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# Non-enzymatic and enzymatic degradation of poly(ethylene glycol)-*b*-poly-(ε-caprolactone) diblock copolymer micelles in aqueous solution

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ABSTRACT

The non-enzymatic and enzymatic degradation behaviors of the monomethoxy-poly(ethylene glycol)-*b*-poly(ε-caprolactone) diblock copolymers (MPEG–PCL) micelles in aqueous solution were investigated by DLS, $^1$H NMR, SEC and HPLC. It is found that the degradation mechanism of MPEG–PCL micelles in aqueous solution in non-enzymatic case is quite different from that in the presence of enzyme. In non-enzymatic case, the degradation induced by acidic catalysis was not found in low pH aqueous solution but the degradation of the micelles occurred under neutral and basic conditions. The degradation of MPEG–PCL micelles first happens near the interface region of the MPEG shell and PCL core, leading to the part detachment of PEG chains. With increasing degradation time, the degradation inside the PCL core with a random scission on PCL chains occurred. Compared with non-enzymatic degradation, the enzymatic degradation of MPEG–PCL micelles is much fast and the degradation rate of MPEG–PCL micelles is proportional to either the micelles or the enzyme concentration in a certain range. Based on the micelle degradation behaviors that we observed, a possible mechanism for the enzymatic degradation of the MPEG-*b*-PCL micelles including PCL core erosion which results in cavitization of micellar core and micellar dissociation is proposed.



## 1. Introduction

Polymer micelles, made of biocompatible and biodegradable amphiphilic block copolymers, such as poly(lactide)–poly(ethylene glycol) (PLA–PEG) [1], poly(caprolactone)–poly(ethylene glycol) (PCL–PEG) [2–4] and poly(caprolactone-*co*-lactide)–poly(ethylene glycol) (PCLLA–PEG) [5,6], have been the subject of scientific attention in recent years. These micelles with a core-shell structure are normally prepared by a self-assembly strategy using amphiphilic block copolymers in a selective solvent [7–11], and such core-shell structure micelles can incorporate lipophilic drugs into their cores and release the drug in a controlled manner at a later stage, making them a potential carrier for poor water solubility drugs [12].

Although these micellar systems have been thoroughly studied on the preparation method, size control, modification, drug loading and drug release behavior [13], the understanding on the degradation behaviors of polymeric micelles is still limited. Moreover, the divergence in their degradation mechanism is obviously present. For example, Li et al. reported the enzymatic degradation of the films prepared by PCL-containing block copolymers [14–19] and they found that the content of PEG in the copolymer would not affect the degradability of the PCL segment [15,16]. However, they have not referred to the degradation of the copolymer micelles yet. Belbella et al. studied the degradation of D,L-PLA nanospheres in different pH solutions and they pointed out that the hydrolysis was much more catalyzed in acidic and basic media with respective "random scission" and chain-end cleavage" mechanisms than in neutral medium [20]. The degradation process of giant and flexible worm micelles prepared from PEO–PCL has also been studied and it was found that the worm micelles could be spontaneously shorten to spherical micelles by chain-end hydrolysis of the PCL [21]. Wu et al. developed a novel method to study the enzymatic biodegradation of PEO–PCL nanoparticles and micelles in the presence of the lipase PS (from *Psedomonas cepacia*) by laser light scattering (LLS) [22–24]. They found that the degradation was a first-order reaction [22–25] and the initial biodegradation rate was independent of the micelles concentration for a given enzyme concentration [22–24]. They also pointed out that the lipase PS "ate" the PCL micelles in a one-by-one manner [22–25], though the conclusion, they

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**Table 1**
The properties of the MPEG–PCL block copolymers and their micelles.

| Sample | NP2K4K | NP4K20K | NP10K30K |
|---|---|---|---|
| CL/EG[a] | 0.77 | 1.93 | 1.16 |
| CL/EG[b] | 0.76 | 1.90 | 1.18 |
| $M_n$[c] | 4.7k | 18.6k | 32k |
| $M_w$[c] | 8.1k | 30.5k | 49.4k |
| $M_w/M_n$[c] | 1.72 | 1.64 | 1.54 |
| Micellar size/nm[d] | 35.4 ± 2.5 | 72.4 ± 2.3 | 92.3 ± 2.1 |
| Micellar size/nm[e] | 49.2 ± 2.8 | 89.4 ± 3.2 | 139.0 ± 4.9 |
| P.D.[f] | 0.17 | 0.21 | 0.12 |

[a] Molar ratio in feed.
[b] Determined by $^1$H NMR.
[c] Determined by SEC.
[d] prepared in ratio of polymer/acetone/water 40/2/40 (mg/mL/mL).
[e] prepared in ratio of polymer/acetone/water 100/2/25 (mg/mL/mL).
[f] P.D.: polydispersity of the micelles.

thought, "should be reconsidered" after introducing a new monitor method of pH in situ examination [24]. Lately, Myrra et al. investigated the chemical and enzymatic degradations of short monodisperse oligo(ε-caprolactone) (OCL) and its amphiphilic block oligomer with short chain PCL and PEG, and they concluded that the PEG-*b*-OCL micelles are stable systems in buffer, and the degradation of OCL was markedly accelerated by the presence of lipase [26]. In our previous work, the non-enzymatic degradation behavior of PEG–PCL triblock copolymer micelles in aqueous solution at room temperature was studied [27].

