Research article

MRC

Received: 19 October 2016 Revised: 29 November 2016 Accepted: 30 November 2016 Published online in Wiley Online Library: 1 February 2017

(wileyonlinelibrary.com) DOI 10.1002/mrc.4558

Assessing 2D electrophoretic mobility spectroscopy (2D MOSY) for analytical applications

Yuan Fang,[a] Pavel V. Yushmanov[b] and István Furó[a]*

Electrophoretic displacement of charged entity phase modulates the spectrum acquired in electrophoretic NMR experiments, and this modulation can be presented via 2D FT as 2D mobility spectroscopy (MOSY) spectra. We compare in various mixed solutions the chemical selectivity provided by 2D MOSY spectra with that provided by 2D diffusion-ordered spectroscopy (DOSY) spectra and demonstrate, under the conditions explored, a superior performance of the former method. 2D MOSY compares also favourably with closely related LC-NMR methods. The shape of 2D MOSY spectra in complex mixtures is strongly modulated by the pH of the sample, a feature that has potential for areas such as in drug discovery and metabolomics. Copyright © 2016 The Authors. *Magnetic Resonance in Chemistry* published by John Wiley & Sons Ltd.

Keywords: NMR; ^{1}H; electrophoretic NMR; 2D DOSY NMR; electrophoretic mobility; self-diffusion; ion; acid; base; LC-NMR

Introduction

Nuclear magnetic resonance spectroscopy possesses unparalleled chemical selectivity that is often exploited for investigating complex molecular mixtures both in conventional areas of chemical analysis[1,2] and in newer fields like metabolomics and drug discovery.[3–5] Yet, spectral resolution endowed to the NMR spectra by the chemical shift and the *J*-coupling turns frequently out as insufficient. If that happens, one can gain additional selectivity by adding another spectral dimension, usually at the cost of longer experimental time. Most often, one performs two-dimensional (2D) experiments that yield, via 2D Fourier transformation (FT), 2D spectra like COSY or HSQC. In such spectra, peak separation is increased by having stretched spectral intensity from a line to a plane on a manner that is most often via *J*-couplings involved, selective to the particular site in a given molecule.

Another option is to modulate spectral peaks by some spectral parameter that differs from molecule to molecule. One such property is the self-diffusion coefficient *D* manifesting itself in various versions of pulsed-field-gradient spin-echo-type (PGSE) experiments.[6] In analytical applications, the acquired extra chemical selectivity is often visualized by 2D DOSY spectra[7,8] where, along one dimension (1D), the spectral peaks are placed according to their corresponding self-diffusion coefficients. Producing such spectra involves not 2D FT along the diffusion/mobility axis but the method termed inverse Laplace transformation (ILT). Namely, the relation between a distribution $p(D)$ of D and the resulting diffusional decay in a PGSE experiment is LT. Yet, inverting LT is, in contrast to FT, an ill-posed mathematical problem with strong sensitivity to experimental noise.[9–13] Practical ILT procedures seek, often via least-square methods, a smooth $p(D)$ that is consistent with the experimental data. Hence, the common core of ILT methods is a fitting procedure whose results are not unique and known to be prone of artefacts.[9,13] Superficially, the outcome is similar to that yielded by 2D FT – a 2D spectrum with peaks whose position in 1D is defined by the chemical shift and in the other dimension by *D*. The advantage with this type of presentation – relative to that by, say, simply marking the peaks in a 1D spectrum with their respective *D* values – is better visualization, particularly for overlapping peaks belonging to molecules with different *D*.[1,2,8,14–17] Note that 2D DOSY is neither the sole nor the automatically preferred option for resolving this latter type of complications.[18–21]

Yet, another option that explores molecular mobility for increased selectivity is coupling NMR directly to analytical systems with flow separation such as in chromatography and capillary electrophoresis.[4,22–34] Such methods, to large part collected under the umbrella of LC-NMR, typically provide a time-dependent series of spectra where, from the point of view of NMR, the increase of resolution arises from limiting the molecular species that at any time contribute to the spectrum. Arranging spectra as time series provides a 2D representation.

