Research Article

Magnetic Resonance in Chemistry

Received: 1 April 2010 Revised: 23 April 2010 Accepted: 27 April 2010 Published online in Wiley Interscience:

(www.interscience.com) DOI 10.1002/mrc.2621

Matrix-assisted diffusion-ordered spectroscopy: mixture resolution by NMR using SDS micelles

Claudio F. Tormena,[a,b] Robert Evans,[a] Stephan Haiber,[c] Mathias Nilsson[a] and Gareth A. Morris[a]*

Diffusion-ordered spectroscopy (DOSY) is a powerful technique for mixture analysis, but in its basic form it cannot separate the component spectra for species with very similar diffusion coefficients. It has been recently demonstrated that the component spectra of a mixture of isomers with nearly identical diffusion coefficients (the three dihydroxybenzenes) can be resolved using matrix-assisted DOSY (MAD), in which diffusion is perturbed by the addition of a co-solute such as a surfactant [R. Evans, S. Haiber, M. Nilsson, G. A. Morris, *Anal. Chem.* 2009, *81*, 4548–4550]. However, little is known about the conditions required for such a separation, for example, the concentrations and concentration ratios of surfactant and solutes. The aim of this study was to explore the concentration range over which matrix-assisted DOSY using the surfactant SDS can achieve diffusion resolution of a simple model set of isomers, the monomethoxyphenols. The results show that the separation is remarkably robust with respect to both the concentrations and the concentration ratios of surfactant and solutes, supporting the idea that MAD may become a valuable tool for mixture analysis. **Copyright © 2010 John Wiley & Sons, Ltd.**

Keywords: NMR; ^{1}H; DOSY; surfactant; micelle

Introduction

NMR spectroscopy is a powerful tool for the assignment of molecular structure, conformation, and relative and/or absolute configuration for pure substances in solution,[1,2] but for mixtures it is often difficult or even impossible to assign resonances to an individual species. Diffusion-ordered spectroscopy (DOSY)[3–5] allows NMR signals of different species to be distinguished by virtue of their different diffusion behaviour. DOSY techniques are typically applied to mixtures containing species of different sizes (i.e. hydrodynamic radii) and hence different diffusion coefficients. The analysis of mixtures of species of similar size (e.g. isomers) by conventional DOSY is difficult and often impossible.[6] However, it has been shown[7–14] that diffusion behaviour in DOSY experiments can be manipulated by adding a co-solute or co-solvent. In principle, the use of micellar surfactants (and/or other co-solvents) in DOSY could afford the NMR spectroscopist the same degree of freedom to separate signals as is enjoyed in the use of liquid chromatography to separate species. Thus, for example, surfactants can be used as co-solutes for the systematic manipulation of diffusion resolution, changing the criteria by which molecules are differentiated in a DOSY experiment, but such methods have attracted surprisingly little attention.[7,15,16]

It was shown recently[17] that micelles, both normal and reverse, can be used as separation agents to distinguish between the isomers of dihydroxybenzene, catechol, resorcinol and hydroquinone, in DOSY experiments, and attention was drawn to the surprisingly large range of conditions over which useful separation was seen. Here a systematic investigation is reported of the range of concentrations and concentration ratios over which diffusion resolution is obtained with SDS co-solute for another simple model system, the monomethoxyphenols (Fig. 1). While the three isomers have, as expected, very similar diffusion coefficients in free aqueous solution (Fig. 1a), the spectra of the *ortho, meta* and *para* isomers are resolved in DOSY experiments when SDS is added (Fig. 1b).

Experimental

All measurements were carried out non-spinning on a 400 MHz Varian INOVA spectrometer, using a 5 mm indirect detection probe, equipped with a *z*-gradient coil producing a nominal maximum gradient of 30 G cm^{-1}. DOSY data were acquired using the Oneshot pulse sequence[18] with a total diffusion encoding pulse duration δ of 5 ms, a diffusion delay Δ of 60 ms and ten nominal gradient amplitudes ranging from 3.0 to 27.3 G cm^{-1}, chosen to give equal steps in gradient squared; each FID was acquired using 32 *k* data points. The experiments were carried out at a nominal probe temperature of 22 °C, with standard VT regulation.