In this paper, we used the MPEG–PCL diblock copolymer micelles as a model to study their non-enzymatic and enzymatic degradation behaviors in aqueous solution at 37 °C by dynamic light scattering (DLS), proton nuclear magnetic resonance ($^1$H NMR), size exclusion chromatography (SEC), high performance liquid chromatography (HPLC) and transmission electron microscopy (TEM) techniques in order to gain further insight into the degradation of PEG–PCL micelles.

## 2. Experimental section

### 2.1. Materials

ε-Caprolactone (ε-CL) (Aldrich, USA) was purified by drying over $CaH_2$ and distilled under reduced pressure. Monomethoxy-poly(ethylene glycol) (MPEG), with molecular weights of 2000, 4000, and 10 000 g/mol were obtained from Jinling Petroleum Co., Jiangsu, China, and vacuum-dried at 50 °C for 24 h before use. Stannous octoate (Sigma) and 6-hydroxycaproic acid (Alfa Aesar) were used as-received. Lipase AY with the activity of about 2 U/mg (lipase from *Candida cylindracea*, Fluka) was purified by freeze-drying. All other chemicals were of analytical grade and used without further purification.

### 2.2. Synthesis of MPEG–PCL diblock copolymers

MPEG–PCL diblock copolymers were synthesized by a ring-opening copolymerization as previously described [5,27]. Briefly, a predetermined amount of CL was added into a polymerization tube containing MPEG and a small amount of stannous octoate (0.1% wt/wt). The tube was then connected to a vacuum system, sealed off, and placed into an oil bath at 130 °C for 48 h. After the polymerization was complete, the crude copolymers were dissolved with chloroform and precipitated into an excess amount of diethyl ether to remove the unreacted monomer and oligomer. The precipitates were then filtered and washed with diethyl ether several times before thoroughly dried at reduced pressure.

### 2.3. Preparation of MPEG–PCL micelles

MPEG–PCL diblock copolymer micelles were prepared by a precipitation method. Copolymer 100 mg was dissolved in 5 mL of acetone, and the solution was added dropwise into 25 mL of distilled water under moderate stirring at 25 °C to produce an aqueous suspension. The acetone in suspension was then removed under reduced pressure or by dialysis in water. The suspension was filtered with a microfilter of pore size 650 nm to remove the polymer aggregates and the larger micelle aggregates.

### 2.4. Degradation of MPEG–PCL micelles

The degradation behaviors were studied by two different ways: with and without the presence of lipase AY. In the non-enzymatic experiment, in each 25 mL bottle, 10 mL suspensions of MPEG–PCL micelles (about 4 mg/mL) was added, and the volume was adjusted to 20 mL with buffer solution ($KH_2PO_4$, 0.1 N pH 4.4). The medium pH can also be adjusted to the required value (4.44, 7.40 and 10.20) by addition of 4 N NaOH. These bottles were then stored at 37 °C in dark. At determined intervals, samples were taken out from the bottles for analyses. In the enzymatic biodegradation experiment, a proper amount of lipase AY was added into the polymeric micelle dispersion to start biodegradation. The biodegradation was conducted at 37 °C both inside the DLS cuvette to in situ measure the effective diameter and light scattering intensity with time and in a big container to collect the samples for other tests simultaneously.

### 2.5. Characterization of copolymers and the micelle degradation products

SEC measurements were performed at room temperature on a Waters 515 systems equipped with a Wyatt Technology Optilab

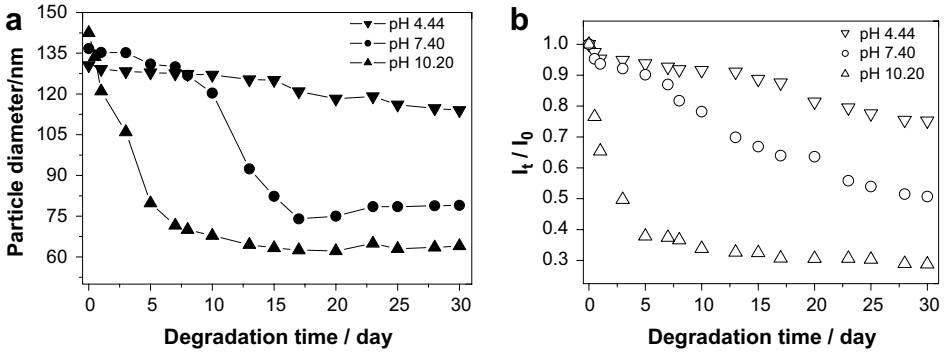


**Fig. 1.** (a) Micellar size and (b) light scattering intensity changes of NP10K30K micelles in different pH media at 37 °C during the non-enzymatic degradation process.

