Magnetic field dependence of residual dipolar couplings measured in dilute liquid crystalline media

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Abstract

The effect of the magnetic field strength on the magnitude of residual dipolar couplings introduced by dilute liquid crystal media was investigated. One-bond heteronuclear residual dipolar couplings and residual deuterium quadrupolar splitting of the water were monitored at various static magnetic fields. It is suggested that the value of residual anisotropic NMR parameters resulting from exchange between ordered and non-ordered molecules decreases with increasing magnetic fields.
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1. Introduction

Residual dipolar couplings (RDCs) became routinely measured NMR parameters in the structure determination of biomolecules [1] and are currently being introduced into organic chemistry [2]. Biological macromolecules are primarily studied at high magnetic fields in order to achieve higher resolution and sensitivity needed in these cases. It is known that the magnitude of the RDC measured for intrinsically aligning molecules—in cases where alignment is due to magnetic anisotropy—is increasing with the square of the magnetic field [3]. For molecules, which do not self-orient in magnetic fields, dilute liquid crystalline media are used to establish anisotropy in their rotational motions. To our knowledge, the influence of the magnetic field on the RDCs introduced by the aligning media has not been investigated in detail so far [2]. The source of alignment in this latter case is entirely different with respect to the self-orienting case as the apparent value of the RDC originates from a fast exchange between ordered and non-ordered molecules dissolved in the medium. The magnetic field dependence of the observed value of RDCs is therefore primarily determined by the exchange phenomena.

The objective of the present study is to investigate the influence of the external static magnetic field on the value of the RDCs in phospholipid based liquid crystal forming systems. To that end one-bond heteronuclear RDC of a model compound and residual quadrupolar couplings (RQC) of deuterated water were measured at various magnetic fields under strict temperature control. The influence of the exchange phenomena on the apparent value of the anisotropic NMR parameter is further evidenced by temperature dependent measurement of the aforementioned parameters.

The model system used contained DMPC/CHAPSO bicelles [4,5] at 7.5, 15, and 30% total liquid crystal concentration. The model compound used in this study is the disaccharide **1** with a disulfide interglycosidic bridge between the sugar units [6] (Scheme 1).

The unusual interglycosidic link connects two six-membered, rigid sugar units, glucose and mannose, in which the possible alignments of CH bond vectors are limited to axial and equatorial orientations. For CH

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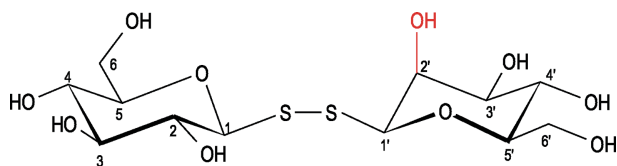
Scheme 1. Structure of the model compound: Glc-S-S-Man.

groups with identical stereochemical arrangement RDCs of comparable magnitude are anticipated, hence reliability and precision of the data can be assessed. The magnitude of the exchange related to the residual quadrupolar coupling of the deuterated water and that of the one-bond ^{1}H–^{13}C heteronuclear RDCs of the ring protons has been monitored at three different magnetic fields, 9.4, 14.1, and 16.4 T corresponding to 400, 600, and 700 MHz ^{1}H frequency, respectively.

1.1. Phospholipid based liquid crystals

Alignment with phospholipid bicelles [7–9] is the most common way to align biomolecules. The bicellar alignment medium usually consists of a long-chain phospholipid and a short-chain phospholipid or a surfactant in an aqueous medium. The long-chain phospholipid forms a stable flat bilayer in the shape of a disc, whilst the short-chain phospolipid or the detergent is located at the edges controlling the size of the disc. The latter components are preventing the long hydrophobic alkyl chains from contact with water. These aggregates adopt a nematic liquid crystalline phase at a given temperature. The individual lipid molecules possess a large magnetic susceptibility anisotropy, which does not cancel out but add up due to the cooperative effect of the long-range order. As a result, the bicelle orients itself in the presence of magnetic field with the plane parallel to the direction of the magnetic field [10–12]. The disk model is, however, subject to controversy. More recently, it was demonstrated that the morphology of these mixtures at concentrations and temperatures used for alignment in the magnetic field are stacked lamellar phases that align with their bilayer normal orthogonal to the field direction [13].

