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Diffusion-ordered spectroscopy on a benchtop spectrometer for drug analysis

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ABSTRACT

The first reported two-dimensional diffusion-ordered spectroscopy (DOSY) experiments were recorded at low field (LF) on a benchtop NMR spectrometer using the BPP-STE-LED (bipolar pulse pair-stimulated echo sequence with a longitudinal eddy current delay) pulse sequence which limits phase anomalies and baseline discrepancies. A LF DOSY map was first obtained from a solution of a model pharmaceutical formulation containing a macromolecule and an active pharmaceutical ingredient. It revealed a clear separation between the components of the mixture and gave apparent diffusion coefficients (ADC) values consistent with those measured from the reference high field experiment. LF DOSY was then applied to a real esomeprazole medicine and several gradient sampling schemes (linear, exponential and semi-gaussian (SG)) were compared. With a pulsed field gradient range of 4–70%, the most reliable results were given by the SG ramp. The resulting LF DOSY map obtained after 2.84 h of acquisition confirmed that the diffusion dimension is of prime interest to facilitate the assignment of overcrowded LF spectra although relevant ADC values could not be obtained in part of the spectrum with highly overlapped signals.

1. Introduction

Although NMR is not considered as a routine tool in the quality control field due to its high purchase and maintenance costs, the ability of this technique to provide a large amount of information (structural, qualitative, quantitative, diffusion) could make it an essential tool in pharmaceutical manufacturing industries and quality control laboratories [1]. In the pursuit of a better analytical performance in terms of resolution and sensitivity, modern high-resolution NMR spectrometers rely on higher and higher static magnetic fields provided by large superconducting magnets. While high resolution NMR is competitive with other analytical techniques -allowing micromolar compound detection-, this bulky and sophisticated instrumentation is not always compatible with demanding industrial environments and usually requires a dedicated room and a trained staff to operate technical and cryogenic maintenance.

As an alternative, a new generation of compact and cryogen-free low-field (LF) spectrometers has emerged [2]. These permanent magnets have reached a sufficient level of maturity to afford a stable and homogeneous enough static magnetic field to resolve the chemical shift information. They can deliver NMR spectra with a sufficient quality to generalize their use in various fields of application such as chemical process monitoring [2–6], food screening [7–9], quality control of dietary supplements [10] and more recently in drug analysis [11]. However, those spectrometers operating at ^{1}H resonance frequencies between 40 and 80 MHz involve a drastic loss of analytical performances. Besides the loss in sensitivity, the reduction of the frequency dispersion leads to two main drawbacks: i) congested spectra with strong signal overlaps, especially in the case of complex mixtures; ii) ubiquitous second order couplings making the analysis of signal patterns a tricky task in the context of structural elucidation. The recent implementation of gradient coils in commercial LF NMR spectrometers opens the way to modern and efficient experiments, which improve the

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LF NMR performance [4] and increase its application potential in a high-throughput industrial context.

Introducing a diffusion dimension based on pulsed field gradient (PFG) experiments is an appealing solution to analyse complex mixtures. Incrementing the intensity of the PFG leads to a signal attenuation described by the well-known Stejskal and Tanner equation [12]. By fitting this attenuation, a virtual separation between the different components of a mixture is obtained, which gives access to their individual sub-spectra. Diffusion Ordered Spectroscopy (DOSY) is a powerful data processing tool introduced by Morris and Johnson [13,14] which allows a two-dimensional (2D) map representation with one dimension corresponding to frequencies and the other to apparent diffusion coefficients (ADC). Several efficient processing algorithms have been suggested to optimize the quality of DOSY experiments, all being gathered in the useful DOSY Toolbox [15], recently updated to GNAT [16].

Diffusion experiments have already been reported at LF especially in the petroleum, polymer and food industries [6,17,18], but they mainly provided either single ADC measurements or diffusion-relaxation correlation maps. Here, we present the first 2D DOSY NMR experiments -whereby diffusion and chemical shifts are mapped together- on a commercial benchtop LF spectrometer equipped with a gradient coil, first on a model sample and then on a real medicine. The performances and limitations of this new approach are discussed, as well as its potential for pharmaceutical analysis.

2. Materials and methods

2.1. Samples

All chemicals were from Sigma-Aldrich (St. Louis, MO, USA). Deuterated solvents were obtained from Euriso-top (Gif-sur-Yvette, France).

The model sample was prepared by mixing 2 mg (i.e. 16 mM) of paracetamol with 5 mg of hypromellose 2910 (4000 cps) in 1 mL of DMSO-d_6.

The generic formulation of Esomeprazole 40 mg from Ranbaxy was bought in a French pharmacy. A tablet was crushed in a mortar and 100 mg of the obtained powder were added to 1 mL of DMSO-d_6. After sonication (5 min) and centrifugation (5 min, 3000 rpm), 700 μL of supernatant and 90 μL of a 10 mM solution of sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl) propanoate (TSP) in DMSO-d_6 (for chemical shift calibration) were introduced in a NMR tube.