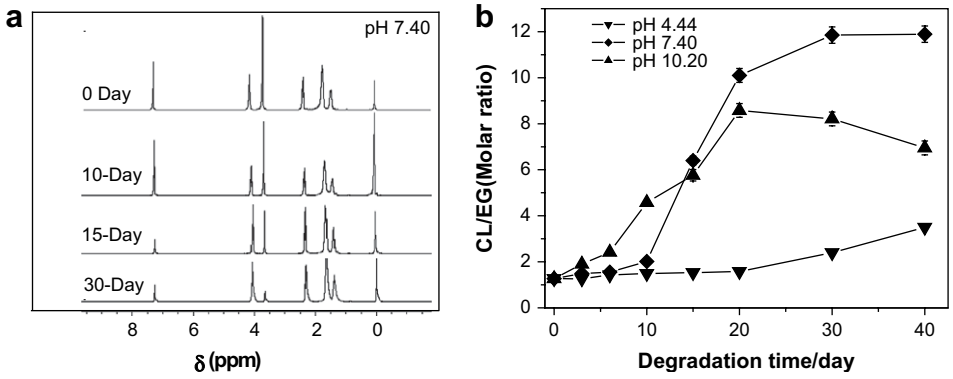


**Fig. 2.** (a) $^1$H NMR spectra of micelles in pH 7.40 PBS: 0, 10, 15 and 30 days. (b) CL/EG ratio as a function of standing times in three different pH PBSs.

rEX refractive index detector. The columns were STYRAGEL HR3, HR4 and HR5 (300 × 7.8 mm) from Waters. HPLC grade THF was used as eluent at a flow rate of 1 mL/min. THF and samples were filtered over a filter with pore size of 0.45 μm (Nylon, Millex-HN 13 mm Syringes Filters, Millipore, US). The columns were calibrated by using polystyrene standards with molecular weights in the range from 900 to $1.74 \times 10^6$ g/mol. MALLS detector (DAWN EOS) was placed between the absorbance detector and the refractive index detector. The molecular weight and molecular weight distribution were determined by SEC/DAWN EOS/Optilab rEX. ASTRA software (Version 5.3.1.4) was utilized for acquisition and analysis of data.

$^1$H NMR (Bruker MSL-300) spectra of the copolymers and the micelle degradation products were recorded in $CDCl_3$ at room temperature. The composition of the samples was estimated from the ratio of the peak areas assigned to PCL and PEG blocks in the NMR spectrum. From the CL/EG molar ratio, we can estimate the number–average molar mass ($M_n$) of the copolymers and the change of the composition of the micelle degradation products.

Mean diameter of the MPEG–PCL micelles and the light scattering intensity of the micelles suspension were determined by DLS method using a Brookheaven BI9000AT system (Brookheaven Instruments Corporation, USA).

Morphological examination of the micelles was conducted using a JEOL (Japan) JEM-100S transmission electron microscope (TEM). One drop of micelle suspension was placed on a copper mesh covered with nitrocellulose membrane and dried in air before being stained with phosphotungstic sodium solution (1% w/v).

HPLC measurements were carried on a Shimadzu LC-10AD (Shimadzu) HPLC system using a Lichrospher C-18, 5 μ, 200 mm × 4.6 mm RP-HPLC analytical column. The mobile phase was consisting of 20/80 acetonitrile (HPLC grade, Merck, Germany)/distilled water. The column was eluted at a flow rate of 1.0 mL/min at 35 °C and the working wavelength was 215 nm by UV absorption. The standardization curve was a straight line and the minimum detectable amount was 0.01 mg/mL of 6-hydroxycaproic acid.

The degradation products were obtained as follows: the samples were first adjusted to pH 2–3 with 2 N HCl solution and then the undissociated micelles were separated from the aqueous phase by centrifugation (Ultra ProTM 80, Du Pont) at 16 000 rpm for 1 h. The solution above the sediment was collected for HPLC measurement, and the sediment was first washed with distilled water three times and then frozen and lyophilized to obtain the dried residual micelle products for NMR measurements. Enzymatic degradation products were treated by acidification with 2 N HCl in order to stop the degradation [28] at designated time and centrifugated before injected into the HPLC system.

## 3. Results and discussion

### 3.1. Characterization of MPEG–PCL copolymers and their micelles

Three MPEG–PCL diblock copolymers, named MPEG2K-PCL4K, MPEG4K-PCL20K, MPEG10K-PCL30K with MPEG molecular weight of 2000, 4000, and 10 000 g/mol, respectively, were synthesized by ring-opening polymerization of ε-caprolactone using MPEG as initiator in the presence of small amount of stannous octoate. Their micelles named NP2K4K, NP4K20K and NP10K30K, respectively, were prepared by precipitation method in the absence of surfactant. Their major physicochemical properties are summarized in Table 1.

As shown in Table 1, the actual CL/EG ratio determined from $^1$H NMR spectra and from the molecular weight detected by SEC is closed to the feeding and designed ones, which means that the ring-opening copolymerization of ε-caprolactone with MPEG is almost complete. The influence on the micelles size includes the

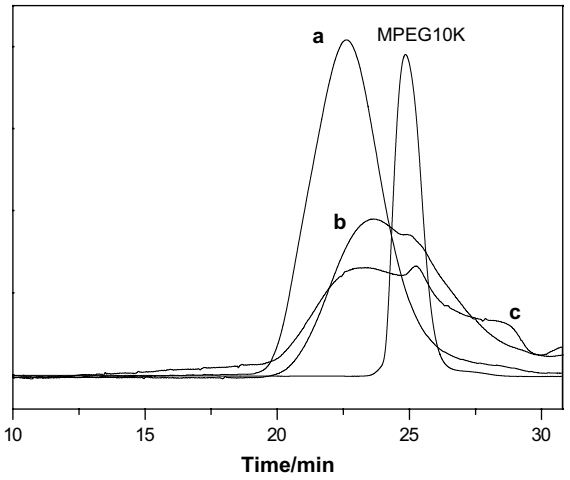


**Fig. 3.** SEC traces of MPEG and NP10K30K micelles in different degradation conditions: (a) 0 day; (b) 40 days in pH 7.40 PBS; (c) 40 days in pH 10.20 PBS.

