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Nanospheres with a smectic hydrophobic core and an amorphous PEG hydrophilic shell: Structural changes and implications for drug delivery

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Abstract

The structure of nanospheres with a crystalline core and an amorphous diffuse shell was investigated by small-angle neutron scattering (SANS), small-, medium-, and wide-angle x-ray scattering (SAXS, MAXS and WAXS), and differential scanning calorimetry (DSC). Nanospheres, 28 to 35 nm in diameter, were prepared from a triblock copolymer with poly(ethylene glycol) (PEG) hydrophilic end-blocks and oligomers of alternating desaminotyrosyl-tyrosine octyl ester (DTO) and suberic acid (SA) as the central hydrophobic block. In the lyophilized nanospheres, the diffraction patterns show that the PEG shell is ~ 10 nm in thickness and crystalline, and the hydrophobic core is ~ 10 nm in diameter with a smectic liquid crystalline texture. In aqueous dispersions, the hydrated PEG forms an amorphous shell, but the crystalline phase in the core persists at concentrations down to 1 mg/ml as evidenced by the sharp MAXS diffraction peak at a d -spacing of 24.4 Å and a melting endotherm at 40 °C. As the dispersion is diluted (< 1 mg/ml), the core becomes less ordered, and its diameter decreases by 50% even though the overall size of the nanosphere remains essentially unchanged. It is likely that below a critical concentration, intermixing of hydrophobic segments with the PEG segments reduces the size and the crystallinity of the core. At these concentrations, the PEG corona forms a eutectic with water. The mechanisms by which the concentration of the dispersion influences the structure of the nanospheres, and consequently their drug-release characteristics, are discussed.

Graphical Abstract

Keywords

Nanospheres; Tyrosine-derived polyarylates; PEG; Structure; X-ray/neutron scattering; Crystalline core; Drug delivery

Introduction

Nanoparticles are widely used to deliver active ingredients in biomedical applications and cosmetics, and to design structures such as photonic devices, dendrimers and biomimetic structures at supramolecular scales [1–4]. The design and the structure of nanospheres made from amphiphilic polymers for drug delivery have been extensively reviewed [3,5,6]. The dynamics of the formation of these particles from self-assembly of polymer chains has been well understood from such studies as the measurement of critical aggregation concentration (CAC; similar to critical micellar concentration, CMC, used in connection with micellar nanospheres). However, the structures of these nanospheres, beyond their core-shell structure, are yet to be elucidated. The effect of polymer concentration, and freeze-drying and storage on the structure of the nanosphere that are known to influence the drug-release characteristics as well as their behavior as nanofluids [7] need to be understood.

There are numerous reports on nanospheres of homopolymers [8], and di- and tri-block copolymers [3,4,9]. In copolymers, coronal block is typically poly(ethylene glycol) (PEG) or also referred to as poly(ethylene oxide) (PEO) and poly(acrylic acid) (PAA); the core block is one of the following polymers: poly(lactic acid) (PLA), poly(ϵ -caprolactone) (PCL), poly(lactic-*co*-glycolic acid) (PLGA), polystyrene (PS), and polybutadiene (PBD). The most widely studied nanospheres from the triblock polymers are from (PEO-PPO-PEO), commonly known as Pluronic® [10], PEG-PCL-PEG [11,12], PEO-PHB-PEO (where PPO is poly(propylene oxide) and PHB is poly(hydroxyl butarate)) [13]. The core-shell structures of some of these nanospheres have been analyzed using x-ray and neutron scattering [14–16]. Crystalline core has been reported in dried or lyophilized nanospheres of diblock copolymers of PCL-PEG [17,18], PCL-PAA [19] and in PEG-PCL-PEG triblock copolymers [20]. Although Glover et al. reported melting of the PCL core in the aqueous dispersions of PCL-PEO [18], the details of the core's structure in nanoparticles in dispersion, appears not to have been investigated.

