NOTE

Modulating the Guest Encapsulation and Release Properties of Multi-Arm Star Polyethylenimine-\textit{block}-Poly(\textepsilon\text{-Caprolactone})

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INTRODUCTION

Guest encapsulation has long been a hot topic due to its widespread application area, such as catalysis,1 drug delivery,2 and reaction vessels.3 The typical encapsulation systems include nanogels,4 microemulsions,5 and micelles derived from surfactants or amphiphilic polymers.6 Among these systems, the one with core-shell structure attracts much attention since it combines two different environments together.2 The core can supply confined microenvironment for the accommodation of guest molecules with similar polarity, while the shell with different polarity helps to stabilize the interface between the core and the external immiscible solvent, leading the guest molecules to be able to dissolve in their poor solvents.

Dendritic polymers including dendrimer and hyperbranched polymer are attracting tremendous interest at present due to their specific spheroid-like shape and multi-functionality, which are powerful motifs in the design of new molecular and supramolecular structures.7,8 On the basis of dendritic polymers, many kinds of amphiphilic dendrimers,9–14 hyperbranched polymers,14–24 and multi-arm star polymers25–34 have been addressed as unimolecular micellar nanocapsules for accommodating guest molecules due to their distinct core-shell structures. Moreover, their guest encapsulation efficiencies were higher than those of their corresponding linear analogs.20–23

The structure of the amphiphilic multi-arm star polymers derived from dendritic polymers can be categorized mainly into two types. Type I is that many linear homopolymer arms are linked together by dendritic polymer. The homopolymer arms are used as the shell for stabilizing the multi-arm star polymers in solvent, and the dendritic polymer having contrast polarity to the arms forms the core supplying microenvironment for the accommodation of guest molecules with the similar polarity.25–28 Type II is that amphiphilic linear block copolymers are used as arms, being linked together by dendritic polymer. The inner and outer blocks of the arms are regarded as the core and shell, respectively, whereas the dendritic polymers are only used as linker for the arms.29–34 When compared with the Type II polymers found in the literature,29–34 the reported Type I polymers usually contained large amount of easily modified functional end-groups, such as hydroxyl groups25–27 and amino groups.28 However, for both Type I and II polymers the modulation of their guest encapsulation and release properties was mainly realized by varying the size of the core or the shell, and nearly no attention has been paid for the effect of end group's modification on them due to the minor content of end-groups.25–24

In the previous article, we reported the Sn(Oct)$_2$ catalyzed ring-opening polymerization of $\textepsilon$-caprolactone (CL) initiated by hyperbranched polyethylenimine (PEI) polymers, leading to core-shell type multi-arm
star block copolymers polyethyleneimine-block-poly (ε-caprolactone) (PEI-b-PCL) with an average arm number in the range of 30–276. The resultant polymers contained large amount of hydroxyl end-groups, which were suitable for further modification. In this article, we will show the effect of the simple modification of the hydroxyl end-groups of PEI-b-PCL polymers on their guest encapsulation property. Moreover, the factors influencing the release rate of the encapsulated guests were also studied in detail.

**EXPERIMENTAL**

**Materials**

Hyperbranched polyethylenimines, PEI1.8K (Polysciences, $M_n = 1800$ g/mol, $M_w/M_n = 1.04$) and PEI10K (Aldrich, $M_n = 10^4$ g/mol, $M_w/M_n = 2.5$) were dried under vacuum prior to use. ε-Caprolactone (CL, 99%, Acros) was distilled from CaH$_2$ under reduced pressure. Tin(II) 2-ethylhexanoate [Sn(Oct)$_2$, 97%] was purchased from Alfa Aesar and used directly. Methyl Orange (MO, >95%) was purchased from Kewei Chemical Company and used directly. Benzoylated cellulose (MWCO 1000) were purchased from Sigma and used directly.

**Nomenclature**

PEIMW-(P(CL)$_y$)$_z$: PEIMW-(P(CL)$_y$)$_z$ represents the multi-arm star copolymer PEI-b-PCL; PEI means hyperbranched polyethyleneimine; MW represents the number-average molecular weight of PEI; P(CL) represents poly(ε-caprolactone); $y$ means the average degree of polymerization per PCL arm ($DP_{arm}$); $z$ indicates the average arm number of the star polymer.

PEIMW-(P(CL)$_y$)$_z$-CX: PEIMW-(P(CL)$_y$)$_z$-CX represents aliphatic acid modified PEI-b-PCL and $X$ means the carbon number of the aliphatic acid used.

