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Self-association of bis-(α,β-D-glucopyranosyl)-polyisobutylene

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Abstract—In this paper, we report the structure and apparent molecular weights of bis-(α,β-D-glucopyranosyl)-polyisobutylene (Gluc-PIB-Gluc) aggregates in $CDCl_3$ by NMR spectroscopy. Analysis of DOSY (diffusion-ordered NMR spectroscopy) experiments of a solution of Gluc-PIB-Gluc showed the presence of aggregates that were corroborated with dynamic light scattering. The structure of the aggregates was also studied by correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) experiments.

Keywords: ^{1}H, ^{13}C NMR; Diffusion-ordered NMR; Self-assembly; Glucose; Polyisobutylene

1. Introduction

It is known that several types of block copolymers form micelle-like aggregates when dissolved in a block-selective solvent.[1–5] Amphiphilic block copolymers, that is, block copolymers possessing hydrophilic and hydrophobic chains are interesting from both academic and practical points of view.[6–15] The self-assembly of amphiphilic block copolymers into various types of polymer aggregates, for example, micelles, vesicules, or rod-like associates can be expected when dissolving them in water (preferred for the hydrophilic blocks) or in organic solvents (preferred for the hydrophobic blocks).[6–15] Similar association phenomenon can also be expected when the hydrophilic blocks are replaced with a relatively large hydrophilic molecules, for example, glucose.[16] In one of our previous papers bis-(α,β-D-glucopyranosyl)-polyisobutylene (Gluc-PIB-Gluc) was synthesized; in this structure the PIB block and the glucose units represented hydrophobic and hydrophilic segments, respectively.[16] A dynamic light scattering study of the solutions of Gluc-PIB-Gluc in water and in tetrahydrofuran revealed the presence of aggregates (average molecular sizes are 280 and 180 nm, respectively). However, similar to other amphiphilic molecules, the aggregation behavior of Gluc-PIB-Gluc is highly dependent on the block-selective solvent used. Although DLS gives information on micellar parameters such as average particle size and particle size distribution, it does not provide any particular information about the structure of the polymer aggregates. Modern NMR methods, such as NOESY and DOSY are able to provide information not only about average particle size but also about the structure of the polymer aggregates formed. Application of DOSY for the determination of sugar size or the molecular weights of the aggregates has also been reported.[17,18] In this article, we report the investigation of the polymer aggregates formed from Gluc-PIB-Gluc in $CDCl_3$, the preferred solvent for the PIB segment, but not the Gluc-portion. The structure of Gluc-PIB-Gluc is shown in Scheme 1.

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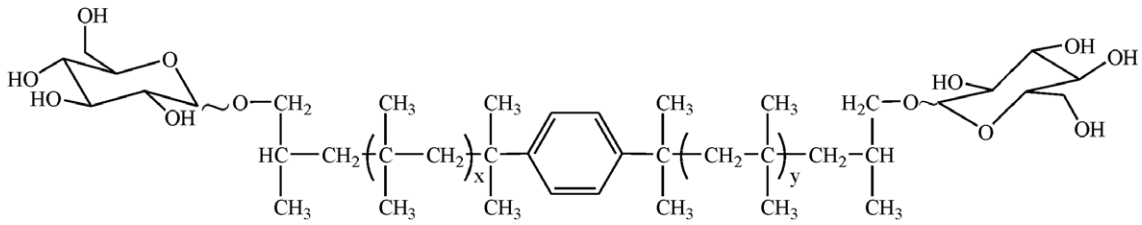

Scheme 1. Structure of bis-(α,β-D-glucopyranosyl)-polyisobutylene (Gluc-PIB-Gluc).

2. Results and discussion

The partial COSY spectrum of Gluc-PIB-Gluc with assignments of the resonances are shown in Figure 1. The unlabelled peaks in Figure 1 belong to the methylene protons of the polyisobutylene chain-ends. In addition, the glucopyranose skeleton protons of the α anomer are of much weaker intensities.