**Table 2**
Molecular Weight of NP10K30K in micelles after degradation.

| Sample | $M_n$ (g/mol) | $M_w$ (g/mol) | $M_w/M_n$ |
|---|---|---|---|
| 0 Day | 32k | 49.4k | 1.54 |
| 40 Days in pH 7.40 | 10.1k | 31k | 3.07 |
| 40 Days in pH 10.20 | 5.2k | 23.8k | 4.58 |

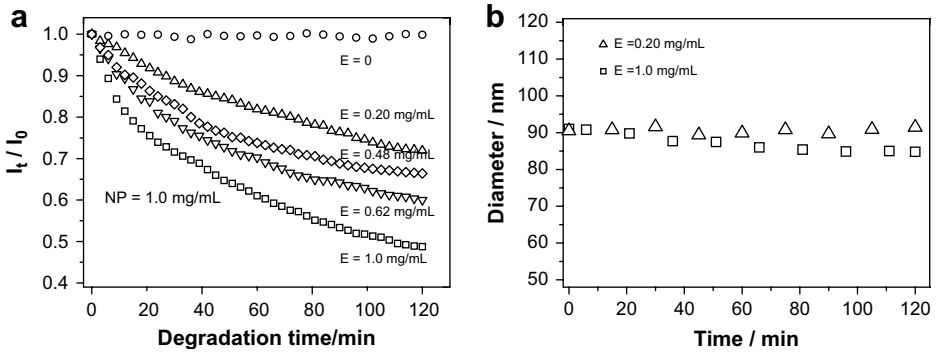


**Fig. 4.** (a) Degradation time dependence of DLS intensity ratio $I_t/I_o$ of NP10K30K micelles at 37 °C in the presence of lipase AY, where the subscripts "0" and "$t$" represent time $t = 0$ and $t = t$, respectively; (b) The degradation time dependence of the micellar diameter during enzymatic degradation.

copolymer composition, the molecular weight, the concentration of the copolymer and the organic solvents. Since NP10K30K micelles have the lowest polydispersity and the largest micelle diameter which are beneficial for monitoring during the degradation experiments, the NP10K30K copolymer micelle solution has been chosen to take the degradation experiment in this work.

### 3.2. Degradation in the absence of lipase AY

Fig. 1 shows the size and light scattering intensity changes of NP10K30K micelles in the aqueous solutions at 37 °C with three different pH values (4.44, 7.40 and 10.20) but without lipase as a function of degradation time. Both the size and the light scattering intensity decreased with the degradation time in all samples. It could be seen that the micelles in pH 4.44 PBS were most stable. After 30 days the change in size was less than 15 nm and the scattering light intensity lowed about 20%. On the other hand, the degradation rate of the micelles was very fast in pH 10.20 PBS. Both the size and scattering light intensity decreased quickly during the first several days. In pH 7.40 PBS, the micelles had a medium degradation progress. After 30 days, some sediment was found in the pH 10.20 and pH 7.40 PBS bottles, thus we stopped DLS characterization but measured the final pH values of these three systems. It is also found that after 30 days, the pH value of original pH 10.20 system was shifted to 9.02 and the pH 7.40 system was shifted to 7.17. The decrease of medium pH indicates that some small acidic molecules are released from micelles to the medium during the degradation procedure.

$^1$H NMR was used to detect the changes in the chemical composition of NP10K30K micelles during the degradation, and the results of $^1$H NMR are shown in Fig. 2. From Fig. 2a, it can be seen that there is no new peak in the spectrum when the degradation time is extended. The peak area at 3.6 ppm, assigned to PEG, decreased with the degradation time in the pH 7.40 PBS. Fig. 2b presents the variation of the molar ratio CL/EO of the caprolactone unit to the ethylene glycol unit of NP10K30K micelles with different degradation times in three different pH PBSs. It can be seen that the CL/EO ratio shows an increase in the whole 40 days in pH 4.44 and pH 7.40 PBS, but in the pH 10.20 PBS, the ratio increased much faster than the two others in the first 10 days, and then became slower than the pH 7.40 one. After 20 days, the CL/EO ratio of the sample in pH 10.20 PBS turnover to decrease. It should be noted that these results represent the sediments collected by centrifugation, including the undissociated micelles and unsoluble polymer chains, not the whole system. Thus, in the residual degradation samples, the PEG component loses especially in the first several days, indicating that the degradation should first mainly occur at the interface region near the shells, which results in a large part of the PEG segments (may contain short PCL chains) detaching to the micelles. After that, the degradation continues on the PCL cores. In the pH 10.20 PBS, the degradation is much faster and after 20 days, the degradation rate of the PCL may exceed the detachment rate of the PEG, leading to the decrease of the CL/EG molar ratio of the undissociated micelles.