PEIMW-HCl-(P(CL)$_y$)$_z$: PEIMW-HCl-(P(CL)$_y$)$_z$ represents HCl treated PEIMW-(P(CL)$_y$)$_z$.

**Polymerization**

In a typical experiment for PEI10K-(P(CL)$_{11,155}$-C5: 3.78 g (33.1 mmol) of CL, 40 mg (0.099 mmol) of Sn(Oct)$_2$ and 0.194 g of PEI10K (3.29 mmol of primary and secondary amine groups) were placed in a flask immersed in an oil bath thermostated at 130 °C. The mixture was stirred vigorously under N$_2$ atmosphere for 24 h. After cooling down, the mixture was dissolved in chloroform, and precipitated in a mixture of solvents of methanol and water (volume ratio is 4:1). The precipitation purification process was repeated twice to result in pure polymer. $^1$H NMR (chloroform-$d$): $\delta = 4.03$ (t, $-\text{COOC}_2\text{H}_5$), 3.62 (t, $-\text{CH}_2\text{CH}_2\text{OH}$), 2.38–3.55 (br, PEI), 2.28 (t, $-\text{CH}_2\text{COOC}_2\text{H}_5$), 1.61 (m, $-\text{COOC}_2\text{H}_4\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}$–), $-\text{COOC}_2\text{H}_4\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 1.35 (m, $-\text{COOC}_2\text{H}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}$–, $-\text{COOC}_2\text{H}_2\text{CH}_2\text{OH}$).

**Esterification of PEI-b-PCL with Aliphatic Acid**

In a typical experiment for the synthesis of PEI1.8K-(P(CL)$_{10,30}$-C5: 0.10 g (0.50 mmol) of DMAP were added into the flask containing the solution of 0.35 g of PEI1.8K-(P(CL)$_{10,30}$ (0.30 mmol of hydroxyl groups) in 5 mL of methylene dichloride. After all the solids were dissolved under vigorous stirring, 53 $\mu$L (0.50 mmol) of pentanoic acid was added. The mixture was gently stirred under N$_2$ atmosphere at room temperature for 24 h. Subsequently, the mixture was cooled in the fridge for an hour and then filtered. After removing the volatiles, the residue was dissolved in a small amount of chloroform, and precipitated in diethyl ether. The precipitant was collected and dried. Yield: 47%.

**Quaternization of the PEI Core with HCl, HBr, and HI**

The chloroform solution of the obtained multi-arm star polymers (0.02 g/mL) was mixed violently with half the volume of HCl, HBr, or HI aq. with the concentration being 10 M. The pH value of upper aqueous phase was kept to be less than 1 after keeping it still for a minute. The mixture was vigorously stirred for 2.5 h. After phase separation, the organic phase was taken out and dried using anhydrous Na$_2$SO$_4$. After removing the volatiles, the residue was dissolved in chloroform, and precipitated in diethyl ether. The precipitant was collected and dried.

**Exchange of Anionic Counter-Ions for the Quaternized Star Polymers**

The multi-arm star polymer quaternized by HI was dissolved in chloroform and enough Na$_2$SO$_4$ aq. was added. The mixture was vigorously stirred for 2.5 h. After phase separation, the organic phase was taken out and dried using anhydrous Na$_2$SO$_4$. After removing the volatiles, the residue was kept at 40 °C in vacuum oven overnight. (The PEI cores of PEI10K-(P(CL)$_{11,155}$-C5 and PEI1.8K-(P(CL)$_{10,30}$-C5 were regarded to contain 78 and 12 anionic counter-ions, respectively.)

**Encapsulating MO Molecules by the Multi-Arm Star Polymers Using Liquid–Liquid Extraction Protocol**

CHCl$_3$ solution (4.5 mL) of multi-arm star polymer with concentration being $1 \times 10^{-6}$ M was added to 4.5 mL of
MO in water ([MO]₀ = 9.0 × 10⁻⁵ M). The solution was shaken for 10 min and kept at room temperature in dark chamber overnight prior to measure. The water phase was measured by UV-Vis spectrum and the remaining dye in water can be calculated according to Lambert-Beer law (The mole extinction coefficients (e) of MO in water at 464 nm was measured to be 2.16 × 10⁴ L/mol/cm). Subsequently, the numbers of dye transferred into CHCl₃ phase could be therefore obtained.