The phase behavior of phospholipid solutions is dominated by the temperature-induced main (order–disorder) phase transition associated with the melting of the lipid hydrocarbon chains. Below the main transition, a basic equilibrium structure is the gel phase. At temperatures above the main transition, lipids arrange in different liquid crystalline structures. The main phase transition occurs between a highly ordered, solid-like gel state and a fluid-like liquid crystalline state as the bilayer is heated. The phospholipid molecules are more disordered in the liquid crystalline phase than in the gel phase where they are packed in more ordered arrays. This disorder is a result of the increased thermal energy,

which produces conformational changes about the C–C bonds along the alkyl chain between staggered arrangements. The magnetic ordering is possible in the fluid-like liquid crystalline state where the bicelles possess sufficient amount of mobility to align themselves. In the presence of an external magnetic field, the solution is isotropic at room temperature in the gel state, while with increasing the temperature phase transition occurs which converts the medium into the liquid crystalline phase, which is partially ordered [14,15].

1.2. Residual anisotropic NMR parameters

The effect of the external static magnetic field on the RDCs was monitored by measuring one-bond ^{1}H–^{13}C heteronuclear RDCs of the dissolved molecules at different magnetic fields. The degree of alignment of the bicelle solution is traditionally studied by observing anisotropic interactions that originate from the molecules that actually comprise the medium [16]: these are the ^{31}P chemical shifts of the polar head group of the phospholipid and the residual quadrupolar couplings (RQC) of the deuterated water. The RQC likewise originate from an exchange between the ordered water molecules in the hydration sphere of bicelles and the bulk water. Therefore, the value of the RQC is expected to show similar tendencies with changes in the magnetic field strength.

Motions of the molecules dissolved in anisotropic media are restricted in a certain direction, hence the distribution of possible orientations become anisotropic. The exact nature of relation between molecular structural features and their alignment properties is still debated. A simple weak collisional model that depends only on the physical barriers provided by the bicelles and on the non-spherical shape of the dissolved molecules has been suggested [14]. It has been observed, however, that the degree of alignment in the anisotropic medium is not only a function of molecular three-dimensional shape, but also depends on the surface charge distribution and the density and degree of magnetic alignment of the bicelles. The molecules dissolved in the medium are assumed to be in fast exchange between the states transiently associated to the aligned assemblies and the unoriented bulk state. The degree of alignment of the dissolved molecules is only slight resulting in narrow lines. The residual dipolar coupling between two nuclei depends on their separation r, and on Θ, the angle between the internuclear vector and the static field:

$$D_{i,j} = \left(\mu_0 \frac{h}{8\pi^2 \langle r_{ij}^3 \rangle} \gamma_i \gamma_j \right) \left\langle \frac{3\cos^2 \Theta_{ij} - 1}{2} \right\rangle.$$

The angle brackets indicate time averaging over instantaneous values of angles and distances with timescales short compared to the reciprocal of the splitting and γ_i, γ_j are the gyromagnetic ratios of the spins.

Deuterated water in the medium provides another probe of orientation. Deuterium is a quadrupolar nucleus and in the presence of oriented bicelles, a small residual quadrupolar coupling results from a fast exchange between the bulk solvent, which is isotropically arranged, and the solvent associated with the bilayer, which is partially oriented. The value of the splitting is predicted to increase linearly with the lipid concentrations according to the shell model [7].

The values of the apparent RDC and RQC are resulting from a fast exchange between aligned and non-aligned molecules. The quadrupolar or dipolar splitting is zero for the bulk of the molecules, which are isotropically distributed, whereas the angular ensemble average is not zero for the molecules, which are in transient interactions with the bicelles, and a non-zero splitting is obtained. It is assumed that the two subpopulations are in fast exchange, therefore this splitting is a weighted average of splittings of the contributing exchanging sites, hence simply scaled by the fraction of population that experiences alignment. It is estimated that the degree of alignment, hence the fraction of oriented molecules is in the order of 0.001%, which scales the full value of the dipolar coupling from the kHz range up to ca. ±50 Hz [7].