In this paper, we investigate the performance of a lesser-known method, 2D mobility spectroscopy (MOSY),[35–41] as an alternative of and complement to, primarily, 2D DOSY and, to some extent, LC-NMR for analysing complex molecular mixtures. The method is based on electrophoretic NMR (eNMR).[42–44] In that technique, an electric field E is applied collinearly to a magnetic field gradient g in a PGSE-like experiment. Charged entities, that is, not only

* *Correspondence to: István Furó, Division of Applied Physical Chemistry, Department of Chemistry, KTH Royal Institute of Technology, Teknikringen 30, SE-10044 Stockholm, Sweden. E-mail: furo@kth.se*

a *Division of Applied Physical Chemistry, Department of Chemistry, KTH Royal Institute of Technology, Teknikringen 30, SE-10044, Stockholm, Sweden*

b *P&L Scientific Instrument Services, Box 1241, SE-18124, Lidingö, Sweden*

molecular ions but also neutral molecules with ions associated to them, attain in this field a steady velocity in proportion to their electrophoretic mobility μ and *E*. The resulting effect is, depending on the used probe geometry, either a cosinusoidal

$$S(E) \propto \cos\phi(E) \tag{1a}$$

or a complex

$$S(E) \propto e^{i\phi(E)} \tag{1b}$$

phase modulation with

$$\phi = \gamma g \delta \Delta_E \mu E \tag{1c}$$

where γ is the magnetogyric ratio, δ is the length of the applied gradient pulses, and Δ_E is the drift time set by the spacing of gradient pulses in the particular selected pulse sequence. As it has been recognized, this type of signal modulation lends itself well to 2D FT processing, and on that manner, one can prepare 2D MOSY spectra[35–41] with one dimension [that is, after suitable re-scaling as indicated by Eqn (1c)] along the electrophoretic mobility μ. This approach is inherently more sensitive than the alternative[45] where chemical selectivity was improved by signal cancellation at set phase values.

Although 2D MOSY has been indicated for applications as presented in the succeeding texts, there were no systematic attempts to demonstrate its performance for chemical analysis. As we are also going to touch upon the succeeding texts, this has in part instrumental reasons that had adversely influenced spectral resolution and sensitivity. In this short report, we show explicitly that with regard to distinguish among acidic, basic or otherwise charged chemical components in a mixture, 2D MOSY provides a performance that, at least in comparison with 2D DOSY, is superior. We also present a novel aspect that arises from the recognition that – because molecular charges and thereby electrophoretic mobilities are dependent on sample pH – 2D MOSY spectra are strongly pH dependent and therefore their resolution can often be optimized by performing the experiment at a suitable pH. While the pH sensitivity of the eNMR signal has been pointed out previously,[39] there has been no explicit suggestion to explore it in analytical applications.

Experimental

Materials

L-aspartic acid, L-serine and L-lysine were purchased from Sigma-Aldrich and used as obtained. An amino acid mixture (Sample 1) was prepared by mixing 3-mM aqueous (in D_2O) solutions of those individual amino acids with each other. This mixture was measured both as is (at approximately pD = 6.2) and at pD 3.0 (adjusted with 1 M DCl) where the pD values were obtained by the customary pD = pH + 0.4 correction of conventional experimental data for pH.

Powder made of tablets of Thomapyrin® Classic containing the active ingredients acetylsalicylic acid, acetaminophen and caffeine were added to D_2O at 12.1 mg/ml (equivalent to 5-mg/ml concentration of acetylsalicylic acid, 1-mg/ml concentration of caffeine and 4-mg/ml acetaminophen, as derived from nominal tablet composition). The drug solution (previously analysed with LC-NMR[24,46]) was then filtered through a nylon membrane of 0.2-μm pore size to dispose of a minor undissolved fraction and used as a stock solution, containing acetaminophen and caffeine at the concentration presented in the preceding texts and acetylsalicylic acid at ~3.3 mg/ml (saturation concentration). The stock solution was subsequently diluted twofold by D_2O (Sample 2, containing ~1.65-mg/ml acetylsalicylic acid, 0.5-mg/ml caffeine and 2-mg/ml acetaminophen, all below their respective solubilities[47–49]) yielding pD = 2.9. At this pH, the MOSY spectrum provided poor chemical selectivity (see discussion in the succeeding texts), and for that reason, the pH was changed to a more basic value of pD = 9.0 by adding NaOD.

