ViscY experiments in phosphoric acid as viscous solvent for the individualization of small molecules within mixtures by spin diffusion

François Pedinielli, Ritchy Leroy, Agathe Martinez, Jean-Marc Nuzillard* and Pedro Lameiras*[a]

[a]Université de Reims Champagne-Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 7312, IFR 53 Biomolécules, BP 1039, 51687 Reims Cedex 02

The problem of the analysis of small molecule mixtures has fostered in the recent years an active search for effective and practical solutions. Application fields include the study of metabolite mixtures and of chemical reaction media. The complexity of the mixtures is a determining factor for the success of mixture component characterization, which is often the main goal of such studies, in academic and industry contexts. Mixture analysis is often restricted to the identification and quantification of known compounds. Standard techniques for the structure determination of unknown compounds involve the hyphenation of physical separation and spectroscopic methods, such as high performance liquid chromatography (HPLC) coupled with NMR (LC-SPE-NMR) or/and mass spectrometry (LC-MS), approaches that are not devoid of limitations. Physical separation by analytical chromatography requires the determination of appropriate conditions by trial and errors, a process that has a cost in equipment, method development time, solvents, and columns. When physical separation does not provide a satisfactory solution to a given problem, NMR must be carried out on the mixtures. Being able to efficiently group the NMR peaks according to the compounds they originate from would reduce the need for chromatographic separation and would thus considerably facilitate the study of natural and synthetic product mixtures. The structure determination of compounds in mixtures by NMR **without performing any physical separation** remains of restricted use to date, even though dedicated methods have been reported in the literature.

In this context, the NMR **ViscY experiments** provided added value for the structural investigation of complex mixtures based on **the use of viscous solvents**. ViscY is a collective name for the NMR experiments that take benefit from spin diffusion in highly viscous solvents for the individualization of the NMR spectra of small molecule mixture components. According to the microviscosity theory of Gierer and Wirtz, the value of **the molecular overall correlation time τ_c** of a compound in solution depends on the medium viscosity. Consequently, when it increases sufficiently, the molecular tumbling of small and medium sized dissolved molecules is slowed so that magnetization exchange by longitudinal cross-relaxation becomes highly efficient and thus favours spin diffusion. As a result, the molecules undergo a **negative nuclear Overhauser effect (nOe) regime** and their resonances can be grouped according to their ability to share magnetization by intramolecular **spin diffusion**. The resonances of the ^{1}H nuclei within the same molecule tend to correlate together in a 2D NOESY spectrum, thus giving access to the **individual** ^{1}H NMR spectra of the mixture components. This topic has been extensively explored during the last four decades: the initial idea was implemented in 1981 [1] using CTFEP, a perfluorinated polymer solvent. In 2008, Simpson *et al*. first introduced the use of CTFEP for mixture analysis. [2] The use of supercooled water to modulate the spin dynamics of small metabolites was published in 2012 by the same author. [3] Our team published articles in 2011, 2016, 2017, 2019 and 2020 about the use of viscous solvents for the creation of ^{1}H and ^{19}F spin diffusion conditions and the use of ^{13}C and ^{31}P nuclei as chemical shift markers. [4]

In this context, the present work focusses on the assessment of two viscous media which were prepared from ortho- phosphoric acid (85 %) by dilution with either D_2O or DMSO-d_6, thus providing solvents blends with slightly different polarities in which all liquid-state NMR experiments can be carried out easily. Two mixtures, one of four dipeptides and one of four low-polarity phosphorus-containing compounds were used as examples.

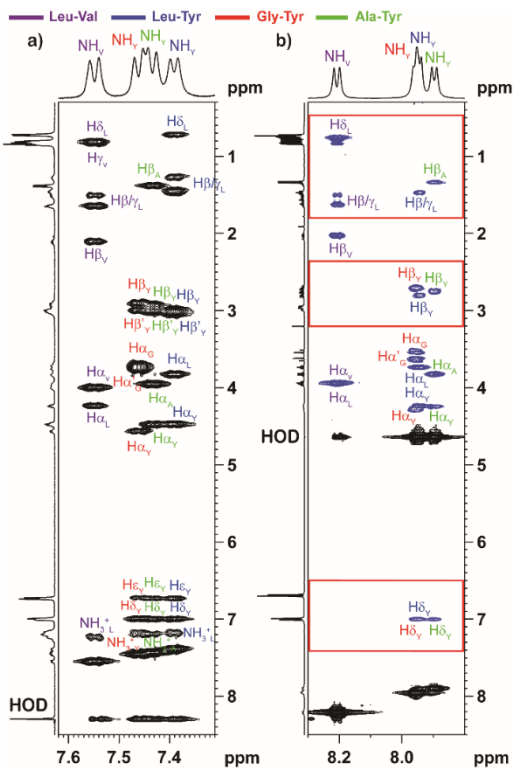

Figure 1. Amide proton region of 2D NOESY spectra of the dipeptide test mixture (20 mM), mixing time (t_m) = 1 s, at 500 MHz (^{1}H), with water suppression by excitation sculpting a) dissolved in phosphoric acid/D_2O (8/2 v/v), at 288 K. b) dissolved in H_2O/D_2O (9:1 v/v), at 298 K. The red frames correspond to spectral regions in which the change in solvent viscosity produces a major effect on the number and sign of observable NOESY cross peaks.

[1] M. Williamson *et al*. *Journal of The Chemical Society, Chemical Communications* **1981**.
[2] A. J. Simpson *et al. Analytical chemistry* **2008**, *80*, 186-194.
[3] H. Farooq *et al. Analytical chemistry* **2012**, *84*, 6759-6766.
[4] *a* P. Lameiras *et al. Journal of magnetic resonance* **2011**, *212*, 161-168, *b* P. Lameiras *et al. Analytical chemistry* **2016**, *88*, 4508-4515, *c* P. Lameiras *et al. Chemistry – A European Journal* **2017**, *23*, 4923-4928, *d* P. Lameiras *et al. Faraday Discuss.* **2019**, *218*, 233-246, *e* F. Pedinielli *et al. Analytical chemistry* **2020**, *92*, 5191-5199, *f* P. Lameiras *et al. Prog. Nucl. Magn. Reson. Spectrosc.* **2021**, *123*, 1-50.