DOSY spectra were constructed in the DOSY Toolbox[19] by standard methods,[3,4] using fitting to a modified Stejskal–Tanner

* *Correspondence to: Gareth A. Morris, School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom. E-mail: g.a.morris@manchester.ac.uk*

a *School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom*

b *Chemistry Institute, University of Campinas, Campinas, Sao Paulo, CP 6154, CEP 13094-971, Brazil*

c *Givaudan, Dept Analyt Res, Huizerstr 28, NL-1411 GP Naarden, The Netherlands*

 Copyright © 2010 John Wiley & Sons, Ltd.

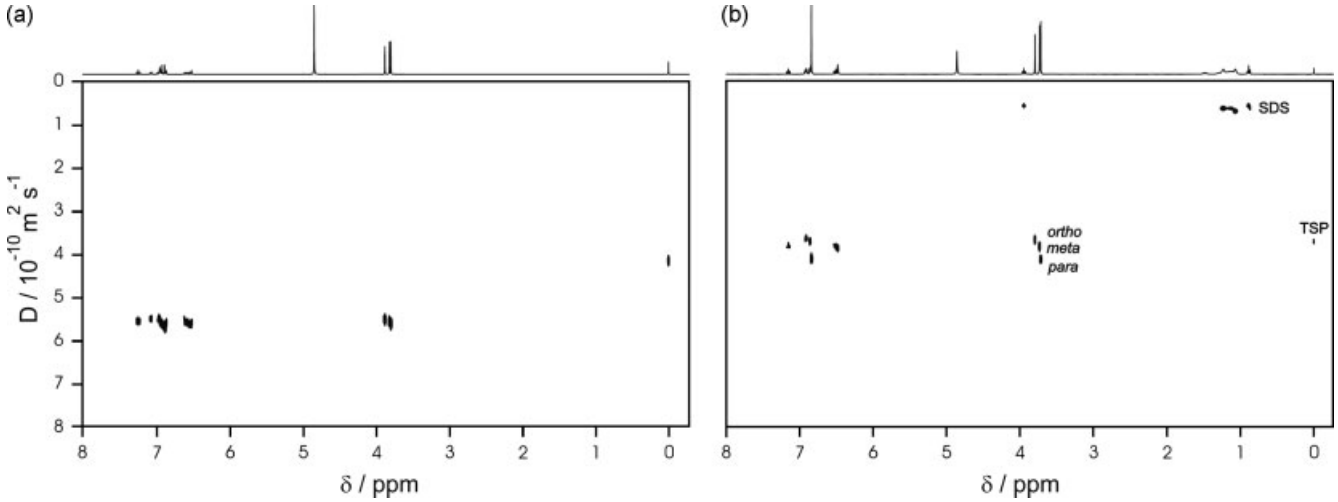

Figure 1. ^{1}H Oneshot[18] DOSY (400 MHz) spectra (with the least attenuated 1D spectra [top]): (a) sample containing 12 mM of each of the methoxyphenol isomers in D_2O, with TSP as reference; (b) sample containing 48 mM of each of the methoxyphenol isomers and 36 mM SDS in D_2O, with TSP as reference. The signals for the 1,2-, 1,3- and 1,4-methoxyphenol isomers are labelled *ortho, meta* and *para*, respectively.

equation parameterised to take into account the effects of pulsed field gradient non-uniformity.[4,20] Reference deconvolution[21] was used to correct for instrument inconsistencies,[22,23] with Gaussian target lineshapes chosen to optimise the resolution of signals.

All chemicals used in this study are commercially available and were used without further purification. A stock solution for methoxyphenol isomers and SDS was prepared in D_2O and diluted as necessary to obtain the concentrations used in this study. Sodium 3-(trimethylsilyl)-1-propanesulfonate (TSP) was used as chemical shift reference at 3.2 mM in each sample.

