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Detection of a π–π complex by diffusion-ordered spectroscopy (DOSY)

Stéphane Viel,[a] Luisa Mannina[a,b,*] and Annalaura Segre[a]

[a]*Istituto di Metodologie Chimiche, C.N.R., Area della Ricerca di Roma, C.P. 10, I-00016 Monterotondo Stazione, Rome, Italy*
[b]*Università degli Studi del Molise, Facoltà di Scienze MM.FF.NN., Dip. S.T.A.T., Via Mazzini 8, I-86170 Isernia, Italy*

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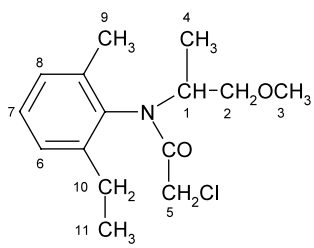
Abstract—Diffusion-ordered NMR spectroscopy (DOSY) has been used in the detection of π–π complexes formed through π–π stacking interactions of a hydrophobic compound in concentrated aqueous solutions. © 2002 Elsevier Science Ltd. All rights reserved.

The formation of complexes through π–π stacking interactions is of the utmost importance in the study of biological systems and also in organic chemistry. As a matter of fact, π–π stacking interactions play a determinant role in molecular structures and conformations, and contribute also significantly to enzymatic specific activity. NMR-based techniques used for the study of such interactions have mainly relied on chemical shifts variations,[1] and on NOE spectroscopy (NOESY and ROESY).[2] To our knowledge, here we report a new way of detecting the presence of π–π stacked complexes in solution by means of diffusion-ordered NMR spectroscopy (DOSY).

Diffusion-ordered 2D NMR (DOSY) experiments[3] have been used for the analysis of a large variety of mixtures,[4] as well as for the characterization of aggregates[5] and, more recently, for the study of intermolecular interactions such as hydrogen bond strength.[6] A DOSY experiment yields a 2D spectrum with NMR chemical shifts in one dimension (horizontal axis) and diffusion coefficients in the other one (vertical axis). Therefore, DOSY spectroscopy allows one to distinguish compounds according to differences in their effective size, provided that their resonances are well resolved in the chemical shift dimension.[7] Thus, a primary requirement for DOSY is that resolution be optimized in the chemical shift dimension. Other basic requirements for DOSY are an undistorted pulsed-field gradient NMR (PFG-NMR) data set obtained with incremented values of the gradient strength and a reliable inversion of the data through an approximate inverse Laplace transformation. In this way, a diffusion spectrum at each chemical shift can be obtained and a global visualization of the diffusion coefficients and, in turn, of the particle sizes is possible.

Metolachlor (see Scheme 1), hereafter referred to as M (1), [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-metoxy-1-methylethyl)acetamide] is a herbicide that belongs to the chemical class of chloroacetamides.[8] M has four stereoisomers; the isomerism is due to a chiral center in the aliphatic chain and a chiral axis between the nitrogen atom and the phenyl group.[9] The structure of this compound has an aromatic ring which, in particular conditions, can give rise to π–π interactions.

Due to its hydrophobic structure, M is soluble in most organic solvents (DMSO-d_6, $CDCl_3$, and CD_3OD) and has only a low solubility in water (about 1.8 mM at 25 °C). In Fig. 1(a), the ^1H spectrum of M in D_2O at a concentration (1.1 mM) lower than the reported solubility and the corresponding assignment[10] (see Table 1) are reported. The ^1H spectra of M in D_2O at concentra-



Scheme 1. Metolachlor (**1**).

Keywords: stacking interaction; π–π complex; diffusion-ordered spectroscopy; DOSY; NMR.