Methods

All ^{1}H-NMR measurements were performed on a Bruker Avance III 500 spectrometer equipped with a standard-bore magnet with a resonance frequency of 500 MHz. The instrumentation used for eNMR measurements (sample cell and filters) was essentially as reported elsewhere,[50] with improvements implemented since. Both 2D DOSY (Figs 1 and 3) and MOSY (Figs 2 and 3) NMR studies were carried out with a conventional 5-mm broadband inverse probe with z-gradient (maximum value of 50 G/cm). The gradient pulses were provided by a Bruker GREAT 60 gradient power supply, while the voltage pulses were delivered by an eNMR 1000 electrophoretic power supply (P&L Scientific Instrument Service, www.plscientific.se, also the source of current sample cell and filters). All experiments were performed at the average temperature (23 °C) attained by the sample as a result of heating (by ~3 °C) during the course of the eNMR experiments.

The eNMR experiments for 2D MOSY were performed by using a double-stimulated echo pulse sequence[51] at a fixed gradient pulse length of δ = 1 ms and a fixed gradient value *g* of either 35 or 45 G/cm. The electric field was incremented from 0 to 264 V/cm in 28 steps, set by incremented voltage and the nominal (calibrated by 10-mM tetramethylammonium bromide solution[50,52]) electrode–electrode distance of 3.60 cm. The drift time Δ_E was set to either 250 or 300 ms. The DOSY experiments were performed with a double-stimulated echo[53] pulse sequence. The gradient pulse length was set to δ = 2 ms, the diffusion time was set to Δ = 200 ms, and the gradient strength *g* was incremented from 2 to 43 G/cm in 32 equal steps.

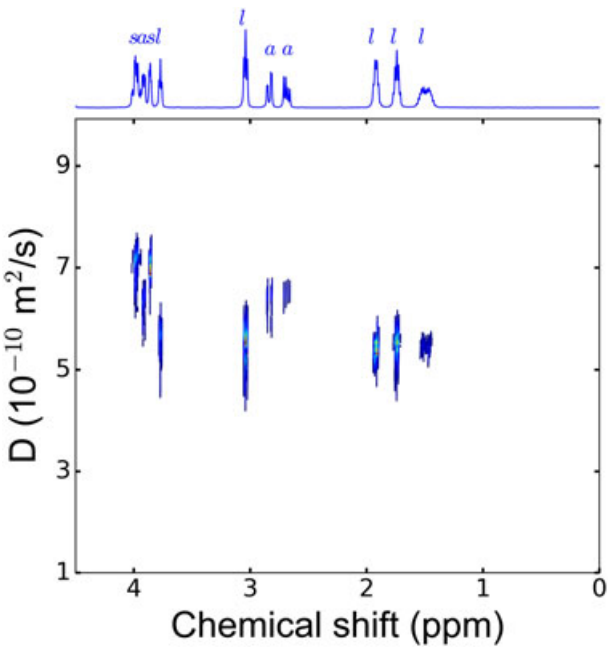

Figure 1. ^{1}H 2D DOSY spectrum of the mixed L-aspartic acid : L-serine : L-lysine (marked in the 1D spectrum by a, s and l respectively) solution (Sample 1) recorded at pD = 6.2 (at pD = 3.0, the spectrum looks very similar).