Results

Simple spectra with sharp lines for all species were seen for all the samples studied, confirming that both solutes and SDS species remain throughout in fast exchange between free and micellar solution on the chemical shift timescale. The effect of SDS concentration on the diffusion of the methoxyphenol isomers was investigated using six samples containing 6.8 mM of each of the three isomers, varying the concentration of SDS from 38 to 230 mM. All six samples were above the critical micelle concentration (CMC) of SDS in D_2O of 7 mM.[24] The apparent diffusion coefficients for methoxyphenol isomers are plotted as a function of SDS concentration in Fig. 2.

As can be seen from Fig. 2, increasing the SDS concentration leads to a significant decrease in diffusion coefficient for all the species present; the diffusion coefficients of the three methoxyphenol isomers remain in a similar ratio over the full range of SDS concentrations used. The decrease in diffusion coefficient for TSP is much less than that for the methoxyphenols, confirming that the decrease in diffusion coefficients for these solutes is due to association with SDS micelles rather than viscosity or obstruction effects. The strongest association (i.e. the lowest diffusion coefficient) is seen for *o*-methoxyphenol, while the weakest is observed for *p*-methoxyphenol; this behaviour is analogous to that seen for the dihydroxybenzene isomers in aqueous SDS.[17] The degree of resolution of the isomer diffusion coefficients, and hence their component spectra in a DOSY

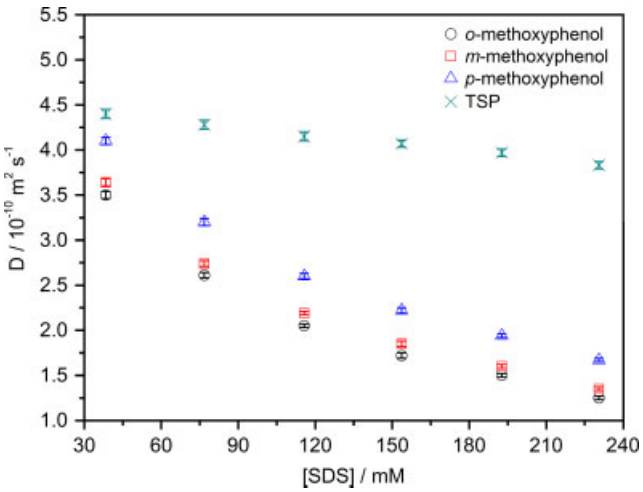

Figure 2. Diffusion coefficients as a function of SDS concentration for a D_2O solution containing 3.2 mM TSP and 6.8 mM of each of the methoxyphenol isomers. Error bars show ±2× estimated standard error.

experiment, is remarkably consistent across the range of SDS concentrations.

The effect of varying the solute–solvent concentration ratio at constant SDS concentration was investigated by measuring diffusion coefficients in a further six samples in which the concentrations of the three methoxyphenol isomers were increased from 12 to 60 mM, while keeping the concentration of SDS fixed well above the CMC at 36 mM (Fig. 3).

Again the separation ratio between the isomers was almost constant over the concentration range studied. However, while the diffusion coefficients of the methoxyphenol isomers increased slightly with concentration, the average diffusion coefficient of SDS was observed to decrease, showing that the presence of the methoxyphenol isomers has a significant effect on the SDS micellisation equilibrium. The addition of both polar and non-polar organic molecules is known to affect the CMC, aggregation number and micelle shape in SDS solutions,[25–27] so further experiments were conducted at low concentrations of SDS. Two series of

Copyright © 2010 John Wiley & Sons, Ltd.