The aggregates formed from Gluc-PIB-Gluc in $CDCl_3$ were studied by NOESY spectroscopy (Fig. 2). The signals due to intra-sugar, through-space interactions are of like phase with respect to the diagonal. This fact indicates that the glucopyranosyl rings lack the mobility expected for the terminal parts of the molecules. Most probably, reverse micelle-formation is responsible for this inflexibility because of the vicinity of the intermolecular glucopyranosyl end-groups. Possible hydrogen bonding and other intermolecular interactions may hinder internal motions within the aggregates. In β-D-glucopyranosides the 1,2-*trans*-diaxial NOE interaction is generally weak (because of the large intra-ring distance) but in case of Gluc-PIB-Gluc the anomeric hydrogen (H1 in Fig. 1) showed a strong interaction with H2, which is probably due to reverse micelle generation. The 1,2-*trans*-diaxial interaction was observed in the NOESY spectrum even at 3.3 mg/mL concentration.

Three NOESY cross peaks are clearly observed for the α anomeric proton in Figure 2. The strongest cross peak at 3.58 ppm is assigned to H2 of the α anomer and the two unassigned cross peaks of lower intensity may arise from inter-ring NOEs. These peaks cannot be unambiguously assigned because of spectral overlap but they must arise from inter-residue effects because of the probable $^4C_1$ conformation of the α anomer. Even in the case of the improbable alternative chair conformation of the α anomer, we would have only two intra-unit NOEs (H1–H2, H1–H5), which are not observed.

Not surprisingly, the β anomeric proton has more NOE cross peaks because it has intra-unit H1–H3 and H1–H5 NOE cross peaks, in addition to inter-ring NOEs. Based on the NOESY spectrum (Fig. 2), it has been proven that the polymer molecules having α-linked glucopyranose units are also aggregated. Furthermore, all carbohydrate–carbohydrate NOE cross peaks in the

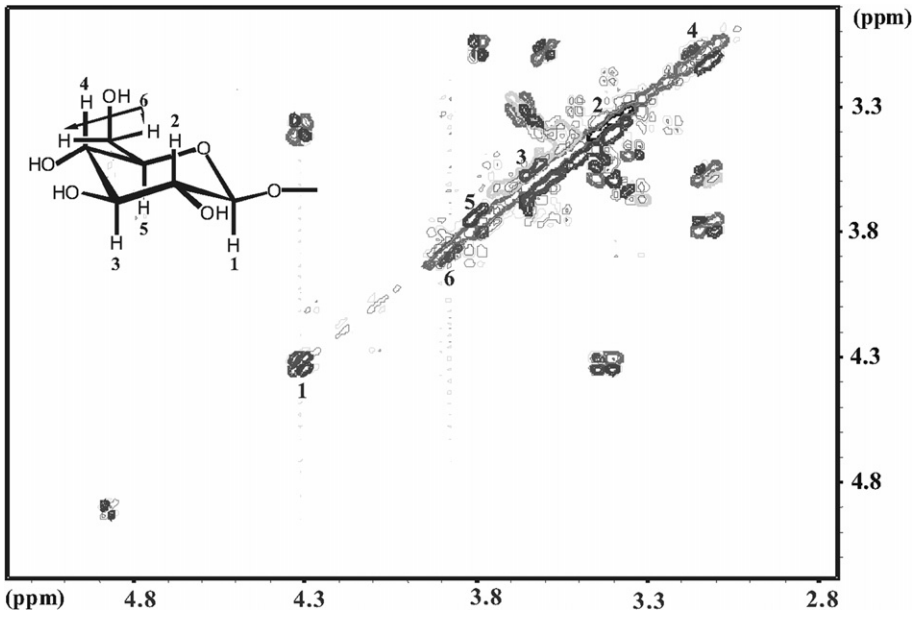

Figure 1. Partial COSY spectrum of bis-(α,β-D-glucopyranosyl)-polyisobutylene with assignments.