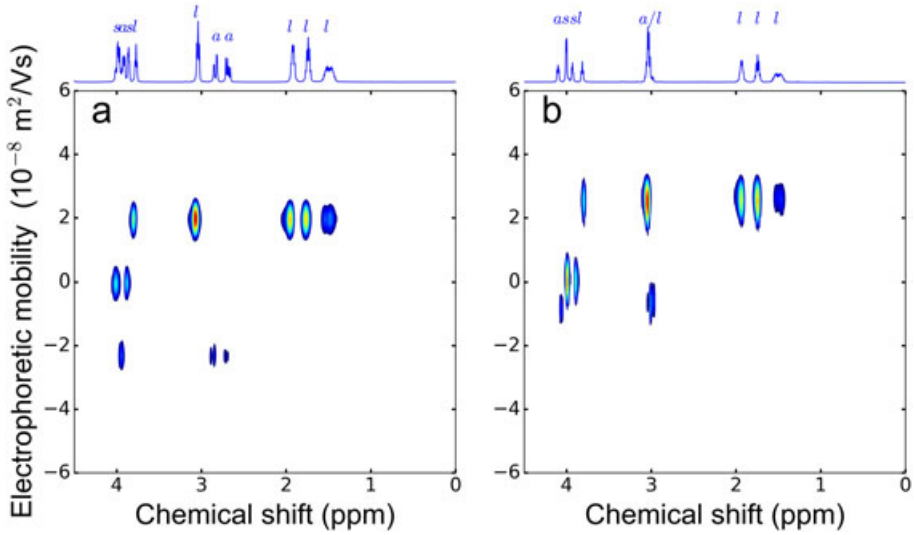

Figure 2. ^{1}H 2D MOSY spectrum of the mixed L-aspartic acid : L-serine : L-lysine solution (Sample 1, the respective peaks marked by a, s and l) recorded at (a) pD = 6.2 and at (b) pD = 3.0.

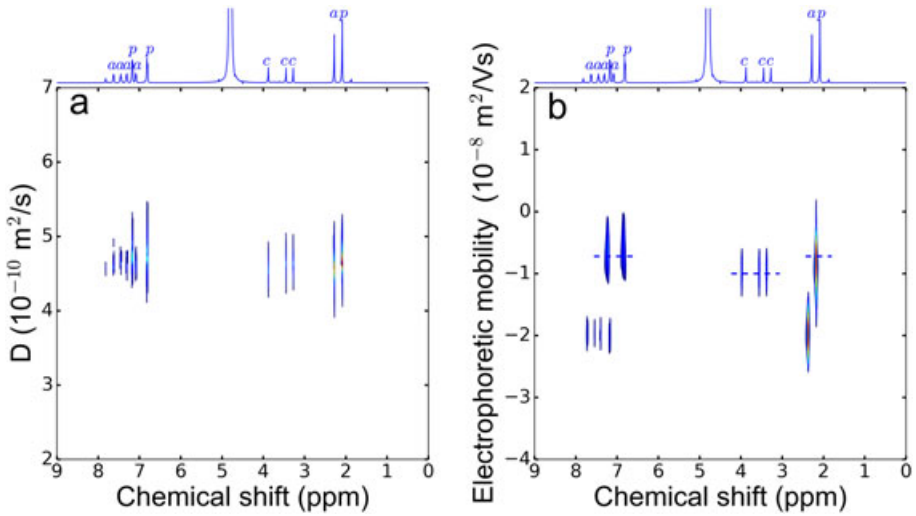

Figure 3. (a) ^{1}H 2D DOSY and (b) MOSY spectra of dissolved Thomapyrin® tablets (Sample 2) recorded at pD = 9.0. The peaks (marked in the 1D spectrum by a for acetylsalicylic acid, c for caffeine and p for acetaminophen) presented arise from the active ingredients; the effect of the dominant water peak has been suppressed by masking, in the 2D spectra, the spectral region of 4.2–5.2 ppm.

In eNMR experiments, the phases of the different peaks are typically measured relative to that of an uncharged reference molecule,[50] in our case, water. Hence, prior to the FT processing along the mobility dimension, the 1D spectra were phase corrected to provide non-dispersive line shape for the water peak. DOSY processing was performed by the commercial routine within the TOPSPIN software supplied by Bruker. The resulted DOSY spectrum was insensitive to the set number of components. The spike suppression factor was set to 1 and the noise sensitivity factor to 4. For 2D DOSY and MOSY, the number of points along the respective mobility dimensions was set to 128 or 256 (by zero filling) respectively.

Results

The samples selected are models that, despite their simplicity, demonstrate how 2D MOSY would perform in metabolomic (the amino acid mixture in Sample 1) and in more conventional analytical (the tablet components in Sample 2) applications.

