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Maximum Entropy Processing of DOSY NMR Spectra

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The measure of translational diffusion using pulsed field gradient NMR has seen a recent renewal of interest with the development of the DOSY experiment (Morris, K. F.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* 1992, *114,* 3139), which has a wide field of application as a noninvasive analytical method. In the case of spectral superpositions or of polydisperse species, the processing of the DOSY experiment requires a Laplace transform analysis because this analysis does not make any assumption on the data. We present here the use of the maximum entropy (MaxEnt) technique for this processing and compare this method with the other approaches currently used in the literature. We found that MaxEnt produces simultaneously an accurate estimate of the amplitudes of the components of the Laplace spectrum and a reliable detection of their positions.

The measure of translational diffusion using pulsed field gradient NMR has seen a recent renewal of interest with the development of the DOSY experiment,[1−4] which has a widespread field of application as a noninvasive analytical method. In this experiment, the separation of species in a mixture relies on an efficient analysis of the exponential decays present in the data set. Least-squares fitting is usually used for this purpose; however, in the case of spectral superpositions, several exponential decays have to be broken down. One may then resort to Laplace transform analysis, which avoids the ill-conditioning problems associated with multiexponential fits. Furthermore, this approach does not make any assumption about the number of exponential components, nor the monodispersity of the species present in the mixture. The CONTIN method[5,6] used previously to perform this analysis[2] does not show a resolution sufficient to permit separation of species of similar sizes in the mixture. The use of the maximum entropy (MaxEnt) method for the processing of DOSY experiments is introduced here and compared with the other approaches currently used in the literature. We show that MaxEnt is the first method in the frame of Laplace transform analysis to permit an efficient separation of the species on the basis of their diffusion coefficients.

Diffusion is analyzed in the DOSY experiment by observing the exponential decay of the NMR signal due to the diffusion taking place between a coding gradient and a decoding one. This decay can be expressed as the Laplace transform of the distribution of the diffusion coefficients δ of the different species:

$$G(s) = \int_{\delta_{\min}}^{\delta_{\max}} A(\delta) \exp(-\delta) \exp(-\delta s)\, \mathrm{d}\delta \qquad (1)$$

where $A(\delta)$ is the diffusion coefficient distribution in the range $\delta_{\min}-\delta_{\max}$, and s is an experimental parameter depending on the gradient intensities and on the different delays of the excitation sequence.[1] In this paper, the function G will be said to be in the direct space, while $A(\delta)$ will be said to be in the reciprocal space. We will also call $A(\delta)$ the Laplace spectrum.

The Laplace transform is linear in $A(\delta)$; however, a direct inversion of eq 1 in order to determine the Laplace spectrum is not feasible, because the solutions that would be obtained would display a very strong dependence on the experimental noise.[7] In the DOSY experiment, two techniques have been used to solve this problem. The first one consists of fitting the measured signal to one or a small number of exponential decays, in a parametric representation of the diffusion distribution $A(\delta)$. The main advantage of this approach is that a least-squares fit can be applied, resulting in simple algorithms and fast processing. This method, however, requires a preliminary estimate of the number of decays in the signal. It is very difficult, with this approach, to decipher the case of several closely related decays, particularly in the presence of noise. Additionally, this representation cannot describe cases where there is a true distribution of diffusion coefficients, for instance, in the case of polydisperse samples such as polymers.

A nonparametric representation of the amplitude distribution, in which the $A(\delta)$ function is described as a complete vector A_i over the range $\delta_{\min}-\delta_{\max}$, is thus much more appealing. It has been proposed to use the CONTIN algorithm[5,6] for the determination of such an amplitude distribution. A disadvantage of this approach is that the obtained accuracy and resolution may be insufficient for the diffusion separation required in NMR.

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Table 1. Results of the Monte Carlo Test Performed on the Three Processing Methods (Least-Square Fit, CONTIN, and MaxEnt)[a]

	no. of successes	damping[c]				amplitude[c]			
		1	2	3	4	1	2	3	4
values		0.098	0.50	2.55	12.99	1000	2000	4000	8000
LS[b] fit	100	0.137 ± 0.017	0.87 ± 0.069	4.26 ± 0.58	16.28 ± 1.95	1458 ± 155	2607 ± 370	4609 ± 740	6334 ± 730
CONTIN	59	0.125 ± 0.0115	0.62 ± 0.103	2.71 ± 0.20	13.13 ± 0.24	1190 ± 110	1869 ± 280	3688 ± 245	7674 ± 170
MaxEnt	89	0.1015 ± 0.0075	0.448 ± 0.047	2.39 ± 0.19	12.11 ± 0.32	877 ± 67	1571 ± 260	4077 ± 540	7122 ± 432

[a] The actual values of damping factors and amplitudes used for simulating the data are given in the first row. For each tested method, the number of successful analyses is given, and the mean values and standard deviation values of damping factor and amplitude are displayed. [b] Least-square. [c] Arbitrary unit.

