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Self-Assembly of PEG and Diester Copolymers: Effect of PEG Length, Linker, Concentration and Temperature

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The formation of micelles in a solvent that is selective for one of the blocks is one of the most important and useful properties of block copolymers. Recently, we have synthesized copolymers of polyethylene glycol and various dimethyl esters, which self-assemble into nano micellar aggregates in aqueous media. These nano micelles are very effective in solubilizing otherwise insoluble drugs. The structure, interaction and size of these nano micelles are very important and critical to design an efficient drug delivery system. In the present work, we have studied the effect of hydrophilic block, PEG length, linker, concentration and temperature on these nano micellar structures and interactions by static light scattering techniques.

Keywords diester copolymers, self assembly

Introduction

Micelles formed by the assembly of amphiphilic block copolymers in aqueous solution have been investigated as potential drug carriers and offer many attractive characteristics (1–3). These nanocontainers are capable of encapsulating hydrophobic drugs in their core, thus improving the drug's water solubility. In addition, the size of copolymer micelles, typically between 20 and 100 nm, is not only effective in avoiding rapid renal exclusion, but is also small enough to avoid undesirable uptake by the reticuloendothelial system (4). Therefore, the circulation of the micellar carrier and encapsulated drug is

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prolonged, but since the micelles are composed of polymer chains that are small enough to be eliminated by renal filtration, the eventual disintegration of the micelle will allow the polymer to be excreted. This is important since the long-term build-up of polymer in the body could lead to toxicity. The size and prolonged circulation of copolymer micelles also facilitates their passive accumulation at pathological sites such as tumors where the vasculture has increased the permeability (5). This selective targeting of the drug carrier is ideal in terms of increasing the effectiveness and lowering the systemic toxicity of the drug payload. The important issue in determining the effectiveness of micellar drug carriers is their ability to encapsulate and deliver drugs in a controlled fashion. This in turn depends on the number of different parameters such as size, shape and stability of the micelles. The self-assembly of block copolymers has emerged as a popular means to produce complex nano-structured materials (6–8). The main advantages of self-assembly include the control over the structural and surface properties of critical importance in biological systems such as shape, size, surface charge, morphology and the availability of ligands.

Recently, we have developed a highly efficient nano-micellar drug delivery system capable of encapsulating hydrophobic, as well as hydrophilic drugs (9, 10). This new approach is based on the formation of nano-micelles by the self-assembly of amphiphilic copolymers in aqueous media. The amphiphilic polymers used in the self-assembly are based on poly(ethylene)glycol and various diesters, synthesized by chemical and enzymatic methods (11, 12). The design of system and synthetic strategy is very flexible and provides a high degree of control over the polymer structures. This has allowed us to tune the properties of the micelle disruption, the critical micelle concentration and size of the micelles. Here we describe the influence of the various parameters such as length of the hydrophilic PEG block, chemical structure of the linker, hydrophobic block, temperature and concentration on the self-assembly of the synthesized copolymers.

Experimental

Materials

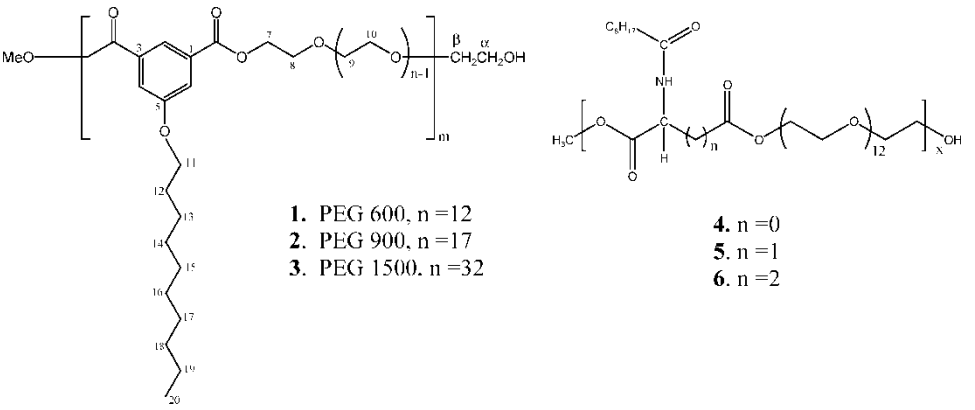
PEG-based amphiphilic copolymers including PEG 600IPdecanyl (**1**), PEG 900IPdecanyl (**2**), PEG 1500IPdecanyl (**3**), PEG 600MALnonanoyl (**4**), PEG 600ASPnonanoyl (**5**) and PEG 600GLUnonanoyl (**6**) were synthesized as reported earlier (9, 12, 13) (Scheme 1). The numbers 600, 900, and 1500 represent the number average molecular weights of PEG blocks, respectively that were used to form the main molecular chains of the copolymers.

Characterization

Static light scattering data was collected on a laser light scattering photometer (Wyatt Technology DAWN Model F) equipped with a 632 nm He-Ne laser as the light source.