Amino acid mixture

Because the self-diffusion coefficient *D* is inversely proportional to the hydrodynamic radius, the peaks from the three similarly sized amino acids are crowded along the diffusion axis in Fig. 1. Hence, 2D DOSY experiments increase chemical resolution only moderately. In contrast, the 2D MOSY spectra in Fig. 2 provide a clear increase of spectral resolution. The charge of the various amino acids depends on the p*K*a values of the different moieties[54] that we present in Table 1. When comparing Figs 2a and 2b, one should be aware of the significant sensitivity of some of the aspartic acid and lysine chemical shift to pH.

Based on the data in Table 1, one expects that, at pD = 6.2 (Fig. 2a), aspartic acid should be negatively charged, serine should be approximately neutral, while lysine should be positively charged.

Table 1. p*K*a values of the various moieties of amino acids in water and their resulting isoelectric points pI

—	αCOOH	NH_3	Side chain	pI
L-aspartic acid	1.88	9.6	3.65	2.77
L-lysine	2.2	9.0	12.5	9.74
L-serine	2.1	9.2	—	5.68

Indeed, the corresponding average peak positions on the mobility axis are -2.3×10^{-8} m^2/Vs, -0.04×10^{-8} m^2/Vs and 2.0×10^{-8} m^2/Vs respectively. As is shown by Fig. 2b, the chemical selectivity is poorer at pD = 3.0, because there, the aspartic acid is close to neutral.

Active ingredients in a tablet

Prescription drugs are often formulated to contain more than one active ingredient. Hence, they can be subject to evaluation by analytical methods. Recently, the performance of a capillary flow electrochromatography system with NMR detection[24,46] has been tested by a prescription drug containing acetaminophen, caffeine and acetylsalicylic acid (and the same drug was also used to test the performance of more classical analytical setups[55]). We chose the same mixture to test the performance of 2D MOSY in a realistic scenario.

Just like in the previous case, the DOSY spectrum in Fig. 3a is not well resolved because the diffusion coefficients involved are rather similar. It remains certainly unclear that three different molecules constitute the mixed solution. The 2D MOSY spectrum recorded at pD = 2.9 (not shown) presents a similarly low resolution along the mobility dimension. On the other hand, three separate peak manifolds arise in the MOSY spectrum in Fig. 3b recorded at pD = 9.0. The performance of MOSY is quite similar to that of NMR-detected capillary flow electrochromatography[24,46]: The peaks arising from acetylsalicylic acid are well resolved, while the separation along the respective mobility dimensions (arrival time or electrophoretic mobility) between the peaks from the other two compounds is smaller, yet well detectable (see dashed lines in Fig. 3b marking the average peak positions -1.0×10^{-8} m^2/Vs for caffeine and -0.7×10^{-8} m^2/Vs for acetaminophen).

Performance, limiting factors and possible improvements

The spectra presented in the preceding texts are improved in several respects relative to 2D MOSY spectra obtained previously. In arrangements that provided cosine modulation ([Eqn (1a)]) of the eNMR signal,[35–37,56] the 2D spectra lose sign sensitivity in the 'mobility' direction. This increases spectral overlap (consider, for example, the aspartic acid and lysine manifolds in Fig. 2a). If we compare performance with that in previous arrangements where the sign was preserved after having detected the complex modulation of the signal,[39–41] the sample holder used here[50] has a far larger filling factor and thereby a far larger signal-to-noise ratio. This leads to improved detection threshold. In addition, we obtain the eNMR signal in a double-stimulated echo experiment[51] where the sign of the electric field is alternated, and thereby, one avoids electrode polarization.