To monitor the changes in the molecular weight of MPEG–PCL diblock copolymers in the micellar degradation process, SEC measurements were carried out for the degradation products of NP10K30K after 40 days of degradation in pH 7.40 and 10.20 PBS, together along the MPEG block. Fig. 3 shows the SEC traces of MPEG10k used in the synthesis of NP10K30K diblock copoymers, and the NP10K30K micelles in different situations. Obviously, the as-prepared NP10K30K micelles and the MPEG10k present a symmetrical SEC curve appearance, while the samples that were degraded for 40 days in pH 7.40 and pH 10.20 PBS display broad and asymmetric peaks with long tails in the SEC curves and even the shoulder peak arising from MPEG10K was observed, indicating that

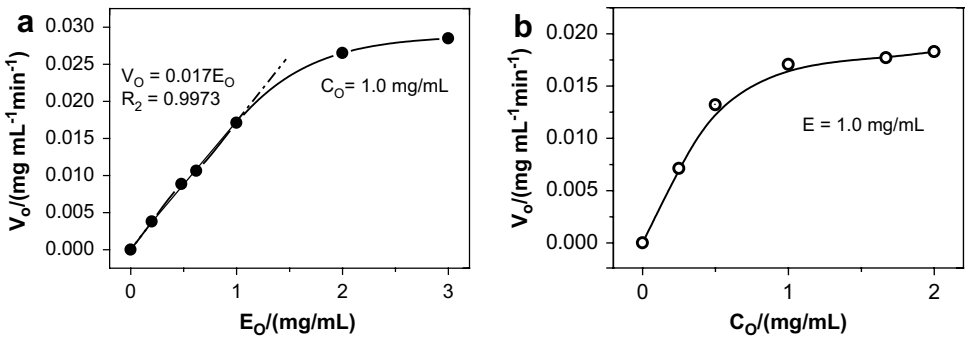


**Fig. 5.** (a) Enzyme concentration dependence of initial degradation rate ($V_0$); (b) initial copolymer concentration dependence of initial degradation rate ($V_0$).

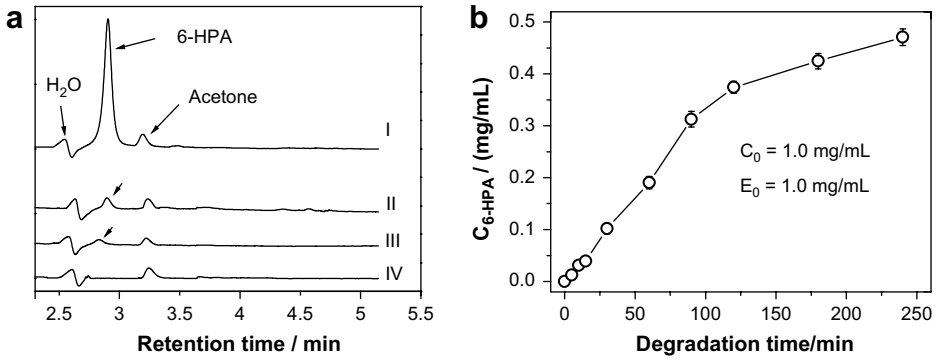


**Fig. 6.** (a) HPLC curves of the micellar degradation products: I. Enzymatic degradation of micelles after 180 min; II–IV: non-enzymatic degradation after 40 days in three pH value PBS, pH 10.20 (II), pH 7.40 (III) and pH 4.44 (IV), respectively. (b) Degradation time dependence of 6-hydroxycaproic acid concentration examined by HPLC, where the particle concentration and the enzyme concentration are both 1.0 mg/mL.

after 40 days, the micelles had obviously degradation that the MPEG10K block including some MPEG with short PCL block detached largely from diblock copolymer micelles in both pH 7.40 and pH 10.20 PBS. The molecular weight and molecular weight distribution of these samples are listed in Table 2. It shows that, after 40 days of degradation, the molecular weight of the NP10K30K micelles decreased and the molecular weight distribution became significantly broad. The degradation rate of the NP10K30K micelles in pH 7.40 PBS was slower than it in pH 10.20 PBS. This is very different from the degradation behavior of DL–PLA nanoparticles, in which, the PLA nanoparticles was very stable in a neutral pH value solution [20].

### 3.3. Degradation in the presence of lipase AY

To study the enzymatic degradation of the micelles, the lipase AY was introduced into the degradation experiment. Since the enzymatic degradation is very fast and thorough, and also the lipase AY was not easy to be removed, the $^1$H NMR and SEC are no more effectual. However, the DLS is a very convenient way to in situ monitor the degradation progress. Compared with the lipase PS used frequently in biodegradation, the enzymatic activity of lipase AY is only tenth of that of lipase PS. We expect that the low activity of lipase AY can help us observe the process of the enzymatic degradation by DLS in more detail. Fig. 4 shows the degradation results conducted inside the DLS cuvette at 37 °C at different concentrations of lipase AY. The changes of the light scattering intensity and micelle size were recorded online. From Fig. 4a, it can be seen that the light scattering intensity of samples drops very fast at the initial stage in most of the enzymatic degradation samples, and only one curve (the enzyme concentration $E = 0.20$ mg/mL) showed a quasi-linear decrease in light scattering intensity with the degradation time. However, it is interesting that the micelle size of the enzymatic degradation samples keeps almost constant with degradation time, as shown in Fig. 4b.