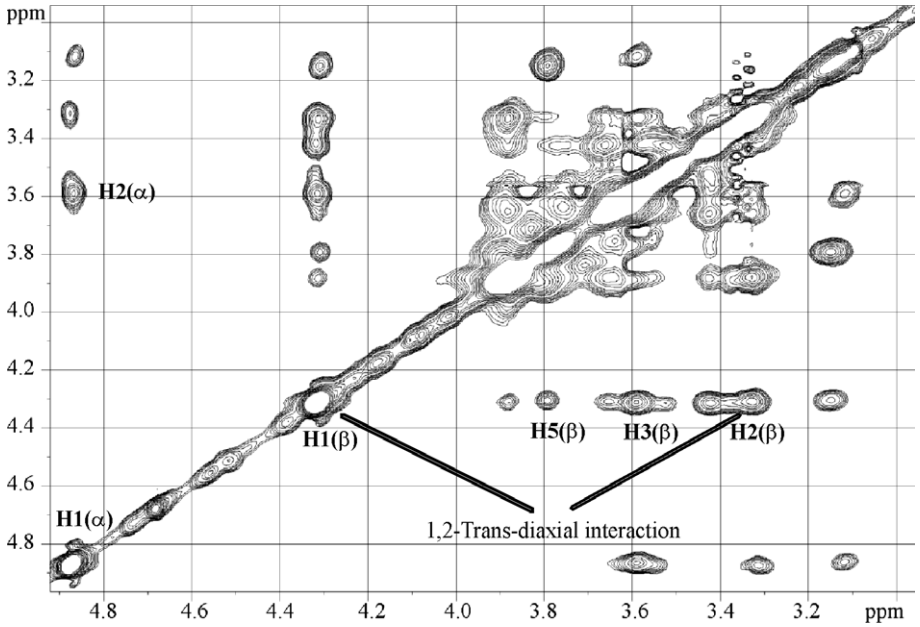

Figure 2. Partial NOESY (150 ms mixing time) spectrum of bis-(α,β-D-glucopyranosyl)-polyisobutylene $c = 16.7$ mg/mL.

3–5 ppm region have like sign with respect to the diagonal (negative NOE range). This fact also supports that the carbohydrate portions of the polymers belong to the less flexible parts of the aggregates.

The partial NOESY spectrum of the methyl and methylene protons of polyisobutylene revealed that the signals corresponding to the methyl and methylene protons of polyisobutylene are of negative phase with respect to the diagonal (positive NOE), which indicates that the polyisobutylene chains are relatively flexible in spite of reverse micelle-formation, even at higher concentrations (Fig. 3).

The molecular weight of the aggregates was studied by DOSY (diffusion-ordered spectroscopy) NMR using tetramethylsilane (TMS) or toluene as the internal reference. The DOSY technique has been successfully applied to the analysis of mixtures,[19] for the characterization of aggregates or carbohydrate aggregates[17,18,20] and for the study of intermolecular interactions.[21] The diffusion data were also processed by Bruker's 'pseudo'