Here we investigate the core-shell structure of the nanospheres from an ABA triblock copolymer with PEG as the hydrophilic end-blocks, and oligomers of alternating monomers desaminotyrosyl-tyrosine octyl ester (DTO) and suberic acid (SA), referred to as oligo(DTO-SA), as the central hydrophobic block (Figure 1) [21]. Using thermal analysis (differential scanning calorimetry, DSC), small-, medium- and wide-angle x-ray scattering (SAXS, MAXS and WAXS) and small-angle neutron scattering (SANS) we study the structural changes that occur in the crystalline core and the core-shell structure in aqueous dispersion of these nanospheres with pH and over a hundred-fold change in concentration.

Experimental

Materials

The triblock copolymers used in this study (Figure 1) were synthesized as described in a previous publications [21,22]. Briefly, the central block was first synthesized by dissolving DTO, suberic acid and 4-(dimethylamino)-pyridine, 4-toluenesulfonic acid (DPTs) in anhydrous dichloromethane under nitrogen and at room temperature. After complete dissolution, diisocarbodiimide (DIC) was added to the reaction mixture, and stirred until the desired molecular weight was reached. PEG(5k) and DIC were then added and stirred for 2 h. The reaction mixture was then filtered through a sintered glass funnel, the filtrate was evaporated, and then precipitated with 2-propanol. This precipitate was dried, dissolved in methylene chloride, and reprecipitated with methanol. The product was isolated by centrifugation, washed with methanol, and dried under vacuum at room temperature. The resulting polymer was characterized by elemental analysis, GPC and ^1H NMR. Nanospheres were obtained by self-assembly as the polymer dissolved in THF was added dropwise into water under mild agitation. Uniform particle size was obtained by filtering the turbid aqueous dispersion sequentially through 0.45, 0.22, and 0.1 μm size syringe filters. All subsequent characterizations were performed using this final filtered preparation. Results from nanospheres made from two polymers with a degree of polymerization (DP) for the B block of 4.6 (DP5) and 20.4 (DP20) will be discussed. The nanospheres were prepared by dissolving the polymer in dimethylformamide (DMF) and dispersing the solution in phosphate buffered saline (PBS).

Nanoparticle aggregates in the dry state were obtained by lyophilizing (bench top lyophilizer; FreeZone 2.5, Labconco, NJ) the nanoparticle dispersions frozen at -80°C .

Methods

Differential scanning calorimetry (DSC) data were obtained on a Mettler DC 823 instrument using heating and cooling rates of $10^\circ\text{C}/\text{min}$ with ~ 10 mg samples in $40\ \mu\text{L}$ aluminum pans.

X-ray diffraction (XRD) experiments were carried out on the beam line DND CAT 5 ID-D (Advance Photon Source, Argonne National Laboratories). The samples were placed in a 1.5 mm diameter quartz capillary for the XRD measurements. XRD data were collected with 9.8 keV ($\lambda = 0.7293\ \text{\AA}$) x-rays using 0.25×0.25 mm slits and three detectors at 199.9, 1012.8 and 8502.5 mm from the sample. The three detectors provided the data at the $0.0014 < q_{\text{SAXS}} < 0.083$, $0.077 < q_{\text{MAXS}} < 0.48$ and $0.39 < q_{\text{WAXS}} < 2.57\ \text{\AA}^{-1}$. Preliminary and confirmatory data were also collected on a rotation anode (Cu radiation, $\lambda = 1.542\ \text{\AA}$) using a Bruker area detector and a sample to film distance of 89 mm.

SANS data were obtained at Oakridge Spallation source on the Extended Q-Range SANS (BL-6).

