Bulgarian Chemical Communications, Volume 49, Special Issue F, (pp. 65 - 69) 2017

Slice selective NMR approach for investigation of distribution phenomena in biphasic samples

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Submitted on July 16, 2016; Revised on October 29, 2016

Distribution phenomena in biphasic systems using slice selective NMR measurements are discussed. Two quantification strategies are compared, demonstrating, that reliable data for the systems $D_2O/CDCl_3$ are easily obtained, even on low field spectrometer.

Keywords: Slice selection, NMR, biphasic samples, quantification

INTRODUCTION

Slice-selective NMR is a relatively new technique, allowing registration of spectral information from different parts of the sample. The idea of spatially resolved spectroscopy is not new and numerous examples can be found in the literature, using specialized equipment [1-3]. In the last years its application on standard NMR equipment is drawing increasing attention due to its easier implementation. It is successfully used to study diverse systems, where the sample composition varies along the NMR tube, including diffusion in polystyrene [4,5] agar gels [6] and ionic liquids [7].

Surprisingly, little is done on its application on biphasic samples. In 2000, Kozminski demonstrates the possibility to record high resolution NMR spectra in the system $D_2O/CDCl_3$ [8]. More recent papers investigate D_2O/olive oil [9] and D_2O/octanol systems [10], but in all cases, no attempt to derive quantitative information is made.

As a continuation of previous investigations, this paper discusses the possibility to quantify biphasic samples from the type chloroform/water and tries to provide guidelines for performing that type of analysis, using the distribution of vanillin as an example.

THEORY AND METHODS

1. Introduction to slice selection

Slice selection is achieved by the simultaneous use of frequency selective pulses and pulsed field gradients (PFG). Applying a gradient with strength G in the direction of the external magnetic field (B_o) induces linear variations in the local magnetic fields, depending on their position (z):

$$B(z) = B_0 + G*z \quad (1)$$

Following the resonance condition, this results in encoding of the spatial position of the spins in their resonance frequencies:

$$\nu(z) = \nu_0 + G*z*\gamma \quad (2)$$

where ν_0 is the resonance frequency in the static field and gamma is the gyromagnetic ratio. Applying a frequency selective pulse during the gradient will then affect only those resonances, which fulfil Eq. 2. When the bandwidth is large enough to cover the whole spectrum of the compound under investigation, this results in the selection of spins from a discrete region of the sample (slice), with thickness Δz, proportional to the ratio of the bandwidth (BW) and the gradient strength:

$$\Delta z = \frac{BW}{G * \gamma}$$

Changing the offset of the selective pulse allows to "scan" different parts of the sample along the direction of the applied gradient (Figure 1).

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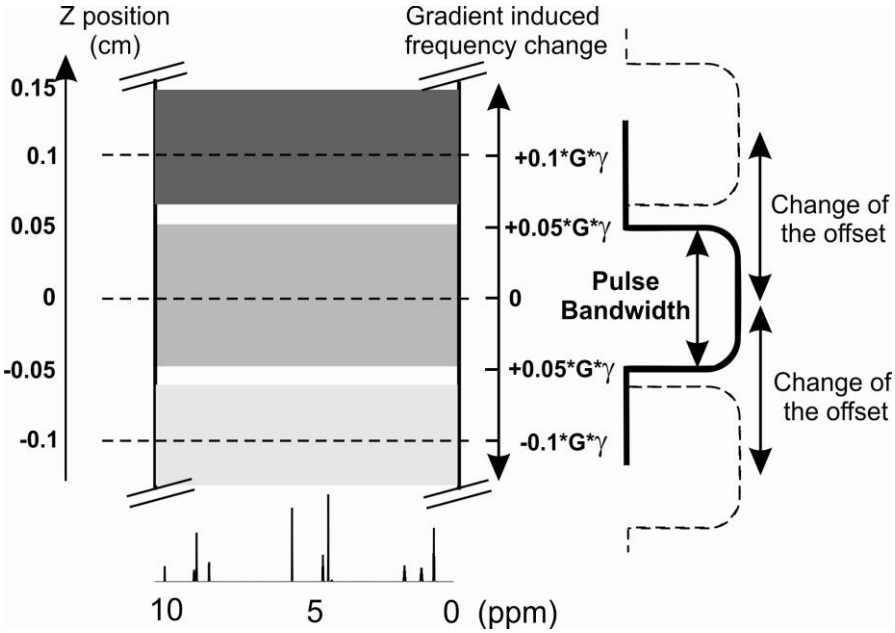

Figure 1. Principal scheme of slice selective NMR. The pulse bandwidth is 0.1*G*γ, resulting in 1 mm slice thickness.