* Corresponding author. Tel.: +3-906-967-2385; fax: +3-906-967-2477; e-mail: mannina@mlib.cnr.it

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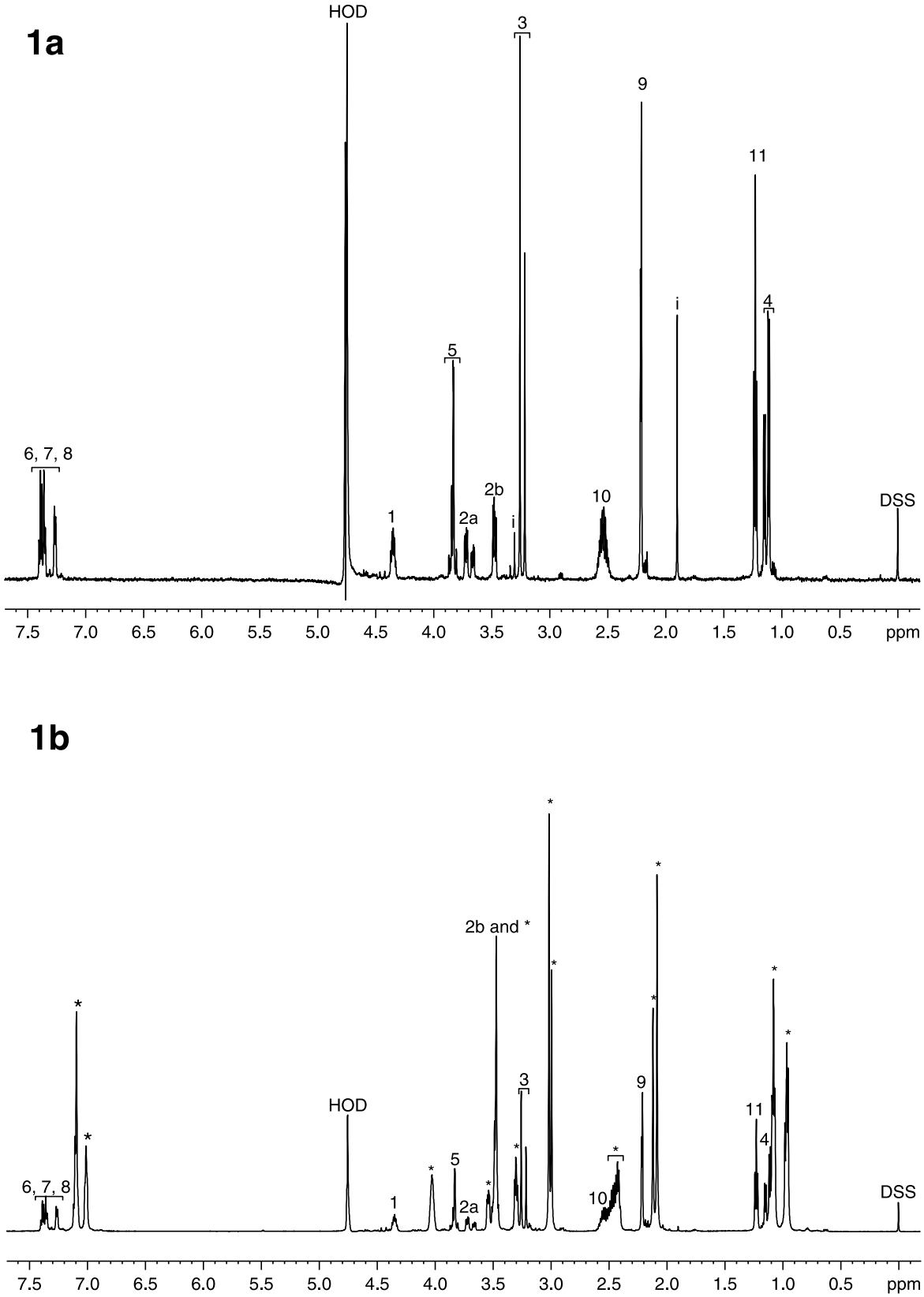

Figure 1. 600.13 MHz ^{1}H spectra of metolachlor solutions in D_2O at (a) 1.1 mM (0.2 mg of M in 600 μL of D_2O) and (b) 11.6 mM (2 mg of M in 600 μL of D_2O). Resonances due to the monomeric compound are labeled with numbers (see Scheme 1); the extra-resonances due to the other compound are labeled with an * whereas impurities are labeled with an i. The spectra were recorded on a Bruker AMX-600 spectrometer at 300.0 K with a soft presaturation of the HOD residual signal. Chemical shifts are reported with respect to 2,2-dimethyl-2-silpentane-5-sulfonate sodium salt (DSS), used as an internal standard.

