THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Reversed Hexagonal Lyotropic Liquid-Crystal and Open-Shell Glycodendrimers as Potential Vehicles for Sustained Release of Sodium Diclofenac

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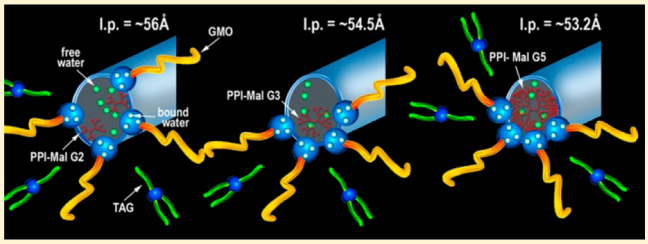
ABSTRACT: The effect of second, third, and fifth generations of poly(propylene imine) glycodendrimers-open maltose shell (PPI-Mal) on reverse hexagonal (H_{II}) mesophase and on the release of sodium diclofenac (Na-DFC) drug was investigated. The H_{II} mesophase comprised glycerol monooleate (GMO)/tricaprylin (TAG) in a weight ratio of 90/10 and 20 wt % water (+0.5 wt % PPI-Mal of each generation) without or with 0.25 wt % (Na-DFC). The microstructural characteristics of these systems were determined by small-angle X-ray scattering; attenuated total reflectance Fourier transform infrared was used to characterize the molecular level interactions and the location of the PPI-Mal. Third-and fifth-generation PPI-Mal, because of their maltose groups, interact mainly with the bulk water within the cylinders of the H_{II} and strongly bind the water molecules, as manifested by the decrease in the lattice parameter and dehydration of the lipid headgroups. Co-solubilization of Na-DFC with the third and fifth generations caused competition of the two host compounds for water binding and induced relocation of the drug from the bulk water to the GMO−water interface. In vitro release of Na-DFC from the H_{II} showed that the release process was faster in the systems with third- and fifth-generation PPI-Mal compared with the control and second-generation systems.



INTRODUCTION

The development of suitable systems that will allow controlled release and transport of drugs to the blood system and into cells is one of the challenges in the field of drug delivery. Many studies have shown the use of lyotropic liquid crystal (LLC) systems that provided sustained release of drug molecules with a range of physicochemical properties.[1−6] LLCs are formed by mixing certain polar lipids with aqueous medium to yield well-defined geometric arrangements of the swollen micelles depending on the chemical structure and concentration of the components.[1−6] Modulating the concentration of the surfactant, aqueous medium, pH, temperature, and other physical parameters has demonstrated the ability to sustain the release of the desired drugs.[1−7]

Dendrimers are macromolecules with a unique branching structure ("treelike") that radiates from a central core and ends with peripheral functional groups.[8−13] Because of their distinctive architecture, dendrimers have generated considerable interest in the fields of drug-delivery vehicles,[14] anticancer therapies,[8] diagnostic imaging,[15] and gene transfections.[16] They are in most cases monodispersed and are of a nanometer size range, while, especially, cationic dendrimers and their polyplexes allow their easier passage through biological membranes.[17−19] Moreover, "host−guest" can take place either in the interior or on the periphery of the dendrimers. Dendrimers provide an excellent platform for the attachment of drugs, solubility modifiers, and cell-specific targeting due to their exterior groups.[20−24]

Nevertheless, for successful implantation in drug-delivery applications, dendrimers have to be nontoxic and nonimmunogenic. It was reported in several studies that dendrimers with positively charged surface groups such as poly(propylene imine) (PPI) and polyamido amine (PAMAM) are prone to destabilizing the cell membrane and causing cell lysis, especially in high generations.[25−29] This injurious effect can be reduced by partial surface modification of the cationic dendrimers with chemically inert entities such as polyethylene glycol, sugar units, or fatty acids.[10,30,31] These modifiers reduce the overall positive charge of dendrimers' surface and greatly enhance the biocompatibility of the dendrimers. Moreover, open- and dense-shell glycodendrimers (sugar unit modifiers) are materials well suited for in vitro and in vivo studies.[32,33]

In previous studies, we have proposed LLC−dendrimer systems combining the advantages of LLC to sustain the release

Received: December 24, 2013
Revised: March 10, 2014
Published: March 11, 2014

dx.doi.org/10.1021/jp4125974 | J. Phys. Chem. B 2014, 118, 4016−4024

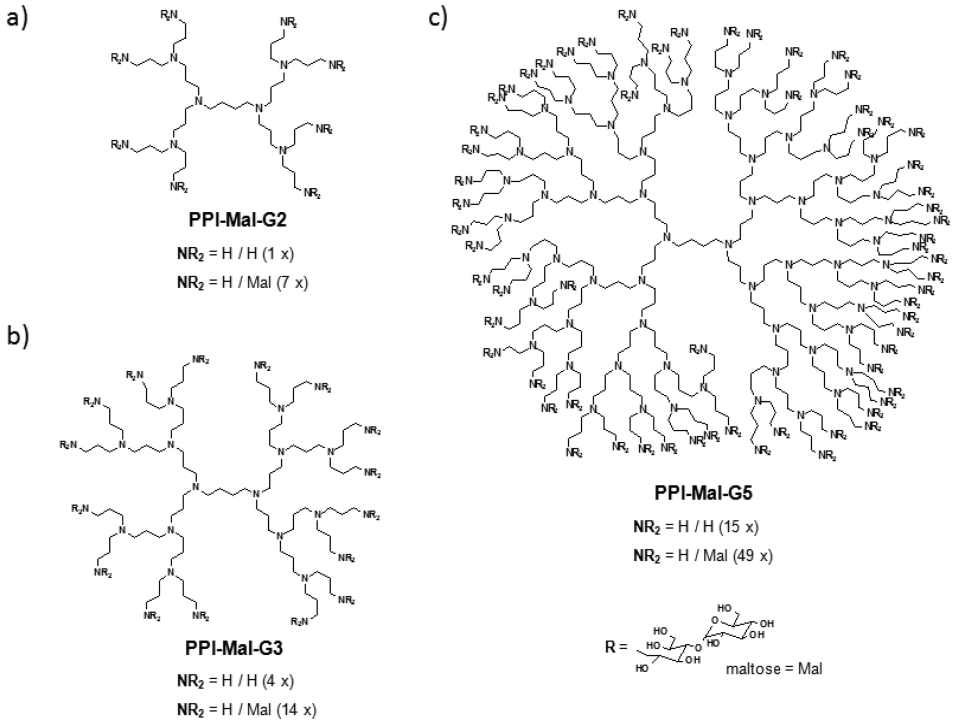


Figure 1. Chemical structure of PPI-Mal of (a) second, (b) third, and (c) fifth generations.

