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Enantiomeric discrimination of water soluble compounds using deuterium NMR in a glucopon/buffered water/*n*-hexanol chiral lyotropic liquid crystal

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Abstract—Enantiomers of water soluble materials can be observed using deuterium NMR spectroscopy in the lyotropic mesophase formed by glucopon/hexanol/buffered water. The orientational properties of this mesophase is evaluated and the dependence of the enantiomeric discrimination on the temperature and the composition of the mesophase is studied.

1. Introduction

The use of chiral liquid crystalline systems as solvents for NMR has proved to be a method of choice for enantiomeric discrimination and analysis.[1] When dissolved in such media, enantiomers do not assume the same orientation.[2] Consequently, all the order dependent NMR interactions are different for enantiomers, namely the chemical shift anisotropy, $\Delta\sigma_i$, the dipolar spin–spin couplings, D_{ij} and the quadrupolar splitting and $\Delta\nu_Q^i$, for spins >1/2 such as deuterium.[3] This technique appears more general than the conventional isotropic methods. One of the reasons lies in the tremendous sensitivity of anisotropic magnetic interactions to molecular orientation, noticeably in the case of quadrupolar splitting. The best results in enantiomeric analysis were obtained using lyotropic liquid crystals made of synthetic homopolypeptides, mainly poly-(γ-benzyl-L-glutamate), dissolved in various organic solvents such as chloroform, dioxane, tetrahydrofuran and dimethylformamide.[4–7] This technique is also efficient for the analysis of mixtures of diastereoisomers even in the case of compounds bearing remote stereogenic carbons.[8]

Recently, we developed a methodology based on the combination of a nonchiral hydrophilic liquid crystal, composed of a cromolin–water mesophase, and β-cyclodextrins as a chiral cage. Such a system proved to be efficient in the discrimination of enantiomers of some small organic molecules.[9]

The mesophases described above are only useful for molecules, which are soluble in organic solvents. Currently, we lack of a water-containing chiral lyotropic liquid crystal suitable for the analysis of water soluble materials.

Only a few aqueous-based liquid crystalline systems efficient in enantiomeric discrimination by NMR have been reported.[10–13] Thus Tracey and Diehl discriminated the enantiomers of L-alanine by ^{1}H NMR using a sodium *n*-decylsulfate/(−)-sodium decyl-2-sulfate/sodium sulfate/decanol/water system.[10] Later, it was shown that such a discrimination could also be achieved using a potassium *N*-dodecanoyl-L-alaninate/decanol/caesium chloride/water mesophase.[11–13] However, this four component system appeared rather unfriendly to use due to the complexity of the phase diagram and to the instability of the mesophase when dissolving some chiral guest molecules.[14]

Recently, Baczko et al. reported the synthesis of new sulfonated L-alanine and L-phenylalanine surfactant derivatives whose association with some chlorinated

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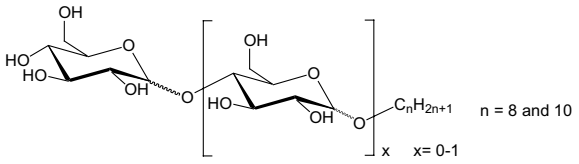
solvents and water led to liquid crystalline systems. The use of such mesophases gave some success in enantiomeric discrimination of 2-deuteroalanine through proton and deuterium NMR.[15]

A few years ago, Rückert and Otting proposed a set of water-based liquid crystalline media of interest as solvents for NMR studies of macromolecules.[16] Among these, the chiral glucopon/hexanol/water system came to our attention. Glucopon is a cheap commercially available aqueous solution of a mixture of alkylpolyglucosides, APG. This mixture is mainly composed of octyl and decyl α- and β-mono and diglucosides (Scheme 1).



Scheme 1.

The phase diagram of the pseudo-ternary system formed by glucopon/*n*-hexanol/buffered water has been established by Stradner et al.[17] In their study, water was replaced by 0.1 M Tris buffer. It reveals the existence of a lamellar phase, L_α, which is stable upon a wide domain of APG and cosurfactant weight fraction.

