

Assignment of ^{13}C resonances of a nematic liquid crystal using off-magic angle spinning

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A novel method for assigning the resonances in the ^{13}C NMR spectrum of a static liquid crystalline sample in its nematic phase is proposed. The method is based on the fact that the carbon chemical shifts in the isotropic phase and in the oriented phase under static and off-magic angle spinning (OMAS) conditions are uniquely related by the tensorial property of the CSA tensor, requiring just one OMAS spectrum and the assignment in the isotropic phase. A computational procedure is proposed to take into account deviations arising out of non-ideal experimental conditions and the assignments are made by identifying the minimum in the differences in the frequencies between calculated and experimental line positions. Practical implementation of the method has also been demonstrated in the case of the liquid crystal *N*-(4-ethoxybenzylidene)-4-*n*-butylaniline. Copyright © 2004 John Wiley & Sons, Ltd.

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INTRODUCTION

The assignment of spectral lines in solution state is fairly straightforward owing to the availability of a host of experimental techniques designed for such studies.¹ However, the assignment of ^{13}C spectral lines in liquid crystalline systems is beset with problems due to the presence of strong anisotropic interactions,^{2,3} requiring entirely different approaches for this purpose. One of the procedures for the spectral assignment of molecules in their oriented phase is to accomplish the assignment in the isotropic phase and then obtain assignments in the oriented phase by correlation of the peaks of the latter with those of the former.^{4–6} Such a correlation can be obtained by manipulating the orientation of the director in variable angle experiments^{7,8} in either a series of 1D experiments⁴ or as 2D experiments.^{5,6} The method based on the manipulation of the director orientation by spinning the sample at various angles⁴ requires recording a large number of 1D spectra at fine intervals of the angle of spinning, necessitated by a possibility of crossover of the line positions of different carbons. In addition, there would also be the problem of signal-to-noise ratio as the spinning angle comes closer to the B_0 field direction for solenoidal r.f. coils.

When the molecular weight and hence the viscosity of the mesogenic molecule increase, the method of orienting the director along the spinning axis becomes less efficient. In such cases, correlation between isotropic and anisotropic ^{13}C resonances can be brought about by imposing a sudden change in the orientation of the sample and taking advantage

of the slow reorientation of the director to its equilibrium position.^{5,6} The pulse sequence involves an initial evolution time t_1 during which the liquid crystal director is along B_0 and a short storage period for the magnetization, when the sample is rapidly turned away from its equilibrium position. The spectrum is scanned during the t_2 period when the sample orientation is at an angle $\theta = 90^\circ$. This gives a peak position of $(\delta_{\text{iso}} + \delta_{\text{aniso}})$ for $\theta = 0^\circ$ and $(\delta_{\text{iso}} - \delta_{\text{aniso}}/2)$ for $\theta = 90^\circ$ along the two axes of the 2D spectrum, from which both the isotropic and anisotropic chemical shifts can be obtained.

This method and also a few other methods^{9–12} which can be used to obtain chemical shift correlations require additional hardware besides having the disadvantage of a relatively poor signal-to-noise ratio due to the intermediate storage period. Alternatively, parallel and perpendicular orientations of the director can be achieved by mixing the system under study with a liquid crystal of opposite diamagnetic susceptibility anisotropy and recording the spectra in both orientations of the director.¹³ A comparison of spectral lines in these two phases with that in the isotropic phase then yields the assignment of the lines. Double-quantum ^{13}C NMR in natural abundance is another approach that can be used for assignment.^{14,15} Recently, a new 2D technique has been proposed in which off-magic angle spinning (OMAS) is combined with chemical shift γ -encoding to obtain the assignments.¹⁶

In this paper, we propose a novel procedure which uses a single OMAS spectrum, to identify all the ^{13}C resonances in the oriented phase. This procedure has the advantage of considerable saving of time in comparison with recording a series of off-magic angle spectra and the 2D methods. We

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demonstrate the application of this procedure on the liquid crystal crystal *N*-(4-ethoxybenzylidene)-4-*n*-butylaniline (EBBA).