The magnetic field dependence of the RDCs is of key importance due to their application in structure determination of molecules, while the RQC of the water is typically used only as a parameter to control the ordering and alignment of the media. However, residual values in dilute liquid crystalline media for both anisotropic parameters originate from an analogous exchange phenomenon, therefore it is expected that their magnetic field dependence can be explained similarly. Furthermore, the RQCs of water can be readily monitored by simple one-dimensional experiments, which are producing reproducible and precise values, while measurement of the RDCs for the dissolved molecule is more time-consuming and the obtained values are less precise.

The dipolar and quadrupolar coupling are independent of the magnetic field. Magnetic field dependence of observed lineshapes resulting from exchange is well known, it is associated to the increasing frequency difference between exchanging sites at higher magnetic fields which in turn affects the exchange situation with respect to the NMR timescale. The chemical shifts of the nuclei in the oriented and the randomly distributed molecules can be assumed to be slightly different due to the residual chemical shift anisotropy for the oriented case. The frequency difference between the exchanging sites is increasing at higher magnetic fields, which shifts the exchange from fast into the direction of medium or slow exchange rate on the NMR timescale. This in turn changes the averaged value of the observed anisotropic NMR parameter, i.e., RDC or RQC.

2. Results and discussion

The influence of the magnetic field was investigated by measuring values of the deuterium RQC of the water and the RDCs of the dissolved molecule at various magnetic field strengths. To verify the tendencies observed, the measurements were repeated on samples with different total liquid crystal concentration. Values were determined at magnetic fields 9.4, 14.1, and 16.4 T corresponding to 400, 600, and 700 MHz ^{1}H frequencies with samples containing 7.5, 15, and 30% total phospholipid and detergent concentration, respectively.

Changes of the deuterium multiplet with increasing magnetic field are shown in Fig. 1 whereas the values are summarized in Table 1. Changes of the deuterium RQC with increasing magnetic field strength are indicated in Fig. 2. The RQC values in higher magnetic field strengths were slightly decreasing. With increasing magnetic field the linewidths tend to broaden slightly. These two observations can be rationalized by considering the magnetic field dependence of the exchange phenomena. The exchange pattern on the NMR timescale is determined by the ratio between the exchange rate and the frequency difference between the signals of the different exchanging sites. The exchange rate can be assumed to be the same since the composition of the samples examined at different magnetic fields was identical and the measurement temperatures were equal. The resonance frequency difference between the two exchanging sites, however, will increase at higher magnetic field which in turn changes the ratio between the exchange rate and frequency difference slightly shifting the exchange pattern on the NMR timescale from fast exchange condition towards intermediate exchange. This explains the decrease of the apparent value of the RQC and the slight increase in linewidths.