Results and Discussion

For the DP5 and DP20 polymers, the concentrations above which the polymers form vesicles, CAC, are 0.011 and 0.0022 mg/mL, respectively. The hydrodynamic diameters of

the DP5 and DP20-derived nanospheres were measured by dynamic light scattering (DLS) [21,22] and were found to be 41 and 68 nm, respectively. Previously published electron microscopy observations [21,23], and x-ray scattering data that will be presented here, show that these particles are spherical, core-shell structured nanospheres.

Core structure in thermal data

DSC scans from the nanospheres, freeze-dried and in aqueous dispersions, and from two controls, PEG_{5k} and the copolymer, are given in Figure 2. The results are summarized in Table 1. The T_m and T_g of the copolymer are in agreement with the previously reported values [22]. The melting transition in the copolymer at 58 °C is due to PEG as indicated by the presence of the signature diffraction peaks of PEG at 1.36 and 1.65 Å⁻¹ in the XRD scan (Figure 2b) [24]. PEG in the copolymer melts at 58 °C, 12 °C below the 70 °C value in the homopolymer. This is because the PEG is a segment in the copolymer are disordered and their crystallite size is small [24]. The main melting transition in freeze-dried sample at 51 °C is due to PEG, as indicated by the presence of PEG diffraction peaks in the XRD scan [24].

The hydrated sample (11.6 mg/ml dispersion) shows a melting peak at 40 °C (Figure 2c). This was unexpected because these are presumably amorphous nanoparticles dispersed in an aqueous medium. PEG was ruled out as the source by examining the DSC scan of hydrated PEG, which did not show a melting peak (supplementary material, S1). Furthermore, XRD pattern of this sample (Figure 2b) shows a sharp diffraction peak at 0.25 Å⁻¹ ($d = 25$ Å) that cannot be attributed to PEG [24,25]. Therefore, we assign the 40 °C melting peak and the 0.25 Å⁻¹ diffraction peak to the non-hydratable crystalline core of the nanosphere. Note that even the freeze-dried nanospheres have a shoulder at 42 °C in the DSC scan and a diffraction peak in the XRD scan indicating that this crystalline core is present even in the freeze-dried samples. The scale bar for the DSC scan in Figure 2a (dry sample) is 5 W/g and that for Figure 2c (11.6 mg/ml dispersion) is 0.02 W/g. Thus, the 40 °C transition that appears as a shoulder in the freeze-dried sample in Figure 2a and as a distinct peak in Figure 2c, both have similar heats of melting that can be attributed to the crystalline core.

Figure 2d shows the DSC scans of dispersions at 5 and 30 mg/ml. The 30 mg/ml dispersion has the 40 °C melting peak. In contrast, the 5 mg/ml sample, instead of the 40 °C melting peak has an exotherm at -35 °C (barely visible at the magnification shown in the figure) followed by a strong melting peak at -22 °C, along with the melting peak of free water near 0 °C. These features are similar to those in PEG_{5k} (supplementary data, S2), and have been investigated extensively with PEGs of different molecular weights [26–29]. Based on these reports, the -35 °C exotherm and the -22 °C endotherm can be attributed to the formation and melting, respectively, of a binary eutectic of PEG and water [26–28]. No such eutectics were observed at higher concentrations of the nanospheres. This suggests that the structure of PEG corona changes upon dilution. The implications of these changes will be discussed in later sections.

Structure of the core in XRD

The characteristics of the crystalline, water-insoluble, hydrophobic core were analyzed using the XRD scans. SAXS, MAXS and WAXS data that were obtained cover three different length scales, which roughly span the mesoscopic (5000 to 50 Å), supramolecular (50 to 10 Å), and molecular (10 to 1 Å) length scales, respectively. Figure 3 shows an example of the data from two concentrations of the DP20 polymer.