If enantiomers of a water soluble compound do not assume the same orientation in glucopon mesophase, then we expect all the NMR order sensitive magnetic interactions to be different. We therefore chose to study deuterated aminoacids, in order to take advantage of the high sensitivity of the quadrupolar interaction of deuterium to order. The purpose of this work is to show that enantiomers of water soluble materials can be observed using deuterium NMR spectroscopy in a glucopon liquid crystalline solvent. The orientational properties of such a mesophase are evaluated and the dependence of enantiomeric discrimination on the temperature and the composition of mesophase is studied.

2. Results and discussion

Commercial glucopon solution has a high pH value to avoid microbial attack. In this study, Tris buffer was used instead of water in order to lower the pH value to 6–8. The composition of mesophases is given in APG and *n*-hexanol weight fractions expressed in percentage.

The orientation of glucopon mesophase in the NMR magnet is not spontaneous and is time consuming. In order to obtain a satisfactory orientation, samples were heated to the isotropic phase (335–345 K depending on APG weight fraction) then gradually cooled in the NMR magnet. Using this procedure, the final orientation, monitored by deuterium quadrupolar splittings, was reached within 20–30 min.

The proton-decoupled deuterium, $^2H–\{^1H\}$, NMR spectrum of a racemic mixture of 2-deuteroalanine dissolved in glucopon mesophase (APG 9.5%, hexanol 4.0%) is shown in Figure 1a. It exhibits two broad signals due to water deuterium observed at a natural abundance level, and four additional sharp signals. The latter were interpreted as two quadrupolar doublets centred on the same chemical shift. In other words enantiomers are discriminated and each of them gives a quadrupolar doublet. In order to confirm this interpretation, a sample with L-2-deuteroalanine (38% ee) was prepared and led to the NMR spectrum shown in Figure 1b. Thus, the use of glucopon mesophase allows the discrimination of the enantiomers of a water soluble compound. Besides, the enantiomeric excess measured from the NMR spectrum using a deconvolution procedure (31% ee) was in quite good agreement with that of the sample.

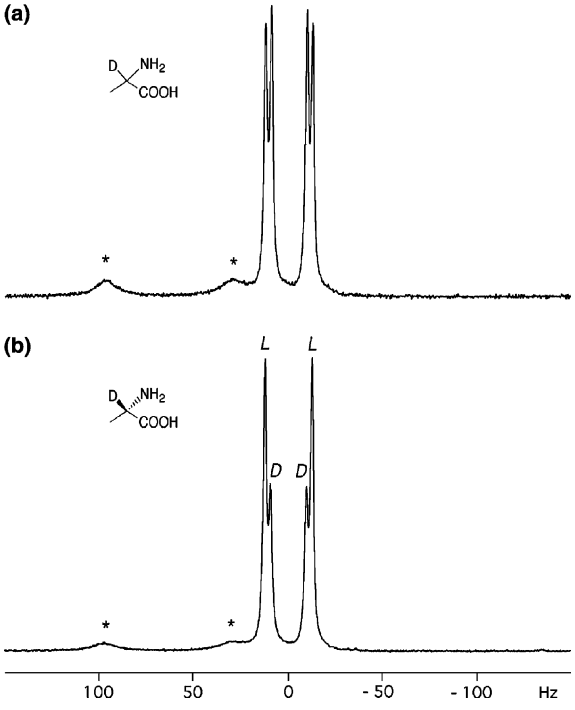


Figure 1. $^2H–\{^1H\}$ NMR spectrum in glucopon mesophase (APG 9.5%, *n*-hexanol 4.0%) at $T = 300$ K of (a) *racemic* 2-deuteroalanine and (b) L-2-deuteroalanine 38% ee. The broad signals marked with * are due to water observed at a natural abundance level.