THEORY

Consider a liquid crystal with a positive diamagnetic susceptibility anisotropy $\Delta\chi$ having N carbon atoms. Let the chemical shift of the k th carbon in this molecule in the isotropic phase be $\delta^k(\text{iso})$, which is known, and let $\delta^k(\text{st})$ be its chemical shift in the spectrum of the static oriented sample which is to be identified. A spectrum of the sample in the oriented phase is also recorded with OMAS. In this case, the angle θ between the spinning axis and the magnetic field may be chosen to be less than the magic angle θ_m , so as to avoid complications arising out of spinning sidebands for $\theta > \theta_m$.^{17,18} θ can be determined accurately from the separation of the deuterium doublet of a sample of oriented C_6D_6 spinning at the same angle.¹⁹ The chemical shift of the k th carbon in the OMAS spectrum, $\delta^k(\theta)$, for $0^\circ \leq \theta \leq \theta_m$ is given by⁸

$$\delta^k(\theta) = \delta^k(\text{iso}) + [\delta^k(\text{st}) - \delta^k(\text{iso})](3 \cos^2 \theta - 1)/2 \quad (1)$$

where $\delta^k(\text{st}) - \delta^k(\text{iso}) = \delta^k(\text{aniso})$ represents the anisotropic part of the chemical shift in the nematic phase. There are two unknowns in Eqn (1), namely $\delta^k(\theta)$ and $\delta^k(\text{st})$. However, Eqn (1) represents a unique trajectory of the chemical shift for each carbon as a function of θ , which is related to the chemical shift tensor of the carbon.⁴ Therefore, a pair of $\delta(\text{st})$ and $\delta(\theta)$, provided by the static and the OMAS spectra, respectively, that satisfy Eqn (1) should identify the correct carbon chemical shifts for the k th carbon.

We have used the above idea to evolve an automated procedure for assignment of spectra in oriented static liquid crystalline samples. The details of the procedure are as follows. We assume that the carbon lines in all the spectra are resolved and that the chemical shifts of all the carbons in the isotropic phase are known. Let $\delta_p(\theta)$, with p ranging from 1 to N , represent the chemical shifts of the N peaks in the experimental OMAS spectrum. We rewrite Eqn (1) as

$$\delta_p^k(\text{st}) = \delta^k(\text{iso}) + \{[\delta_p(\theta) - \delta^k(\text{iso})]/[(3 \cos^2 \theta - 1)/2]\} \quad (2)$$

From Eqn (2), we obtain $\delta_p^k(\text{st})$, which is a manifold of N calculated chemical shifts corresponding to carbon k obtained using the experimental OMAS spectrum. One of the values in this manifold will correspond to the correct chemical shift $\delta^k(\text{st})$ of the k th carbon, which can be identified by comparison with the experimental spectrum of the static oriented sample. This is illustrated diagrammatically in Fig. 1.

In practical situations, there will be deviations from the ideal case shown in Fig. 1 and the spectra will also be much more crowded. For this purpose, we have devised the following numerical procedure for identifying the lines in the static spectrum. Each of the N values of $\delta_p^k(\text{st})$ obtained from Eqn 2 are compared with the chemical shifts $\delta_e(\text{st})$ of the N peaks observed in the experimental static spectrum and differences $(\Delta\delta)_{pe}^k = |\delta_p^k(\text{st}) - \delta_e(\text{st})|$ are then calculated. There will be N^2 such differences and the least of these values will identify the resonance of carbon k in the static spectrum.