In the MAXS segment ($0.1 < q < 0.5 \text{ Å}^{-1}$), the 0.25 Å^{-1} peak ($d = 25 \text{ Å}$), which was seen in Figure 2a, is intense. A second order reflection of this peak could also be discerned at $q = 0.5 \text{ Å}^{-1}$ ($d = 12.5 \text{ Å}$) in this scan, and in all the scans of concentrated dispersions. These two features show that nanospheres have an internal structure with a d -spacing of 25 Å. On the basis of the DSC results discussed earlier, we assign this to the crystalline packing of the polyarylate chains in the core of the nanosphere. This peak gets weaker (decrease in integrated intensity) and broader (increase in the full-width at half-maximum) in dilute dispersions, and is not detectable at the lowest concentrations (0.07 mg/ml) that was measured. These changes, which are due to the changes in the crystalline structure of the core, are accompanied by large changes in the WAXS region of the scattering pattern ($0.4 < q < 2.6 \text{ Å}^{-1}$). The peak at 1.5 Å^{-1} , which corresponds to the 4.3 Å interchain spacing in both the core and the corona, is replaced by a peak at 2.0 Å^{-1} , which corresponds to the 3.1 Å oxygen-oxygen correlations in water [30]. This 2.0 Å^{-1} peak is present after complete subtraction of the background scattering from the aqueous medium. Therefore, we attribute this peak to the structured water in the PEG-water complex, a eutectic that was identified in the DSC scans. Apparently, as water diffuses into the corona and dominates the XRD pattern, the disorder in the core increases as indicated by the weakening of its crystalline peak.

Structure of the nanosphere by SAXS

The scattering curve in the low-angle region ($0.001 < q < 0.1 \text{ Å}^{-1}$) has an interference peak at $q \sim 0.015 \text{ Å}^{-1}$ that can be easily seen in the $I(q) \cdot q$ vs. q plots (Figure 4a). This corresponds to an interparticle distance of 71 nm between the nanospheres. The intensity at $q < 0.005 \text{ Å}^{-1}$ is most likely due to particles larger than nanospheres ($> 200 \text{ nm}$), or a small fraction of aggregates. This central diffuse scattering increases considerably at lower concentrations, suggesting that there could also be polymersomes.

Guinier plots of the SAXS data (Figure 4b) show two clearly defined slopes (see also supplementary S3). On the basis of TEM images and simulated curves (see the next paragraph), the slope of the low-angle segment ($0.0001 < q^2 < 0.0008 \text{ Å}^{-2}$) was assigned to the size of the whole particle, and that of the high-angle segment (typically $0.002 < q^2 < 0.006 \text{ Å}^{-2}$) to the size of the core. These two dimensions are listed in Table 2. In some of the data, it was possible to fit another Guinier slope with an R_g of $\sim 9 \text{ Å}$ at even higher q values ($0.1 < q < 0.17 \text{ Å}^{-1}$; data not shown). This could be due to unimers. It has been known that vesicles, unlike polymersomes, are in a dynamic equilibrium with unimers

The SAXS curves were further analyzed by fitting them to core-shell scattering using Irena Software [31] to confirm the core-shell structure and the dramatic decrease in the core size

at lower concentrations. Examples of the fits are given in Figure 4b–c. The simulated values were in agreement with the Guinier values, although the actual numbers were different (supplementary information, S3): the total diameter was higher and the core diameter was lower in the Guinier fit. This could be because in the Guinier analysis, the low-angle segment (representing the sizes of the whole particle) is weighted by the larger particles, and the high angle segment (representing the sizes of the core) is affected by the contrast of the shell relative to the core and the medium. Since modeling uses several adjustable parameters (solvent density, core and shell densities, core size and shell thickness, and particle size distribution), and Guinier fit has no adjustable parameters, we used the Guinier analyses to parameterize the data.