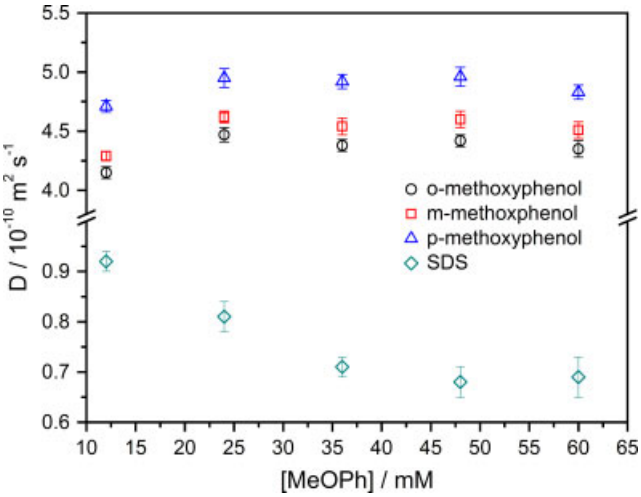

Figure 3. Diffusion coefficients as a function of methoxyphenol concentration for a 36 mM solution of SDS in D_2O containing 3.2 mM TSP and equal concentrations of the methoxyphenol isomers varying from 12 to 60 mM. Error bars show ±2× estimated standard error.

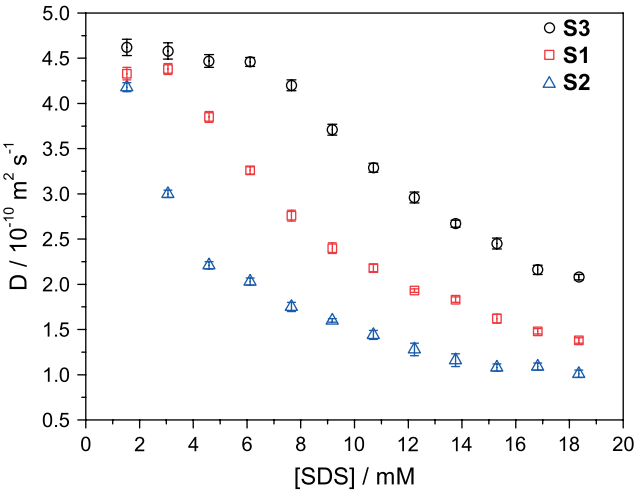

Figure 5. Variation of SDS diffusion coefficient with concentration for sample series **S1** (14 mM of each methoxyphenol), **S2** (35 mM of each methoxyphenol) and **S3** (no methoxyphenol). Error bars show ±2× estimated standard error.

samples (**S1** and **S2**), containing respectively 14 and 35 mM of each of the methoxyphenols, were used, while the concentration of SDS was varied from 1.5 to 18 mM. The diffusion coefficients of the methoxyphenol isomers are shown in Fig. 4.

Figure 4 shows that the differential effect of SDS on the diffusion of the methoxyphenol isomers persists well below the nominal SDS CMC of 7 mM; statistically significant differences in diffusion are seen at 8 mM in series **S1** (14 mM methoxyphenol) and at 4 mM in **S2** (35 mM methoxyphenol). This suggests strongly that the methoxyphenols act to encourage micellisation, lowering the SDS CMC to an extent that depends on methoxyphenol concentration. While the small variation of methoxyphenol diffusion coefficient with concentration in the absence of SDS shows little if any evidence of association, Figs 2–4 show both that SDS micelles can incorporate relatively high mole fractions of methoxyphenol and that methoxyphenols are quite effective at encouraging the formation of SDS micelles.

The effect of the methoxyphenols on the micellisation of SDS can be seen more clearly in Fig. 5, which compares the variation of SDS diffusion coefficient with SDS concentration for the sample series **S1** and **S2** and for a third series (**S3)** for SDS alone with no methoxyphenol.

The variation of diffusion coefficient with concentration in the absence of methoxyphenols, series **S3**, shows, as expected, a roughly constant diffusion coefficient until just below the literature CMC of 7 mM, followed by the expected decline as the proportion of free SDS decreases and that in micelles increases. A plot of diffusion coefficient against the inverse of concentration (not shown) takes the form of two straight lines intersecting at the CMC, as expected for a simple associative model of free SDS molecules in equilibrium with micelles of uniform size. Series **S2**, and to a lesser extent **S1**, shows significant deviations from this behaviour. In series **S1,** the apparent CMC is around 3 mM, and in **S2** it is below 2 mM, but the behaviour is a much less good match to the simple associative