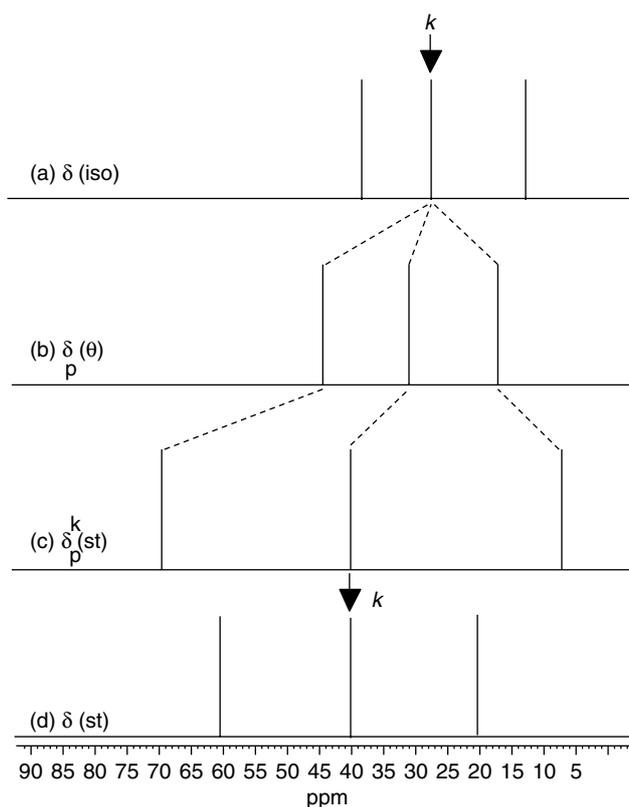


Figure 1. Illustration of the procedure used for assigning the spectrum of static oriented liquid crystalline sample using the spectrum in the isotropic phase with the help of an OMAS spectrum. A three-line spectrum is used as an example. Corresponding to carbon k in the isotropic spectrum (a), a set of three line positions shown in (c) are calculated using Eqn (2), using all three lines of the OMAS spectrum (b). Of these, only one has the exact frequency as a line in the spectrum of the static sample (d), which is then assigned to carbon k .

In this procedure, the possibility of ambiguous assignments arises. A fully automated approach to resolve all the ambiguities would be to compare all possible combinations of calculated frequencies with the experimental spectra and perform a χ^2 test for each of such assignment. However if the ambiguities are few in number, it might be simpler to follow the strategy described below. For example, we consider the case of a line e' in the spectrum of the static sample having a chemical shift $\delta_{e'}$ being assigned to two different carbons l and m having isotropic chemical shifts $\delta^l(\text{iso})$ and $\delta^m(\text{iso})$. This will happen if both $(\Delta\delta)_{pe'}^l$ and $(\Delta\delta)_{pe'}^m$ are minimum in the manifold of $\Delta\delta$ s calculated for carbons l and m , respectively [for simplicity of notation, we use here p as a dummy index which will correspond to any one of the N calculated values obtained from Eqn (2), whereas the superscript identifies a specific peak in the isotropic spectrum and the index e in the subscript corresponds to a specific line in the experimental static spectrum]. In such a case we may assign e' to the carbon giving a lower value of $\Delta\delta$ and find an alternative assignment for the other carbon. We have used another strategy, which we consider could be more reliable because the differences $\Delta\delta$ would be more significant, reducing the

probability for error in assignment. This is to look for alternative assignments for both carbons l and m by examining the second minimum difference in the two manifolds. Let these be given by $(\Delta\delta)_{pe''}^l$ and $(\Delta\delta)_{pe''}^m$ with, say, $(\Delta\delta)_{pe''}^l$ being less than $\Delta\delta_{pe''}^m$. This means that the assignment of carbon l to line e'' is more likely than the assignment of carbon m to line e'' . Therefore, carbon l is assigned to line e'' . The assignment of carbon m is reverted back to line e' . The above algorithm has been automated and implemented using a C language program (this program, AROMAS_LC, is available on request from the authors). The results are illustrated for the liquid crystal EBBA.