Based on DLS theories [22–24,29–31], the intensity of light scattered by a suspension of particles with diameter $d$ is proportional to the number of particles $N$, the square of the particle mass $M$, and the particle form factor $P(q,d)$ which depends on particle size, scattering angle, index of refraction, and wavelength:

$$I = \sum_{i=1}^{N} M_i^2 P(q,d) \tag{1}$$

The invariation in particle diameter during the degradation time, as shown in Fig. 4b, reveals that the form factor $P$ is a constant for the degradation system. Thus, the decrease in light scattering intensity of samples can be attributed to the decrease of either the particle mass $M$ or the number of the particles $N$, i.e.

$$I \propto M^2 N \tag{2}$$

The enzymatic degradation progress may be quite complex according to Wu's assumption [22–25]: for a single nanoparticle, the degradation process by the lipase (the lipase first penetrate the PEG shell to the PCL core, and then degrade it quickly) was so fast that the DLS can only detect the remaining undegraded nanoparticles but not detect mass change of the micelles. In other words, the enzymatic degradation likes a one by one process, i.e. enzyme eats the nanoparticles in a one-by-one fashion. Based on this assumption, the decrease in the light scattering intensity was attributed only to the decrease of the number of particles $N$, or the concentration $C$ ($C = N \times M$, $M$, the mass of remained micelles). Thus, the initial biodegradation rate ($V_0$) together with the degradation percent can be calculated conveniently from the intensity changing curves. Here, $V_0$ is defined as $[dC_t(I)/dt]_{t\to 0}$ and the degradation percent as $(1 - I_t/I_0)$.

Fig. 5a shows that the initial degradation rate $V_0$, determined from Fig. 4, first increased linearly with the enzyme concentration. However, when $E_0 > 1.0$ mg/mL, the points of $V_0$ deviated from the linearity, suggesting that the lipase may be excessive for the micelles or become less efficient. The straight line can be fitted by the formula: $V_0$ (mg/mL$^{-1}$/min$^{-1}$) $= 0.017\,E_0$, when

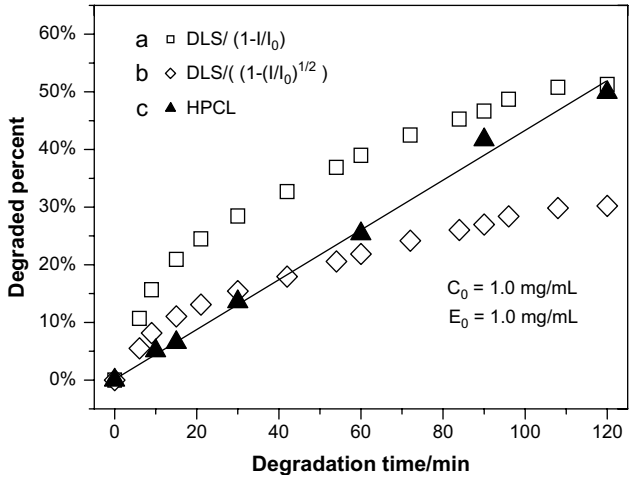


**Fig. 7.** Degradation time dependence of degraded percents of NP10K30K micelles, where the data were obtained by DLS and HPLC.

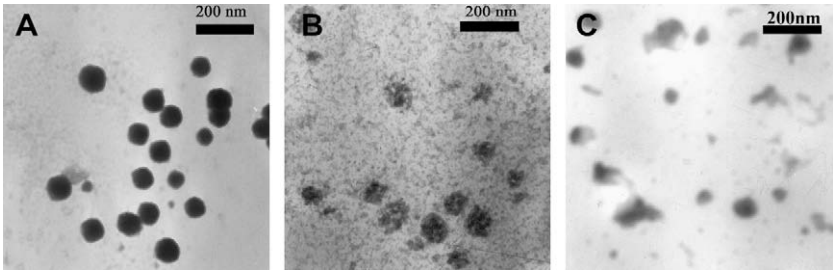


**Fig. 8.** TEM images of NP10K30K micelles: fresh sample (A) and sample during the degradation in the presence of lipase AY (B) as well as degradation products (C) at pH 7.40, 30 days without the presence of lipase.

$E_0 < 1.0$ mg/mL. Fig. 5b shows that for a given lipase AY concentration ($E = 1.0$ mg/mL), the initial degradation rate $V_0$ increased with the initial micelles concentration. When $C_0$ is more than 1.0 mg/mL, $V_0$ increased only a little, which means that the concentration of the micelles is excess for the enzymatic degradation system and $V_0$ start to be independent with $C_0$, following the zero-order kinetics. Thus, from Fig. 5, the degradation rate of the micelles is proportional to either the micelle or the enzyme concentration in a certain range, that is, the degradation of micelles follows first-order kinetics. When the concentration of micelles or enzyme is excess, the degradation rate of the micelles becomes independent to it and the degradation of micelles follows zero-order kinetics.