The effect of temperature on the $^2H–\{^1H\}$ NMR spectrum was studied for this sample and the results obtained are summarized in Table 1. This table contains the quadrupolar splitting for each enantiomer, $\Delta\nu_Q^1$ and $\Delta\nu_Q^2$, and the average half-height linewidth, LW measured on the signal of each isomer. On cooling the sample in the magnet, the pure oriented phase appeared around 335 K and was found stable down to 280 K. Below this temperature, a severe line broadening was observed. Quadrupolar splittings of both enantiomers

Table 1. Quadrupolar splittings, $\Delta\nu_Q^1$ and $\Delta\nu_Q^2$, and average linewidth, LW, measured by ^2H–$\{^1H\}$ NMR spectroscopy for enantiomers of 2-deuteroalanine

	APG/HexOH (wt %)	Solute	T (K)	$\Delta\nu_Q^1$ (Hz)	$\Delta\nu_Q^2$ (Hz)	LW (Hz)	$\Delta\nu_Q^1 - \Delta\nu_Q^2$ (Hz)
1	9.5/4.0	*racemic*	300	25	19	2.4	4
2	9.5/4.0	L-*enriched*	330	19 (L)	16 (D)	1.6	3
3			320	22 (L)	18 (D)	1.8	4
4			300	25 (L)	19 (D)	2.3	6
5			290	25 (L)	19 (D)	2.9	6
6			280	25 (L)	18 (D)	3.5	7
7	18.5/6.8	*racemic*	320	58	45	3.8	13
8			300	62	45	3.7	17

The composition of the mesophase is expressed in APG and *n*-hexanol (HexOH) weight percent.

vary only slightly as the temperature decreases and seem to reach a limit at 300 K. The increase of the enantiomeric discrimination reflected by $(\Delta\nu_Q^1 - \Delta\nu_Q^2)$ is somehow counterbalanced by an increase in the linewidth.

It is not obvious to study the effect of the weight fraction of APG on the NMR spectrum. Indeed, increasing this parameter results in the onset of a biphasic system.[17] Thus one has to increase both APG and *n*-hexanol weight fractions for the system to remain in the L_α phase. We therefore prepared a sample with a high APG content (APG 18.5% hexanol 6.8%) (Table 1, entry 3). This resulted in:

(a) An enlargement of the domain of stability of the liquid crystalline phase towards high temperature. Thus, for this sample, the L_α phase was found stable up to 342 K.
(b) An increase in the quadrupolar splittings, in the enantiomeric discrimination and in the linewidth. Nevertheless, the orientation of this sample in the NMR magnetic field was far longer than when using a smaller APG weight fraction. Such an orientation time was found rather inconsistent with a routine NMR analysis technique.

Another water soluble aminoacid has been studied using deuterium NMR in glucopon mesophase: 3,3-dideuterophenylalanine. For this compound one expects a quadrupolar doublet for each of the diastereotopic deuterons in each enantiomer. Thus, for a racemic mixture, four quadrupolar doublets of equal intensity should be observed. The ^2H–$\{^1H\}$ NMR spectrum for this compound in a glucopon mesophase (APG 6.2%, hexanol 3.0%) is shown in Figure 2. The inner doublet corresponds to the signal of water in natural abundance. The outer doublet with the largest intensity corresponds to

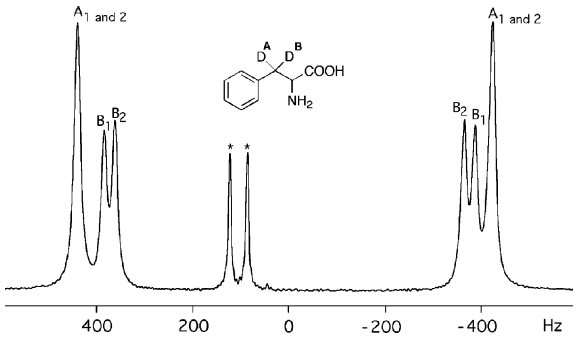


Figure 2. ^2H–$\{^1H\}$ NMR spectrum in glucopon mesophase (APG 6.2%, *n*-hexanol 3.0%) at $T = 300$ K of racemic 3,3-dideuterophenylalanine. The broad signals marked with * are due to water observed at a natural abundance level. The diastereotopic deuterons are labelled A and B.

the signal of the less shielded deuterium in the molecule. On this site, there is no enantiomeric discrimination. The last two doublets are due to signal of the second deuterium, one for each enantiomer. That enantiomers are not discriminated simultaneously on all the deuterium atoms is a known phenomenon.[4] This indicates that the absolute value of the order parameter associated with the carbon–deuterium bond of the nondiscriminating deuterium is the same for these enantiomers. Four liquid crystalline samples were prepared for this deuterated phenylalanine with different APG and *n*-hexanol content. The results obtained are summarized in Table 2. As already observed, increasing both APG and *n*-hexanol content in the mixture results in an increase of the overall orientation of the solute. It also induces a dramatic raise in the linewidth.