To prove the influence of the exchange phenomena on the apparent value of the deuterium RQC, temperature dependence of the magnitude of the deuterium RQC at different magnetic fields was compared. The temperature dependence was examined only for the 15% total liquid crystal concentration sample. The change of the deuterium RQC with temperature is shown in Fig. 3. After the phase transition temperature, the splitting roughly linearly increases with the temperature. Following the initial linear regime the increase of splittings slows down resulting in a saturation type curve. Our qualitative interpretation of this behavior is the following: the temperature increase will increase the kinetic energy of the bicelle forming lipid molecules which initially helps to overcome the energy barrier needed for the re-orientation of the bicelles into the ordered liquid crystalline phase. Later, the increased kinetic energy, however, acts against the magnetic field-induced ordering. Comparison of the curves obtained at 400 and 700 MHz shows a constant difference between the values of the deuterium RQC.
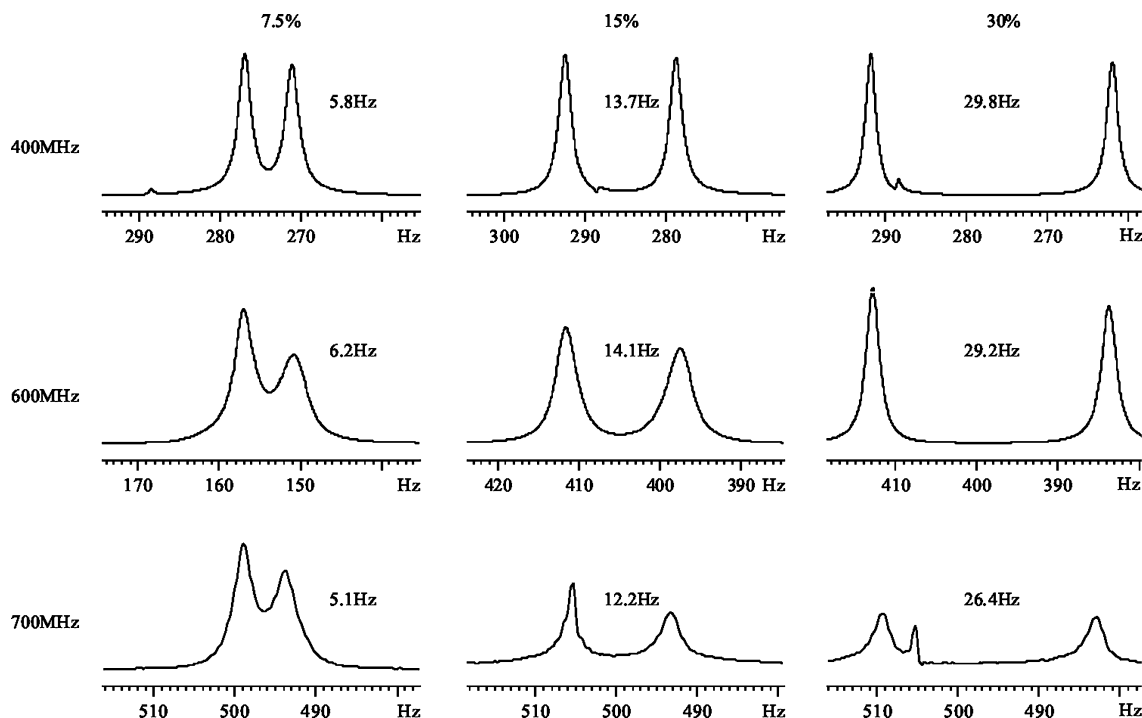
Fig. 1. Change of residual deuterium quadrupolar splitting with the concentration of the liquid crystal (7.5, 15, and 30% in rows) and the magnetic field (400, 600, and 700 MHz in columns).

Table 1
Change of deuterium quadrupolar splitting with the liquid crystal concentration and the magnetic field

^{2}D RQC (MHz)	7.5%	15%	30%
400	5.8	13.7	29.8
24,600	6.2	14.1	29.2
700	5.1	12.2	26.4
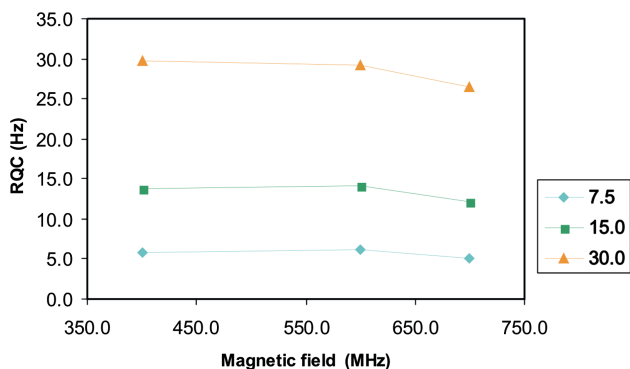
Fig. 2. Deuterium residual quadupolar splitting versus increasing magnetic field.

Fig. 3. Change of deuterium RQC with temperature at 400 and 700 MHz.