EXPERIMENTAL

Experiments were carried out on a Bruker DSX 300 NMR spectrometer operating at a carbon resonance frequency of 75.47 MHz using a 5 mm variable-angle spinning DOTY probe. The angle of spinning is set accurately by recording the deuterium spectrum of C_6D_6 dissolved in a liquid crystal and monitoring the deuterium doublet.¹⁹ The proton decoupled ^{13}C spectrum of a static sample of EBBA was recorded in its nematic phase at 320 K. The MAS and the OMAS ^{13}C spectra of EBBA at the same temperature were also recorded with the sample spinning at 2 kHz. The assignment of the spectrum at the magic angle was taken to be the same as that of the spectrum in the isotropic phase, which was

obtained with the help of standard 2D experiments. The ^{13}C spectra recorded at the magic angle and at $\theta = 46.7^\circ$ along with the spectrum of the static sample are shown in Fig. 2.

RESULTS AND DISCUSSIONS

As an illustration of the method used, the three methylene carbons α , β and γ of EBBA are considered. Any one of the three peaks in the chemical shift range 20–40 ppm in the experimental spectrum [Fig. 2(c)] can correspond to either α , β or γ resonances. We start with the α -carbon and combine the isotropic chemical shift for the α -carbon with the three peaks in the OMAS spectrum in Eqn (2) to generate three calculated line positions for $\delta_{\alpha p}^{\text{st}}$. Similarly, three lines each are calculated corresponding to β and γ . The chemical shift differences of these three lines with each of the three experimental lines in Fig. 2(c) are shown in Table 1. From these differences, it is seen that for the γ -carbon the assignment of the peak in the oriented phase is straightforward, since the peak at 23.3 ppm provides the minimum difference ($\Delta\delta$). The assignment for the α - and β -carbons is not straightforward since the peak at 27.3 ppm has the minimum difference for both the carbons in their respective manifolds. To resolve this difficulty, $\Delta\delta$ for other lines in the two manifolds are considered. The next higher minimum values of $\Delta\delta$