The maximum entropy technique (MaxEnt) has already been successfully used for inverse Laplace transform in domains where a nonparametric representation is required: quasi elastic light scattering[8] time-resolved spectroscopy[9,10] or NMR relaxation,[11] for instance.

The MaxEnt method determines from an experimental vector (D_i, $i = 1...p$) the most probable inverse Laplace transform (A_i, $i = 1...n$). This is done by maximizing a function:

$$Q = S - \lambda\chi^2 \tag{2}$$

where λ is a Lagrange multiplier, $S = -\sum_{i=1}^{n} A_i/F \log(A_i/F)$ is called the entropy of the vector (A_i, $i = 1...n$), and χ^2 represents the Euclidean distance between the data and the Laplace transform of A_i. F is a normalization constant, taken here equal to $\sum_{i=1}^{n} A_i$. The correct A_i solution maximizes the function Q at a value of λ for which the χ^2 value is equal to p, the number of experimental data points.

Several algorithms have been proposed to solve iteratively this optimization problem.[12−16] We use here the generalized iterative fixed-point algorithm (GIFA),[14] which is based on the fixed-point algorithm proposed by Gull and Daniell,[12] stabilized by an additional line search. In this algorithm, the λ value is controlled by monitoring the angles between the dQ, dS, and dχ^2 vectors, and by using a γ parameter given by the user:

$$\lambda = \gamma \frac{\mathrm{d}S\ \mathrm{d}S}{\mathrm{d}S\ \mathrm{d}\chi^2} \tag{3}$$

The MaxEnt Laplace inversion has been implemented in the Gifa software,[17] which includes already all the controls for 1D, 2D, and 3D NMR spectroscopy as well as MaxEnt spectral analysis. Experimental data points can be either regularly spaced in direct space or measured at arbitrary locations. Points in the reciprocal space are logarithmically sampled between δ_{min} and δ_{max}. The total processing time is proportional to $i \times n \times p$, where i is the number of iterations, n is the size of experimental data, and p is the size of the inverse Laplace transform.

EXPERIMENTAL SECTION

MaxEnt was compared to least-squares fit and CONTIN methods by running a Monte Carlo simulation on a data set of four exponential decays with damping factors between 0.1 and 13.0, and amplitudes between 1000 and 8000 (Table 1). One hundred synthetic realizations of the data set were simulated using an additive stationary noise, with a level equal to 0.1% of the first data point. The data set was simulated on 40 geometrically sampled points, generated in a such way that the last point is at the noise level. Each realization was then analyzed successively with the three methods.

The least-squares fit was based on the Levenberg−Marquardt algorithm.[18] Starting by fitting a single decay, the number of decays was progressively doubled until the four decays were estimated. The doubling was performed by replacing each term (A, δ) by two new terms ($0.5A$, 2δ) and ($0.5A$, 0.5δ). When using CONTIN, the input parameters were set to the values provided in the literature.[6] In the MaxEnt processing, convergence was stopped at 20 000 iterations, in case the criterion ($\chi^2 = p$) was not reached; γ was set to 4 in all computations. The Laplace spectra obtained from CONTIN and MaxEnt were reconstructed on 100 points. These reconstructions were considered successful if four local extrema could be found in the spectra and were rejected otherwise. Positions and amplitudes of the different components were then evaluated by a simple Gaussian fit.

A 2D DOSY spectrum was acquired on a mixture composed of sodium dodecyl sulfate, adenosine 5′-triphosphate, and glucose, all 0.1 M in D_2O. The experiment was performed at 35 °C on a Bruker AMX 400 spectrometer. The LED sequence was used,[19] with bipolar gradients.[20] The total gradient duration was 3 ms,