Table 1. Assignment of the ^{1}H spectrum of the monomeric and polymeric forms of metolachlor in D_2O (11.6 mM) at 300.0 K

		Monomeric form						Polymeric form					
		SR/*RS*			*SS*/*RR*								
		δ (ppm)	*m*	J_{H-H} (Hz)	δ (ppm)	*m*	J_{H-H} (Hz)	δ (ppm)	*m*	J_{H-H} (Hz)	δ (ppm)	*m*	J_{H-H} (Hz)
1	CH	4.351	m	–	4.351	m		4.025	m	–	4.025	m	–
2	CH_2	Ha 3.664	dd	10.3; 5.0	Ha 3.722	dd	10.0; 4.7	Ha 3.497	dd	9.4; 5.3	3.541	m	–
2		Hb 3.476	m	–	Hb 3.476	m		Hb 3.299	m	–	3.299	m	–
3	CH_3	3.215	s	–	3.257	s		2.994	s	–	3.017	s	–
4	CH_3	1.150	d	7.0	1.112	d	6.8	0.973	d	6.5	0.957	d	7.0
5	CH_2	3.838	m	–	3.838	m		3.478	m	–	3.478	m	–
6	CH	7.353	d	7.0	7.353	d	7.0	7.087	m	–	7.087	m	–
7	CH	7.383	dd	7.6; 7.0	7.383	dd	7.6; 7.0	7.109	m	–	7.109	m	–
8	CH	7.263	d	7.6	7.263	d	7.6	7.005	m	–	7.005	m	–
9	CH_3	2.218	s	–	2.210	s		2.119	s	–	2.084	s	–
10	CH_2	2.541	m	–	2.541	m		2.44	m	–	2.44	m	–
11	CH_3	1.227	t	7.5	1.227	t	7.5	1.076	t	7.0	1.080	t	7.6

Chemical shifts are reported with respect to DSS used as an internal standard.

tions lower than the reported solubility and the corresponding ones in organic solvents (data not shown) show similar patterns. Pairs of diastereoisomers can be clearly distinguished: for example, the two doublets at 1.112 and 1.150 ppm are due to the CH_3 in position 4 (see Scheme 1) of the *SS*/*RR* and *SR*/*RS* enantiomeric pairs, respectively. In order to obtain the separation of the enantiomeric forms, it is necessary to add a chiral selector such as cyclodextrins.[10]

In aqueous solutions of M at concentrations higher than the reported solubility, some opalescence is apparent; the ^{1}H spectra of such solutions, hereafter referred to as concentrated solutions, show the presence of new resonances. The observation of these new resonances indicates that M is soluble in water at a much higher concentration (roughly about a factor ten) than the reported solubility. Leaving concentrated samples at room temperature for several days, some precipitation occurs. The NMR spectrum of these solutions does not change anymore, meaning that the solution has become stable, and confirms the presence of new resonances possibly due to the formation of irregular aggregates, dimers or even polymers. In Fig. 1(b), the ^{1}H spectrum of M in D_2O at 11.6 mM is reported with the corresponding assignment (see Table 1).

It is worth noting that all extra resonances, whose relative intensities increase with M concentration, are upfield shifted from the corresponding M signals, as again given in Table 1. This observation is in agreement with the formation of a π–π complex;[11] in fact, in a π–π complex, the center of the magnetic anisotropy is shifted out of the aromatic plane toward the center of the complex. This is the only way to cause an upfield shift generalized to all resonances including those of the aromatic protons themselves.[12] All observed resonances are sharp and well resolved, which rules out the possibility of irregular aggregates. Therefore, all these observations suggest that, in concentrated solutions, molecules of M tend to form dimers or polymers, which minimize the exposition of the aromatic rings to water through the formation of π–π stacking interactions and hence contribute to increasing M solubility.