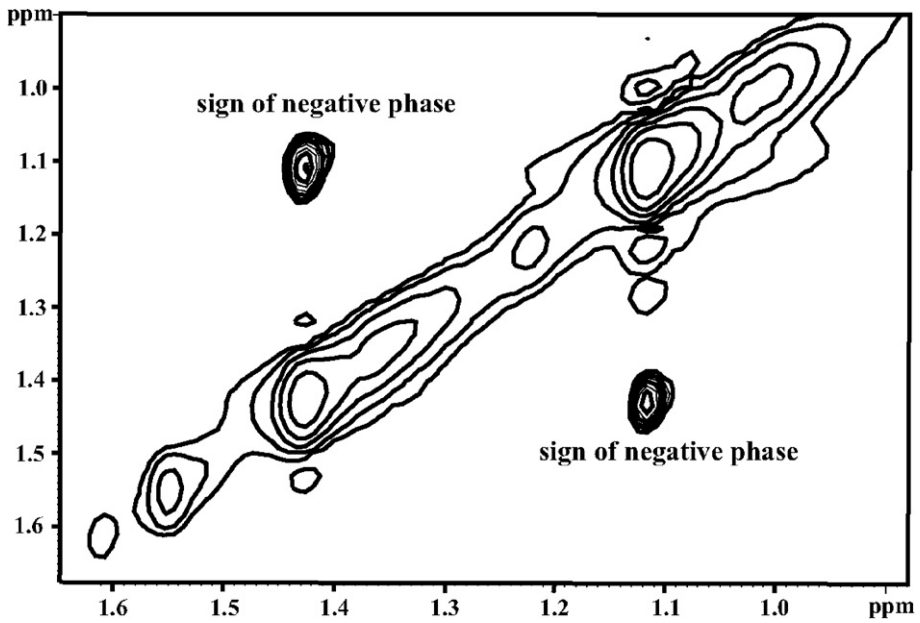

Figure 3. Partial NOESY spectrum (200 ms mixing time) of bis-(α,β-D-glucopyranosyl)-polyisobutylene. The region of methyl and methylene protons of polyisobutylene $c = 167$ mg/mL.

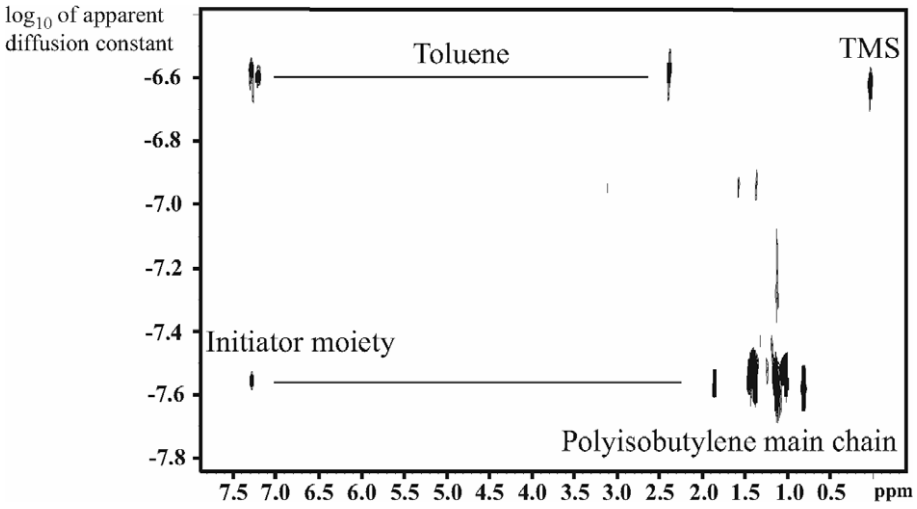

Figure 4. ^{1}H DOSY spectrum of bis-(α,β-D-glucopyranosyl)-polyisobutylene $c = 16.7$ mg/mL, reference compound: toluene.

inverse Laplace transformation routine (Bruker XWINNMR 2.6 DOSY package, adjustable parameters: STE, biexp mode, offset fit = YES, Si1 = 128, Sdev = 0.04, solution bonds = 1–1000, solution grid = log-quad). The result is shown in the DOSY spectrum (Fig. 4).

It can be seen that TMS and toluene appear at nearly the same apparent diffusion constant at a $\log_{10}$ scale due to their similar molecular weights (88 and 92 g/mol, respectively) and the mass of the polymer aggregates is much larger than that of the individual polymer. Molecular mass of Gluc-PIB-Gluc was referenced to toluene, because of its faster relaxation compared to TMS. Because Bruker's 'pseudo' inverse Laplace transformation routine is considered to be less accurate than exponential fitting of Stejskal–Tanner equation (Eq. 3), the latter was used to evaluate diffusion constants (Fig. 5).

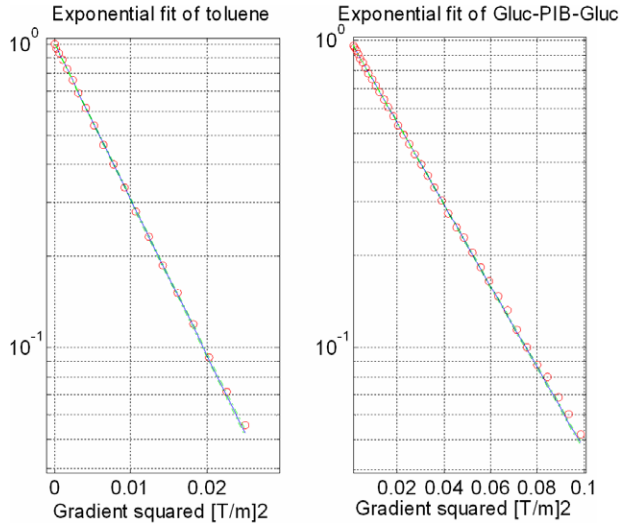

Figure 5. Single exponential fit of toluene and bis-(α,β-D-glucopyranosyl)-polyisobutylene with the 5% error bounds.