Encapsulating MO Molecules by the Multi-Arm Star Polymers Using Solid-Liquid Extraction Protocol

A 5 mg of MO was added to the CHCl₃ solution of multi-arm star polymer with concentration being 1 × 10⁻⁶ M. The mixture was violently stirred for 14 h, and then was filtered to remove the insoluble solid. The absorbance data of the solution were obtained using a UV–Vis spectrophotometer. The molar extinction coefficient of the MO encapsulated by the polymers in CHCl₃ at 428 nm was found to be 2.59 × 10⁴ L/mol/cm.

Release Study of MO Molecules Encapsulated by the Multi-Arm Star Polymers

A solution of the MO-encapsulated polymer (24 mg) in THF (1 mL) was dialyzed at 37 °C using a dialysis tube (Benzoylated cellulose tubing, MWCO 1000) in Phosphate Buffered Saline (PBS) with pH = 7.4 (100 mL). At scheduled time intervals, about 3 mL of the outer PBS solution was transferred into a UV-vis cuvette, and its UV-vis spectrum was characterized.

Characterization

¹H NMR spectra were recorded on a Varian INOVA 500MHz spectrometer, operated at 500 MHz. The chemical shifts are given in parts per million (ppm). UV-vis spectra were obtained from Purkinje General (China) T6 UV/Vis Spectrophotometer. FTIR spectra were recorded on a Nicolet 5DXC FTIR spectrometer. Dynamic light scattering (DLS) measurements were performed using the BrookHaven BI-200SM DLS instrument at 25 °C with 532.0 nm laser light, and the light collection at 90 °C. The particle size was calculated from COTTIN method.

RESULTS AND DISCUSSION

Syntheses of Aliphatic Acid Ester Terminated PEI-b-PCL

Commercially available PEIs with Mₙ = 1800 and 1 × 10⁴/g/mol, namely, PEI1.8K and PEI10K, are hyperbranched polymers resulted from the cationic ring-opening polymerization of aziridine, which contains primary, secondary, and tertiary amine functional groups. In this article, based on PEI1.8K and PEI10K, a series of amphiphilic multi-arm star copolymers, PEI-b-PCL, with an average arm number in the range of 30–160 were prepared using the method reported previously.²⁵,²⁶ The obtained PEI-b-PCL star copolymers contain large amount of polar hydroxyl end-groups, which are suitable for the further modification with one kind or several kinds of desired functional groups. Herein, we transformed the polar hydroxyl end-groups of PEI-b-PCL into the apolar aliphatic acid ester groups using the DCC and DMAP catalyzed esterification reaction between PEI-b-PCL and the corresponding aliphatic acid at room temperature under N₂ atmosphere (Scheme 1). All the aliphatic acids used here were linear, including acetic acid (C2), propanoic acid (C3), pentanoic acid (C5), octanoic acid (C8), and dodecanoic acid (C12). All the reaction proceeded at very mild condition and the obtained products were purified through the simple precipitation in diethyl ether solvent.

The purified polymers were characterized by FTIR spectrometry. The disappearance of the broad peak centered at 3418 cm⁻¹ originating from stretching bond frequency (O-H) pointed out the successful modification of the hydroxyl groups of PEI-b-PCL polymers. PEI-b-PCL polymers before and after being modified with different aliphatic acids were also characterized by ¹H NMR spectra (Fig. 1). When compared with the ¹H NMR spectrum of PEI-b-PCL [Fig. 1(A)], new signal coming from the terminal methyl protons of aliphatic chains can be seen clearly in all the ¹H NMR spectra of aliphatic acid modified PEI-b-PCL, indicating the successful modification of the hydroxyl groups of PEI-b-PCL into aliphatic acid ester groups. Furthermore, the location of these signals coming from the short aliphatic chains is obviously different with that from the long aliphatic chains due to the different environments. For instance, the signal appears at 2.01, 1.10, 0.88, 0.84, and 0.84 ppm for acetic acid, propanoic acid, pentanoic acid, octanoic acid, and dodecanoic acid modified PEI-b-PCL, respectively [Fig. 1(B–F)]. The signal of methylene protons adjacent to the terminal hydroxyls of PEI-b-PCL [Fig. 1(A)] can not be seen in the aliphatic acid modified PEI-b-PCL, thus it can be deduced that nearly all the hydroxyl groups of PEI-b-PCL can be effectively transformed into the aliphatic acid ester groups under the experimental condition.