The chemical equilibrium involving monomers and dimers or polymers is a slow process with respect to the NMR time scale; in fact, the ^{1}H spectrum of M in concentrated solutions show the resonances due to the two species. A possible method to study the monomer–dimer equilibrium is based on the detection of exchange as a function of the temperature, as previously reported by Petersen et al.[1] on disodium guanosine 5′-monophosphate. This method can be used when the energy gap between the involved species is such as to enable the detection of exchange as a function of the temperature. In the case of M, however, no exchange process could be detected in the temperature range under study (300–353 K) since no coalescence nor broadening of the ^{1}H resonances with temperature is observable. Moreover, by increasing the temperature, the monomer solubility increases and the chemical equilibrium is shifted toward the monomer. Therefore, in the case of M, the previously outlined method cannot be applied. In order to identify the π–π complex, diffusion-ordered spectroscopy has been used.

A proton-detected DOSY of a concentrated M aqueous solution (16.9 mM) has been performed (see Fig. 2). The ^{1}H spectrum of the M solution is shown in the horizontal projection: all signals are well resolved and can be classified according to their self-diffusion coefficients[13] ($D_{Monomer} = 4.9 \pm 0.1 \times 10^{-10}$ m^2/s and $D_{Complex} = 2.8 \pm 0.1 \times 10^{-12}$ m^2/s). The DOSY spectrum allows us to clarify two important points. Firstly, it shows that all extra resonances are characterized by the same self-diffusion coefficient, which means that they all are due to the same compound. Secondly, the self-diffusion coefficient of this compound appears to be much lower, about two orders of magnitude, than that