Structure of the nanosphere in SANS

SANS experiments were carried out to independently determine the sizes of the core and the corona by contrast matching. Data were collected at different ratios of D₂O to H₂O (100, 50 and 15 % D₂O) so as to gradually mask the scattering from PEG domains. The radius of gyration (R_g) of the particles were determined by Guinier analysis [32]. Dilution did not change the R_g . Since the particles have been found to be spherical by transmission electron microscopy (TEM) [21,23,33], their diameter (D) was determined using the relation $D^2 = 5 R_g^2/3$. The results are summarized in Table 2. The particle sizes did not change significantly in the 100 and 50 % D₂O samples. However, the size decreased dramatically at 15% D₂O as indicated by the large decrease in slope (Figure 5). This smaller size with 15 % D₂O results from the masking of the density of the outer shell by the medium, and corresponds to the core with unmatched contrast. These results show the presence of low-density PEG shell and a high-density core.

The physical size of 35 nm for the DP20 nanosphere (Table 2) is in agreement with the TEM size of 39 nm [23]. The parameters of the nanospheres obtained from SANS and SAXS, are compared in Table 2, and clearly show the presence of distinct core and shell domains. The core size determined in SANS is by matching the contrast of the outer shell, and thus depends on the extent to which D₂O diffuses into the shell. The core size in SAXS is determined from the tail end of the scattering curve, and reflects the location of the interface between the crystalline core and the diffuse shell. The two results are therefore not expected to be in quantitative agreement: the core size by SANS is expected to be larger than that by SAXS as seen in Table 2. Because the total particle diameter is the same in both the techniques, the shell thickness is smaller in SANS. Since extensive data were collected by SAXS, we will use the X-ray core diameter while acknowledging that this is the lower limit.

The core diameter of at higher concentrations in DP20 nanosphere is larger than the crystallite size determined from the width of the MAXS peak (10.6 vs. 8 nm). This is expected because of the crystalline disorder that is invariably present, the size determined from the width of the diffraction peak is always lower than the true size.

Model for the nanosphere

The results presented thus far are captured in the model shown in Figure 6. The three characteristic diameters of the nanosphere, that of the core, corona and the hydrodynamic

shell, are given in Figure 6a. The gross features of the structure are similar to that typically found in nanospheres of amphiphilic molecules with a hydrophobic core and a hydrophilic shell. However, we find that the structure changes dramatically with concentration.

The length of the DTO-SA central block in DP20 nanospheres, calculated using $\sim 25 \text{ \AA}$ for the monomer length (based on known bond distances and angles), is 51 nm. This is 5 to 10 times diameter of the core depending on the concentration (Table 2). The length of the central block in DP 5 nanosphere is 11.5 nm, or 1.4 to 2.6 times the core diameter. In both instances, the middle polyarylate block has to fold upon itself to form the core. Molecular simulations confirm the formation of these chain folds in the core [34]. Based on the accepted theories of polymer crystallization, it is most likely that the core is composed of folded chain crystalline lamellae. Most interestingly, the d-spacing of the peak observed in MAXS is $24.4 \text{ \AA}$, almost the same as the length of the monomer. In WAXS, there is only the diffuse peak corresponding to an interchain distance of $4.3 \text{ \AA}$. These features are characteristic of a smectic liquid crystalline phase. Thus, the core is made of layers in which the monomers assemble to form a $24.4 \text{ \AA}$ thick layer as indicated in Figure 6. Since the core diameter of the nanospheres is 5 – 10 nm, we estimate two to four layers of molecules in the core. Since the interchain separation is $4.3 \text{ \AA}$, we could have ~ 20 stems in the lamellae that span the diameter of the core. We note that the core polymer is amorphous, and forms a layered, smectic-like liquid crystalline structure when constrained to form a core in these nanospheres. In other nanospheres in which PCL is the central block, the semicrystalline nature of PCL gives rise to the crystalline core.