as well as enabling the dendrimer to pass through the membrane to the target cell with the encapsulated/conjugated drug. We solubilized high concentrations of PPI of the second generation within lamellar (L_α), reverse cubic (Q^G), and reverse hexagonal (H_{II}) mesophases.[34−36] The dendrimers caused phase transition and enabled a controlled and sustained release of the guest molecule gallic acid from the LLC into excess water. In another study we demonstrated the effect of gradual generations of PPI (second, third, and fourth) to sustain and control the release of Na-DFC drug from a Q^G mesophase.[36]

In this work, we present a new LLC−dendrimer system based on solubilization of open-shell dendritic glycopolymers as biocompatible[37,38] and also multifunctional globular architecture. The glycodendrimer based on dendritic PPI scaffold with maltose residues instead of the amino groups is also attributed to lower cationic (surface) charge in comparison with parental PPI dendrimers.[37] We demonstrate the structural behavior of H_{II} mesophase composed of glycerol monooleate (GMO), tricaprylin, and water (weight ratio of 72/8/20, respectively) with the solubilization of PPI glycodendrimer (at pH 7) and the effect on the release of sodium diclofenac (Na-DFC) drug.

Na-DFC is a nonsteroidal anti-inflammatory drug (NSAID) that is popular in treating pain and inflammation via topical, intravenous, and oral formulations. Na-DFC consumed dosage for osteoarthritis or rheumatoid arthritis is 50 mg orally two to three times a day or 75 mg orally twice a day. By controlling the release with the use of dendrimer, the uptake per day can also be reduced.

In recent work, we have already demonstrated that with higher generations of the dendrimer (PPI) the release of Na-DFC out of the mesophase was sustained. Here we examined how the modification in the surface of the dendrimer, namely, addition of the maltose units, changes the structural interactions and profile release of the same guest molecule.

EXPERIMENTAL SECTION

Materials. Distilled GMO, monoolein consisting of 97.1 wt % monoglycerides, 2.5 wt % diglycerides, and 0.4 wt % free glycerol (acid value 1.2, iodine value 68.0, melting point 37.5 °C), was purchased from Riken (Tokyo, Japan). Tricaprylin (triacylglycerols, TAG; assay 97 to 98%) was obtained from Sigma Chemical (St. Louis, MO). Na-DFC was purchased from Sigma (St. Louis, MO). D_2O (D, 99.9%) was purchased from Cambridge Isotope Laboratories (Andover, MA). Phosphate-buffered saline (PBS) was purchased from Sigma Chemical. D-(+)-Maltose monohydrate and borane-pyridine complex (8 M in THF) (BH_3·Pyr) were purchased from Fluka (Germany). Sodium tetraborate decahydrate was purchased from Sigma-Aldrich (Germany). The PPI dendrimers of second, third, and fifth generations (PPI-G2, PPI-G3, and PPI-G5) were supplied by SyMO-Chem (Eindhoven, The Netherlands). All chemicals were used without further purification.

Synthesis of Glycodendrimers (glyco-PPI). The synthesis and characterization of open-shell PPI glycodendrimers decorated with maltose units were carried out as described in previous studies.[38,39] In brief, the precursor (second, third, or fifth generation of PPI dendrimer), maltose monohydrate (360.31 g/mol), and borane−pyridine complex were taken up in a sodium borate buffer (25 mL, 0.1 M). Molar ratio between one peripheral amino group on each PPI dendrimer and maltose unit is 1:1 for all conversion steps. The solution was stirred at 50 °C for 7 days. The crude product was purified twice by dialysis with deionized water for 4 days to ensure the capture of impurities. The solid product was obtained by freeze-drying. The degree of maltose substitution on each dendritic PPI scaffold was determined by ^{1}H NMR approach.[27] The chemical composition of each glycodendrimer (PPI-Mal G2, PPI-Mal G3, and PPI-Mal G5) is presented in Figure 1.

Sample Preparation. The composition of each LLC system (empty and loaded) was as follows: In the case of H_{II} loaded

dx.doi.org/10.1021/jp4125974 | J. Phys. Chem. B 2014, 118, 4016−4024

with different PPI dendrimers, 0.5 wt % PPI-Mal (second, third, and fifth generations) was solubilized in water prior to its incorporation into the H_{II} mesophases. The H_{II} mesophase was composed of 72 wt % GMO, 8 wt % TAG (9:1 weight ratio), and 20 wt % water. In the systems with Na-DFC, 0.25 wt % was solubilized into the mesophase to yield 71.775 wt % GMO, 7.975 wt % TAG, 0.25 wt % Na-DFC, and 20 wt % water.

The hexagonal liquid crystals were prepared by mixing weighed quantities of GMO, TAG, and Na-DFC while heating to ∼70 °C. An appropriate quantity of preheated water (with glycodendrimer) at the same temperature was added, and the samples were stirred for 10 min and cooled to 25 °C.

Small-Angle X-ray Scattering (SAXS). SAXS measurements were used to identify the structure of the LLC containing GlycoPPI-G2, G3, and G5 and Na-DFC. The scattering experiments were performed using Ni-filtered Cu K_α radiation (0.154 nm) from an Elliott rotating anode X-ray generator that operated at a power rating of 1.2 kW. The X-ray radiation was further monochromated and collimated by a single Franks mirror and a series of slits and height limiters and measured by a linear position-sensitive detector. The samples were held in 1.5 mm quartz X-ray capillaries inserted into a copper block sample holder. The temperature was maintained at 25 ± 1 °C with exposure time of ca. 20−60 min. The camera constants were calibrated using anhydrous cholesterol. The scattering patterns were desmeared using the Lake procedure implemented in home-written software.[40]

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR). An Alpha P model spectrophotometer, equipped with a single reflection diamond ATR sampling module, manufactured by Bruker Optik (Ettlingen, Germany), was used to record the FTIR spectra (GMO/water with PPI and Na-DFC). The spectra were recorded in 50 scans, with spectral resolution of 2 cm^{-1}, at room temperature. The absorbance intensities reported here were reproducible to ±0.005.

ATR-FTIR Data Analysis. Multi-Gaussian fitting has been utilized to resolve individual bands in the spectra. The peaks were analyzed in terms of peak frequency, width at half-height, and area.

Release Experiments. The release experiments of Na-DFC from H_{II} mesophase were performed according to Rizwan et al.[41] as follows: 500 mg of LLC containing 0.25 wt % Na-DFC with 0.5 wt % PPI-Mal (second, third, and fifth generations) was prepared, covered with 10 mL of PBS (pH 7.4), and kept at 25 °C.

Each system was compared with its reference (without PPI-Mal) to exclude the contribution of other components to the release. Samples of 2 mL of the PBS were collected at constant times, and the Na-DFC and PPI concentrations of each sample were determined by a Cary 100 Bio UV−vis spectrophotometer (Varian, Palo Alto, CA). The Na-DFC concentrations were calculated according to the absorption at 275 nm. Each sampling was followed by insertion of 2 mL of fresh PBS. Throughout the experiment, each vial containing the LLC floating in excess of PBS was gently shaken before sampling 2 mL for UV measurements.

