Journal of Magnetic Resonance 212 (2011) 161–168

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Glycerol and glycerol carbonate as ultraviscous solvents for mixture analysis by NMR

Pedro Lameiras [a], Leslie Boudesocque [a], Zéphirin Mouloungui [b], Jean-Hugues Renault [a], Jean-Michel Wieruszeski [c], Guy Lippens [c], Jean-Marc Nuzillard [a,*]

[a] Université de Reims Champagne-Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 6229, IFR 53 Biomolécules, BP 1039, 51687 Reims Cedex 02, France
[b] Université de Toulouse, INRA, INP, Laboratoire de Chimie Agro-Industrielle, ENSIACET, 4 Allée Emile Monso, 31030 Toulouse, France
[c] Université des Sciences et Technologies de Lille 1, Unité de Glycobiologie Structurale et Fonctionnelle, CNRS UMR 8576, 59655 Villeneuve d'Ascq Cedex, France

ARTICLE INFO

Article history:
Received 25 May 2011
Revised 21 June 2011
Available online 1 July 2011

Keywords:
Glycerol
Glycerol carbonate
Ultraviscous solvents
NOESY
Spin diffusion
Mixture analysis

ABSTRACT

NMR of weakly polar analytes in an apolar ultraviscous solvent has recently been proposed for mixture analysis as a pertinent alternative to the DOSY experiment. The present article reports the first use of glycerol and glycerol carbonate as polar solvents for the NMR analysis of a model mixture of dipeptides. This work demonstrates the high potentiality of these solvents for the analysis of mixtures made of polar and potentially bioactive compounds. Medium-sized molecules slowly reorient in glycerol and glycerol carbonate under particular temperature conditions, so that solute resonances may show spin diffusion in NOESY spectra, thus opening the way to mixture analysis. Glycerol and glycerol carbonate have turned out to be ultraviscous solvents of choice for the individualization of four structurally close mixed dipeptides: Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr by means of 1D and 2D NOESY experiments. Selective sample excitation and signal detection were implemented to eliminate the intense proton signals of the non-deuterated solvents. Moreover, the recording of a multiplet selective 2D NOESY–TOCSY has shown that the analytical power of NMR in highly viscous solvents is not limited to the extraction of mixture component 1D subspectra but may also yield some supplementary information about atom connectivity within components.

1. Introduction

NMR spectroscopy plays a leading role among the spectroscopic techniques that are devoted to structure elucidation of unknown low-molecular weight organic molecules. Chemical purity is generally considered as a necessary prerequisite in this context. Purification and NMR spectroscopy can be carried out at the same time if a special LC–NMR probe is available. Sensitivity problems are then solved by the use of very high magnetic fields, of ultra-cooled probe coils and by sample concentration on solid adsorbents [1,2]. In some instances, however, separation is not possible, and NMR must be performed on mixtures. Mixture analysis by NMR is most often restricted to known compound identification and quantification. Finding the structure of unknown compounds within mixtures is a demanding exercise because the NMR response set has to be split into subsets, each one being related to a single mixture component. The task becomes all the more challenging when accidental peak superimposition occurs. Being able to assign each peak to a specific compound would reduce the necessity of chromatographic separation and would therefore greatly enhance the efficiency of synthetic and natural product chemists.

The DOSY experiment is presently considered the best way to make this dream possible. NMR signals are sorted according to the value of the translational diffusion coefficient of each molecule constituting the mixture [3–7]. However, 2D DOSY spectra have a limited resolution in the diffusion domain and therefore NMR signals from structurally close molecules may be difficult to group according to their compound of origin. The efficiency of the DOSY experiment can be enhanced under particular conditions by addition of auxiliary ingredients to the sample, such as solid chromatographic phases [8–13] or aggregate forming molecules [14,15]. Molecular mobility is then modulated by specific interactions between the analytes and the sample additive, thus resulting in significantly different measured diffusion coefficients.