Comparing the performance of 2D MOSY experiments with that of 2D DOSY is rather straightforward: 2D MOSY is inherently superior in those cases when the molecules involved are charged. In addition, charge can often be strongly modulated by pH, while there exist no straightforward option for doing so with the hydrodynamic radius on which the self-diffusion coefficient depends on. The advantage for 2D MOSY comes at the cost of extra instrumentation (sample holders, filters and power supply for voltage pulses[50]). Yet, this extra instrumentation is a lot less extensive (and significantly less expensive) than that used by comparable techniques collected under the umbrella of LC-NMR.[28–30] In particular, there is no inherent need for dedicated probes because there is no flow through the sample space but rather a stagnant sample column within which the ions move alternately up and down. The gradient strength in conventional probes is typically sufficient. Sample handling[50] is also far simpler, with the sample in a conventional NMR tube into which the electrodes are inserted from the top.

Regarding limitations, the 2D MOSY experiment requires electric field pulses applied to the sample that requires electric wiring from the sample space to a power supply. This has two effects (several other deleterious effects such as noise pickup have already been dealt with[50]). First, metallic wires with their characteristic magnetic susceptibility within the sample volume have a negative influence on the field homogeneity. Second, sample spinning is not permitted. Both effects decrease spectral resolution along the chemical shift dimension. There are ways to counteract this. First, one can arrange the wires around the tube in a symmetric fashion that should minimize field inhomogeneity. Second, one may use sliding galvanic contacts that would permit sample spinning. If custom-made components, such as a custom-made sample tube with one electrode led through the tube bottom, are tolerable, then one can skip wiring through the sample, the arrangement used in this study,[50] and move the wires out and away of the sample tube.

Regarding resolution along the electrophoretic mobility direction, signal loss caused by thermal convection caused in turn by Joule heating is the main limiting factor. The loss is increasing by increasing electric field strength, and this effect dominates the line width after FT along the mobility dimension. Thermal convection can be effectively reduced by decreasing the cell diameter[57]; indeed, capillaries have been proposed and used to counteract thermal convection in eNMR.[58] Heating effects are also alleviated if the subsequent scans are recorded with the electric field incremented (that is, instead of continually recording all signals at a given electric field value). In addition, heating can be reduced by a judicious choice of the applied voltage in relation to the selected gradient strength. To keep sample heating low with preserved phase modulation, one must reduce E (heating is $\propto E^2$) and Δ_E (on which heating depends linearly) while increasing by an inverse factor the $g\delta$ product in Eqn (1c). The problem is that this leads to an increased diffusional decay $\propto \exp[-\gamma^2 g^2 \delta^2 D(\Delta_E - \delta/3)]$ of the signal. Hence, both solutions (reducing tube diameter without having the rf coil adjusted to it and choosing suitable parameters) may reduce signal strength. Yet, just like in LC-NMR, one can use microcoils[29,30] to reduce the loss of signal strength, while another option is to use conventional probes with conventional tubes containing capillary bundles.[58,59] In general, sample heating and the presence of diffusion as a limiting factor are a concern shared with related LC-NMR experiments.[29,30]

Conclusions

We present here a direct comparison of 2D MOSY and 2D DOSY experiments in simple mixtures of either amino acids or drug components. As the spectra in the preceding texts clearly illustrate, in the selected mixtures with charged molecular components, the

performance of the 2D MOSY experiment is superior. One goal with 2D NMR spectra of any form is to extend the resolving power of NMR spectroscopy. This is achieved if different molecules exhibit different unique 2D spectral patterns. As we demonstrate, this particular aspect – to produce unique patterns for different molecules in 2D MOSY – is aided by being able to change the sample pH. Related LC-NMR methods provide results that are qualitatively similar. Yet, instrumental simplicity such as the use of conventional probes and the ease of sample handling such as conventional NMR tubes for the samples should promote the application of 2D MOSY in a range of applications.

Acknowledgements

This work has been supported by the Swedish Research Council VR. Y.F. acknowledges the China Scholarship Council (CSC) for the doctoral fellowship support.