RESULTS AND DISCUSSION

Impact of PPI-Maltose Concentration on the Mesophase Structure. SAXS Study. SAXS measurements were conducted to elucidate the effect of three generations of open-shell cationic PPI-Mal (second, third, and fifth) at pH 7 on the lattice parameter of hexagonal phase composed of GMO/tricaprylin/water mixtures. In all mixtures, empty as well as loaded with 0.5 wt % PPI-Mal, the SAXS profiles provided evidence of the existence of three diffraction peaks, indexed as (10), (11), and (20) reflections of a 2D H_{II} phase. The lattice parameters (a) of all mixtures were calculated and are summarized in Table 1. SAXS results indicated a minor decrease in the lattice parameter in the presence of PPI-Mal second-generation dendrimer (from 57.3 ± 0.5 Å in the original control system to 56 Å) and a noticeable decrease from 57.3 ± 0.5 Å to 54.5 and 53 Å, induced by the third- and fifth-generation molecules, respectively (Figure 2). The shrinking of the lattice parameter indicating a partial dehydration of the hydrophilic heads of monoglyceride is probably due to competition for water binding between the PPI-Mal and the polar moieties of the surfactant.

Table 1. Lattice Parameters (α) of the H_{II} Systems Unloaded and Loaded with 0.5 wt % PPI-Mal of Second, Third, and Fifth generations with and without 0.25 wt % Na-DFC at Room Temperature

	no Na-DFC Å ± 0.5 Å	+Na-DFC Å ± 0.5 Å
blank	57.3	54
0.5 wt % PPI-Mal-G2	56	51.5
0.5 wt % PPI-Mal-G3	54.5	52.5
0.5 wt % PPI-Mal-G5	53	53.5

The presence of sugar-based guest molecules in the water domains has been studied by several researchers.[42,43] Saturni et al.[42] demonstrated a strong shrinking of the lattice parameter of *Pn3m* cubic mesophase induced by the solubilization of trehalose sugar molecules in the water domains. Mezzenga et al.[43] reported on the order-to-order transitions of LLCs formed by self-assembled monogylcerides (Dimodan U/J) and water in the presence of polysaccharides of various molecular weights. They showed that the presence of the sugar molecules in the aqueous domain leads to the favorable $L_\alpha \rightarrow$ cubic $\rightarrow H_{II}$ shift in lower temperature than without them. Also, they stated that because glucose is a very hydrophilic compound, which is barely partitioned in the lipid tails, it interacts mainly with the water molecules and changes the equilibrium of water molecules in the cavities of LLC phases. In addition, water molecules hydrate the polar heads of the surfactants. Thus, water molecules are in competition for binding events in LLC phases where sugar molecules are stronger hydrogen bond acceptors as headgroups of surfactants used in such multi-component systems. Moreover, the very hydrophilic sugar molecules act as a "water pump", as they reduce the lattice parameter of the mesophases (Figure 2).

We calculated the molar ratios of water and PPI-Mal (of second, third, and fifth generations) in the H_{II} phase. At 0.5 wt % of additive, the mole ratios for water/PPI-Mal are ∼33 620/1, 68 820/1, and 255 240/1 for second, third, and fifth generations, respectively, meaning that one molecule of PPI-Mal-G2 solubilizate is accommodated within 33 620 molecules of water. As the generation of the glycodendrimer increases, the number of water molecules that surround and bind the dendrimer is increased.

Finally, we can conclude that although the mole quantity of the glycodendrimers compared with water is extremely small, they induce major competition for water binding with the GMO polar headgroups, and the interaction of the high (third

dx.doi.org/10.1021/jp4125974 | J. Phys. Chem. B 2014, 118, 4016−4024

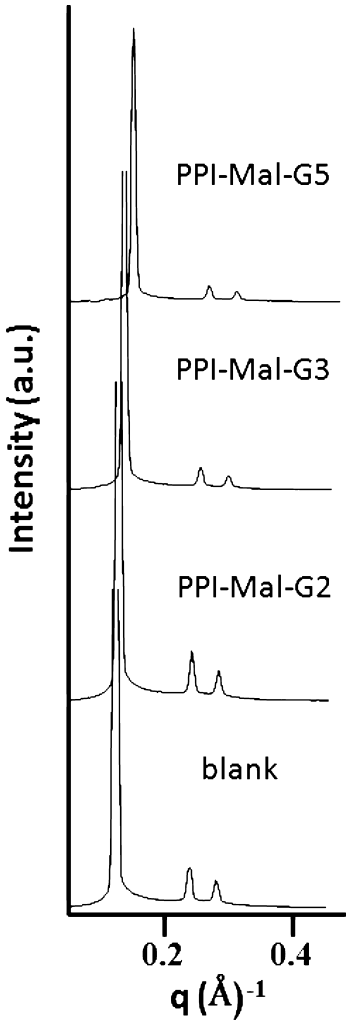


Figure 2. Small-angle X-ray diffraction patterns of the H_{II} blank, 0.5 wt % PPI-Mal-G2 loaded, 0.5 wt % PPI-Mal-G3 loaded, and 0.5 wt % PPI-Mal-G5 loaded mixtures (composed of 72 wt % GMO, 8 wt % tricaprylin, and 20 wt % aqueous phase) at room temperature.

and fifth) and hydrophilic generations with water is stronger, as was seen by SAXS.

On the basis of Mezzenga et al.'s observations, we can further conclude that the glycodendrimer molecules interfere with the water resulting in intermolecular bonding, thereby decreasing the lattice parameter and acting as a "water pump", where water molecules strongly drain glycodendrimer macromolecules of higher generations.

Following this interpretation, the PPI-Mal guest molecules are thought to interact mainly with the aqueous domains of the H_{II} system and the surfactant headgroups. We addressed this issue directly by performing ATR-FTIR analysis, as described in the following section.

ATR-FTIR Experiment. The samples loaded with 0.5 wt % PPI-Mal second, third, and fifth generations were explored. To monitor the location of the PPI-Mal in the hosting mesophase, we analyzed three distinct functional groups' regions (reflecting possible interactions with the system components) in each ATR-FTIR spectrum. The regions are of: (1) the hydroxyl groups derived from the water in the cylinders, (2) water surfactant interface, and (3) the hydrophobic region containing the lipophilic acyl chains.