Sample Preparation

The solutions of the amphiphilic copolymers, **1–6** were prepared by dispersing them in distilled water with gentle stirring for 30 min, followed by sonication for 15 min. The concentrations of the samples were varied from 0.1 mg/ml to 100 mg/ml. The sample solutions were purified by passing through a Millipore 0.20 μm filter.



Scheme 1. Amphiphilic copolymers synthesized by enzymatic catalysis.

Results and Discussion

The amphiphilic copolymers, **1–6** when dissolved in water above their critical micelle concentration (CMC) (Table 1), aggregate to form nano-micelles. The CMC values were determined by static light scattering and found independent on the size of the hydrophilic segment PEG as no significant change in CMC values were observed in going from PEG 600 to PEG 1500 (in the case of copolymers **1–3**). On the contrary, the CMC was highly dependent on the linker molecule used for copolymerization. The copolymers **1–6** have two different classes of linker molecules, aromatic linker (in the case of **1–3**) and aliphatic amino acid based linker molecules (in the case of copolymers **4–6**). As shown in Table 1, the copolymers **1, 4, 5**, and **6** having the same hydrophilic segment PEG 600 but with different linkers have very different CMC values. Copolymer **1**, having an aromatic linker, has the lowest CMC value, whereas for the copolymers **4–6** having aliphatic linker molecules, the CMC value increases with an increase in the molecular weight (or size) of the linker molecule.

The size of the nano-micelles formed by the self-assembly of copolymers **1–6** varied both with hydrophilic, as well as with hydrophobic, segments. In the case of copolymers **1–3** having the same aromatic diester as the linker, the radius of gyration (Rg) increases with the increase in size of the hydrophilic segment, (PEG size) and was found to be 17.85, 19.40, and 29.6 nm for PEG 600, 900, and 1500, respectively (Table 1, entry 1, 2, and 3).

Table 1
Critical micelle concentration and radius of gyration of copolymers **1–6**

Entry	Nanospheres	Rg (nm)	CMC (mmol)
1	(IP-PEG)600 **(1)**	17.85	3.17×10^{-5}
2	(IP-PEG)900 **(2)**	19.40	2.66×10^{-5}
3	(IP-PEG)1500 **(3)**	29.60	2.88×10^{-5}
4	(MAL-PEG)600 **(4)**	13.4 ± 3.4	5.08×10^{-5}
5	(ASP -PEG)600 **(5)**	18.8 ± 4.2	2.24×10^{-4}
6	(GLU-PEG)600 **(6)**	30.6 ± 3.5	5.89×10^{-4}

Similarly, in the case of copolymers **4–6** having the same hydrophilic segment, PEG 600, the radius of gyration increases with the increase in the molecular weight of the hydrophobic linker molecule *i.e.* from **4** to **5** and then to **6** (Table 1, entry 4, 5, 6). It was also observed that the particle size of the nano-micelle formed by the self-assembly of copolymer **1** was smaller as compared to the nano-micelles formed by the copolymers with aliphatic linker, **4**, **5**, and **6** (except in case of **4** the Rg was very close to **1**) having same hydrophilic segment, *i.e.* PEG 600 suggesting tighter packing in case of copolymer **1**. Based on these observations, we concluded that the nano-micelles formed by the self-assembly of copolymer having an aromatic diester as the linker are more stable as compared to the micelles formed by the copolymers having aliphatic linker molecules. Similar observations were noted in our earlier work on the encapsulation of various drugs in these nano-micelles (10). This may be due to the contribution of both hydrophobic and π-π interaction during the micellization process in case of copolymers **1–3** having aromatic diester as the linker molecule compared to copolymers **4–6** having aliphatic linker molecules.

In order to substantiate these observations that micelles formed by the copolymers having aromatic diesters as linker molecules are more stable as compared to those with aliphatic linker molecules, we have studied the effect of temperature and concentration on the stability of the micelles formed by the copolymers **1** (with aromatic linker) and **5** (with aliphatic linker). Figure 1 shows the effect of temperature on the radius of gyration and turbidity (which is directly related to voltage at 90° detector) for the copolymer **1**. It was observed that the radius of gyration and intensity of voltage at 90° detector increases slowly with increase in temperature until 55°C, and after that, there was a sudden jump in the radius of gyration as well as in voltage and the solution becomes highly turbid. This sudden increase was attributed to the disruption of micelles above 55°C leading to a highly turbid solution. Similar observations were noted in the aqueous solution of copolymer **5** (having aliphatic linker) with temperature (Figure 2) but at a lower temperature of 38°C as compared to 55°C in the case of copolymer 1 (with aromatic linker). This further strengthens the higher stability of micelles formed by the copolymers **1–3**, having aromatic linker compared to micelles formed by copolymers **4–6**, having aliphatic linker molecules.