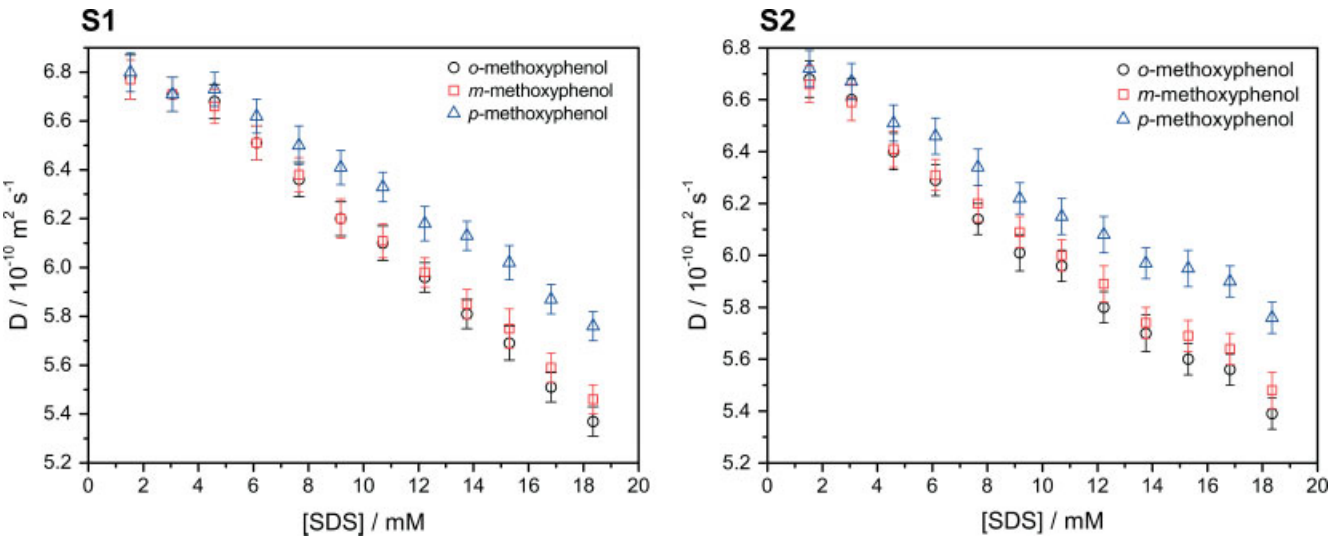

Figure 4. Diffusion coefficients for methoxyphenol isomers as a function of SDS concentration for solutions containing (**S1**) 14 mM of each isomer and (**S2**) 35 mM. Error bars show ±2× estimated standard error.

Copyright © 2010 John Wiley & Sons, Ltd.

model: clearly, the presence of the solutes enhances association. The remarkable wide range of solute and surfactant concentrations over which the presence of SDS allows DOSY to resolve the spectra of the methoxyphenol isomer is thus in part attributable to the methoxyphenols encouraging the formation of SDS micelles at concentrations below the nominal CMC.

Discussion

Micellar SDS solutions can be used in matrix-assisted DOSY to exploit the competition for solute between the free aqueous and the micellar environment to resolve the NMR spectra of mixtures of isomers, systems that are difficult or impossible to resolve by normal DOSY. Successful resolution of isomer spectra is seen over a very wide range of concentrations of solutes and of surfactant, even in some cases at surfactant concentrations below the normal CMC and in solutions where the surfactant concentration is significantly less than that of the solutes. The observation that the component signals in a mixture can be resolved even at low concentrations of SDS and with high ratios of solute to surfactant, due to the presence of a solute encouraging the formation of SDS micelles, is of immediate practical significance, as these conditions allow the amplitudes of interfering SDS signals and/or the cost of perdeuteriated SDS surfactant if used, to be minimised.[15] The results presented here provide further evidence for the versatility and robustness of matrix-assisted methods in mixture analysis by DOSY.