A recent publication by Simpson and coworkers proposed an alternative approach that is based on spin diffusion [16]. By dissolving the sample in a highly viscous medium, the chlorotrifluoroethylene (PCTFE) polymer, relaxation falls into the very slow molecular tumbling regime where spin diffusion becomes very efficient: a 2D ^{1}H–^{1}H NOESY spectrum with a long mixing time can then correlate together the chemical shifts of the ^{1}H nuclei that belong to the same molecule. All the rows (or columns) in the spec-

* Corresponding author. Fax: +33 3 26 91 31 66.
E-mail address: jm.nuzillard@univ-reims.fr (J.-M. Nuzillard).

1090-7807/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2011.06.021

trum that are identical thus contain the expected 1D spectrum of a single mixture component. This method was able to separate the spectra of strychnine and brucine, two structurally close alkaloids.

The PCTFE polymer, when mixed with perdeuterated organic solvents, has been previously used to modify the NOE responses of small or medium molecules [17–20]. This polymer does not impair the sample analysis as it is nearly chemically inert. As an aprotic solvent it can be used when dealing with lipophilic molecules, however, its low dielectric constant ($\varepsilon = 2.6$ at 25 °C) [21] does not favor the dissolution of polar compounds. This issue has been addressed in our study, and we wish to report the use of two small molecules, glycerol (GL) or glycerol carbonate (GC), used for the first time in the NMR analysis of a mixture of polar compounds.

Both GL and GC are viscous solvents with a high dielectric constant: $\varepsilon = 42.5$ [21] for GL and $\varepsilon = 109.7$ [22] for GC at 25 °C. Glycerol is a co-product of the industry scale manufacturing of biofuels, soaps and surfactants from triglycerides. Glycerol production currently exceeds demand. For this reason, new developments and new markets for glycerol are being actively explored. Glycerol has many applications such as being a hydrophilic component in neutral surfactants and an emulsifier in food, cosmetics and pharmaceuticals. In the domain of organic chemistry, one of the most attractive transformations of glycerol is the synthesis of glycerol carbonate [23,24]. This latter compound may play a central role in the near future for the industrial-scale production of solvents, lubricants and surfactants from renewable carbon sources. The existence of such ton-scale markets is not incompatible with the search for highly specific niche applications.

Medium-sized molecules slowly reorient in GL and GC under particular temperature conditions and therefore present a negative NOE regime. Spin diffusion can be observed in these conditions, so that all resonances of the protons within the same molecule correlate together in a 2D NOESY spectrum, thus opening the way to mixture analysis. In this context, this work focused on the assessment of GL and GC for the individual NMR characterization of four structurally close dipeptides: Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr within a single mixture. The major experimental pitfall of this approach was the mandatory elimination of the strong ^{1}H signals of GL and GC. Failing to correctly achieve this would have resulted in spectra in which solvent signals obscured solute signals. The first experiments that involved GL were carried out in perdeuterated GL. It is worth noting that this commercially available solvent is at most 99% D-enriched and is very expensive, about 100 Euros per gram. Therefore, 1D and 2D selective NOESY experiments that incorporate solvent suppression were implemented.

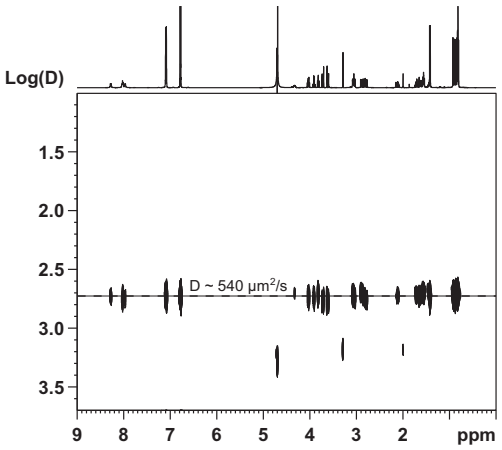

Fig. 1. ^{1}H DOSY spectrum of the dipeptide test mixture in water at 298 K. Data was acquired with the stebpgp1s19 pulse sequence. The diffusion time (Δ) was 50 ms and the gradient pulse length (δ) was 2 ms. The size of the raw data set was 32×8192. The gradient intensity values were equally spaced from 2% to 95%. Water suppression was achieved by a 3-9-19 pulse sequence with 1 ms gradient pulses of −20% intensity (WATERGATE). The DOSY spectrum was calculated using the Bruker TOPSPIN Software. Inverse Laplace transformation in the indirectly detected dimension was carried out by means of the MaxEnt algorithm. Log (D) was calculated with D expressed in $(\mu m)^2/s$.