The Einstein–Stokes equation (Eq. 1) relates the translational self-diffusion coefficient D of an individual molecule to its hydrodynamic radius at infinite dilution, where k is the Boltzmann constant, η is the viscosity of the solvent and r_{hs} is the hydrodynamic radius of the spherical molecule

$$D = \frac{kT}{6\pi\eta r_{hs}} \tag{1}$$

Then, according to Waldeck et al.,[22] molecular masses and diffusion constants of the unknown particles and those of the reference compound can be related with the restrictions of spherical approach and dilute solutions (Eq. 2) where M_1 and M_{ref} are the molecular weight of the aggregates and the internal reference compound, respectively, and D_1 and D_{ref} is the diffusion coefficient of the aggregates and the reference compound, respectively

$$\frac{M_1}{M_{ref}} = \left(\frac{D_{ref}}{D_1}\right)^3 \tag{2}$$

The apparent molecular weight of the aggregate (65 ± 6 kDa) was obtained using Eq. 4 (see Section 3.3). Diffusion constants of the polymer aggregates were obtained from single exponential fits of the decaying CH_2 main chain signal (Eq. 1). The molecular weight obtained for the Gluc-PIB-Gluc aggregates in $CDCl_3$ corresponds to an aggregation number of approximately 30. It was also found that at low concentration (3.3 mg/L) aggregates with relatively small aggregation number (ca. 4) were formed.

The dependence of the molecular weight of the aggregates on the Gluc-PIB-Gluc concentration indicates an open association mechanism for the polymer-aggregate formation.[23] In addition, based on the NOESY experiments, due to the lack of mobility of the glucopyranosyl rings and higher flexibility of the PIB chains in the

aggregates, it can be suggested that the glucopyranosyl rings form the core, while the PIB chain constitutes the shell.[23]

3. Experimental

3.1. Materials

$CDCl_3$ was obtained from Aldrich (Germany); all other chemicals were obtained as described previously.[16]

3.2. Synthesis of bis-(α,β-D-glucopyranosyl)-polyisobutylene (Gluc-PIB-Gluc)

Gluc-PIB-Gluc was synthesized as described[16] from the corresponding dihydroxy-telechelic polyisobutylene HO-PIB-OH. The number-average molecular weight (M_n) of HO-PIB-OH was determined by ^{1}H NMR spectroscopy, size exclusion chromatography, and MALDI-TOF spectrometry; the values obtained were 1980, 1910, and 1950 g/mol, respectively. The M_n and M_w/M_n values of Gluc-PIB-Gluc were 2300 g/mol and 1.1, respectively.

3.3. Diffusion NMR experiments

Translational diffusion measurements were performed with a Bruker DRX 500 instrument at 300 K using Bruker's 'ledbpgs2s' stimulated echo DOSY pulse sequence including bipolar and spoil gradients. This was extended with a 2×4 ms spin echo period before detection period to suppress baseline shift (Fig. 6). Sine shaped gradient strength was linearly incremented in 32 steps between 2% and 95% of the upper limit of 0.5 T/m B_0 field gradient of the 5 mm inverse broadband probehead. The diffusion delay was always 100 ms, and the gradient pulse duration was typically 2–3 ms. Correction for gradient shape was considered according to the sine shape and square gradient area ratios ($2/\pi$), and apparent diffusion coefficients were obtained using a single exponential fit of Stejskal–Tanner equation (Eq. 3)[24]

$$\ln(R) = -\gamma^2 D G^2 \{\delta^2(\Delta - \delta/3) + B\} \qquad (3)$$

where R is the ratio of detected signal intensity in the presence and absence of gradients, D is the translational self-diffusion coefficient, γ is the gyromagnetic ratio of the considered nucleus, G is the B_0 gradient strength, δ is the gradient pulse length and Δ is the diffusion delay. Because we measured the diffusion constants using short gradient pulses, the B correction factor was disregarded.[24]

