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NMR diffusometry data sampling optimization for mixture analysis

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ABSTRACT

NMR diffusometry is a powerful but challenging method to analyze complex mixture. Each component diffuses differently, from the faster small species to the slower large species, corresponding to different signal attenuation. However, the method is highly sensitive to the quality of the acquired data and the performance of the processing used to resolve multiexponential signals influences. Adapting the signal decay sampling to the mixture composition is one way to improve the precision of the measure. In this work, we propose a prediction tool, based on the calculation of the Cramér-Rao lower bound to minimize the variance of diffusion coefficient estimation in order to determine the optimal number of diffusion gradient steps, the best diffusion gradient sampling (among linear, exponential, quadratic and sigmoidal ones) and the optimal maximum diffusion factor. The tool was validated experimentally on a unimer/micelle solution of sodium dodecyl sulfate and on Caelyx®, a commercial liposomal preparation containing a mixture of pegylated-liposomes and sucrose.

1. Introduction

The analysis of drug pharmaceutical impurities remaining from the formulation or generated from their degradation [1] is a major issue of safety as well as drug counterfeiting detection [2,3]. The analysis of such mixture components distribution is quite challenging and Nuclear Magnetic Resonance (NMR) diffusometry [4–8] appears as a powerful and challenging method to virtually separate the mixture components. With ^{1}H 2D NMR pulsed field gradient (PFG) acquisition, diffusion coefficients are estimated related to chemical shift by the observation of the exponential decay of the NMR signal due to the self-diffusion behavior taking place between the two gradients of magnetic field [9]. In a mixture, each component diffuses differently from the faster small species to the slower large species. Thus, each component can be separated based on its diffusion coefficient. When in addition, a molecule is involved in various molecular association such as monomer, polymer, micelle, vesicle or particle, signal overlapping appears. This spectral overlapping complicates the processing as the contribution of each species to the multiexponential signal attenuation needs to be extracted [10–12]. The accuracy of estimates will strongly depend on both the quality of the acquired data set [13,14] and the processing method [15,16] used. In the present paper, we will focus only on the influence of the acquisition parameters by adapting the signal decay sampling to the mixture composition in order to improve the precision of the measure.

2. Theory

Among important acquisition parameters, the sampling pattern of the diffusion sensitizing gradients is of importance. Dynamic range in the diffusion dimension is determined by the diffusion weighting factor b, $b = \gamma^2.g^2.\delta^2.(\Delta-\delta/3)$ with γ being the gyromagnetic ratio, δ, g and Δ being respectively the gradient duration, intensity and separation. In case of a mixture study with potentially large orders of diffusion coefficient magnitude among species, it is particularly important to optimize the diffusion gradient sampling scheme in order to measure accurately all involved species contributions. Linear, exponential or quadratic gradient spacing are usually pre-programmed in NMR systems. N gradient steps are used ranging between the minimum gradient value g_{min} and the maximum gradient value g_{max}. The i^{th} gradient magnitudes are respectively:

$$g_i = g_{min} + (i-1)\left(\frac{g_{max}-g_{min}}{N-1}\right) \quad \text{(linear)} \qquad (1)$$

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$$g_i = e^{(\ln(g_{min})+(i-1).(\ln(g_{max})-\ln(g_{min}))/(N-1))} \quad \text{(exponential)} \tag{2}$$

$$g_i = g_{min} + \sqrt{\frac{(i-1)}{(N-1)}}.(g_{max}-g_{min}) \quad (quadratic) \tag{3}$$

Recently, sigmoid pattern has been also proposed to deal with the diffusion coefficient heterogeneity encountered in complex formulation and to improve the measurement accuracy [13]. In this case, the i^{th} gradient magnitude is:

$$g_i = g_{min} + (1/(1+e^{\lambda.(\frac{1}{2}-\frac{i}{N})})).(g_{max}-g_{min}) \tag{4}$$

where λ is a positive parameter controlling the curve inflexion.

The gradient shape ramp determines how densely the signal is sampled at high or weak gradients strengths. Compared to linear spacing, exponential one with its denser sampling at low gradient magnitude is more adapted to fast moving molecules while quadratic one with its denser sampling at high gradient magnitude is more appropriate to slow moving molecules. Sigmoidal spacing with both denser sampling at high and low gradient strengths has previously demonstrated its efficiency for mixtures of slow and fast diffusing compounds.

We hereby proposed a method to optimize the design of diffusion gradient sampling, based on prior information about the range of diffusion coefficient values likely to be encountered in the mixture. The sampling strategy was optimized according to the Cramér-Rao Lower Bound (CRLB) theory [17]. It minimizes the variance of parameter unbiased estimates. Briefly, the CRLB theory states that the minimum variance $\sigma(\theta_i)$ of a parameter θ_i of an unbiased estimator is equal to diagonal terms of the inverse of the Fisher information matrix. It corresponds to the best achievable precision of parameter estimates.

$$\sigma(\theta_i) = \sqrt{(F^{-1})_{ii}} \tag{5}$$

The cross-terms of the Fisher information matrix are defined as:

$$F_{ij} = \frac{1}{\sigma_G 2}\sum_n \left(\frac{\delta S(\theta)}{\delta \theta_i}\cdot\frac{\delta S(\theta)}{\delta \theta_j}\right) \tag{6}$$

where σ_G is the standard deviation of a zero-mean Gaussian noise, S is the function dependent on the set of parameters θ and the sum is over all sampling points, n. Indexes i and j run over all the parameters that determine S.