For a given APG weight fraction, quadrupolar splittings and therefore the order induced on the solute increase

Table 2. Quadrupolar splittings for the diastereotopic deuterons A ($\Delta\nu_Q^A$) and B ($\Delta\nu_Q^{B1}$ and $\Delta\nu_Q^{B2}$), and average linewidth, LW, measured at 300 K by ^2H–$\{^1H\}$ NMR spectroscopy for enantiomers of (±)-3,3-dideuterophenylalanine

	APG/HexOH (wt %)	$\Delta\nu_Q^A$ (Hz)	LW (Hz)	$\Delta\nu_Q^{B1}$ (Hz)	$\Delta\nu_Q^{B2}$ (Hz)	LW (Hz)	$\Delta\nu_Q^{B1} - \Delta\nu_Q^{B2}$ (Hz)
1	6.2/2.8	816	26	731	688	23	43
2	6.2/3.0	865	17	773	728	18	45
3	9.4/3.2	1322	33	1184	1113	34	71
4	9.4/3.4	1361	40	1213	1145	43	68

The composition of the mesophase is expressed in APG and *n*-hexanol (HexOH) weight percent.

with an increase of *n*-hexanol content in the sample. It appears that the order parameter of the lyotropic mesophase increases with *n*-hexanol content. In contrast the effect of the alcohol content on the linewidth is rather unclear. Thus for the 6.2% glucopon sample, increasing the alcohol content results in a decrease in the linewidth whereas an opposite behaviour is observed with the 9.4% glucopon sample (Table 2, entries 3–4). At this time, we have no explanation for this phenomenon but we think that it might be related to the complexity of the phase diagram of this pseudo-ternary mixture.

3. Conclusion

In conclusion we have shown that water soluble enantiomers exhibit different ordering properties when dissolved in glucopon liquid crystal. This allowed the enantiomers of two aminoacids to be discriminated through proton-decoupled deuterium NMR. Although the deuterium NMR linewidth are not yet totally satisfactory, an estimation of the enantiomeric excess is possible. Presently, we are working on the optimization of the linewidth and the chiral discriminating power of this inexpensive lyotropic mesophase. Besides the behaviour of enantiomers of other classes of water soluble compounds is under investigation.

4. Experimental

4.1. Materials

Glucopon 215 CS UP was purchased from FLUKA as a 65% aqueous solution of alkylpolyglucosides. Tris-(hydroxymethyl)-aminomethane hydrochloride (99%) was purchased from Acros Organics. Amino-acids, 2-deuteroalanine (98.8% D), L-2-deuteroalanine (98% D) and 3,3-dideuterophenylalanine (98% D) were purchased from Isotec™. All these materials were used without further purification.

4.2. Sample preparation

A mother solution of APG is prepared by diluting the commercial glucopon to 20–10% using a 0.1 M tris buffer solution (tris-(hydroxymethyl)-aminomethane hydrochloride) serving as a source of APG. An adequate amount of this solution is weighted into an NMR tube completed with buffered water and hexanol. After a vigorous shaking, 2–5 mg of solute are added. The sample is again vigorously shaken till obtaining a homogeneous viscous solution. The composition of the samples indicated in the text were calculated without taking into account the solute whose weight fraction was less than 1%.

4.3. Deuterium NMR measurements

^{2}H–$\{^{1}H\}$ NMR spectra were recorded at 61.4 MHz on a Bruker DRX-400 spectrometer equipped with a selective deuterium probe. Temperature was controlled using a BVT3000 variable temperature unit. Proton broad-band decoupling was achieved using the WALTZ-16 composite pulse sequence. 5000–10000 scans were necessary to obtain a good signal to noise ratio.

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