Only the peaks that were altered by the incorporation of glycodendrimers are discussed (Figure 3). The absorption band around at 2500 cm^{-1}, which was assigned to the O−D stretching mode of the D_2O molecules (ν_{OD}), was monitored to gain insight regarding the interaction associated with the surfactant polar headgroups and glycodendrimer molecules. The absorption band at 3200−3600 cm^{-1}, which is usually attributed to the O−H stretching modes (ν_{OH}), was used to characterize the interactions of the O−H groups of the GMO headgroups and D_2O molecules in the outer part of the interface (Figure 3). The stretching modes of the CO−O (ester at position ∼1180 cm^{-1}), which are sensitive to conformational changes at the interface and headgroup region, were also monitored. The "free" and "bound" carbonyl groups (C═O at 1720−1740 cm^{-1} wavenumbers) were also identified and considered to provide data on the conformational modification at the interface area.[44,45]

No changes were detected in the band correlated to the GMO lipophilic area (region III) (2800−2900 cm^{-1}) upon glycodendrimer embedment. It is known that these bands are sensitive to the conformation of the surfactant hydrocarbon chain; thus we concluded that the embedment of the PPI-Mal of the different generations had no impact on the lipophilic region because their location is isolated and far from this area.[45]

The absorption band at ∼2500 cm^{-1}, attributed to the O−D stretching mode (ν_{O-D}) of the water (region I), demonstrated a minor but visible shift of 3 cm^{-1} toward lower wavenumbers (from 2500 cm^{-1} in the empty mesophase to 2497 cm^{-1})

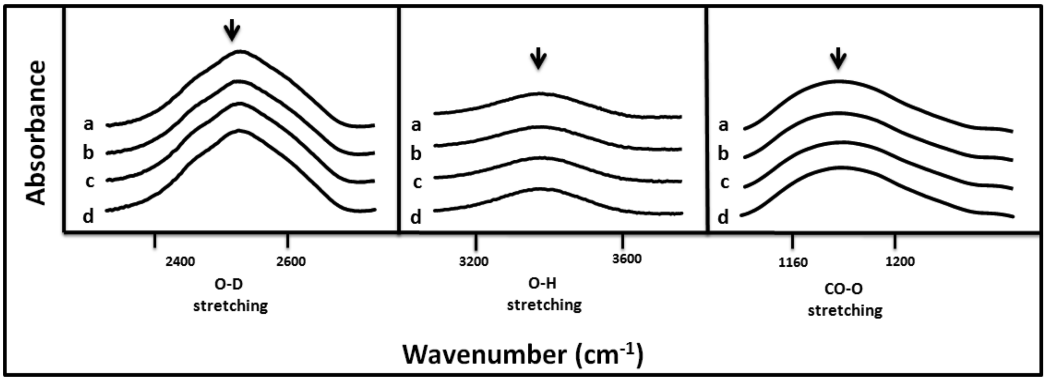


Figure 3. ATR-FTIR spectra, at 25 °C, for the empty (a) and PPI-Mal- (second (b), third (c), and fifth (d) generations) loaded H_{II} mesophases (composed of 72 wt % GMO, 8 wt % tricaprylin, and 20 wt % aqueous phase in the frequency ranges: (from left to right) 2400−2600 cm^{-1} corresponding to O−D stretching, 3200−3600 cm^{-1} corresponding to O−H stretching, and 1160−1200 cm^{-1} corresponding to CO−O stretching.

dx.doi.org/10.1021/jp4125974 | J. Phys. Chem. B 2014, 118, 4016−4024

Table 2. Resolved ATR-FTIR Bands for GMO/Tricaprylin/D_2O System Containing GMO/Tricaprylin at a Constant Weight Ratio of 9:1, 20 wt % D_2O, and 0.5 wt % PPI-Mal in Second, Third, and Fifth Generations

	blank (no PPI-Mal)	0.5 wt % PPI-Mal-G2	0.5 wt % PPI-Mal-G3	0.5 wt % PPI-Mal-G5
band	wavenumber (cm^{-1})	wavenumber (cm^{-1})	wavenumber (cm^{-1})	wavenumber (cm^{-1})
O−D stretching	2500	2499	2498	2497
O−H stretching	3399	3398	3397	3395
CO−O stretching	1181	1181	1180	1179

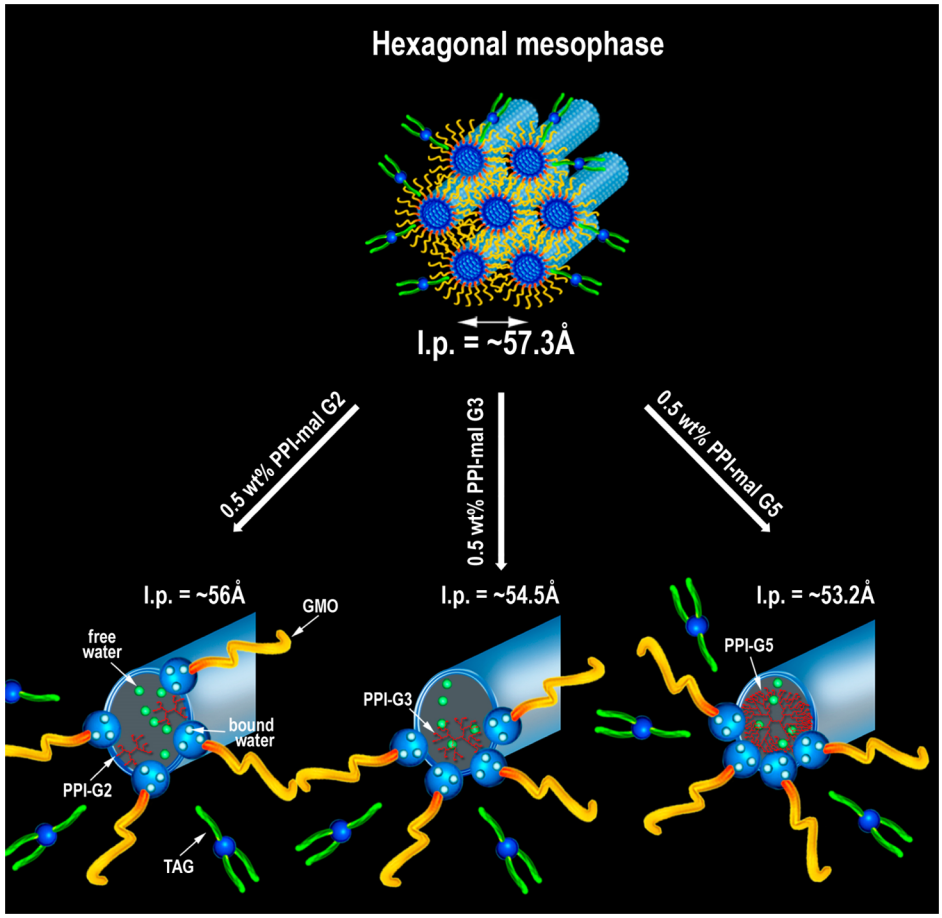


Figure 4. Schematic representation of the H_{II} mesophases loaded with PPI-Mal of second, third, and fiftth generations. (l.p. represents the lattice parameter.).