In the presence of a pair of gradients of magnetic field, the 2-parameter model [9] for diffusion NMR signal attenuation of a single component solution with a diffusion coefficient D may be written as:

$$S(b) = S_0.e^{-b.D} \tag{7}$$

where S(b) and S_0 are respectively the signal amplitude in the presence and absence of diffusion gradients. The parameters θ_1 and θ_2 therefore correspond respectively to S_0 and D.

According to the CRLB theory, the minimum variance of D parameter estimate is expressed as [18]:

$$\sigma^2 D = \sigma_G 2.\left(\sum_{i=1}^{N}\begin{bmatrix} \frac{\delta S(b_i)}{\delta So}\cdot\frac{\delta S(b_i)}{\delta So} & \frac{\delta S(b_i)}{\delta So}\cdot\frac{\delta S(b_i)}{\delta D} \\ \frac{\delta S(b_i)}{\delta D}\cdot\frac{\delta S(b_i)}{\delta So} & \frac{\delta S(b_i)}{\delta D}\cdot\frac{\delta S(b_i)}{\delta D} \end{bmatrix}\right)^{-1} \tag{8}$$

where b_i is the diffusion factor for the i^{th} gradient amplitude and σ_G the standard deviation of the signal.

$$\sigma^2 D = \sigma_G 2.\begin{bmatrix} \sum_{i=1}^{N} e^{-2.b_i.D} & -\sum_{i=1}^{N} S_o.b_i.e^{-2b_iD} \\ -\sum_{i=1}^{N} S_o.b_i.e^{-2b_iD} & \sum_{i=1}^{N} S_o 2.b_i 2.e^{-2.b_iD} \end{bmatrix}^{-1} \tag{9}$$

The coefficient of variation $\frac{\sigma_D}{D}$ for a single molecular species can then be expressed as:

$$\left(\frac{\sigma_D}{D}\right)^2 = \frac{1}{SNR^2}\cdot\frac{A}{A\Gamma - B^2} = \frac{1}{SNR^2}.p^2(b_1.D, b_2.D, \ldots, b_N.D) \tag{10}$$

where $A = \sum_{i=1}^{N} e^{-2b_iD}$, $B = \sum_{i=1}^{N} b_iD.e^{-2b_iD}$, $\Gamma = \sum_{i=1}^{N} (b_iD)^2.e^{-2b_iD}$ and the signal to noise ratio $SNR = S_o/\sigma_G$. p can be defined as the uncertainty factor which is proportional to the relative standard deviation of the estimated parameter D.

For a solution with two components that differ in molecular sizes, the sampling was optimized in a similar manner. Optimal diffusion factors $\{b_i\}$ are chosen to minimize $\sum_{j=1}^{2} \frac{\sigma_{D(j)}}{D(j)}$ with j = 1 or j = 2 for respectively the fast or the slow diffusing specie. Assuming a SNR sufficiently high, the optimal design was therefore obtained for $\{b_i\}$ which minimize the function P representing the total uncertainty factor:

$$P = p(b_1.D(1), b_2.D(1), \ldots, b_N.D(1)) + p(b_1.D(2), b_2.D(2), \ldots, b_N.D(2)) \tag{11}$$

The uncertainty factor was first calculated theoretically and then measured experimentally on a unimer/micelle solution of sodium dodecyl sulfate (SDS). Finally, a simulation tool to choose the adequate gradient sampling for a given mixture was proposed and validated on a solution of liposome and sucrose.

3. Material and methods

3.1. Diffusion curve sampling optimization

The uncertainty factor P was calculated for a gradient amplitude g varying from 2.408 to 45.742 G/cm using MATLAB® (Mathworks) for various number of gradient sampling points (N = 32, 64 and 128), different maximum diffusion factors (δ from 4 to 8 ms corresponding to the maximum b value, b_{max}, from 47,769 to 189,793 s/mm² for Δ = 200 ms) and different gradient patterns (linear, exponential, quadratic and sigmoidal with an arbitrary λ = 7.7 chosen to give a smooth variation in the gradient profile) and for two estimated diffusion coefficients D = 0.8 10^{-4} mm²/s (SDS micelle) and 2.5 10^{-4} mm²/s (SDS unimer).

The validation was performed on a solution of 4.88 mg/mL (16.9 mM) SDS dissolved in 500 μL of D_2O for locking. The concentration is 2.4 times higher than the theoretical critical micellar concentration (7 mM) [19].

3.2. Gradient sampling prediction tool for mixture

The objective is to determine the best gradient sampling scheme for a solution of two compounds with hydrodynamic radii ranging from 1 to 50 nm (corresponding to diffusion coefficients from 3.45 10^{-6} to 1.72 10^{-4} mm²/s at 293 K in a water solution). For each combination of two solutes and each value of sampling points (32, 64 or 128), the optimal gradient profile (linear, exponential, quadratic or sigmoidal) and the optimal b_{max} (ranging from 11,982