Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (7): 60-64
Published online November 20, 2015 (http://www.cellmolbiol.com)
Received on September 12, 2015, Accepted on November 11, 2015.
doi : 10.14715/cmb/2015.61.7.10

Encapsulation and in vitro release of erythromycin using biopolymer micelle

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Abstract

An amphiphilic block copolymer poly(ethylene glycol)-block-poly[2-(2-methoxyethoxy)ethyl methacrylate] (PEG-*b*-PMEO$_2$MA) was prepared and the polymer micelle was applied to encapsulate erythromycin. The Critical Micelle Concentration (CMC) of PEG-*b*-PMEO$_2$MA was determined by the fluorescent probe pyrene. The effects of addition of erythromycin on encapsulation efficiency and drug loading content were investigated. Drug release was also studied in a phosphate buffer solution with a pH of 7.5. The CMC of PEG-*b*-PMEO$_2$MA is 0.065 mg/mL when the monomer ratio of the hydrophobic block PMEO$_2$MA to the hydrophilic block PEG is equal to 6:4. The encapsulation efficiency and drug loading were 87.1% and 16.8%, respectively, as the loading content of erythromycin in polymeric micelle is equal to 28%. After erythromycin is loaded into the micelle, the size of PEG-*b*-PMEO$_2$MA micelle becomes approximately thrice the size of unloaded micelle. The loading micelles stably release erythromycin within 180 hours in phosphate buffer, suggesting that the micelle loaded with erythromycin have a good sustained-release effect.

Key words: Amphiphilic block polymer, polymeric micelle, erythromycin, encapsulation, drug release.

Introduction

Erythromycin is an antibiotic, it has been used to treat a number of bacterial infections including: tonsillitis, scarlet fever, diphtheria, gonorrhea and Lester disease among others (1). The chemical structure of erythromycin is given in Figure 1. Due to its strong hydrophobicity, hydrogenated castor oil and absolute ethyl alcohol were often used in clinical treatment, which results in severe allergic reactions and tissue toxicity to patients (2). In view of this, the following measures are usually taken to solve the problems in clinical erythromycin administration: 1. Improve the solubility of erythromycin to avoid the use of co-solvents. 2. Increase the life of erythromycin to decrease the dosage. 3. Enhance its targeting and reduce the side effects of the drug.

Due to the difference in solubility of the hydrophobic and hydrophilic blocks, amphiphilic polymer can form micelle with unique "core-shell" structure in aqueous solution by self assembly. Within a micelle, the hydrophobic chains assemble to form a core which can solubilize lipid drugs, and the hydrophilic chains form a shell to stabilize and protect the micelle. Because the CMC of an amphiphilic polymer can be 1000 times lower than CMC of a small-molecular surfactant, the polymer micelle is formed at very low polymer concentration to largely avoid the escape of drugs. The hydrophilic shell and smaller particle size effectively avoid phagocytosis of the polymer micelle by reticulo-endothelial system in human body, which favors long circulation time of the micelle in blood (3,4). Furthermore, small size of the polymer micelle favors the retention and accumulation of micelle inside targeting tissues (i.e. tumor tissues), which forms passive targeting to tumor tissues (5,6).

Amphiphilic copolymer has many advantages, such as easy synthesis, low cost and high level of industrialization. Therefore, this polymer has a prospect future in many fields. In this study, an amphiphilic copolymer PEG-*b*-PMEO$_2$MA, poly(ethylene glycol)-block-poly[2-(2-methoxyethoxy)ethyl methacrylate] was synthesized and the polymer micelle was used as drug carrier for erythromycin. Although there are many researches about drug loading with block copolymers such as poly(ethylene glycol)-block-poly(D, L-lactic acid), poly(2-ethyl-2-oxazoline)-block-poly(ε-caprolactone) and poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) (7-11). few studies related to PEG-*b*-PMEO$_2$MA micelle as drug carrier have been reported. To the best of our knowledge, this is the first attempt on loading erythromycin with PEG-*b*-PMEO$_2$MA. This study provides a fundamental research for future application of PEG-*b*-PMEO$_2$MA in drug delivery and release.