Guest Encapsulation Property of the Multi-Arm Star Polymers

PEI-b-PCL is composed of hydrophilic PEI core and hydrophobic PCL shell, which can be used as inverted micellar nanocapsule to accommodate hydrophilic guest molecules.²⁵,²⁶ It has been known that enlarging the polarity difference between core and shell of the nanocapsules helps to enhance their guest encapsulation capacities.²⁵,²⁶ To raise the guest encapsulation capacity of PEI-b-PCL, reducing the polarity of the PCL shell or...
increasing the polarity of the PEI core can be adopted. First, we transformed the polar hydroxyl end-groups of PEI-b-PCL into the apolar aliphatic acid ester groups to see whether its guest encapsulation capacity could be improved due to the polarity reduction of the PCL shell. The water-soluble anionic dye probe, namely MO, was utilized here as the guest molecules.

Liquid–liquid extraction protocol, that is the chloroform solution of the star nanocapsules and the aqueous solution of the anionic hydrophilic MO guests were mixed together, was adopted first for evaluating the MO encapsulation capacities of the obtained nanocapsules. Their guest encapsulation capacities were studied from two aspects, that is the molar ratio and weight ratio of the encapsulated MO molecules to nanocapsules. From Table 1 it can be seen that transforming the hydroxyl end-groups of PEI-b-PCL polymers into pentanoate groups (PEI-b-PCL-C5) can effectively enhance their guest encapsulation capacity at the aspect of both the molar ratio and weight ratio. Subsequently, the effect of the modification of the terminal groups of PEI-b-PCL on other factors influencing the guest encapsulation capacity was studied. Increasing the PCL arm length of the star nanocapsules was also a way to enlarge the polarity difference between core and shell due to the decrease of the shell polarity. However, only the molar ratio of the encapsulated guests to nanocapsules could be improved to some extent when the DP_n(arm) of PCL arm was raised, whereas the weight ratio became much worse (Fig. 2). As far as the practical application is concerned, weight ratio is more meaningful than molar ratio. Thus, the star nanocapsules with shorter hydrophobic PCL arm was preferred for the more efficient guest encapsulation. In this context, the star nanocapsules with the shortest PCL arm, that is DP_n(arm) being about 10 was focused on. Increasing the PEI core size could effectively enhance the guest encapsulation capacity of both PEI-b-PCL and PEI-b-PCL-C5 at the aspect of the molar ratio [Fig. 2(A)]. At the aspect of the weight ratio it was more effective for PEI-b-PCL-C5 with DP_n(arm) being about 10 and the content of the encapsulated MO could be raised from 4.5 to 5.5%, whereas it was almost ineffective for PEI-b-PCL and the content of the encapsulated MO was nearly same to be around 4% [Table 1 and Fig. 2(B)]. Just like the modification of the end-groups of PEI-b-PCL, quaternizing the PEI core with HCl could also effectively enhance their guest encapsulation capacity at the aspect of both the molar ratio and weight ratio (Comparing Nos. 4 with 7 in Table 1). After Quaternization, the MO encapsulation capacity of PEI-b-PCL-C5 could be enhanced from 5.5 to 10.7%, whereas that of PEI-b-PCL was only raised from 4.0 to 7.3%, indicating that the modification of the hydroxyl end-groups and the quaternization of the PEI core of PEI-b-PCL have concerted effect on enhancing their guest encapsulation capacity.

Anionic counter-ions existed in the core of the quaternized nanocapsules. Thus, we further studied the effect of anionic counter-ions on their guest encapsulation capacity. The quaternized nanocapsules with halogen counter-ions were prepared through the direct acidification of the PEI core with the corresponding monoprotic acid, that is HCl, HBr, and HI. The color of the HCl and HBr treated polymers was white or yellowish, whereas the polymer treated with HI was brown. To compare the difference between the univalent and bivalent anions, SO_4^{2-} was used. If the H_2SO_4 aq. was used
directly, especially when adequate quantity was added, the counter-ions would be the univalent HSO₄⁻, not the bivalent SO₄²⁻. Therefore, the ion-exchange method was adopted: The polymer acidified by the HI was dissolved in chloroform, which exhibited brown color. Then the transparent excess Na₂SO₄ aq. was added and the mixture was stirred vigorously for 2.5 h. After phase separation, the organic phase became colorless, indicating that the iodide anions of the polymers were replaced by the sulfate anions.