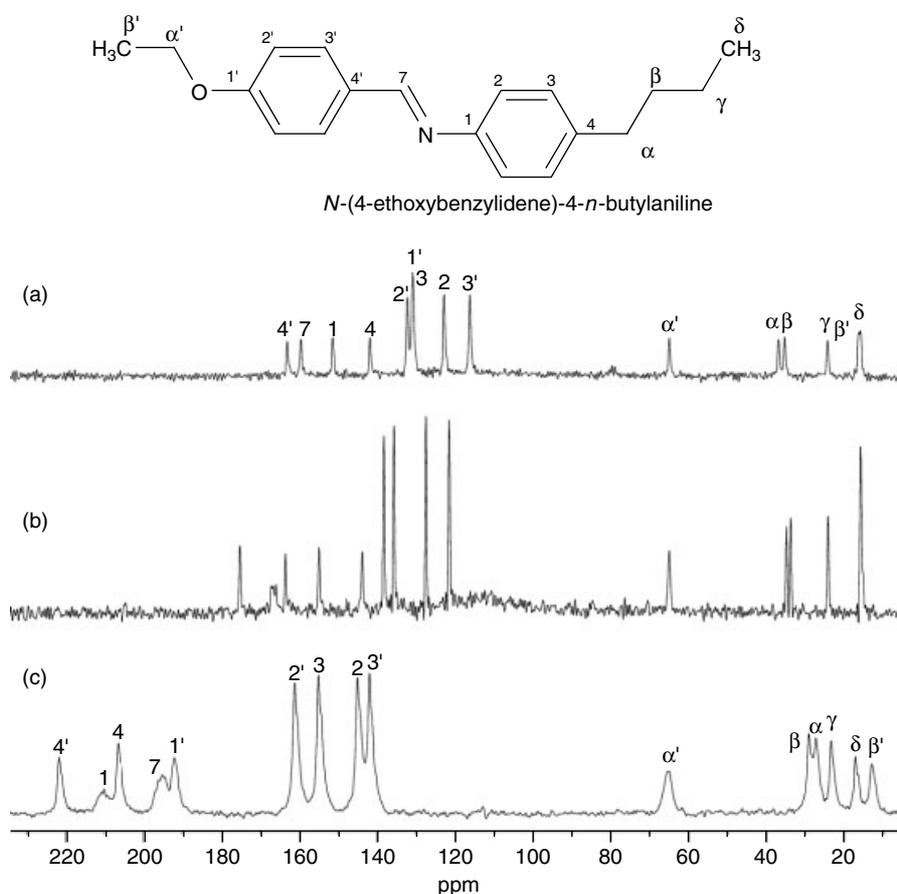


Figure 2. Proton decoupled ^{13}C spectra of EBBA recorded on a Bruker DSX-300 NMR spectrometer at 75.47 MHz using a 5 mm DOTY variable-angle probe at 320 K. (a) MAS spectrum; (b) OMAS spectrum with $\theta = 46.67^\circ$; (c) spectrum of the static sample.

for α - and β -carbons are 1.5 and 1.1, corresponding to peaks at 23.3 and 28.9 ppm, respectively. Since in this case $\Delta\delta$ for β is smaller than $\Delta\delta$ for α , it is justifiable to assign the peak at 28.9 ppm to β and for α we assign the peak at 27.3 ppm. Hence the final assignment for the α -, β - and γ -carbons are 27.3, 28.9 and 23.3 ppm, respectively.

The above procedure was carried out for all the carbons of EBBA and the final assignments are shown in Table 2. In order to confirm these results, OMAS spectra at a number of angles were obtained and are shown in Fig. 3. Similar spectra were obtained by Teeaar *et al.*⁴ In their spectra, the methyl resonances (δ , β') and also the α - and β -carbon resonances were not resolved whereas they were resolved in our experiment. The variation of the chemical shift of the carbons with the angle of spinning shows interesting behavior, with the trajectories of α and β and also as those of β' and δ crossing over each other [Fig. 3(b)]. The crossover of the methyl resonances on going from the isotropic to the nematic

Table 1. Assignment of α -, β - and γ -carbon peaks of EBBA in the oriented phase

Isotropic $\delta^k(\text{iso})$ (ppm)	$\delta_p(\theta)$ at 46.67°		Experimental δ_e (st) (ppm)	Difference $(\Delta\delta)_{pe}^k$ (ppm)	Oriented phase (ppm)
	Calculated $\delta_p^k(\text{st})$ (ppm)	Experimental δ_e (st) (ppm)			
36.9 ppm (α)	34.97	27.2	27.3	0.1 ^a	27.3
			28.9	1.7	
			23.3	3.9	
	33.9	21.8	27.3	5.5	
			28.9	7.1	
			23.3	1.5 ^b	
24.1	-25.25	27.3	52.6		
		28.9	54.2		
		23.3	48.6		
35.5 ppm (β)	34.97	33.1	27.3	5.8	28.9
			28.9	4.2	
			23.3	9.8	
	33.9	27.8	27.3	0.5 ^a	
			28.9	1.1 ^b	
			23.3	4.5	
24.1	-19.3	27.3	46.6		
		28.9	48.2		
		23.3	42.6		
24.3 ppm (γ)	34.97	76.1	27.3	48.8	23.3
			28.9	47.2	
			23.3	52.8	
	33.9	70.8	27.3	43.5	
			28.9	41.9	
			23.3	47.5	
24.1	23.7	27.3	3.6		
		28.9	5.2		
		23.3	0.4 ^a		

^a Smallest difference used for assigning the peak in the oriented phase.

^b Next higher difference used in case there is a conflict in assignment.