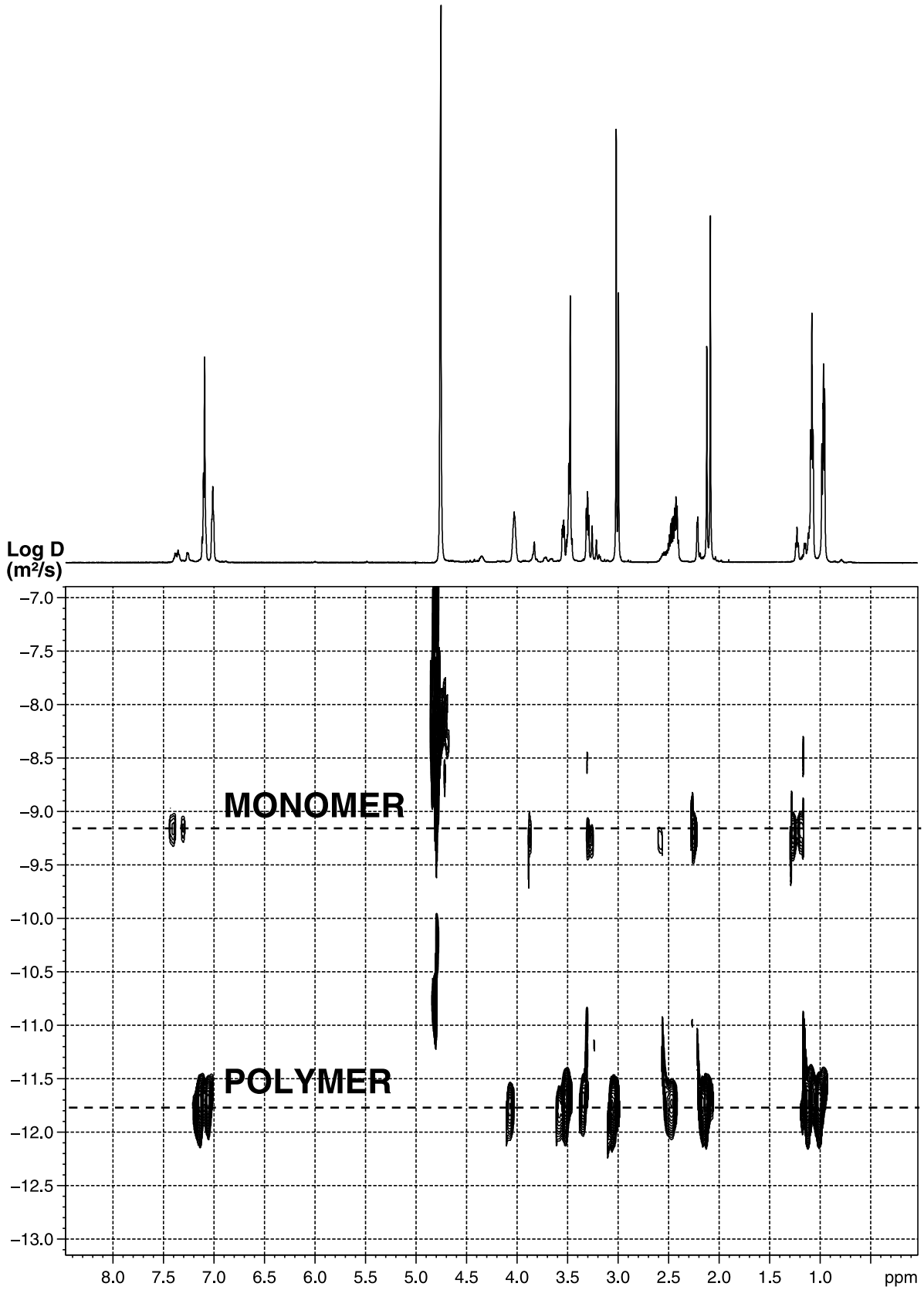

Figure 2. [1]H-detected DOSY spectrum of a 16.9 mM metolachlor solution. The spectrum was recorded at 300.0 K on a Bruker AMX-600 instrument operating at 600.13 MHz with a Bruker z-gradient probehead. The stimulated echo-pulse sequence using bipolar gradients with a longitudinal eddy current delay (BBLED) was used. The strength of the gradient pulses, of 2.2 ms duration, was incremented in 16 experiments, with a diffusion time of 100 ms and a longitudinal eddy currents delay of 5 ms.

of M. This large difference does not agree with the hypothesis of a simple dimer; in fact, a dimeric complex has only a slightly larger effective size than the monomeric species and is thus expected to be associated with a value of self-diffusion coefficient that is not too distant from that of the monomeric species. Therefore, the extremely low diffusion coefficient observed for the compound rules out the possibility of a simple dimer.[14]

Having excluded the possibility of irregular aggregates and that of simple dimers, the possibility of high molecular weight regular polymers held together by π–π and hydrophobic interactions has to be considered. A set of 2D NOESY experiments were then performed, using very different contact times (10–400 ms). The presence of high molecular weight polymers was confirmed by the following observations:[15]

- NOESY cross peaks of the monomeric species appear negative and do not show spin diffusion effects even at rather long contact times;
- NOESY cross peaks of the polymeric species appear positive and show strong spin diffusion effects even at rather short contact times;
- At very short contact times (10ms) no spin diffusion is present and a set of strong, well defined NOESY cross peaks can be observed.

In conclusion, the presence of a polymeric complex has been confirmed showing that diffusion-ordered NMR spectroscopy can be used to detect the formation of π–π stacked complexes. Further work is necessary to obtain the structure of the polymeric compound; to this purpose, a similar study will be performed using pure enantiomeric species.

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13. In the limited range of concentrations considered in this study (4–17 mM), the self-diffusion coefficient of the complex changes with values spanning from $D_{\text{Complex}} = 1.1\pm0.1\times10^{-11}$ m^2/s to $2.8\pm0.1\times10^{-12}$ m^2/s.
14. Viscosity measurements have shown that solutions viscosity does not change significantly in the range of concentrations considered in this study (10^{-3}–10^{-2} M), and remains almost equal to that of D_2O.
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