Figure 3 shows the effect of Cl⁻, Br⁻, I⁻, and SO₄²⁻ counter-ions on the encapsulation capacity of the quaternized PEI10K-(P(CL)₁₁)₁₅₅-C₅. It is clear that no matter what the counter-ions are, the quaternized nanocapsules always show a better guest encapsulation capacity than the unquaternized one, due to the increase of the polarity of the PEI core. The univalent counter-ions, Cl⁻, Br⁻, and I⁻ have a similar effect on the MO encapsulation capacity of the star nanocapsules. After the univalent counter-ions are replaced by the bivalent SO₄²⁻ counter-ions...
counter-ions, the guest encapsulation capacity of the quaternized nanocapsule is reduced. This phenomenon can be explained as follows: \(\text{SO}_2/\text{CO}_4\) ion interacts with the quaternized PEI core much stronger than halogen anion because one \(\text{SO}_2/\text{CO}_4\) ion interacts simultaneously with two ammonium cations of the quaternized PEI core, whereas one halogen anion interacts only with one. Thus, it is more difficult for the MO molecule having one univalent sulphonate functional group to substitute the bivalent \(\text{SO}_2/\text{CO}_4\) ion than the univalent halogen ion.

From the above discussion it is known that the modification of the terminal hydroxyl groups of PEI-\(b\)-PCL through the esterification with pentanoic acids can significantly enhance its guest encapsulation capacity. We wondered whether the chain length of the aliphatic acid used also influenced the encapsulation capacity of

### Table 1. MO Encapsulation Capacities of Star Polymers Before and After Modification with Pentanoic Acid

<table>
<thead>
<tr>
<th>No.</th>
<th>Nanocapsule(^a)</th>
<th>(N)</th>
<th>(W(%))</th>
<th>(N)</th>
<th>(W(%))</th>
<th>(\Delta N)</th>
<th>(\Delta W(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PEI1.8K-(P(CL)<em>{10})</em>{30}</td>
<td>4.3</td>
<td>3.9</td>
<td>5.3</td>
<td>4.5</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>PEI1.8K-(P(CL)<em>{21})</em>{30}</td>
<td>4.9</td>
<td>2.2</td>
<td>6.0</td>
<td>2.6</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>PEI1.8K-(P(CL)<em>{30})</em>{30}</td>
<td>4.8</td>
<td>1.5</td>
<td>8.3</td>
<td>2.4</td>
<td>3.5</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>PEI10K-(P(CL)<em>{11})</em>{155}</td>
<td>25.2</td>
<td>4.0</td>
<td>36.7</td>
<td>5.5</td>
<td>11.5</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>PEI10K-(P(CL)<em>{23})</em>{155}</td>
<td>31.5</td>
<td>2.5</td>
<td>38.5</td>
<td>3.0</td>
<td>7.0</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>PEI10K-(P(CL)<em>{34})</em>{160}</td>
<td>31.6</td>
<td>1.7</td>
<td>40.1</td>
<td>2.3</td>
<td>8.5</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>PEI10K-HCl-(P(CL)<em>{11})</em>{155}</td>
<td>47</td>
<td>7.3</td>
<td>71.9</td>
<td>10.7</td>
<td>24.9</td>
<td>3.4</td>
</tr>
</tbody>
</table>

\(^a\)PEIMW-(P(CL)_{y})_{z}; PEI means hyperbranched polyethylenimine; MW represents the molecular weight of PEI; P(CL) represents poly(ε-caprolactone); \(y\) means the degree of polymerization per arm; \(z\) means the arm number of the star polymer; PEIMW-HCl-(P(CL)_{y})_{z} represents HCl treated PEIMW-(P(CL)_{y})_{z} polymer.

\(^b\)MO represents methyl orange; \(N\) means the average number of MOs transported into one polymer capsule; \(W\) means the average weight ratio of transported MOs to the polymer capsules; \(W = N \times 327.3 \div M_{n}(\text{nanocapsule})\).

\(^c\)HPEI-b-PCL-C5 means pentanoic acid modified HPEI-b-PCL.

![Figure 2](image)

**Figure 2.** The amount of encapsulated MO molecules to nanocapsules versus DP\(_n\) of PCL arms of the star nanocapsules in the aspect of (A) molar ratio and (B) weight ratio.

