4 are different,[35] we were curious if this would render methanol-d_4 a good diffusion resolution enhancing medium.

Indeed, the same mixture was clearly resolved in the new solvent (Fig. 2C). All compounds were unambiguously separated on the diffusion axis, and all signals from each compound could be easily correlated. This is of particular value in mixtures of substituted aromatic compounds that often comprise multiple isolated spin systems. It demonstrates a clear utility of DOSY in analysis of mixtures of isomers, wherein the number of components may be difficult to determine.Diffusion of all compounds is influenced by the viscosity of the three solvents with methanol-d_4 producing, on average, the medium *D* values. Surprisingly, the fastest diffusing analyte **8** displays in methanol-d_4 a diffusion coefficient that is 48% larger than the slowest diffusing analyte **5**. In the first approximation, the diffusion coefficients of two similar molecules are expected to be inversely proportional to the cube roots of their MWs[36]:

$$\frac{D_1}{D_2} = \sqrt[3]{\frac{MW_2}{MW_1}} \qquad (2)$$

If Eqn (2) held, then one would expect a threefold difference in MW.[37] In reality, analytes **5** and **8** differ in MW by one unit only,

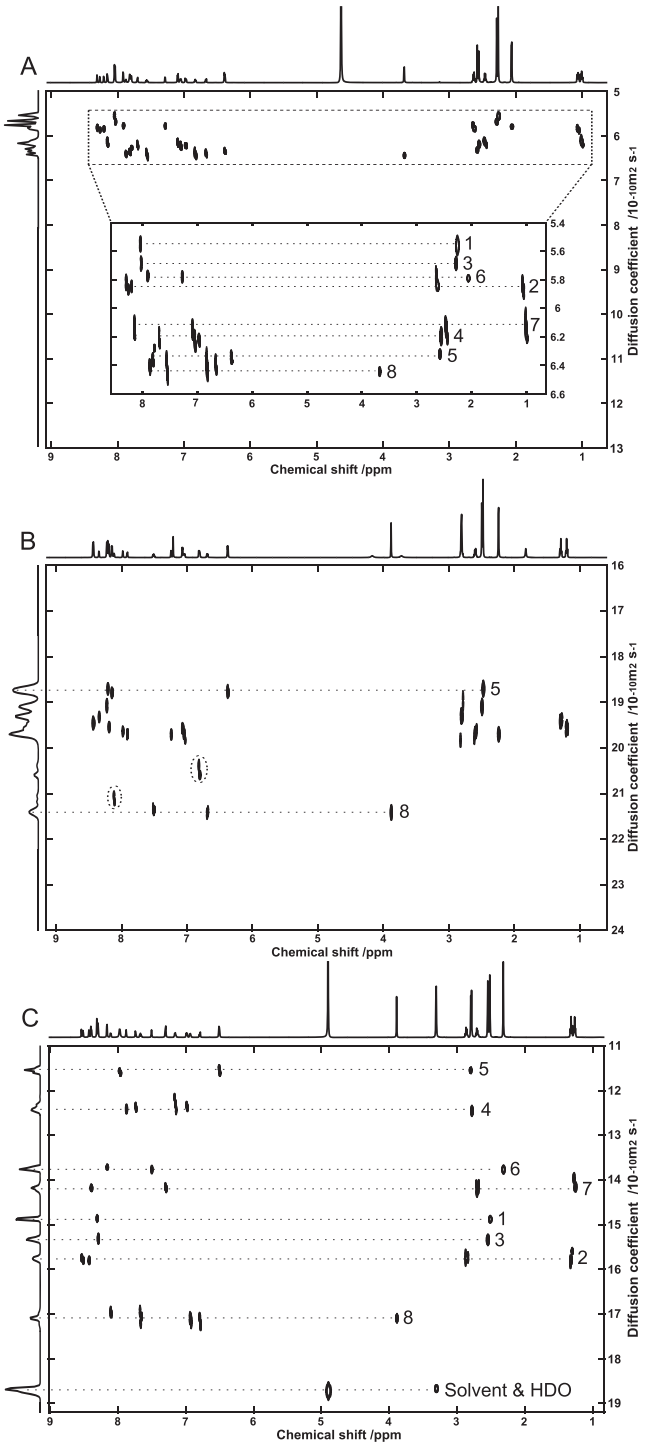

Figure 2. DOSY plots of mixtures of compounds **1**–**8** in (A) D_2O, (B) chloroform-d and (C) methanol-d_4. Concentration of all analytes 10 mM. Diffusion coefficients of solvents fall outside of the displayed region in (A) and (B). Experiments were run at 20 °C with the Oneshot45 pulse sequence,[19] corrected for gradient nonuniformity[20] and fitted to monoexponential decay with the DOSY Toolbox MATLAB script.[21] Further experimental details are available in the supporting information.

and surprisingly, it is the 'heavier' compound that has the larger diffusion coefficient.

We speculate that the underlying reasons for this observation are twofold. Firstly, Eqn (1) is reliable only when the solutes are at least five times the radius of solvent molecules,[37] and deviations are to

be expected when this condition is not met.[38] Secondly, in a coordinating solvent such as methanol, different analytes may have different solvation spheres. Although Eqn (1) is strictly correct only for spherical particles, Eqns (1) and (2) should still hold qualitatively if the analytes are of similar shape.[39] In this case, all analytes are nitrogenous heteroaromatics, but their alkyl substitution patterns and hence shapes are sufficiently different to have an influence on hydrogen bonding and solvation.[40]

We suggest that the diffusion coefficients in Fig. 2C are primarily governed by a combination of electronic and steric factors that influence the size of hydrogen bonding aggregates with the solvent. When the same sample was diluted tenfold, the observed diffusion coefficients did not change, demonstrating that interanalyte influences do not contribute noticeably (Fig. S4). This suggests that, when analytes and the solvent do not differ sufficiently in size, one should be very careful when interpreting diffusion data in alcohol solvents and when trying to correlate diffusion coefficients to molecular properties.

Conclusions

We have demonstrated herein how the interplay of the physical–chemical properties of analytes, hydrogen bonding and diffusion can be used to one's advantage. Clearly, the ability to enhance diffusion resolution by alcohol solvents extends beyond dihydroxybenzenes and is a much more general phenomenon that can also accommodate heteroaromatic compounds that are valuable in the chemical industry. While in most DOSY applications size similarity of solvent and analytes has been considered as something to avoid, we have demonstrated herein how the solvation effects can be used to one's advantage. Although solvent mixtures may allow to tune resolution,[31] excellent results were obtained by using methanol-d_4, a common deuterated solvent. One may think of this solvent as a resolution modifier in the diffusion dimension, just as benzene is used for a similar purpose in the spectral dimension. The clean spectral and diffusion resolution of eight compounds within a MW range of three units demonstrates the potential of DOSY in chemical analysis, achieving component discrimination of a mixture that would be challenging for any analytical technique.

Experimental

Details on NMR experiments, chemicals, materials and sample preparation are available in the supporting information.

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Supporting information

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