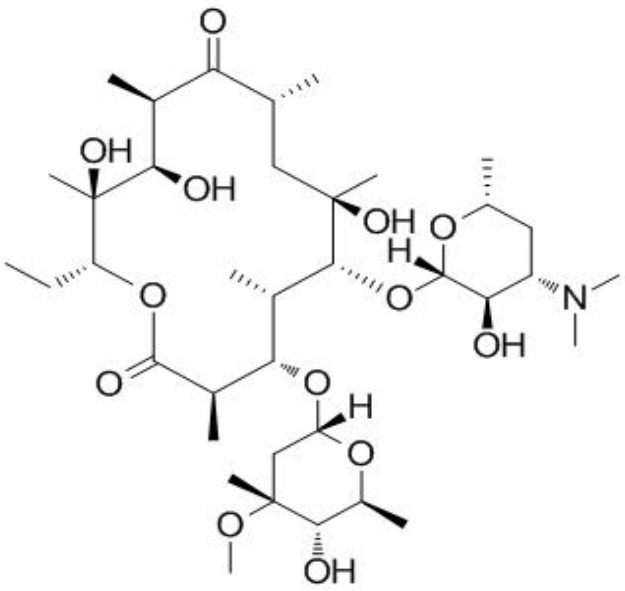

Figure 1. Molecular structure of erythromycin.

Materials and methods

Materials

Erythromycin (99.5%) was purchased from Wuxi Shengguo Biotechnology Co Ltd. 2-Bromoisobutyryl bromide (Aldrich, 98%), 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA, Aldrich, 95%), magnesium sulfate (MgSO4, Aldrich, 99.5%), 2,2'-bipyridine (Aldrich, 98%), toluene (Aldrich, 99.9%) were used as received. 1,4-dioxane, anhydrous diethyl ether, tetrahydrofuran (THF), N,N-dimethylformamide (DMF), ethyl alcohol, orthoboric acid, sodium hydroxide, dichloromethane and pyrene were purchased from Sinopharm Chemical Reagent (Jiangsu) Co Ltd. PEG with one end terminated by methyl group (Polymer Source Co. Ltd.) was used as received. MEO_2MA was distilled two times prior to use in order to remove inhibitors and trace water. CuBr (Aldrich, 99%) was washed with deionized water, acetic acid (Fisher, ACS reagent, glacial), ethanol, and ether in that sequence and then dried in vacuum and stored under nitrogen before use.

Synthesis of amphiphilic block copolymer PEG-b-$PMEO_2MA$

PEG macroinitiator was synthesized in the first step according to the following procedures: PEG (4.5 g, 0.9 mmol) was dissolved in distilled THF (20 mL) in a dried flask followed by circulating with nitrogen. Triethylamine (TEA) (0.23 mL, 1.6 mmol) was then added under nitrogen. The flask was immersed in an ice bath and after 5 min 2-bromoisobutyryl bromide (0.41 mL, 3.3 mmol) was injected via a glass pipette into the reaction flask. The reaction was stirred at room temperature for 24 h. During the reaction, a yellow precipitate was formed and was removed by centrifugation after reaction. Magnesium sulfate was added to remove any traces of moisture during the centrifugation process. A clear solution was collected and was precipitated 3-4 times into *n*-hexane, the precipitate was collected and dried under vacuum to yield PEG macroinitiator.