The RDCs of **1** at different magnetic fields are summarized in Table 2. The changes of the one-bond heteronuclear RDC corresponding to all CH groups in the model compound for 15 and 30% total liquid crystal concentration against different magnetic fields are shown in Fig. 4. At 7.5% total liquid crystal concentration, the precision of the data is hardly smaller than the
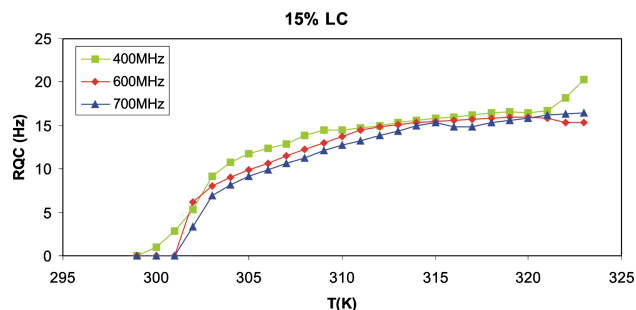
effect to be measured, hence this sample condition was excluded from the consideration of the magnetic field dependence of the RDCs. To increase the effect to be measured, the liquid crystal concentration was therefore increased to 15 and 30% and measured for all CH bond vectors of both sugar units.

The values measured at 400 MHz are in all cases larger than that of determined at 700 MHz. Out of 20 measured dipolar contributions nine cases show a monotonically decreasing curve over all three magnetic fields. The values measured at 600 MHz are larger than those of at 400 MHz in six cases (pairs are shown in italics in Table 2), nevertheless both values are still larger than the value determined at 700 MHz for that given CH moiety. In another five cases, the values at 700 MHz are larger than those at 600 MHz (pairs are

Table 2
One-bond heteronuclear residual dipolar couplings for the CH moieties in Glc-S-S-Man for different liquid crystal concentrations and magnetic field strengths

Assignment	Total liquid crystal concentration					
	15%			30%		
	400	600	700	400	600	700
G1	*4.9*	*5.8*	3.2	10.5	8.4	7.8
G2	4.5	0.4	−0.7	7.2	3.3	1.1
G3	6.5	*−0.2*	*1.2*	8.1	0.1	0.1
G4	8.50	6.1	3.7	*12.6*	*16.5*	*5.9*
G5	*3.4*	5.6	3.3	*7.9*	*8.2*	*6.6*
M1	*8.6*	*10.5*	7.3	18	16.7	16.2
M3	7.6	6	4.4	15.8	*15.1*	*15.5*
M4	14.3	*5.4*	*8.1*	20.3	16.8	15.7
M5	12.4	*6.1*	*8.9*	20.5	*17*	*17.3*
M2	0.8	0.4	−0.1	2.2	2.7	0.3

shown in italics in Table 2), but again both of them are smaller than the value at 400 MHz.

The reliability of the data set can also be assessed by comparing the magnitudes of RDCs for CH bond vectors with similar orientations. The ring geometries of the constituent monosaccharide units in the oligosaccharide can be considered rigid, therefore only two feasible orientations are present: axial and equatorial. All five CH bond vectors are axial in the glucose unit for which the corresponding RDC values are expected to be of comparable magnitude, while there are four axial CH bonds in mannose together with an equatorial C2–H2 group, hence the former ones are expected to be alike, while the last one is anticipated to display a value dissimilar to the values for axial CH vectors. An average RDC value can be calculated for the axial CH bond vectors in glucose and mannose at a given liquid crystal concentration measured at a specific magnetic field as shown in Table 3. The average of deviations from the average RDC value is suitable for assessing the precision of the measurement and to assess the reliability of the data for the axial bond vectors as listed in Table 4. In the glucose unit the RDCs for the axial G1, G2, G3, G4, and G5 bond vectors at 400 MHz are showing relatively similar values at all three total liquid crystal concentrations, while at 600 and 700 MHz the magnitude of RDCs for the G2 and G3 vectors have smaller values than those of the other axial CH vectors. The deviations listed in Table 4 range from 0.9 to 4.5 Hz for the glucose with an average of 2.1 Hz. In the mannose unit, the RDCs for the axial bond vectors are displaying values fairly alike for a given liquid crystal concentration measured at a specific magnetic field with the exception of the value for M5 at 7.5% and 700 MHz which is significantly smaller than the other values for these conditions. In contrast, the RDC for the M2 bond vector in mannose is dissimilar compared to the average values for the axial CH groups at all liquid crystal concentrations and magnetic fields, thus reflecting the equatorial orientation of the

respective proton and confirming the reliability of the data for the mannose unit. The deviations for the axial groups of the mannose part listed in Table 4 range from 0.6 to 2.6 Hz with an average of 1.5 Hz which is considerably better than that for the glucose unit.