![Figure 3](image)

**Figure 3.** Effect of the anionic counter-ions of the quaternized PEI10K-(P(CL)_{11})_{155}-C5 on its MO encapsulation capacity.
Thus, we modified PEI10K-(P(CL)11)155 and PEI10K-(P(CL)23)155 by linear aliphatic acids with different carbon number. From Figure 4, it can be seen that the polymers capacity for the MO encapsulation is enhanced significantly with increasing the carbons of the aliphatic acids at the aspect of molar ratio, especially for the polymer with shorter PCL arm. As far as weight ratio is concerned, the aliphatic acids with carbons ranging from 3 to 12 has only minor effect on the polymer PEI10K-(P(CL)22)155 with longer PCL arm. Whereas, as for the polymer PEI10K-(P(CL)11)155 with shorter PCL arm, the carbon number of the employed aliphatic acid has obvious influence on its encapsulation capacity. The weight ratio increases following the increase of the carbon number from 2 to 8. Aliphatic acids having 8 and 12 carbons have similar effect on the encapsulation capacity of PEI10K-(P(CL)11)155 at the aspect of weight ratio.

For comparison, the MO encapsulation capacities of the obtained nanocapsules were also evaluated by the solid–liquid extraction protocol, that is excess MO molecules in solid state were mixed with the chloroform solution of the multi-arm star nanocapsules. From Table 2, it is obvious that the obtained nanocapsules can encapsulate more MO guest molecules using the liquid–liquid extraction protocol than that using the solid–liquid protocol. What the liquid–liquid extraction protocol mainly differs from the solid–liquid extraction protocol is the existence of water in the system, thus, it can be deduced that water enhances the uptake of the anionic hydrophilic MO molecules by the nanocapsules. It was proposed that additional ionic interaction with anionic guests was introduced due to the partial protonation of the basic PEI core by water, which led more anionic guests to be encapsulated. However, from our experiment this assumption is not the main reason since the star nanocapsule with protonated PEI core (No. 2 in Table 2) can also encapsulate much more MO guest molecules using the liquid–liquid extraction protocol than that using the solid–liquid extraction protocol. In the literature it was also proposed that besides the ionic interaction, the presence of water might lead to aggregate formation, which helped to encapsulate more guest molecules. To make sure whether aggregates were formed, we measured the obtained nanocapsules in chloroform by DLS in the absence and presence of water, respectively (Fig. 5). It is obvious that without water, the nanocapsules in chloroform are in the unimolecular micelle state and the average diameter is around 13 nm [Fig. 5(A)]. When water exists in the system, besides the unimolecular micelles aggregates with diameter around 100 nm appear, implying that the star nanocapsules encapsulate the MO guest molecules not only in the unimolecular micelle form, but also in the aggregate state.

![Figure 4](image.png)

**Figure 4.** The encapsulation capacity of PEI-b-PCL modified by aliphatic acids with different carbon number.

Table 2. Comparison of Liquid–Liquid Protocol with Solid–Liquid Protocol for the MO Encapsulation Capacities of Star Polymers

<table>
<thead>
<tr>
<th>No.</th>
<th>Nanocapsule</th>
<th>MO Encapsulation Using Liquid–Liquid Protocol&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MO Encapsulation Using Solid–Liquid Protocol&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>W (%)</td>
</tr>
<tr>
<td>1</td>
<td>PEI10K-(P(CL)11)155-C5</td>
<td>36.7</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>PEI10K-HCl-(P(CL)11)155-C5</td>
<td>71.9</td>
<td>10.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> PEIMW-(P(CL)<sub>y</sub>)<sub>z</sub>-C5: PEI means hyperbranched polyethylenimine; MW represents the molecular weight of PEI; P(CL) represents poly(e-caprolactone); y means the degree polymerization of per arm; z means the arm number of the star polymer; C5 means that PEIMW-(P(CL)<sub>y</sub>)<sub>z</sub> was modified by pentanoic acid; PEIMW-HCl-(P(CL)<sub>y</sub>)<sub>z</sub>-C5 represents HCl treated PEIMW-(P(CL)<sub>y</sub>)<sub>z</sub>-C5 polymer.