The fact, that only part of the sample is observed marks the main drawback of the slice selection approach – its lower sensitivity, proportional to the thickness of the slice. However, this also offers the opportunity to record series of quantitative spectra at greatly reduced times, using interleaved schemes of acquisition [6,11], making the technique particularly suitable for quantification of systems which change in time.

2. Choice of pulse sequence

The use of interleaved acquisition requires only part of the sample to be irradiated at a time, which is usually the case, when only frequency selective pulses are used. In this respect, the slice selective excitation is often the experiment of choice. Consisting of only one pulse, it offers the best sensitivity with minimum signal losses due to diffusion or transversal relaxation processes. However, when applied to biphasic samples, it leads to the presence of artefacts, often observed in the magnetic resonance imaging when change in the magnetic susceptibility is present (Figure 2) [12]. Spectra with much higher quality are acquired when using a slice selective version of the spin echo. The presence of a refocusing pulse flanked by two spoil gradients effectively supresses the susceptibility artefacts, yielding clean spectra with excellent phase and baseline properties.

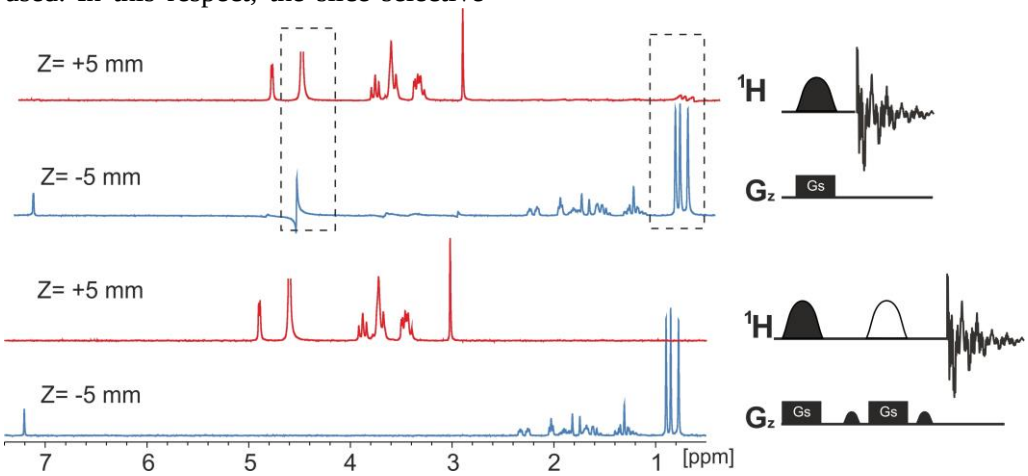

Figure 2. Comparison between slice selective excitation (top) and spin echo (bottom). The corresponding pulse sequences are shown on the right with filled and open shapes corresponding to excitation and refocussing pulses. The used sample is 20 mM α-cyclodextrin in D_2O, layered on 50 mM camphor in $CDCl_3$.

A drawback of the spin echo is the lower signal to noise ratio, compared to the excitation scheme, as the signal intensity depends on transverse relaxation properties of the system and the two spoil gradients also add possible diffusion losses [13]. To minimize those unwanted effects, the echo time is set to 1 ms, resulting in decrease of the sensitivity by approximately 50%, compared to the excitation experiment. Attempting to obtain higher sensitivity, two additional approaches were also examined – a mixed scheme, consisting of slice selective excitation element, flanked by two nonselective 180° pulses, [14] and the LOCSY experiment [9]. In both cases susceptibility artefacts were observed, which limits the choice to the spin echo.

3. Quantification strategies

Commonly, quantification in the slice selective NMR is done by conversion of the measured absolute integral values in concentrations using calibration curves. With samples of known concentration, series of spectra with identical experimental parameters are acquired and separate curves for each slice are built. Although time consuming, this method gives excellent precision, as it accounts for possible diffusion and/or relaxation processes during the experiment.

The presence of two different phases suggests that separate calibration curves for each of the solvents are needed. This is true for high-field spectrometers, which are usually more sensitive to solvent change. In this case, however, the optimal 90^{O} pulse is practically the same - 14.1 μs for D_2O and 14.0 μs for $CDCl_3$, resulting in identical intensity profiles (Figure 3).

The excellent agreement allows us to use single calibration curve in the current study, but performing that type of measurements on high-field instruments may require separate quantification for each solvent.