On the basis of these considerations, tendencies displayed at M1, M3, M4, and probably M5 at liquid crystal concentrations 15 and 30% are regarded to be the most important with respect to deducing magnetic field dependency of RDCs and they are therefore highlighted with bold lines in Fig. 4. The RDCs are decreasing with increasing magnetic field, similar to the tendencies obtained for the deuterium RQCs. Since the RDCs originate from exchange between the oriented and the non-aligned state, this tendency can be explained likewise as in the case of the magnetic field dependence of the deuterium RQCs. Thus, it can be assumed that the aligned and the non-aligned molecules have different chemical shifts and the frequency difference between their signals is larger at higher magnetic fields, which shifts the exchange pattern on the NMR timescale from fast to intermediate exchange. This in turn results in smaller apparent value of the RDC.

The temperature dependence of the RDCs at different magnetic fields was also examined, although for only one liquid crystal concentration and for only one CH moiety. The magnetic field dependence of the dipolar contribution for the CH groups in the model compound was measured by using the BIRD modified F_1 coupled HSQC [17] which ensures precise determination of small RDCs. Determination of the temperature dependence with this method, however, would be extremely time-consuming. The F_2 coupled version of the technique is complicated by strong coupling effects resulting in asymmetric multiplets as demonstrated in our previous contribution [17]. The anomeric Glc-CH-1 multiplet is, however, well separated from its coupling partner, the Glc-CH-2, in the ^{1}H dimension, therefore second order effects do not compromise the extraction of the dipolar
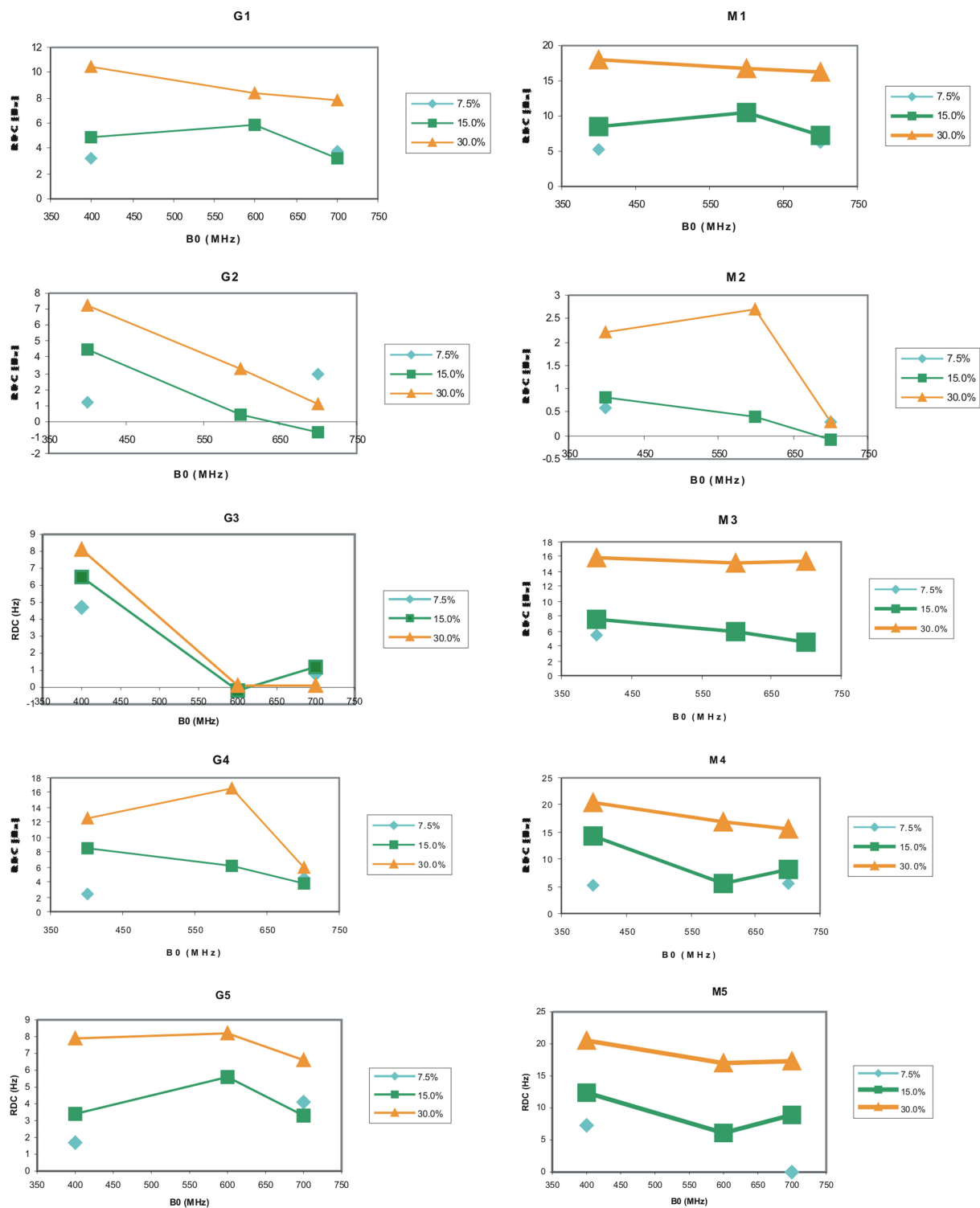
Fig. 4. Dependence of one-bond heteronuclear residual dipolar couplings on the magnetic field at different liquid crystal concentrations.