(Figure 3, Table 2). This shift suggests stronger hydrogen bonds between the glycodendrimers and the water.[3] In addition, a downward shift from 3399 cm^{-1} (original mixture) in ν_{O-H} toward 3395 cm^{-1} (fifth-generation mixture) was recorded. It is well-established that the stronger the hydrogen interactions between the GMO polar region and the D_2O, the lower the frequency of ν_{O-H} and ν_{O-D}.[44,46,47]

A decrease of 2 cm^{-1} from 1181 cm^{-1} in the original and second generation mixture of the CO−O functional groups (region II) toward 1179 cm^{-1} loaded with third- and fifth-generation mixtures was recorded (Figure 3 and Table 2). Hübner and Mantsch[48] and Razumas et al.[49] showed that the low-frequency wavenumber of the CO−O band can be associated with a deviation from the dihedral angle of 180° in this segment, induced by torsional motions or by a small population of gauche conformers near the sn-1 CO−O single bond.[48,49] Therefore, we suggest that the lower frequency positions of the CO−O band upon PPI-Mal encapsulation corresponds to more disordered states of the GMO in the vicinity of the GMO−water interface. No significant change was recorded for the C═O groups, suggesting a replacement of the hydrogen bond of GMO and water by GMO−glycodendrimer interactions.

Hence, it was indicated that mainly PPI-Mal of the third and fifth generations interfered with the water (D_2O) region, with effects on the GMO molecules in the interfacial region, including the ester moiety. The decrease in the lattice parameter upon the addition of glycodendrimer, as detected by the SAXS measurements, is consistent with the dehydration of the GMO hydroxyl groups, that is, breakage of the hydrogen bonds between water and GMO polar groups that is caused by the presence of highly polar dendrimer. In addition, an interaction of the polar groups of the dendrimer with GMO hydroxyls also occurred. Moreover, the modification within the vicinity of the GMO−water interface implies a less ordered state with increased content of *gauche* conformations, which is again consistent with shrinkage of the H_{II} cylinders.

dx.doi.org/10.1021/jp4125974 | J. Phys. Chem. B 2014, 118, 4016−4024

Thus, the ATR-FTIR results indicate that PPI-Mal is confined within the aqueous domains of the H_{II} cylinders, stabilized by hydrogen bonds with water. (See the schematic illustration in Figure 4.) Larger third- and fifth-generation molecules led to a noticeable break of the H bonds in the H-bond donor part of the GMO polar heads (OH) and a decrease in the frequency of the H-bond acceptor bands of the GMO polar heads (CO-O). Bearing in mind the structural impact of the dendrimers on the host mesophase, we further investigated its influence on the release of the encapsulated Na-DFC.

Impact of PPI-Maltose Generation on the Release of Na-DFC. 0.25 wt % Na-DFC was solubilized into the empty system and was cosolubilized (loaded) with second, third, and fifth generations of PPI-Mal mixtures to examine the macromolecules' impact on its release from the H_{II} hosting mesophase. The loading of the drug was set to 0.25 wt % as in our previous study of parental PPI-G2, PPI-G3, and PPI-G4 without maltose decoration. This allowed relevant comparison between the two types of dendrimers: one with maltose residue and one without it. Also, this is the maximum load of Na-DFC that can be solubilized with the dendrimer in the mesophase.

Solubilization and cosolubilization of Na-DFC into the empty and PPI-G2-Mal loaded H_{II} mesophase caused a decrease in the lattice parameter from 56 to 54 Å (empty) and 51 Å (PPI-G2-Mal), suggesting its intercalation mainly in the bulk water of the cylinders, as was shown in our previous publication.[50] However, upon cosolubilization of Na-DFC with the higher glycodendrimer generations, that is, third and fifth, an increase in the lattice parameter from ~51 Å toward 52.5 Å and 53.5 Å, respectively, took place. Considering the fact that the glycodendrimers tend to decrease the lattice parameter (as was seen previously, first section), the obtained increase in the lattice dimensions upon the cosolubilization with Na-DFC can be attributed to the drug molecule. A possible explanation for this complex interacting multicomponent system is that the competition for water binding with the macromolecules caused Na-DFC to move from the bulk water toward the water−GMO interface and thereby enlarge the radius of the water channels (Table 1). A comparison of the lattice parameter values with those of the system without Na-DFC shows that at low generation of dendrimer (second) the interaction of the dendrimer with the aqueous domains is less strong than that of the third and fifth generations. Thus, in the presence of second generation dendrimer, Na-DFC molecules can still interact with the bulk water region. However, at the higher generations (third and fifth), dendrimers bind the water strongly and the Na-DFC is forced to be intercalated within the lipid−water interface. This phenomenon was seen in our previous publication,[50] when Na-DFC was cosolubilized with PPI of second, third, and fourth generations (with no maltose decoration). In those systems, Na-DFC was intercalated in the bulk water in the systems, while the fourth-generation dendrimer was preferentially populated in the GMO−water interface. In this study, the glycodendrimers are very hydrophilic, and we can see the opposite trend of interacting Na-DFC at the GMO−water interface. Furthermore, it should be noted that although Na-DFC was populated in the GMO−water interface, as was shown by Cohen-Avrahami et al.,[51] here the drug can be located in either the GMO−water interface (third and fifth generations) or in the bulk water (empty and second generation). This can be explained by the fact that in Cohen-Avrahami's study[51] Na-DFC was solubilized in high concentrations (up to 5 wt %), in comparison with our previous and present study where Na-DFC is solubilized in low concentration (0.25 wt % of the total formulation). Thus, the solubilization of the drug is below its solubility limitation and it can be located in the bulk water.

The Na-DFC cumulative release profiles from the four mesophases are depicted in Figure 5, illustrating the difference

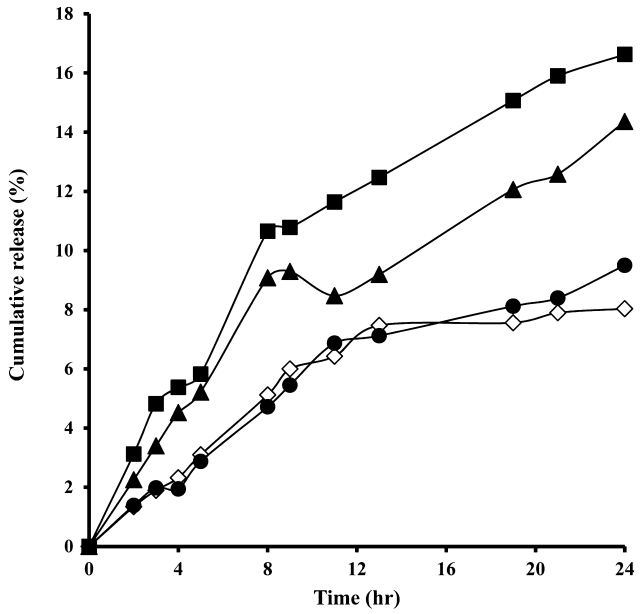


Figure 5. Release profile of Na-DFC from the H_{II} mesophases (composed of 72 wt % GMO, 8 wt % tricaprylin, and 20 wt % aqueous phase): release from the empty system (◇), PPI-Mal-G2- (●), PPI-Mal-G3- (▲), and PPI-Mal-G5- (■) loaded mixtures at room temperature.