References

[1] R. Cao, A. Nonaka, F. Komura, T. Matsui. *Food Chem.* **2015**, *171*, 8–12.
[2] L. A. Cardoza, A. K. Korir, W. H. Otto, C. J. Wurrey, C. K. Larive. *Prog. Nucl. Magn. Reson. Spectrosc.* **2004**, *45*, 209–238.
[3] M. E. Bollard, E. G. Stanley, J. C. Lindon, J. K. Nicholson, E. Holmes. *NMR Biomed.* **2005**, *18*, 143–162.
[4] O. Corcoran, M. Spraul. *Drug Discov. Today* **2003**, *8*, 624–631.
[5] J. Griffin. *Current Opin. Chem. Biol.* **2003**, *7*, 648–654.
[6] E. O. Stejskal, J. E. Tanner. *J. Chem. Phys.* **1965**, *42*, 288–292.
[7] C. S. Johnson. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 203–256.
[8] Y. Cohen, L. Avram, L. Frish. *Angew. Chem. Int. Ed.* **2005**, *44*, 520–554.
[9] M. Prato. *Arch. Comput. Meth. Engin.* **2009**, *16*, 109–160.
[10] A. Al-Shuaibi. *Integral Transforms and Special Functions* **2001**, *11*, 225–232.
[11] R. E. Bellman, R. E. Kalaba, J. Lockett, *Numerical Inversion of the Laplace Transform*, Elsevier, New York, **1966**.
[12] B. Davies, B. Martin. *J. Comput. Phys.* **1979**, *33*, 1–32.
[13] K. L. Kuhlman. *Numerical Algorithms* **2013**, *63*, 339–355.
[14] S. Glanzer, K. Zangger. *Chemistry* **2014**, *20*, 11171–11175.
[15] A. A. Colbourne, S. Meier, G. A. Morris, M. Nilsson. *Chem. Commun.* **2013**, *49*, 10510–10512.
[16] A. K. Rogerson, J. A. Aguilar, M. Nilsson, G. A. Morris. *Chem. Commun.* **2011**, *47*, 7063–7064.
[17] J. C. Cobas, P. Groves, M. Martin-Pastor, A. De Capua. *Curr. Anal. Chem.* **2005**, *1*, 289–305.
[18] B. Antalek. *Concepts Magn. Reson.* **2002**, *14*, 225–258.
[19] C. Barrere, P. Thureau, A. Thevand, S. Viel. *J. Magn. Reson.* **2012**, *216*, 201–208.
[20] P. Stilbs. *Eur. Biophys. J. Biophys. Lett.* **2013**, *42*, 25–32.
[21] P. Stilbs, K. Paulsen, P. C. Griffiths. *J. Phys. Chem.* **1996**, *100*, 8180–8189.
[22] S. L. Eldridge, A. K. Korir, C. E. Merrywell, C. K. Larive, in *Advances in Chromatography* (Eds: E. Grushka, N. Grinberg), CRC Press, Boca Raton, **2008**, pp. 351–390.
[23] P. Gfrorer, J. Schewitz, K. Pusecker, E. Bayer. *Anal. Chem.* **1999**, *71*, 315A–321A.
[24] P. Gfrorer, J. Schewitz, K. Pusecker, L. H. Tseng, K. Albert, E. Bayer. *Electrophoresis* **1999**, *20*, 3–8.
[25] P. A. Keifer, in *Annual Reports on NMR Spectroscopy* (Ed: G. A. Webb), Elsevier, Amsterdam, **2007**, pp. 1–47.
[26] A. M. Wolters, D. A. Jayawickrama, J. V. Sweedler. *J. Nat. Prod.* **2005**, *68*, 162–167.
[27] A. M. Wolters, D. A. Jayawickrama, A. G. Webb, J. V. Sweedler. *Anal. Chem.* **2002**, *74*, 5550–5555.
[28] Y. F. Huang, C. C. Huang, C. C. Hu, H. T. Chang. *Electrophoresis* **2006**, *27*, 3503–3522.
[29] A. G. Webb. *Magn. Reson. Chem.* **2005**, *43*, 688–696.
[30] D. A. Jayawickrama, J. V. Sweedler. *J. Chromatogr. A* **2003**, *1000*, 819–840.