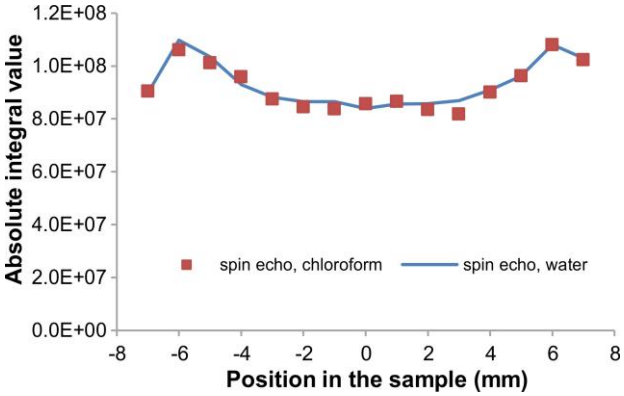

Figure 3. Intensity profiles, obtained for 40 mM vanillin samples in D_2O and $CDCl_3$.

An alternative approach, that could greatly simplify the quantification process in such cases, is the internal standardization. It relies on the assumption, that the measured ratio of two compounds is independent on the experimental parameters, if no signal losses, due to diffusion or relaxation are present. Additionally, when biphasic samples are studied, care should be taken to homogeneously spread the standard in both phases, or, preferably, different standards for each phase should be used. In this respect, hexamethyldisloxane (HDMSO) and tetramethylammonium bromide (TMAB), commonly used for chemical shift referencing, present good compounds for quantification. They have relatively long T2 relaxation times and diffusion coefficients, similar to vanillin, ensuring minimal signal loss during the spin echo experiment (Table 1).

Table 1. Diffusion and relaxation parameters of vanillin, HDMSO and TMAB.

	Chloroform sample		D_2O sample	
Concentration (mM)	Vanillin	HDMSO	Vanillin	HDMSO
D (m^2/s) $*10^{-10}$	13.5	15.2	5.7	8.7
T1 (s)	4.23*	2.32	1.52*	7.45
T2 (s)	3.76*	2.06	1.38*	6.31

* Values for the CH_3O- group

RESULTS AND DISCUSSION

Following the already mentioned considerations, biphasic sample, containing 40 mM vanillin in the D_2O layer was prepared, and its distribution between the two phases is measured. Preliminary investigations revealed that the opposite approach is not practical, as the higher solubility of vanillin in chloroform [15] results in much slower diffusion in the water phase. To ensure good temperature stability, the sample is kept in the magnet for the time of all measurements. Figure 4 summarizes the results, which prove that both methods - using calibration curve and using internal standard yield satisfactory agreement, with difference in the calculated concentrations of approximately 5 %.

Significant dependence of the vanillin distribution from the air flow, used to maintain the sample temperature is observed (Figure 5). This result is unexpected, as it is known, that increasing the airflow results in better temperature stability, at least in the active volume of the coil [16]. However, recent study demonstrate, that often the sample temperature at the bottom of the tube differs from the one in the observable volume, thus promoting convection flows along the tube [17].

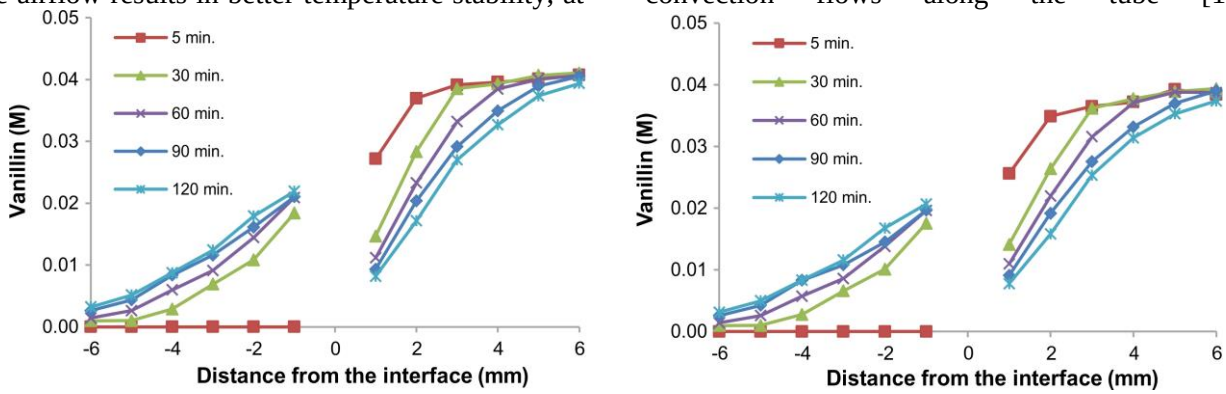

Figure 4. Concentration profiles of vanillin in $D_2O/CDCl_3$ sample at different times. The concentrations are calculated, using calibration curve (left) and internal standard (right). The initial solution was 40 mM vanillin in D_2O. Due to signal broadening, data for the slice, containing the interface is not shown.