In the DP20 polymer, using an estimated volume of $\sim 0.35 \text{ nm}^3$ for the monomer, we find that there are about 75 chains in the 10 nm diameter core, and about 150 PEG chains in the corona. This many PEG chains can be easily accommodated on the surface of a 10 nm core. Since PEG chains can be helical, a 7-unit helix with a repeat of $19.5 \text{ \AA}$, 5 kDa PEG is $\sim 32 \text{ nm}$ in length [34]. Therefore, given that the thickness of the corona is 10–15 nm, PEG also has to form of folded chains. These features are illustrated in Figures 6b and 6c. Figure 6b shows the model for a freeze-dried nanosphere in which the PEG corona is shown as crystalline lamellae on the basis of the PEG diffraction peaks seen in the WAXS patterns (Figure 2b). Upon hydration, the PEG corona becomes amorphous, even though the core remains crystalline (Figure 6c). The thickness of the hydrodynamic shell is 16 nm in DP 5, and 29 nm in DP20. Thus, it is possible that in the larger nanosphere, some of the PEG chains can span the hydrated shell. Although the DLS data shows a broad hydrodynamic size distribution (polydispersity ~ 0.2 determined from assuming Gaussian distribution), the linearity of the Guinier plot of the x-ray scattering data suggest a highly monodisperse suspension ($0.01 < q < 0.025 \text{ \AA}^{-1}$; $R^2 = 0.999$ in most instances; see Supplementary S3). Such differences between hydrodynamic diameter and the particle diameter become even more apparent in the following discussion on the changes that occur as the concentration of the dispersion is changed.

The data in Table 2 show that increasing the length of the central block that occurs with increase in DP results in an increase in the core diameter: 33% (from 8.0 to 10.6 nm) at high concentration and 28% (from 4.4 to 5.3 nm) at low concentrations. This increase in the core diameter is expected considering the stiffness of the aryate segments. Surprisingly, the

thickness of the shell increases from 10 to 12 nm (increase by 23 % and 28%, at low and high concentrations, respectively), and is accompanied by an increase in the hydrodynamic diameter even when the PEG chain length remains unchanged at 5 kDa.

Effect of polymer concentration

It was noted earlier in the discussion of the MAXS data (Figure 3) that the crystalline order in the core decreases considerably at low concentrations (< 1 mg/ml). This concentration is almost 500 times the CAC, and is therefore unrelated to the onset of particle formation. The decrease in the crystalline order in the core with concentration is accompanied by changes in the diameters of the core and the nanosphere (Figures 4b and Table 2). These changes, plotted as a function of concentration in Figure 7 for the DP20 dispersion, show that as the dispersion is diluted, the core diameter decreases by almost half at ~ 1 mg/ml, while the overall diameter decreases only by $\sim 5\%$. In terms of volumes, the ratio of the core volume to the total particle volume decreases by a factor of six, from 2.5% at high concentrations (> 2 mg/ml) to 0.4% at low concentrations (< 1 mg/ml) (Table S4). In other words, the volume of the shell is 240 times the core volume at low concentration and 40 times the core volume at higher concentrations in both the polymers. Because the PEG size is constant at 5 kDa, these comparisons show that shell is more diffuse at low concentrations than at high concentrations. This diffuse shell affects the internal structure of the nanosphere.

The hydrodynamic radius remains essentially unchanged at 65 nm over $100\times$ dilution (16 to 0.16 mg/mL, lowest concentration for which the data are available). This hydrodynamic diameter is only slightly smaller than the interparticle distance of 71 nm observed at high concentrations in SAXS. Dilution increases this distance considerably, thereby weaken the PEG-PEG inter-coronal interactions, and as a result strengthen the intra-coronal interactions. The appearance of the diffraction peak at $2 \text{ \AA}^{-1}$ in WAXS scans (Figure 3) at these concentrations is accompanied by a melting endotherm in the DSC scans (Figure 2). We attribute these two features to a PEG-water complex in the hydrated PEG corona that occurs as a consequence of the intra-coronal interactions. Additionally, the loss of the crystalline features in the core (absence of a core melting peak at 40°C in the DSC scans, Figure 2d and weak $25 \text{ \AA}$ peak in the MAXS scan, Figure 3), suggests interdiffusion of the core hydrophobic segments and the shell hydrophilic segments (Figure 6d). It is possible that in dilute dispersions, as a fraction of the hydrophobic core segments diffuses into the hydrophilic PEG corona, the size of the distinctly identifiable core decreases without changing the overall diameter of the nanosphere. Thus, the interaction of the corona with water appears to play an important role both in determining the size as well as the stability of the nanosphere.