Acknowledgements

Support from the Engineering and Physical Sciences Research Council (grant references EP/E057888/1 and EP/E05899X), the Givaudan Strategic Research Fund, and the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for a fellowship Programa Novas Fronteiras (C.F.T. grant reference 09/02736-9) is gratefully acknowledged.

References

[1] J. H. Simpson, *Organic Structure Determination using 2D NMR Spectroscopy: A Problem-Based Approach*, Academic Press: Burlington, **2008**.
[2] G. Kummerlöwe, B. Luy, *Ann. Rep. NMR Spectrosc.* **2009**, *68*, 193.
[3] C. S. Johnson, *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 203.
[4] G. A. Morris, in *Encylopedia of Nuclear Magnetic Resonance, Advances in NMR*, vol. 2, Eds D. M. Grant, R. K. Harris, John Wiley & Sons Ltd: Chichester, **2002**, pp 35.
[5] Y. Cohen, L. Avram, L. Frish, *Angew. Chem., Int. Ed.* **2005**, *44*, 520.
[6] H. Barjat, G. A. Morris, S. Smart, A. G. Swanson, S. C. R. Williams, *J. Magn. Reson., Ser. B* **1995**, *108*, 170.
[7] K. F. Morris, P. Stilbs, C. S. Johnson, *Anal. Chem.* **1994**, *66*, 211.
[8] J. S. Gounarides, A. D. Chen, M. J. Shapiro, *J. Chromatogr. B* **1999**, *725*, 79.
[9] P. Hodge, P. Monvisade, G. A. Morris, I. Preece, *Chem. Commun.* **2001**, 239.
[10] B. A. Begotka, J. L. Hunsader, C. Oparaeche, J. K. Vincent, K. F. Morris, *Magn. Reson. Chem.* **2006**, *44*, 586.
[11] S. Viel, F. Ziarelli, S. Caldarelli, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9696.
[12] G. Pages, C. Delaurent, S. Caldarelli, *Angew. Chem. Int. Ed.* **2006**, *45*, 5950.
[13] J. S. Kavakka, I. Kilpeläinen, S. Heikkinen, *Org. Lett.* **2009**, *11*, 1349.
[14] K. F. Morris, B. A. Becker, B. C. Valle, I. M. Warner, C. K. Larive, *J. Phys. Chem. B* **2006**, *110*, 17359.
[15] M. E. Zielinski, K. F. Morris, *Magn. Reson. Chem.* **2009**, *47*, 53.
[16] S. A. Kingsbury, C. J. Ducommun, B. M. Zahakaylo, E. H. Dickinson, K. F. Morris, *Magn. Reson. Chem.* **2010**, *48*, 184.
[17] R. Evans, S. Haiber, M. Nilsson, G. A. Morris, *Anal. Chem.* **2009**, *81*, 4548.
[18] M. D. Pelta, G. A. Morris, M. J. Stchedroff, S. J. Hammond, *Magn. Reson. Chem.* **2002**, *40*, S147.
[19] M. Nilsson, *J. Magn. Reson.* **2009**, *200*, 296.
[20] P. Damberg, J. Jarvet, A. Gräslund, *J. Magn. Reson.* **2001**, *148*, 343.
[21] G. A. Morris, H. Barjat, T. J. Horne, *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, *31*, 197.
[22] M. Nilsson, M. A. Connell, A. L. Davis, G. A. Morris, *Anal. Chem.* **2006**, *78*, 3040.
[23] M. Nilsson, G. A. Morris, *Magn. Reson. Chem.* **2006**, *44*, 655.
[24] S. Lapenna, A. R. Bilia, G. A. Morris, M. Nilsson, *J. Pharm. Sci.* **2009**, *98*, 3666.
[25] P. Lianos, J. Lang, C. Strazielle, R. Zana, *J. Phys. Chem.* **1982**, *86*, 1019.
[26] A. Malliaris, *Adv. Colloid Interface Sci.* **1987**, *27*, 153.
[27] P. A. Hassan, S. R. Raghavan, E. W. Kaler, *Langmuir* **2002**, *18*, 2543.

 Copyright © 2010 John Wiley & Sons, Ltd.