PEG-*b*-$PMEO_2MA$ was then synthesized by atom-transfer radical polymerization (ATRP): The PEG macroinitiator (0.5 g, 0.1 mmol), MEO_2MA (1.35 mL, 7.32 mmol), and 2,2'-bipyridine (45.1 mg, 0.29 mmol) were dissolved in ethanol (5 mL) under nitrogen atmosphere in a round bottom flask that had been dried by inflaming under vacuum. The catalyst CuBr (30 μL, 0.14 mmol) was added. After addition of the catalyst, the flask was filled with nitrogen and stirred at room temperature for overnight. After 24 h, the reaction was terminated by air. After evaporation of ethanol, the resulting oily mixture was dissolved in methanol, then the solution was passed through a short silica gel column (neutral, 40-60 μm) against methanol eluent to remove the copper complex. The methanol was then removed with a rotary evaporator. The yielding solution was concentrated and then precipitated in 500 mL anhydrous diethyl ether. The precipitate was filtered, re-dissolved in THF and re-precipitated in anhydrous diethyl ether. The process was repeated several times to remove impurities. After the final precipitation, the solid was dried under vacuum to yield the final polymer product PEG-*b*-$PMEO_2MA$. The product was then characterized by ^{1}H NMR and GPC.

Preparation of erythromycin-loaded polymeric micelle

Erythromycin-loaded polymeric micelle was prepared by dialysis. PEG-*b*-$PMEO_2MA$ polymer (20 mg) and a certain amount of erythromycin were dissolved into 10 mL DMF. The solution was added dropwise to 25 mL ultrapure water under stirring, and the stirring was kept for 24 h. The mixture was then loaded into a dialysis bag and dialyzed against Millipore Water for 3-4 days. The water was refreshed 3 times per day. The solution was centrifuged for 10 min at 5,000 r/min. The erythromycin-loaded polymeric micelle aqueous solution was obtained from the supernatant. The unloaded polymer micelle solution was prepared according to the same procedures.

Pyrene fluorescence

Pyrene saturated solution (C_{py} = 1x10^{-6} mol/mL) was prepared using 0.01 mol/L sodium hydroxide aqueous solution. PMA was dissolved in pyrene solution to prepare polymer solutions having various PMA concentrations, orthoboric acid was added to adjust pH value to 9.5. Fluorescence emission spectra were acquired on a PTI fluorometer equipped with an Ushio UXL-75Xe Xenon arc lamp and PTI 814 photomultiplier detection system, emission spectra were acquired by exciting the samples at 335 nm. The I_1/I_3 ratios were determined from the intensity of the first, I_1, and third, I_3, peaks in the fluorescence spectrum of the pyrene monomer taken at 375 and 384 nm, respectively.

Standard curve for erythromycin UV spectra

Erythromycin was dissolved in ethyl alcohol to prepare a standard stock solution having an erythromycin concentration of 0.1 mg/mL. A series of erythromycin solutions were prepared by dilution of the stock solution using ethyl alcohol. The UV-vis spectra of samples were acquired on a Cary 100 UV-Vis spectrophotometer.

Encapsulation efficiency and drug loading content determination

The solubility of erythromycin in water is very low. During dialysis, the organic solvents were separated and the erythromycin excluded from polymeric micelle were precipitated out. The precipitate was collected and dissolved in a known volume of ethyl alcohol, the amounts of erythromycin excluded from micelle can be calculated according to the absorbance at 482 nm. The encapsulation efficiency of erythromycin is obtained by eq 1.

$$EE = \frac{M_i - M_e}{M_i} \times 100\% \quad (1)$$

where *EE* is encapsulation efficiency of erythromycin in %, M_i is initial mass of erythromycin in mg, M_e is mass of the erythromycin excluded from micelle in mg.
Erythromycin loading content is calculated by eq 2.

$$LC = \frac{M_i - M_e}{M_i} \times 100\% \quad (2)$$

where *LC* is erythromycin content in polymer micelle in %, M_p is initial mass of PEG-*b*-$PMEO_2MA$ in mg.