[31] D. L. Olson, M. E. Lacey, A. G. Webb, J. V. Sweedler. *Anal. Chem.* **1999**, *71*, 3070–3076.
[32] K. Pusecker, J. Schewitz, P. Gfrorer, L.-H. Tseng, K. Albert, E. Bayer, I. D. Wilson, N. J. Bailey, G. B. Scarfe, J. K. Nicholson, J. C. Lindon. *Anal. Commun.* **1998**, *35*, 213–215.
[33] J. Schewitz, P. Gfrörer, K. Pusecker, L.-H. Tseng, K. Albert, E. Bayer, I. D. Wilson, N. J. Bailey, G. B. Scarfe, J. K. Nicholson. *Analyst* **1998**, *123*, 2835–2837.
[34] R. Subramanian, W. P. Kelley, P. D. Floyd, Z. J. Tan, A. G. Webb, J. V. Sweedler. *Anal. Chem.* **1999**, *71*, 5335–5339.
[35] Q. He, C. S. Johnson. *J. Magn. Reson.* **1989**, *81*, 435–439.
[36] Q. He, W. Lin, Y. Liu, E. Li. *J. Magn. Reson.* **2000**, *147*, 361–365.
[37] Q. He, Y. Liu, T. Nixon. *J. Am. Chem. Soc.* **1998**, *120*, 1341–1342.
[38] K. F. Morris, C. S. Johnson. *J. Magn. Reson. A* **1993**, *100*, 67–73.
[39] U. Böhme, U. Scheler. *Chem. Phys. Lett.* **2007**, *435*, 342–345.
[40] U. Böhme, C. Vogel, J. Meier-Haack, U. Scheler. *J. Phys. Chem. B* **2007**, *111*, 8344–8347.
[41] K. Huber, U. Scheler. *Current Opin. Colloid Interf. Sci.* **2012**, *17*, 64–73.
[42] P. C. Griffiths, A. Paul, N. Hirst. *Chem. Soc. Rev.* **2006**, *35*, 134–145.
[43] M. Holz. *Chem. Soc. Rev.* **1994**, *35*, 165–174.
[44] U. Scheler, in *Encyclopedia of Magnetic Resonance* (Eds: R. K. Harris, R. Wasylishen), Wiley, Chichester, **2012**. doi:10.1002/9780470034590.emrstm9780470030154.pub9780470034592
[45] S. R. Heil, M. Holz. *Angew. Chem. Int. Ed.* **1996**, *35*, 1717–1720.
[46] J. Schewitz, K. Pusecker, P. Gfrorer, U. Gotz, L. H. Tseng, K. Albert, E. Bayer. *Chromatographia* **1999**, *50*, 333–337.
[47] K. D. Rainsford, *Aspirin and Related Drugs*, CRC Press, Boca Raton, **2004**.
[48] R. A. Granberg, Å. C. Rasmuson. *J. Chem. Eng. Data* **1999**, *44*, 1391–1395.
[49] A. Cesaro, E. Russo, V. Crescenzi. *J. Phys. Chem.* **1976**, *80*, 335–339.
[50] F. Hallberg, I. Furó, P. V. Yushmanov, P. Stilbs. *J. Magn. Reson.* **2008**, *192*, 69–77.
[51] E. T. Pettersson, I. Furó, P. Stilbs. *Concepts Magn. Reson.* **2004**, *22A*, 61–68.
[52] M. Bielejewski, M. Giesecke, I. Furó. *J. Magn. Reson.* **2014**, *243*, 17–24.
[53] A. Jerschow, N. Müller. *J. Magn. Reson.* **1997**, *125*, 372–375.
[54] M. Fleck, A. M. Petrosyan, *Salts of Amino Acids*, Springer, Cham (Switzerland), **2014**.
[55] V. Pucci, R. Mandrioli, M. A. Raggi, S. Fanali. *Electrophoresis* **2004**, *25*, 615–621.
[56] Q. He, D. P. Hinton, C. S. Johnson. *J. Magn. Reson.* **1991**, *91*, 654–658.
[57] Lord Rayleigh. *Philos. Mag.* **1916**, *32*, 529–546.
[58] Q. H. He, Y. M. Liu, H. H. Sun, E. C. Li. *J. Magn. Reson.* **1999**, *141*, 355–359.
[59] T. Iwashita, T. Konuma, E. Harada, S. Mori, K. Sugase. *Magn. Reson. Chem.* **2016**, *54*, 729–733.