In order to further investigate the degradation behavior of NP10K30K micelles either in the presence of lipase AY or not, HPLC was used to analyze the degradation products and the degradation extent. In the HPLC curves of the enzymatic degradation samples (Fig. 6a), 6-hydroxycaproic acid was detected, which is the monomer product of PCL hydrolysis, no other significant degradation product like dimmers or other water-soluble oligomers was found, which suggested that the enzymatic degradation was fast and thorough. Fig. 6b shows the product 6-hydroxycaproic acid (6-HPA) concentration with the enzymatic degradation time, from which it can be seen the concentration of 6-HPA degraded from micelles increases linearly with the degradation time in the first 120 min, and then gets a little slow. It also reveals that the activity of the lipase AY has no obvious decrease during a long degradation time (more than 120 min) and the degradation was effectively stopped by adding 2 N Hcl. After 4 h, about 65% amount (mass) of PCL has been degraded into 6-HPA (considering the mass ratio of PCL/MPEG is 3/1).

Fig. 7 shows the degradation percent based on DLS and HPLC measurements in the presence of lipase AY, respectively. The degradation percent determined by HPLC is based on the molar ratio of measured 6-HPA at different degradation times to CL unit in PCL in the original system, which reflects how many CL monomers are degraded from the PCL chains. It can be seen that the degradation rate of the micelles measured by DLS is much faster than that by HPLC in the early degradation stage. To explain this phenomenon, we should reconsider the relationship between the light scattering intensity in DLS and the micellar degradation progress. Previously, we supposed that the DLS cannot detect the mass change of the micelles during the degradation procedure, thus the mass of the particles did not decrease before the dissociation of the micelles and the decrease of the light scattering intensity was attributed only to the decrease of the micelles number ($I = \sum_{i=1}^{N} M_i^2 P(q,d)$), ($I \propto N$). However, such ideal one-by-one process may not happen in our case.

Now, let us consider another ideal case that the degradation happen on all of the micelles with a same degradation rate simultaneously and the number of particles does not change during the initial degradation stage, then the decrease of the light scattering intensity can be attributed to the decrease in particle mass ($I \propto M^2$), and the degradation percent can be presented as $1 - \sqrt{I_t/I_0}$ instead of $1 - I_t/I_0$. Here we neglect the mass of the water-soluble MPEG and lipase AY which attach to the micelles because the water-soluble parts contribute only a little to the DLS intensity. After this treatment, the result obtained by DLS in Fig. 7 is quite close to the one by HPLC at the initial degradation stage. The degradation kinetic relationship should be shifted to $V_0$ (mg/mL$^{-1}$/min$^{-1}$) $= 8.73 \times 10^{-3} E_0$ ($E_0 < 1.0$ mg/mL, $C_0 = 1.0$ mg/mL).

The real degradation progress of the micelles in this case may be between these two ideal assumptions. We should note that, the activity of lipase AY (~2 U/mg) was much lower than that of lipase PS (~30 U/mg) used in the previous work [22–25], which means that the degradation process by lipase AY may slow enough to be observed by DLS. Thus, the mass lost and the dissociation of the micelles may take place simultaneously. In other words, the degradation happen on all or most of the micelles during the degradation time and the dissociation of the micelles may follow a one-by-one fashion due to different

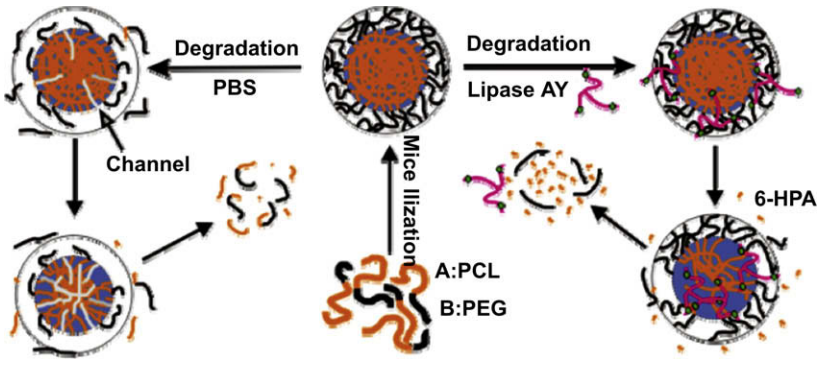


**Fig. 9.** Schematic representation of non-enzymatic and enzymatic degradation mechanisms of MPEG–PCL diblock copolymer micelles.

degradation rates of micelles individually. It is possible that during the initial degradation stage, the decrease in the light scattering intensity is mainly attributed to the decrease of the particle mass. When the degradation of the micelles reaches to a certain degree, the micelles get loose and dissociate. It can be seen from the TEM images shown in Fig. 8 that the fresh micelles of NP10K30 in aqueous solution are compact with a spherical shape. However, during the enzymatic degradation, the micelles get loose gradually and the cavity in the center of some micelles can be seen. But, before the dissociation, the diameter of the undissociated micelles has no obvious change. On the other hand, in the absence of enzyme, the mean diameter of micelles decreases and polydispersity index in size increases.