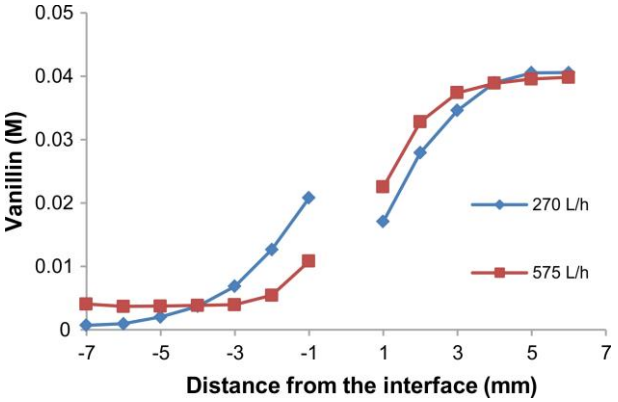

Figure 5. Comparison between the distribution of 40 mM vanillin in $D_2O/CDCl_3$ after 30 min. Air flows of 270 and 575 L/h were used.

EXPERIMENTAL

All experiments were conducted on Bruker DRX 250 spectrometer (5.87 T magnet), operating at 250 MHz ^1H frequencies, equipped with 5 mm BBO probe with z-gradient coil. The temperature was maintained at 298 K, using Bruker B-VT 2000 temperature unit with airflow of 270 L/h. All spectra were referenced against tetramethylammonium bromide (TMAB, 3.15 ppm) and hexamethyldisoloxane (HDMSO, 0 ppm) for water and chloroform, respectively.

Slice selection is performed, using 2088.5 μs G4 Gauss-Cascade [18] excitation pulse and 621.7 μs RSnob [19] for refocusing, both of them with bandwidth of 3751 Hz. Combined with gradient strength of 8.81 G/cm they produce 1 mm slices. Changing the frequency offset for those pulses from 22506 Hz to -22506 Hz with a step of 3751 Hz, results in 13 adjacent slices. The strength of the spoil gradients used in the spin echo sequence is 16.05 G/cm. The spectra are acquired with 4 scans in interleaved mode of acquisition for a total experimental time of 4 min.

Typical samples were prepared by using 250 μl of chloroform, containing HDMSO in standard 5 mm NMR tube. 250 μl D_2O solution of vanillin (40 mM) was carefully layered on top of it so that both layers remain separated. The volume of both solvents was chosen so that the interface appears in the center of the coil as determined by the Bruker sample depth gauge.

CONCLUSIONS

Slice-selective NMR experiments on $D_2O/CDCl_3$ biphasic samples provide a quick and easy technique to obtain quantitative data on distribution processes. On low field instruments, quantification is relatively simple, as no significant difference between the NMR behaviour of the two solvents is observed. As a proof of principle it is shown, that the use of internal standard can be a good alternative to the calibration curve method, which could simplify the quantification on high-field instruments, which are usually more sensitive to changes in the solvent.

***Acknowledgments**: The author acknowledges projects DFNP-147/2016 (Program for career development of young Scientists, BAS), UNA-17/2005, DRNF-02-13/2009 for financial support and Prof. Svetlana Simova for help in the preparation of the manuscript.*

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ПРОСТРАНСТВЕНО СЕЛЕКТИВНА ЯМР СПЕКТРОСКОПИЯ ЗА ИЗСЛЕДВАНЕ НА ПРОЦЕСИ НА РАЗПРЕДЕЛЕНИЕ В ДВУФАЗНИ СИСТЕМИ

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Постъпила на 16 юли, 2016 г. коригирана на 29 октомври, 2016 г.

(Резюме)

Статията разглежда процеси на разпределение в двуфазни системи, изследвани с помощта на пространствено селективна ЯМР спектроскопия. Сравнение между две стратегии за получаване на количествени данни показва, че получаването на надеждни данни за системи от типа $D_2O/CDCl_3$ е лесно, дори на спектрометър с ниска работна честота.