Implications for drug release

Nanospheres of the type used in this study are used to deliver hydrophobic drugs. A challenge in the development of nanoparticles for drug delivery is to make their core amorphous to facilitate incorporation of the drug in the core, and to be able to control the drug release rate. The nanoparticles based on PCL have a crystalline core [17–20]. Glover et al. took advantage of this to demonstrate that melting of the core at physiological temperatures can trigger the drug release in the body [18]. The nanosphere composition that

is investigated here shows increased disorder at lower concentrations that can similarly promote drug release.

It is known that drugs are incorporated into nanospheres during self-assembly as the core is formed. However, what is not clear is how the drugs are released from the hydrophobic core and transported through the hydrophilic shell. The structural changes in the nanosphere that are reported here are useful in understanding this process and consequently in designing nanospheres that release drugs at the desired rates. The presence of a crystalline core of rigid arylate hydrophobic segments, and the lack of a “cavity” into which the drug can be loaded inside the nanosphere explain why the diameter of the spheres remains unchanged at 65–75 nm irrespective of the up to 40% loading of cyclosporine A [23]. Absence of PEG-PEG interactions between neighboring nanospheres at low concentrations, and the consequent interdiffusion of the core and shell segments that destabilize the core, can dramatically affect drug release from nanospheres.

The model developed here for the nanosphere can be used to explain the 30 to 60% decrease in the release camptothecin and paclitaxel at basic pH. The SAXS data from DP20 nanosphere at several pH values are shown in Table 3. The data in the table show that the hydrodynamic size of the particles decreases significantly at alkaline pH, with a corresponding small change in nanosphere diameter. These changes in the diameter of the particles are accompanied by clear differences in the diffraction data in WAXS. The WAXS data in Figure 8 show the appearance of the $2\text{ \AA}^{-1}$ peak at alkaline pH values. We previously attributed this peak to the formation of a PEG-water complex in the shell. It is possible at alkaline pH values, the PEG-water complex in the shell inhibits the release of the drug from the hydrophobic core. At lower pH values, in the absence of the bound water in the corona, it is possible that the drug diffuses through the PEG corona, which has some hydrophobic characteristics when little bound water is present.

Conclusions

Nanospheres with stiff middle hydrophobic blocks that were studied here have a liquid crystalline core even in aqueous dispersions. The PEG hydrophilic shell is crystalline when dry, and becomes amorphous when hydrated. Data from nanospheres of different core segmental lengths show that changes in the structure of the core affect the structure of the shell and the hydrodynamic radius. Similarly, changes in the shell structure that occur as a result of changes in concentration, affect the structure of the core. The changes in the interactions of the PEG corona with the surrounding water that occurs upon dilution causes interdiffusion of the core and the shell segments, and decreases in the size and the crystallinity of the core. Consistent with published observations, unlike polymersomes, nanospheres are in constant state of flux, and this dynamic nature of the nanospheres can affect their drug release characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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PEG-*b*-oligo(desaminotyrosyl tyrosine octyl ester diacid)-*b*-PEG
 $[(C_6H_4)-(C_2H_4)-CONHCH-CH_2-(C_6H_4)COO(CH_2)_6CO] + [COO(CH_2)_7CH_3]$
 DTO-suberic acid (DTO-SA)

Figure 1.
 Chemical structure of the triblock copolymers used in this study.

Figure 2.