### 3.4. Two possible degradation models

The degradation mechanisms of MPEG–PCL micelles with and without enzyme are schematically illustrated in Fig. 9. Considering there is no obvious change on micelle size during the degradation and a rapid decrease in light scattering intensity during the initial degradation stage, the enzymatic degradation may follow three steps: first, the lipase attach to the surface of the particles and penetrate the MPEG shell; then the lipase degrade the PCL cores gradually, leaving loose or hollow particles, but the integration and size of micelle have no obvious change; at last, when the degradation reaches to a certain degree, the particles dissociate. While during the non-enzymatic degradation, the degradation behavior first happens near the interface region of the PEG shell and PCL core, leading to the detachment of PEG chains (may contain short PCL chains). With increasing degradation time, cracks and channels create on the PCL core and the degradation continues on the PCL core with a random scission on PCL chains because there is no considerable amount of 6-HPA found in the HPCL curves (Fig. 6a).

## 4. Conclusions

In this work, the degradation behavior of the micelles was investigated by DLS, $^1$H NMR, SEC, HPLC and TEM techniques. In non-enzymatic case, the degradation rate of micelles in low pH (4.44) is much slower than it in neutral pH (7.40) and high pH (10.20) media, and the non-enzymatic degradation behavior first happens near the interface region of the PEG shell and PCL core, leading to the detachment of part PEG chains from micelles, and then the degradation of PCL core takes place with a random scission. While in the enzymatic degradation case, the degradation rate of micelles is proportional to either the micelle or the enzyme concentration in a certain range. The behavior of enzymatic degradation is different from that of non-enzymatic degradation. At the initial stage, the lipase first degrades the PCL cores gradually, leaving loose or hollow particles, and then the dissociation of micelles happens with the degradation progress.

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## References

[1] Riley T, Govender SS, Xiong CD, Garnett MC, Illum L, Davis SS. Colloids Surf B Biointerfaces 1999;16:147.
[2] Ge HX, Hu Y, Jiang XQ, Cheng DM, Yuan YY, Bi H, et al. J Pharm Sci 2002;91:1463.
[3] Allen C, Han J, Yu YS, Maysinger D, Eisenberg A. J Controlled Release 2000;63:275.
[4] Hu Y, Jiang XQ, Ding Y, Ge HX, Yuan YY, Yang CZ. Biomaterials 2002;23:3193.
[5] Zhang LY, Hu Y, Jiang XQ, Yang CZ, Lu W, Yang YH. J Controlled Release 2004;96:135.
[6] Hu Y, Jiang XQ, Ding Y, Zhang LY, Yang CZ, Zhang JF, et al. Biomaterials 2003;24:2395.
[7] Chu B. Langmuir 1995;11:414.
[8] Xu R, Winnik M. Macromolecules 1991;24:87.
[9] Liu T, Zhou Z, Wu C, Chu B, Schneider DK, Nace VM. J Phys Chem B 1997;101:8808.
[10] Zhao Y, Liang HJ, Wang S, Wu C. J Phys Chem B 2001;105:848.
[11] Zhao Y, Hu T, Lv Z, Wang S, Wu C. J Polym Sci Polym Phys Ed 1999;37:3288.
[12] Kwon GS, Okano T. Adv Drug Deliv Rev 1996;21:107.
[13] Kataoka K, Harada A, Nagasaki Y. Adv Drug Deliv Rev 2001;47:113.
[14] Liu LJ, Li SM, Garreau H, Vert M. Biomacromolecules 2000;1:350.
[15] Li SM, Garreau H, Pauvert B, McGrath J, Toniolo A, Vert M. Biomacromolecules 2002;3:525.
[16] He F, Li SM, Vert M, Zhuo RX. Polymer 2003;44:5145.
[17] Huang MH, Li SM, Vert M. Polymer 2004;45(26):8675.
[18] Li SM, Pignol M, Gasc F, Vert M. Macromolecules 2004;37(26):9798.
[19] He F, Li SM, Garreau H, Vert M, Zhuo RX. Polymer 2005;46(26):12682.
[20] Belbella A, Vauthier C, Fessi H, Defissaguet JP, Puisieux F. Int J Pharm 1996;129:95.
[21] Geng Y, Discher DE. J Am Chem Soc 2005;127:12780.
[22] Wu C, Gan ZH. Polymer 1998;39:4429.
[23] Gan ZH, Jim Y, Li M, Zhao Y, Wang S, Wu C. Macromolecules 1999;32:590.
[24] Nie T, Zhao Y, Xie Z, Wu C. Macromolecules 2003;36:8825.
[25] Lam HF, Gong XJ, Wu C. J Phys Chem B 1531;2007(7):111.
[26] Myrra GC, Cornelus FN, Ruud V, Leo GJD, Daan JAC, Wim EH. J Pharm Sci 2008;97(1):506.
[27] Hu Y, Zhang L, Cao Y, Ge H, Jiang X, Yang C. Biomacromolecules 2004;5:1756.
[28] Pronk W, Boswinkel G, Riet KV. Enzyme Microb Technol 1992;14:214.
[29] Chu B. Laser light scattering. 2nd ed. New York: Academic Press; 1991.
[30] Wu C, Siddiq M, Woo KF. Macromolecules 1995;28:4914.
[31] Bo S, Yang H, Chen T. Funct Polym 1991;4:147.