The appearance of turbidity in the aqueous solution of copolymers **1–6** with the increase in temperature is interesting and intriguing, as we expected that after disruption of the micelles, the copolymer being water soluble, should have stayed in solution irrespective of whether micelles forms or not. Similar behavior was observed earlier by

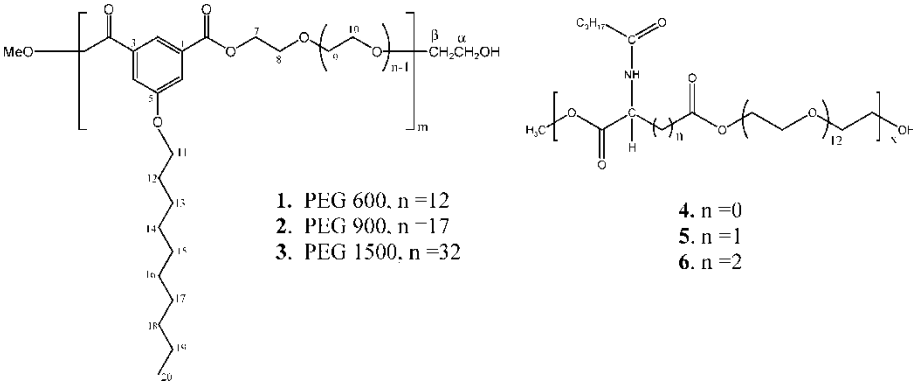


Figure 1. Radius of gyration (Rg) and intensity of scattered light with temperature for copolymer **1**.

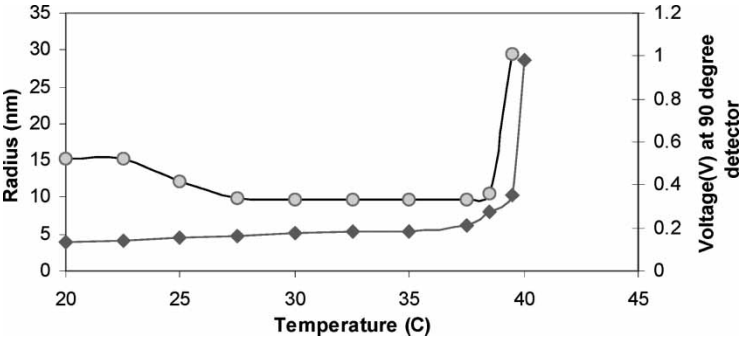


Figure 2. Radius of gyration (Rg) and intensity of scattered light with temperature for copolymer.

Francois et al. (14, 15) during the micellization of mono- and difunctionalized PEG's. The turbidity in the aqueous solution of **1–6** may be due to a decrease in the solubility of PEG chain in water with the increase in temperature. This is in accordance with solubility diagrams of polyethers in aqueous solutions which states that "at low concentrations, the PEG-water system presents a closed-loop solubility diagram" with a critical solution temperature (LCST), whereas when the temperature increases above the LCST, the solvent gets weak for PEG chains and phase separation occurs as a result of the attraction between different PEG chains (16, 17).

Figure 3a and 3b show the effect of concentration on the turbidity point with the increase in temperature on the aqueous solutions of copolymers **1** and **5**, respectively. Both copolymer solutions showed a sharp increase in the intensity of light at lower concentrations with the increase in temperature. Whereas, at higher solution concentrations, the change in intensity of scattered light was gradual with the increase in temperature. This may be due to the fact that in dilute solutions, the chances of PEG molecules to interact with each other are much lower even above LCST. However, once they have enough energy they form a big particle by the complete disruption of the micelles leading to sharp increase in turbidity or intensity of scattered light. Whereas in concentrated solutions, a small increase in temperature will be enough for the interaction of the different PEG chains due to higher concentration, leading to the gradual build up of bigger particles rather than disrupting the micelles and then forming a big particle.

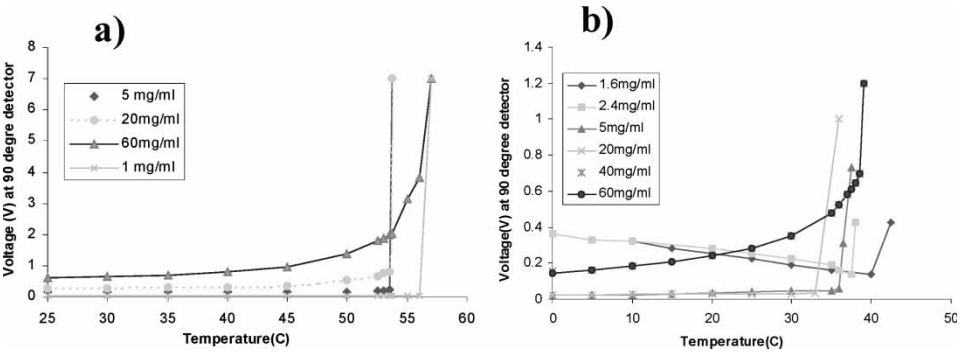


Figure 3. Effect of concentration on the intensity of scattered light with an increase in temperature a) in the case of copolymer **1** and; b) in the case of copolymer **5**.

Conclusions

The micelles formed by the self-assembly of the synthesized copolymers in aqueous media are highly stable. The size (Rg), CMC and stability of the micelles is highly dependent on the structure of the copolymers and can easily be tailored in a controlled fashion for specific application.

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