Table 2. ^{13}C chemical shifts of EBBA in ppm in isotropic, OMAS and static conditions

Carbon	$\sigma_{\text{isotropic}}$	$\sigma_{\text{OMAS}} (46.7^\circ)$	σ_{Static}
4'	163.1	175.2	221.6
7	159.1	166.6	195.0
1	151.6	163.6	210.1
4	141.5	154.9	206.4
2'	132	138.2	161.2
1'	131.4	143.7	191.9
3	130.5	135.6	155.1
2	122.6	127.4	144.9
3'	116.3	121.4	141.8
α'	65.0	65.0	65.0
α	36.9	34.9	27.3
β	35.5	33.9	28.9
γ	24.3	24.1	23.3
β'	16.2	15.2	12.9
δ	15.4	15.8	17.1

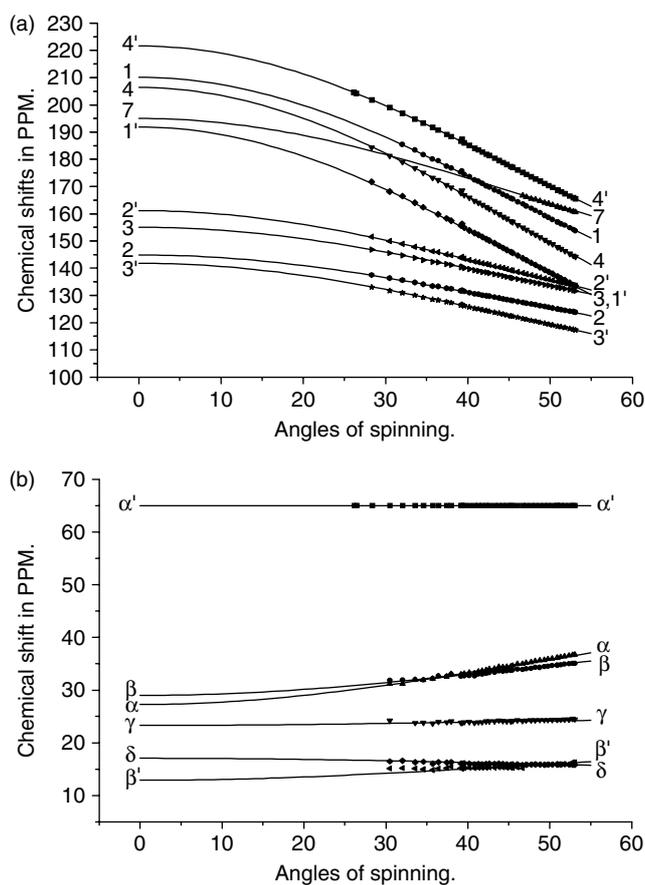


Figure 3. Variation of ^{13}C chemical shifts of EBBA as a function of the spinning angle: (a) aromatic region; (b) aliphatic region.

phase has also been seen earlier and understood on the basis of the order parameters of the respective carbon sites.²⁰ From these trajectories of the chemical shifts extrapolated to $\theta = 0^\circ$ shown in Fig. 3, the assignment of the lines in the static sample can be obtained. A comparison of the results in Fig. 3

with the values of chemical shifts listed in Table 2 confirms that the method proposed in this paper provides the correct assignments for all the carbons, including those cases where the trajectories crossover, such as δ and β' , α and β , $1'$ and $2'$ and also 4, 1 and 7 carbons.

CONCLUSION

We have proposed a method for obtaining ^{13}C chemical shift assignments of liquid crystal molecules in their oriented phases from the 1D OMAS spectrum recorded at just one angle and using a simple algorithm. We have also demonstrated the technique for the case of EBBA. In developing the present procedure, we assumed that the resonances are resolved in all the spectra. In practice, it would be useful to have the OMAS spectra at more than one angle, so that even if there are overlaps in a particular region for one angle, they should be resolved for the other angles. It might be mentioned that the spinning angle θ should be known fairly accurately, as otherwise it might lead to wrong or ambiguous assignments. In view of the availability of techniques which can provide the angles with accuracy, we believe that the proposed method will prove to be a useful alternative.

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