in release rates from the empty and glycodendrimer-loaded mesophases. As seen in Figure 5, the release of Na-DFC to PBS from hexagonal mesophase loaded with third and fifth generations of PPI-Mal is slightly increased in comparison with the release for the empty and loaded second-generation systems. After 24 h, the cumulative release of the drug was 14% and 17 ± 0.3% from the H_{II} mesophase loaded with the third- and fifth-generation mixtures, respectively, and only 8% and 9 ± 0.3% from the original H_{II} and H_{II} loaded with second generation systems, respectively. It is obvious from the results that the Na-DFC molecules that are presumably located mainly in the water−lipid interface in the presence of third- and fifth-generation dendrimer mixtures can diffuse out of the H_{II} mesophase more easily, compared with the control mesophase and second-generation dendrimer systems, where the drug molecules are in the bulk water.

It was interesting to find out if the dendrimers (without Na-DFC) are also released from the inner phase to the outer water solution. The release profile of the dendrimers in the absence of Na-DFC was determined. PPI-Mal G2, G3, and G5 were hardly released. Only 2% of second-generation PPI-Mal was released after 24 h compared with practically no release of third and fifth generations. These results are consistent with the SAXS and ATR-FTIR analyses, stating that the strong hydrogen bonds between third- and fifth-generation mixtures with the aqueous domains of the bulk water are probably responsible for Na-DFC confinement within the interface and thus for the slightly enhanced release in comparison with those of second-generation glycodendrimer.

dx.doi.org/10.1021/jp4125974 | J. Phys. Chem. B 2014, 118, 4016−4024

Interestingly, it is noteworthy that the tendency of the high-generation glycodendrimer to release Na-DFC to the water is faster than the PPI dendrimer with no maltose.[36] In our previous work,[36] we demonstrated a different release profile of Na-DFC from liquid-crystalline cubic phase (Q^G) in the absence and in the presence of PPI dendrimer of second, third, and fourth generations. Solubilization of 25 wt % PPI (from the aqueous phase) into the Q^G mesophase led to phase transition from $Q^G \rightarrow H_{II}$ mesophase. SAXS observation showed an increase in the lattice parameter as the generation of the dendrimer increased. It was revealed that as the PPI generations increased the release of Na-DFC was significantly sustained, accompanied by lag time in the case of third and fourth generations. In this work, the role of the dendrimer in controlling the release is opposite because of the maltose residues that caused the dendrimer to strongly bind bulk water in comparison with regular PPI (with no maltose), which interacted mainly with the GMO−water interface.[36]

In the presence of the glycodendrimers, it was demonstrated that with higher generations of the PPI macromolecule faster release of Na-DFC from the H_{II} mesophase was obtained. Thus, for practical applications, one can design a drug carrier by changing the size (generation) of the dendrimer to control the drug release. (See Figure 5.)

CONCLUSIONS

We have investigated the self-assembly of second, third, and fifth generations of PPI-dendrimer modified with maltose residues within reverse hexagonal columnar hexagonal (H_{II}) mesophase. We used small-angle X-ray scattering to assess structural changes and infrared spectroscopy to provide insight into the nature of molecular interaction between PPI-Mal and the lipids. Finally, we followed the release of Na-DFC as well as the dendrimers from the mesophase loaded with the three generations into excess water by UV−vis absorption spectroscopy and studied the impact of the glycodendrimer generation on the release.

The encapsulation of PPI-Mal in the water channels was tailored by water draining from the bulk water stabilized via hydrogen bonding. This intermolecular interaction was identified by changing H-bonding interactions at GMO−water interfaces by ATR-IR study. Thus, this leads to dehydration of the lipid polar heads and a decrease in the water channel diameter.

Co-solubilization of Na-DFC into the H_{II} mesophase containing the glycodendrimers revealed different interactions of the drug with the components: While the location of the drug was found to be in the bulk water in the system without glycodendrimer and with second-generation macromolecules, intercalation of the drug within the interfacial area of GMO−water interface is suggested in the presence of the third- and fifth-generation dendrimers.

The different Na-DFC locations within the H_{II} water channels induced by third and fifth generations of PPI-Mal impacted the release profile of Na-DFC. The release process was found to be slightly rapid in the systems with PPI-Mal of third and fifth generations, corresponding to their strong interactions with the water channels, which imposed relocation of Na-DFC to the interfacial area. Thus, the release of the drug out of the mesophase was easier. Furthermore, no release of PPI-Mal of these generations from the system without Na-DFC was observed.

For the second-generation mesophase without Na-DFC, 2% of the dendrimer is released to the buffer with 9% of the Na-DFC. These findings manifested the feasibility of finding the second PPI-Mal location toward the GMO−interface rather than in the bulk water, in contrast with what was seen in the third and fifth generations.

In opposition to our previous work[36] where PPI dendrimers enabled sustained release of the guest molecules from LLCs, here we showed that utilization of the PPI glycodendrimers provided an additional means in the design of drug-delivery systems, where slightly increased drug release is desired at a lower level (<20%) in comparison with those releasing drug molecules from drug−polymer complexes in water and buffer solutions.[52]

To control the drug release, one can use the H_{II}-host system with glycodendrimers of varied generations, so the higher the generation of the PPI glycodendrimers, the faster the release of the drug.

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Notes

The results presented in this manuscript are part of the dissertation of L.B.-C. as partial fulfillment of the Ph.D. in Chemistry.

The authors declare no competing financial interest.

REFERENCES

(1) Boyd, B. J.; Whittaker, D. V.; Khoo, S. M.; Davey, G. Lyotropic liquid crystalline phases formed from glycerate surfactants as sustained release drug delivery systems. *Int. J. Pharm.* **2006**, *309*, 218−226.

(2) Mulet, X.; Boyd, B. J.; Drummond, C. J. Advances in drug delivery and medical imaging using colloidal lyotropic liquid crystalline dispersions. *J. Colloid Interface Sci.* **2013**, *393*, 1−20.

(3) Amar-Yuli, I.; Adamcik, J.; Blau, S.; Aserin, A.; Garti, N.; Mezzenga, R. Controlled embedment and release of DNA from lipidic reverse columnar hexagonal mesophases. *Soft Matter* **2011**, *7*, 8162−8168.

(4) Negrini, R.; Mezzenga, R. pH-Responsive Lyotropic Liquid Crystals for Controlled Drug Delivery. *Langmuir* **2011**, *27*, 5296−5303.