The exact equation for sine gradients was calculated by Eq. 4 as described by Berger and co-workers,[25] where g is the maximum gradient strength

$$\ln(R) = -\gamma^2 D g^2 \delta^2 \frac{4\Delta - \delta}{\pi^2} \qquad (4)$$

Instead of this equation, we used the original Stejskal–Tanner equation ($B = 0$); however, the average gradient strength of a sine-bell gradient was applied (the ratio is $2/\pi$ with respect to a square gradient). Using our approach with the pertinent experimental parameters, the diffusion constants obtained were virtually indistinguishable from the results obtained by the latter formula.

Application of internal standard compensates for viscosity changes between different samples and to some extent against experimental errors within the same sample.[25] Using toluene (2 mg/600 μL) as internal standard, the apparent mass referenced to the standard was obtained. The 90° ^{1}H pulse was typically 11 μs. Typical DOSY experiments lasted 0.25–1 h depending on the acquired scans (8–32) and increments (32). Processed 1D spectra were baseline corrected and intensities of the main peak and the reference were fitted (Eq. 1) with an in-house written MATLAB code. Signals below 5% of the starting intensity were omitted. Gradient calibration has been carried out using 500 μL of D_2O/H_2O (99:1) sample doped with 0.5 mg of $CuSO_4$ at 25 °C, using a nominal $D = 2.299 \times 10^{-9}$ m^2/s value.[26] Disregarding relaxation for the diffusion standard in Eq. 3 caused only a 1.3% error. Because the experiments are normalized to the smallest gradient experiment, and the diffusion delay is constant, errors due to relaxation can be safely neglected.

DOSY experimental problems of convection[27–30] were negligible due to the limited sample volume and an air

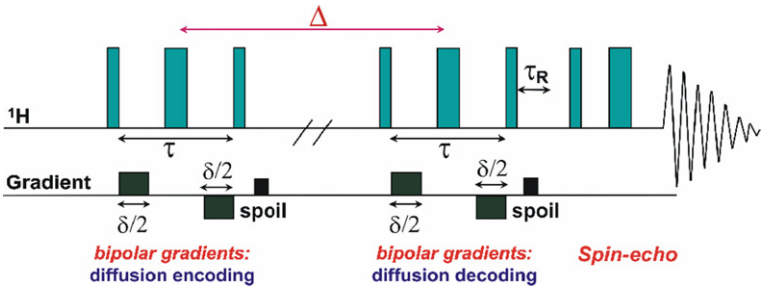

Figure 6. Bruker's 'ledbpgs2s' stimulated echo DOSY pulse sequence including bipolar and spoil gradients extended with a spin echo period.

flow of 0.2 L/s of the variable temperature control unit. Furthermore, diffusion experiments of polymer standards of polystyrene (22 kDa) and PEG (35 kDa) showed no deviations from single exponential decay of the main signal that was observed in the high gradient range. The absence of convection problem could also be proven by using different diffusion times. In these cases, the obtained diffusion constants—processed by single exponential fitting—agreed within 5%. DOSY spectra were generated using the manufacturer's biexponential fitting routine.

4. Conclusions

A detailed NMR study of bis-(α,β-D-glucopyranosyl)-polyisobutylene in $CDCl_3$ was accomplished. Based on NOESY experiments, the terminal glucopyranosyl part of the molecule is inflexible in contrast to the 'internal' polyisobutylene chain, indicating that the interaction that holds the molecules together in the aggregate is mainly due to the glucopyranosyl rings. We have estimated the molecular weight of the aggregates at various concentrations applying DOSY NMR. We found that the apparent molecular weight of the aggregates increases significantly with the concentration.

Acknowledgments

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