2. Results and discussion

The self-diffusion coefficients of the dipeptides in the Leu–Val, Leu–Tyr, Gly–Tyr, and Ala–Tyr mixture were first measured in order to evaluate their ability to differentiate the mixture components [25]. The ^{1}H DOSY experiment was performed in water using WATERGATE solvent suppression (Fig. 1) [26]. The diffusion coefficients of the dipeptides are approximately identical ($D \sim 540\ \mu m^2/s$). The individual components of the mixture could not be clearly discriminated according to their translational

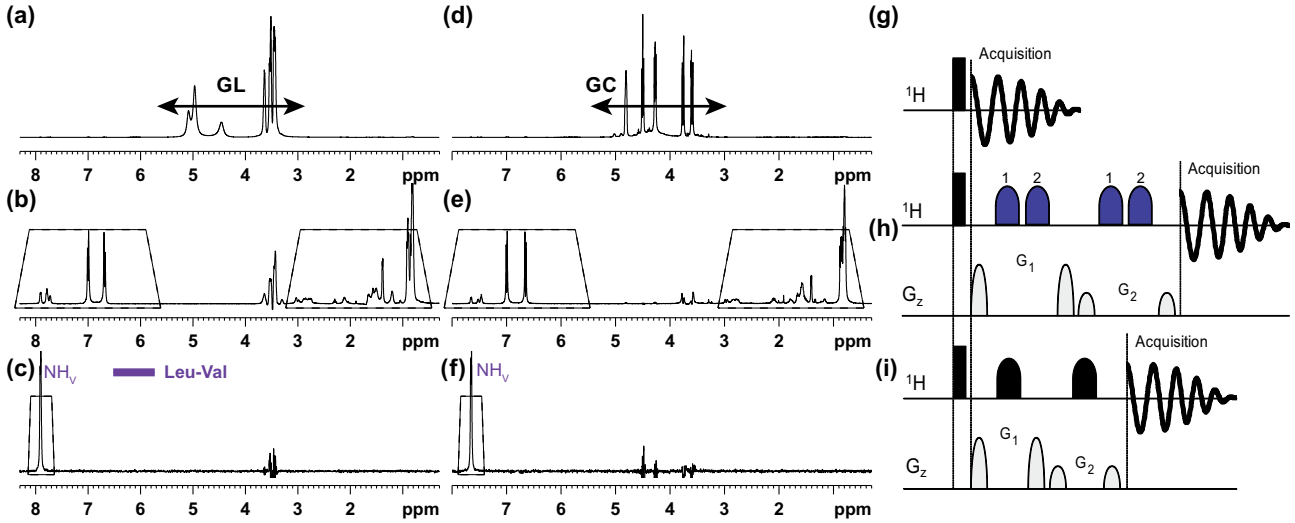

Fig. 2. 1D ^{1}H spectra (8 scans) and corresponding NMR pulse sequence of the dipeptide test mixture in GL (a, b, c, 318 K) and in GC (d, e, f, 288 K). G_1:G_2 = 70:30. The FIDs (32 k points, spectral width = 4500 Hz) were processed with LB = 0.3 Hz and zero-filled to 32 k points. (a, d, g) Non-selective excitation and detection. (b, e, h) Selective detection of two resonance bands. The 4 ms I-BURP-2 pulses cover 1250 Hz (dotted trapezium). The "1" and "2" labels respectively indicate their application to the high and low chemical shift regions. (c, f, i) Selective excitation of the valine amide proton doublet of Leu–Val (dotted trapezium) using a 20 ms, 1% truncated, 180° Gaussian pulse.