contribution. The temperature dependence curves of the one-bond RDCs of the anomeric Glc-CH-1 multiplet for the oriented sample containing 15% total liquid crystal concentration were chosen to be determined at three different magnetic fields and are indicated in Fig. 5. The values show significantly higher noise than the values of the deuterium RQC, but the tendency of obtaining lower values at higher magnetic fields can be observed. This confirms the effect of the exchange on the apparent value of the RDC.

Table 3
Average RDC values for axial CH bond vectors for a given liquid crystal concentration and measured at a three different magnetic fields

MHz	7.5%	15%	30%
Glucose			
400	2.6	5.6	9.3
600	—	3.5	7.3
700	3.2	2.1	4.3
Mannose			
400	5.9	10.7	18.7
600	—	7.0	16.4
700	4.1	7.2	16.2

Table 4
Average of deviations of RDCs calculated for the CH bond vectors with identical axial orientation for a given liquid concentration measured at different magnetic fields

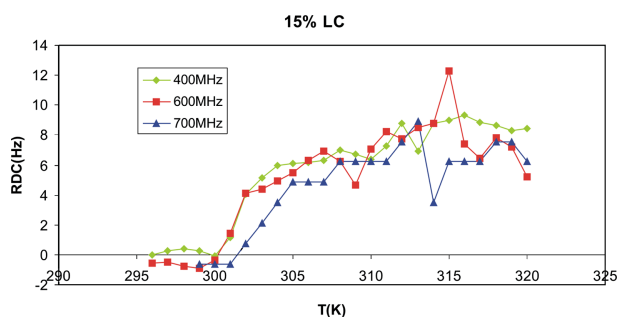| MHz	7.5%	15%	30%
Glucose			
400	0.9	1.6	1.8
600	—	2.8	4.5
700	1.1	1.5	3.0
Mannose			
400	1.2	2.6	1.8
600	—	1.8	0.7
700	2.0	1.4	0.6

Fig. 5. Temperature dependence of one-bond heteronuclear RDC at different magnetic fields.

3. Conclusion

In summary, the anisotropic residual NMR parameters result from exchange and decrease at higher magnetic fields. The overall trends observed for the magnetic field dependence of the deuterium RQC and the one-bond heteronuclear RDC were similar and confirmed each other. This magnetic field dependence is opposite to the case of intrinsic alignment induced by the magnetic field where the degree of alignment and the magnitude of the RDCs are increasing with the square of the magnetic field. Measuring RDCs in dilute liquid crystalline media at higher magnetic fields can therefore be disadvantageous in case of small values.

4. Experimental

4.1. Sample preparation

For alignment, bicelles were formed from a mixture of dimyristoyl-DL-α-phosphatidylcholine 1,2-ditetradecanoyl-rac-glycerol-3-phosphocholine (DMPC) and a detergent, 3-(cholamidopropyl) dimethylammonio-2-OH-1-propensulfonate (CHAPSO) in the ratio of 1:3.5 with various total concentrations [4,5].