<sup>b</sup> MO represents methyl orange; N means the average number of MOs transported into one polymer capsule; W means the average weight ratio of transported MOs to the polymer capsules; \( W = N \times 327.3 / M_n(\text{nanocapsule}) \).
Guest Release Property of the Multi-Arm Star Polymers

The guest release property of the obtained star nanocapsules was studied at 37 °C in the PBS solution with pH = 7.4. From Figure 6, it can be seen that free MO molecules are released very fast and this release process finishes after around 6 h [Fig. 6(A)]. After being encapsulated by the unmodified PEI-b-PCL polymers, MO molecules are released in a sustained way. The influence of their PCL arm length on the MO release rate is minor and all the MO molecules can be released into the PBS solution after 40 to 50 h [Fig. 6(A)]. Furthermore, the PEI core size has also negligible effect on the MO release rate of PEI-b-PCL polymers [Fig. 6(B)]. After transforming the polar hydroxyl groups into the apolar aliphatic acid ester groups, the release rate becomes slow and it will take about 100 h for all the MOs to be released (Fig. 7). Moreover, the difference of the terminal aliphatic chain length renders limited effect on the release rate. The different MO release rate exhibited by the PEI-b-PCL before and after modification by aliphatic acid can be explained as follows: although PEI-b-PCL polymers are not soluble in water, their plenty of hydrophilic hydroxyl groups render them relatively hydrophilic, leading the PCL chains to be loosely packed around the PEI core. The shell formed by the PCL chains with hydroxyl end-groups may contain many irregular pores that are big enough and do not interfere with the passing MO molecules. Thus, the shell thickness, that is the PCL length, will have nearly no effect on the diffusion rate of the entrapped MO molecules in the PEI core across the PCL shell. After transforming the hydrophilic hydroxyl groups into the apolar groups, the PCL chains become totally hydrophobic, which will collapse in water and be packed densely around the PEI core. Thus, the size of the irregular pores in the PCL shell will become small enough and have interaction with the passing MO molecules in some extent, limiting their diffusion rate.

From Figure 7, we can know further that the release rate of MO guest molecules can be prolonged by the quaternization of the PEI core. The total release time of MO encapsulated by PEI10K-(P(CL)11)155-C5 is about 100 h, whereas the quaternized PEI10K-(P(CL)11)155-C5 can prolong it to be around 240 h. Why quaternized PEI10K-(P(CL)11)155-C5 makes MO be released slower than the unquaternized one? One plausible reason is that at pH = 7.4, the PEI core of PEI10K-(P(CL)11)155-C5 is only partially protonated, whereas that of HCl treated PEI10K-(P(CL)11)155-C5 is fully protonated. Thus, the interaction of the anionic MO molecules with HCl treated PEI10K-(P(CL)11)155-C5 is much stronger than that between MO and PEI10K-(P(CL)11)155-C5, leading that MO molecules are released much slower. From the above results it can be known that the guest release time can be well controlled in a wide range by simple modification of the terminal groups of PCL shell.
CONCLUSIONS

Amphiphilic multi-arm star block copolymers PEI-b-PCL could be used as inverted micellar nanocapsule to accommodate hydrophilic guest molecules. Their guest encapsulation and release properties were modulated by the further modification of the PEI core and PCL shell. As for the modification of PCL shell reducing the PCL arm length and transforming the plenty of polar hydroxyl end-groups into the apolar aliphatic acid ester groups were two effective method of enhancing the guest encapsulation capacity of the obtained nanocapsules. Moreover, the guest encapsulation capacity of the aliphatic acid modified PEI-b-PCL increased with increasing the carbon number of aliphatic acid. As far as the PEI core was concerned, quaternizing the PEI core was the very efficient way to increase the guest encapsulation capacity. The guest encapsulation capacity of the quaternized star nanocapsule was almost constant when the anionic counter-ions were univalent, whereas it was reduced after the univalent anionic counter-ions were replaced by the bivalent ones. Furthermore, raising the size of PEI core, quaternizing the PEI core, and modifying the end-groups with apolar groups showed concerted effect on raising the guest encapsulation capacity of PEI-b-PCL. Liquid–liquid extraction protocol helped the star nanocapsules to load more MO guests than the solid–liquid extraction protocol. Furthermore, DLS revealed that the star nanocapsules encapsulated the MO guest molecules not only in the form of unimolecular micelle, but also in the aggregate state when liquid–liquid extraction protocol was adopted. The MO guests encapsulated by the star nanocapsules could be released in a sustained way and the release rate was adjustable. The MO molecules encapsulated by PEI-b-PCL nanocapsule were released completely after 40–50 h no matter what the PCL arm length was, whereas the full release time could be prolonged to be around 100 and 240 h using the unquaternized and quaternized star nanocapsule terminated with apolar aliphatic acid ester groups, respectively.

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