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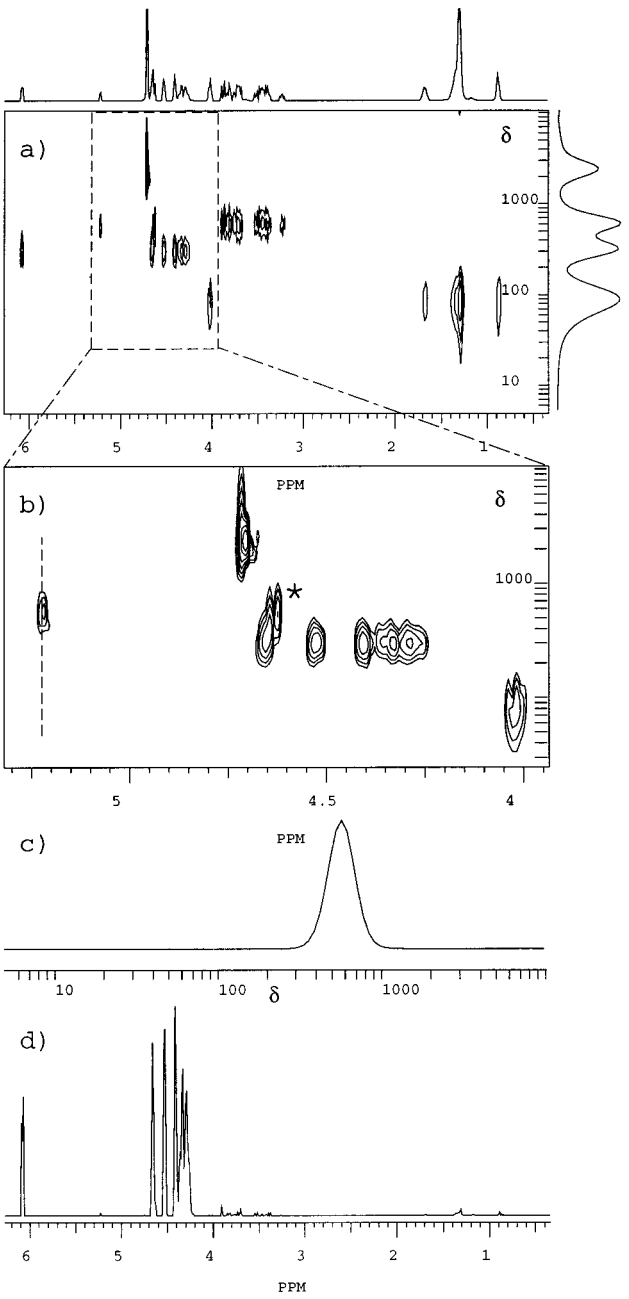

Figure 1. 2D DOSY spectrum of the mixture of SDS, ATP, and glucose; all species are 0.1 M in D_2O. Diffusion axes are labeled in $\mu m^2\ s^{-1}$. (a) The DOSY spectrum of the sample. The projections are displayed on both sides of the 2D spectrum. The aromatic protons of ATP resonating around 8.5 ppm are not shown for the sake of clarity. (b) Central region of the same DOSY spectrum. The asterisk indicates the superposition of the H2′ proton of ATP with the anomeric proton of β-glucose. (c) Diffusion profile extracted from the DOSY spectrum at 3.72 ppm, corresponding to a glucose line. (d) 1D spectrum extracted from the DOSY spectrum at the ATP diffusion coefficient.

and the diffusion delay was 100 ms. Gradient intensities were varied geometrically from 1 to 44 G/cm. We acquired 28 1D spectra, with 32 scans/experiment. The MaxEnt approach was then applied to these data. All the columns for which the initial value was larger than 50 times the noise level were processed in the diffusion axis; the others were set equal to zero. The noise level was assumed to be constant and was measured on a signal-free region of the spectrum. Laplace inversion was calculated by MaxEnt on 100 points, with a maximum of 1000 iterations. Total computing time took 50 min on an R5000 SGI machine. Measured diffusion coefficients are 2360, 590, 300, and 81 $\mu m^2\ s^{-1}$ for HDO, glucose, ATP, and SDS micelles, respectively.

RESULTS

The Monte Carlo test shows the superiority of the nonparametric methods in comparison to the least-squares method. Although the number of decays is included in the latter, it appears to be a much less accurate method than CONTIN and MaxEnt. MaxEnt seems to be more efficient than CONTIN in evaluating the weak components of the signal. The overall amplitude ratios are better respected by MaxEnt, at the expense of systematic underestimation of amplitudes. The MaxEnt method seems to have difficulties coping with transient components, as revealed by the slight inaccuracy on the damping observed for component 4 (Table 1); indeed, this component reaches the noise level on the sixth data point. The somewhat lower rate of success observed for CONTIN can be related to its tendency to oversmooth the estimated distribution, as already described.[21,22]

The DOSY spectrum obtained with MaxEnt (Figure 1) shows that this technique can successfully be used in this case. The overall result compares favorably with equivalent studies presented in the literature.[2] Each species in the mixture is perfectly separated (Figure 1d). Monodisperse species present very narrow diffusion profiles (Figure 1c). The zoom extract (Figure 1b) proves that the method performs well at the low signal-to-noise level encountered on the wings of the peaks. The signals of the anomeric proton H1′ of β-glucose and of the proton H2′ of ATP are separated, even though they are superimposed in the spectrum (asterisk in Figure 1b).

The procedure presented here has several advantages over other methods used for the processing of DOSY spectra. First, the MaxEnt processing makes it possible to obtain high-quality spectra over a wide range of experimental conditions. Resolution, as well as accuracy and reliability, appears to be optimum. Second, no parameter input is needed from the user, except an estimate of the noise level. The MaxEnt processing also benefits from the complete user interface of the Gifa software[17] and has been made available with the current release of Gifa (ftp://www.cbs.univ-montp1.fr/pub/gifa_v4).

The method described here for inverting the Laplace transform in a stable manner can probably be used successfully for other Laplace problems encountered in NMR, such as relaxation measurement.

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