The model compound used in this study was a disaccharide with a special type of interglycosidic linkage comprising of a disulfide bridge connecting a glucose and a mannose at the anomeric position; both have β orientation as depicted in Scheme 1.

The substance to be investigated was dissolved in 0.7 ml D_2O. One milligram of NaN_3 and one milligram of DSS were added to the solution. Weighted amount of DMPC was dissolved in the previous solution resulting in a milky, but still easily flowing, liquid. The solution was carefully homogenized by vortexing, then a premeasured amount of CHAPSO was added. By adding the detergent the consistency changes giving a semi-transparent, opalescent gel, which practically does not flow. The mixture was homogenized by vortexing. Thorough homogenization is crucial for obtaining a stable liquid crystal phase, however mixing using ultrasound should be avoided. The presence of microscopic inhomogeneities was checked by centrifuging at 4000*g* for 5 min. If centrifugation yielded phase separation, additional cycles of vortexing/centrifuging were repeated. If the sample formed one, homogeneous, semi-transparent, slightly opalescent phase, it was cooled on ice which reduces viscosity and makes it easier to transfer to the NMR tube by using a Pasteur pipette. Upon warming above 325 K, irreversible disruption of the phase was observed in several cases. A stable medium is perfectly clear, but extremely viscous under 299 K and becomes milky above 299 K where thermal-induced phase transition from to gel to liquid crystal occur.

4.2. NMR measurements

All experiments were performed on Bruker DRX-400, 600, and Avance 700 spectrometers (Bruker AG, Rheinstetten, Germany) equipped with TBI z-gradient probes. All spectra were processed with XWINNMR 3.1 (Bruker AG, Karlsruhe, Germany).

The temperature for measurements under isotropic conditions was set to 297 K while for anisotropic conditions to 308 K. The nominal temperature displayed at the spectrometer unit was calibrated by using ethylene glycol [18].

The deuterium signal of the solvent, which is usually used for frequency-field locking, was split by the residual quadrupolar coupling. Locking was performed on one

signal components by manually optimizing parameters of the lock channel. Magnetic field homogenizing was executed by shimming on ^{1}H FID integral.

^{2}H spectra were recorded using 1 scan with switching out the field-frequency lock. The resonance frequency of ^{2}H signal showed significant drift between measurements conducted under unlocked conditions, hence the chemical shift scales of spectra presented are obscured by the drift of the main static field.

For the measurement of ^{1}H, ^{13}C residual dipolar couplings F_1 coupled gradient selected, sensitivity enhanced HSQC spectra modified with a G-BIRD module [17] were used. The spectral window in the indirect dimension was typically 20 ppm giving a frequency span of 2000 Hz at 400 MHz, 2500 Hz at 600 MHz, and 3500 MHz at 700 MHz. Acquiring 4K t_1 experiments yielded ca. 0.49, 0.61, and 0.85 Hz spectral resolution, respectively. Columns of the 2D map were extracted and Fourier transformed. Linear prediction and zero filling were used to increase digital resolution, the time domain data were apodized with squared cosine window function prior to 2D Fourier transformation to yield a digital resolution of 0.25, 0.30, and 0.43 Hz. Each 1D trace was individually phase corrected. Peak maxima were identified using the peak picking utility of the XWINNMR 3.1 program.

Measurement of RDCs displayed at various temperatures was performed by using non-decoupled gradient selected sensitivity enhanced HMQC. Quadrature detection in the indirect dimension was achieved by using the echo–antiecho method. The dipolar contribution was extracted only for the Gly-C1 multiplet, where strong coupling effects in the ^{1}H dimension [17] can be safely excluded due to the separation of the anomeric multiplet from the multiplet of the Glc-C2. 4k points of the FID were acquired resulting in an acquisition time of 1s giving 0.5 Hz FID resolution. The time domain data were zero filled to 8K digital points, multiplied by a shifted squared sine function. After Fourier transformation rows corresponding to the Glc-C1 multiplet were extracted and individually phase corrected.

Acknowledgment

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