(a) DSC scans of PEG_{5k}, and from DP20 copolymer and freeze-dried DP20 nanosphere. (b) Wide-angle XRD scans of the samples used for the DSC scans. (c) DSC scan of the 11.6 mg/ml nanodispersion used in Figure 1b. (d) DSC scans of 5 and 30 mg/ml dispersions, different from those used in Figures b and c. The inset shows a segment at 10X y-scale.

Figure 3.

(a) Scheme of the XRD measurement. (b) Overlay of the three segments of the XRD scans at two different concentrations. The two sets of curves have been offset vertically for clarity. The inset shows that the MAXS peak is still visible at low concentrations, but is much broader.

Figure 4.

(a) $I(q) \cdot q$ vs. q plot to show the interference peak at high concentrations. (b) Guinier plots of the SAXS data for DP20 polymer at high and low concentrations. (c–d) Simulated curves at high and low concentrations.

Figure 5.
SANS data from DP20 at two different D₂O concentrations.

Figure 6.

(a) A model based on the SAXS data in Table 2. The dimensions shown are for the DP20 polymer-based nanospheres. (b) The structure in freeze-dried nanosphere. (c) Structure of the nanosphere at concentrations > 1 mg/ml. (d) Illustration of a diffuse interface between the core and the shell at concentrations < 1 mg/ml.

Figure 7.

Effect of concentration on the core structure. (a) Total diameter or the corona diameter. (b) Core diameter. (c) The crystallite size of the core. (d) The d-spacing between the crystalline stems in the core. Top row figures are from SAXS data, and the bottom row from the MAXS data. Curves are drawn free-hand through the data points to show the trend.

Figure 8.
WAXD data showing the effect of pH. Left: High concentration. Right: Low concentration.

Table 1

DSC results

Sample	$T_g(^{\circ}\text{C})$	$T_m(^{\circ}\text{C})$	$T_{\text{Exo}}(^{\circ}\text{C})$	$T_{\text{cc}}(^{\circ}\text{C})$
PEG _{5k}		69		40
DTO-SA[22]	21			
DTO-SA- <i>co</i> -PEG _{5k}	10	58		
Freeze-dried nanospheres		42 and 51	120	
Nanosphere dispersion (30 mg/ml)		40		

T_g - Glass transition temperature. T_m - Melting point. T_{ch} - Crystallization temperature upon heating. T_{cc} - Crystallization temperature upon cooling. T_{Exo} - Temperature of the exothermic peak upon heating

Table 2

Comparison of the various dimensional characteristics (all dimensions in nm) of the nanosphere at low- and high-concentrations. Low: The values are average of measurements at 0.3 and 0.6 mg/ml for DP 4.6, and at 0.07, 0.15 and 0.29 mg/ml for DP 20.4. High: The values are average of measurements at 2.3, 5.8 and 11.5 mg/ml for DP 4.6, and at 1.2, 2.3 and 11.6 mg/ml for DP 20.4.

DP	Conc.	D _h	PDI	SAXS				SANS		
				Inter-particle distance	Particle diameter	Core diameter	Corona thickness	Particle diameter	Core diameter	Corona thickness
4.6	low				26.9	4.4	11.3			
	High	40.7	0.086	55	28.0	8.0	10.0	27.5	14.8	6.3
20.4	Low				34.2	5.3	14.5			
	High	67.8	0.22	71	35.1	10.6	12.2	33.7	17.0	8.4

Abbreviations: DP- degree of polymerization of the DTO segments. D_h- hydrodynamic diameter from light scattering, PDI – Polydispersity index. D_g- geometric diameter from SAXS. The interparticle distance is from SAXS data obtained from concentrated dispersions.

Table 3

Dimensions of the nanospheres (DP 20.4) at three pH values.

pH	Hydrodynamic diameter (nm) Estimated error ± 1 nm	Particle diameter (nm) Estimated error ± 0.17 nm
5	66.2	33.4
7.4	65.8	33.4
10	55.6	32.8