(5) Negrini, R.; Mezzenga, R. Diffusion, Molecular Separation, and Drug Delivery from Lipid Mesophases with Tunable Water Channels. *Langmuir* **2012**, *28*, 16455−16462.

(6) Clogston, J.; Caffrey, M. Controlling release from the lipidic cubic phase. Amino acids, peptides, proteins and nucleic acids. *J. Controlled Release* **2005**, *107*, 97−111.

(7) Cohen-Avrahami, M.; Libster, D.; Aserin, A.; Garti, N. Penetratin-induced transdermal delivery from H_{II} mesophases of sodium diclofenac. *J. Controlled Release* **2012**, *159*, 419−428.

(8) Wolinsky, J. B.; Grinstaff, M. W. Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Adv. Drug Delivery Rev.* **2008**, *60*, 1037−1055.

(9) Klajnert, B.; Pastucha, A.; Shcharbin, D.; Bryszewska, M. Binding properties of polyamidoamine dendrimers. *J. Appl. Polym. Sci.* **2007**, *103*, 2036−2040.

(10) Ciolkowski, M.; Palecz, B.; Appelhans, D.; Voit, B.; Klajnert, B.; Bryszewska, M. The influence of maltose modified poly(propylene imine) dendrimers on hen egg white lysozyme structure and thermal stability. *Colloids Surf., B* **2012**, *95*, 103−108.

(11) Chen, Y.; Wang, M.; Fang, L. Biomaterials as novel penetration enhancers for transdermal and dermal drug delivery systems. *Drug Delivery* **2013**, *20*, 199−209.