2.2. HF NMR spectroscopy

HF NMR spectra were recorded on a Bruker 500 MHz AVANCE spectrometer equipped with a 5 mm TCI cryoprobe set at 298 K. The maximum gradient strength this probe was able to deliver was 0.541 T/m (i.e. 54.1 G/cm). After calibration of the 90° radiofrequency pulse, diffusion experiments were recorded by using the bipolar pulse pair-stimulated echo sequence with a longitudinal eddy current delay (BPP-STE-LED) [19]. This sequence is based on a stimulated echo with unbalanced bipolar gradient pulses to remove unwanted magnetization and a LED delay to limit eddy currents. The duration of each gradient pulse (δ/2) was 1.5 and 3.0 ms for the model sample and the pharmaceutical formulation respectively while the diffusion time (Δ) was set at 200 and 500 ms. The spoiler gradients were either 600 μs or 2 ms at −17.13 and −13.17% of the maximum gradient strength. All the gradient pulses had a sinusoidal shape to limit eddy currents. The gradient recovery time was 300 μs for the model sample and 500 μs for the esomeprazole drug and the relaxation delay was 3.5 s and 3.0 s, respectively. A total of 10 or 16 spectra with different gradient strengths were recorded. The gradient strength ranged from 2 to 95% of the maximum gradient intensity. The gradient sampling was chosen from a home-written au-program and was semi-gaussian (i.e. sigmoid shape). Four dummy scans were recorded at the beginning of the experiment followed by 16 transients per gradient strength recorded in 32 K data points. The total experimental time was 16.67 min for the model sample and 23.38 min for the esomeprazole sample.

2.3. Benchtop NMR spectroscopy

LF NMR experiments were performed on a Spinsolve benchtop spectrometer commercially available from Magritek (Germany), working at a frequency of 43.62 MHz via a permanent magnet based on a Halbach design [20,21]. This equipment includes a gradient coil along the B_0-axis (i.e. along the transverse plane of the NMR tube) which can generate a maximum field gradient of 0.16 T/m (i.e. 16 G/cm) and was operated through the programming interface Prospa 3.2. (Magritek, Germany).

The diffusion experiments were performed with the BPP-STE-LED sequence schematized in Fig. 1 [19]. Moreover, the following 16-step phase cycling was used:

ϕ_1 = x; ϕ_2 = x; ϕ_3 = 2x, 2 (−x); ϕ_4 = 4x, 4 (−x), 4y, 4 (−y); ϕ_5 = x; ϕ_6 = 2 (x, −x), 2 (−x, x), 2 (y, −y), 2 (−y, y); ϕ_7 = 4x, 4 (−x), 4y, 4 (−y); ϕ_{rx} = x, 2 (−x), x, −x, 2x, −x, −y, 2y, −y, y, 2 (−y), y.

This phase cycling enforces the coherence transfer pathway 0 → +1 → −1 → 0 → +1 → −1 → 0 → −1. All the diffusion experiments presented in the article share the following features. The diffusion dimension was sampled through 32 increments recorded with 80 scans separated by a total repetition time of 4 s involving a total experiment duration of 2.84 h. The FIDs contained 8192 points separated by a dwell time of 200 μs leading to a detection time of 1.64 s. The flip angle of 90° was achieved by a pulse length of 6.7 μs at 0 dB. The diffusion gradient pulses were applied during 2 ms (δ/2) with a trapezoidal shape including gradient ramps of 200 μs. The gradient sampling was chosen from a home-written script and

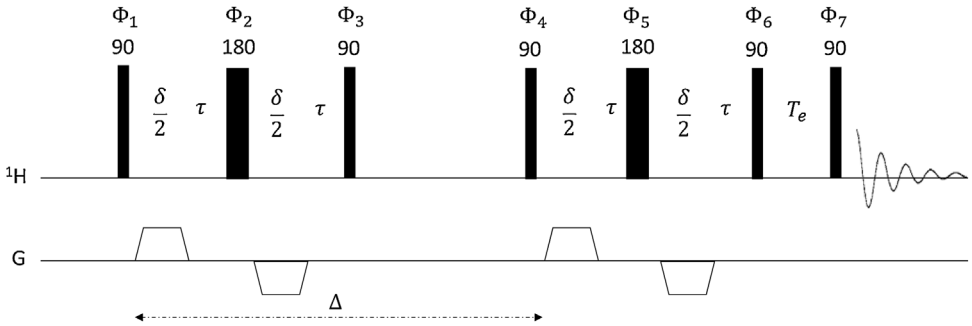

Fig. 1. Schematic BPP-STE-LED pulse sequence. Φx indicate pulse phases, δ/2 is the duration of each trapezoidal gradient, τ is the recovery delay, Te is the time involved within the LED block. Δ is the diffusion time. Phase cycling and parameters values are reported in the experimental part.