(12) Webster, D. M.; Sundaram, P.; Byrne, M. E. Injectable nanomaterials for drug delivery: Carriers, targeting moieties, and therapeutics. *Eur. J. Pharm. Biopharm.* **2013**, *84*, 1−20.
(13) Tian, W.-d.; Ma, Y.-q. Theoretical and computational studies of dendrimers as delivery vectors. *Chem. Soc. Rev.* **2013**, *42*, 705−727.
(14) Nanjwade, B. K.; Bechra, H. M.; Derkar, G. K.; Manvi, F. V.; Nanjwade, V. K. Dendrimers: Emerging polymers for drug-delivery systems. *Eur. J. Pharm. Sci.* **2009**, *38*, 185−196.
(15) Gajbhiye, V.; Kumar, P. V.; Tekade, R. K.; Jain, N. K. PEGylated PPI dendritic architectures for sustained delivery of H(2) receptor antagonist. *Eur. J. Med. Chem.* **2009**, *44*, 1155−1166.
(16) de las Cuevas, N.; Garcia-Gallego, S.; Rasines, B.; de la Mata, F. J.; Guijarro, L. G.; Munoz-Fernandez, M. A.; Gomez, R. In vitro studies of water-stable cationic carbosilane dendrimers as delivery vehicles for gene therapy against HIV and hepatocarcinoma. *Curr. Med. Chem* **2012**, *19*, 5052−5061.
(17) Ionov, M.; Ciepluch, K.; Klajnert, B.; Glinska, S.; Gomez-Ramirez, R.; Javier de la Mata, F.; Angeles Munoz-Fernandez, M.; Bryszewska, M. Complexation of HIV derived peptides with carbosilane dendrimers. *Colloids Surf., B* **2013**, *101*, 236−242.
(18) Witvrouw, M.; Fikkert, V.; Pluymers, W.; Matthews, B.; Mardel, K.; Schols, D.; Raff, J.; Debyser, Z.; De Clercq, E.; Holan, G.; Pannecouque, C. Polyanionic (i.e., polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (Reverse transcriptase/integrase) in the virus replicative cycle. *Mol. Pharmacol.* **2000**, *58*, 1100−1108.
(19) Klajnert, B.; Cortijo-Arellano, M.; Bryszewska, M.; Cladera, J. Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases. *Biochem. Biophys. Res. Commun.* **2006**, *339*, 577−582.
(20) Heegaard, P. M. H.; Pedersen, H. G.; Flink, J.; Boas, U. Amyloid aggregates of the prion peptide PrP106−126 are destabilised by oxidation and by the action of dendrimers. *FEBS Lett.* **2004**, *577*, 127−133.
(21) Klajnert, B.; Cortijo-Arellano, M.; Cladera, J.; Bryszewska, M. Influence of dendrimer's structure on its activity against amyloid fibril formation. *Biochem. Biophys. Res. Commun.* **2006**, *345*, 21−28.
(22) Yabbarov, N. G.; Posypanova, G. A.; Vorontsov, E. A.; Obydenny, S. I.; Severin, E. S. A new system for targeted delivery of doxorubicin into tumor cells. *J. Controlled Release* **2013**, *168*, 135−141.
(23) Garcia-Vallejo, J. J.; Ambrosini, M.; Overbeek, A.; van Riel, W. E.; Bloem, K.; Unger, W. W. J.; Chiodo, F.; Bolscher, J. G.; Nazmi, K.; Kalay, H.; Van Kooyk, Y. Multivalent glycopeptide dendrimers for the targeted delivery of antigens to dendritic cells. *Mol. Immunol.* **2013**, *53*, 387−397.
(24) Jain, N. K.; Mishra, V.; Mehra, N. K. Targeted drug delivery to macrophages. *Exp. Opin. Drug Delivery* **2013**, *10*, 353−367.
(25) Fischer, M.; Appelhans, D.; Schwarz, S.; Klajnert, B.; Bryszewska, M.; Voit, B.; Rogers, M. Influence of surface functionality of poly(propylene imine) dendrimers on protease resistance and propagation of the scrapie prion protein. *Biomacromolecules* **2010**, *11*, 1314−1325.
(26) Yoo, H.; Juliano, R. L. Enhanced delivery of antisense oligonucleotides with fluorophore-conjugated PAMAM dendrimers. *Nucleic Acids Res.* **2000**, *28*, 4225−4231.
(27) Klajnert, B.; Appelhans, D.; Komber, H.; Morgner, N.; Schwarz, S.; Richter, S.; Brutschy, B.; Ionov, M.; Tonkikh, A. K.; Bryszewska, M.; Voit, B. The influence of densely organized maltose shells on the biological properties of poly(propylene imine) dendrimers: New effects dependent on hydrogen bonding. *Chem.—Eur. J.* **2008**, *14*, 7030−7041.
(28) Pietsch, T.; Appelhans, D.; Gindy, N.; Voit, B.; Fahmi, A. Oligosaccharide-modified dendrimers for templating gold nanoparticles: Tailoring the particle size as a function of dendrimer generation and -molecular structure. *Colloids Surf., A.* **2009**, *341*, 93−102.
(29) Appelhans, D.; Oertel, U.; Mazzeo, R.; Komber, H.; Hoffmann, J.; Weidner, S.; Brutschy, B.; Voit, B.; Ottaviani, M. F. Dense-shell glycodendrimers: UV/Vis and electron paramagnetic resonance study of metal ion complexation. *Proc. R. Soc. London, Ser. A* **2010**, *466*, 1489−1513.
(30) Ciepluch, K.; Ziemba, B.; Janaszewska, A.; Appelhans, D.; Klajnert, B.; Bryszewska, M.; Fogel, W. A. Modulation of biogenic amines content by poly(propylene imine) dendrimers in rats. *J. Physiol. Biochem.* **2012**, *68*, 447−454.
(31) Eurich, D.; Bahra, M.; Berg, T.; Boas-Knoop, S.; Biermer, M.; Neuhaus, R.; Neuhaus, P.; Neumann, U. Treatment of hepatitis C-virus-reinfection after liver transplant with silibinin in nonresponders to pegylated interferon-based therapy. *Exp. Clin. Transplant.* **2011**, *9*, 1−6.
(32) Filimon, A.; Sima, L. E.; Appelhans, D.; Voit, B.; Negroiu, G. Internalization and intracellular trafficking of poly(propylene imine) glycodendrimers with maltose shell in melanoma cells. *Curr. Med. Chem.* **2012**, *19*, 4955−4968.
(33) Ziemba, B.; Matuszko, G.; Appelhans, D.; Voit, B.; Bryszewska, M.; Klajnert, B. Genotoxicity of poly(propylene imine) dendrimers. *Biopolymers* **2012**, *97*, 642−648.
(34) Bitan-Cherbakovsky, L.; Libster, D.; Aserin, A.; Garti, N. Complex dendrimer-lyotropic liquid crystalline systems: Structural behavior and interactions. *J. Phys. Chem. B* **2011**, *115*, 11984−11992.
(35) Bitan-Cherbakovsky, L.; Libster, D.; Ottaviani, M. F.; Aserin, A.; Garti, N. Structural behavior and interactions of dendrimer within lyotropic liquid crystals, monitored by EPR spectroscopy and rheology. *J. Phys. Chem. B* **2012**, *116*, 2420−2429.
(36) Bitan-Cherbakovsky, L.; Aserin, A.; Garti, N. Structural characterization of lyotropic liquid crystals containing a dendrimer for solubilization and release of gallic acid. *Colloids Surf., B* **2013**, *112*, 87−95.
(37) Klementieva, O.; Benseny-Cases, N.; Gella, A.; Appelhans, D.; Voit, B.; Cladera, J. Dense shell glycodendrimers as potential nontoxic anti-amyloidogenic agents in Alzheimer's disease. Amyloid-dendrimer aggregates morphology and cell toxicity. *Biomacromolecules* **2011**, *12*, 3903−3909.
(38) McCarthy, J. M.; Moreno, B. R.; Filippini, D.; Komber, H.; Maly, M.; Cernescu, M.; Brutschy, B.; Appelhans, D.; Rogers, M. S. Influence of surface groups on poly(propylene imine) dendrimers antiprion activity. *Biomacromolecules* **2013**, *14*, 27−37.
(39) Mkandawire, M.; Pohl, A.; Gubarevich, T.; Lapina, V.; Appelhans, D.; Roedel, G.; Pompe, W.; Schreiber, J.; Opitz, J. Selective targeting of green fluorescent nanodiamond conjugates to mitochondria in HeLa cells. *J. Biophotonics* **2009**, *2*, 596−606.
(40) Lake, J. A. An iterative method of slit-correcting small angle x-ray data. *Acta Crystallogr.*. **1967**, *23*.
(41) Rizwan, S. B.; Hanley, T.; Boyd, B. J.; Rades, T.; Hook, S. Liquid crystalline systems of phytantriol and glyceryl monooleate containing a hydrophilic protein: Characterisation, swelling and release kinetics. *J. Pharm. Sci.* **2009**, *98*, 4191−4204.
(42) Saturni, L.; Rustichelli, F.; Di Gregorio, G. M.; Cordone, L.; Mariani, P. Sugar-induced stabilization of the monoolein Pn3m bicontinuous cubic phase during dehydration. *Phys. Rev. E* **2001**, *64*.
(43) Mezzenga, R.; Grigorov, M.; Zhang, Z. D.; Servais, C.; Sagalowicz, L.; Romoscanu, A. I.; Khanna, V.; Meyer, C. Polysaccharide-induced order-to-order transitions in lyotropic liquid crystals. *Langmuir* **2005**, *21*, 6165−6169.
(44) Nielsen, L.; Khurana, R.; Coats, A.; Frokjaer, S.; Brange, J.; Vyas, S.; Uversky, V. N.; Fink, A. L. Effect of environmental factors on the kinetics of insulin fibril formation: Elucidation of the molecular mechanism. *Biochemistry* **2001**, *40*, 6036−6046.
(45) Snyder, R. G.; Strauss, H. L.; Elliger, C. A. C-H Stretching modes and the structure of normal-alkyl chains 0.1. long, disordered chains. *J. Phys. Chem.* **1982**, *86*.
(46) Amar-Yuli, I.; Wachtel, E.; Shalev, D. E.; Aserin, A.; Garti, N. Low viscosity reversed hexagonal mesophases induced by hydrophilic additives. *J. Phys. Chem. B* **2008**, *112*, 3971−3982.
(47) Amar-Yuli, I.; Wachtel, E.; Shalev, D. E.; Moshe, H.; Aserin, A.; Garti, N. Thermally induced fluid reversed hexagonal (H-II) mesophase. *J. Phys. Chem. B* **2007**, *111*, 13544−13553.

(48) Hubner, W.; Mantsch, H. H. Orientation of specifically 13C=O labeled phosphatidylcholine multilayers from polarized attenuated total reflection FT-IR spectroscopy. *Biophys. J.* **1991**, *59*, 1261−1272.
(49) Razumas, V.; Larsson, K.; Miezis, Y.; Nylander, T. A cubic monoolein cytochrome c water phase: X-ray diffraction, FT-IR, differential scanning calorimetric, and electrochemical studies. *J. Phys. Chem.* **1996**, *100*, 11766−11774.
(50) Cherbakovsky-Bitan, L. LLC-Dendrimer System: Structural Behavior, Interactions and Drug Release. Ph.D. Dissertation, The Hebrew University of Jerusalem, 2013.
(51) Cohen-Avrahami, M.; Libster, D.; Aserin, A.; Garti, N. Sodium diclofenac and cell-penetrating peptides embedded in H-II mesophases: Physical characterization and delivery. *J. Phys. Chem. B* **2011**, *115*, 10189−10197.
(52) Polikarpov, N.; Appelhans, D.; Welzel, P.; Kaufmann, A.; Dhanapal, P.; Bellmann, C.; Voit, B. Tailoring uptake and release of ATP by dendritic glycopolymer/PNIPAAm hydrogel hybrids: first approaches towards multicompartment release systems. *New J. Chem.* **2012**, *36*, 438−451.