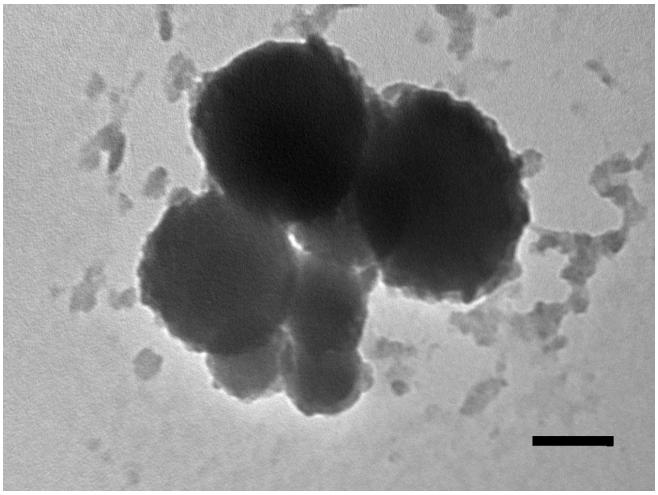

Figure 2. TEM image with a polymer concentration of 0.075 mg/mL. Scale bar = 0.2 μm.

Simulation experiment for erythromycin release in vitro

Aqueous solution (10 mL) of erythromycin-loaded polymeric micelle was transferred to a dialysis bag with a pipette, the bag was then sealed and immersed in 100 mL phosphate buffer solution with a concentration of 0.2 mol/L (pH = 7.5). The simulation experiment for erythromycin release in vitro was performed with an agitator in water bath at 37°C. 50 mL solution was taken with a pre-set time interval for analysis, 50 mL fresh phosphate buffer solution was then supplemented.

The 50 mL solution containing released erythromycin was extracted two times using 5 mL dichloromethane. The extraction liquid was collected and evaporated at room temperature to give erythromycin residue. The obtained solid was dissolved in ethyl alcohol and the absorbance of the solution at 482 nm was measured. The concentration of erythromycin was then calculated by Beer–Lambert law. The accumulative release rate of erythromycin is calculated by eq 3.

$$RR = \frac{V_d \sum_1^{n-1} C_i + V_r C_n}{m_p} \times 100\% \quad (3)$$

where *RR* is accumulative release rate of erythromycin in %, V_d is displaced volume (50 mL) of phosphate buffer solution, V_r is volume (100 mL) of release solution, C_i is concentration of release solution from the i^{th} sampling in mg/mL, n is total number of sampling times, m_p is mass of erythromycin loaded in polymeric micelle.

Results and Discussion

Characterization of PEG-b-PMEO$_2$MA

A PMEO$_2$MA and PEG molar ratio of 6.12:3.85 (approximately 6:4) was obtained by analysis of ^{1}H NMR spectrum. Analysis GPC trace obtained with PEG-*b*-PMEO$_2$MA indicates that number-average molecular weight (M_n) and weight-average molecular weight (M_w) of the synthesized polymer are 25,500 g/mol and 31,000 g/mol, respectively, with a polydispersity index (PDI) of 1.22. Figure 2 shows transmission electron microscopy (TEM) image of PEG-*b*-PMEO$_2$MA at a concentration of 0.075 mg/mL, PEG-*b*-PMEO$_2$MA spherical micelles with a hydrophobic PMEO$_2$MA core surrounded by a hydrophilic PEG shell can be observed.

CMC determination

Figure 3 shows steady-state fluorescence emission spectrum of pyrene at a polymer concentration of 0.085 mg/mL, five peaks can be observed from the spectrum. Because the ratio of the first peak (λ_1 = 375 nm) and the third peak (λ_3 = 384 nm) is strongly dependent with the polarity of solvent, the I_1/I_3 ratio obtained at different polymer concentration can be used to probe the polarity of the micro-environment in where pyrene is located (12,13).

Figure 4 shows that the I_1/I_3 ratio remains constant at low polymer concentration, indicating that the polarity of the micro-environment solubilizing pyrene does not change because no micelle has been formed yet. When lg*C* reaches -1.20, the I_1/I_3 ratio increases dramatically due to formation of polymer micelles, pyrene is solubilized into the micelles which results in a change in solvent polarity. The onset polymer concentration is 0.065 mg/mL, suggesting that CMC of PEG-*b*-PMEO$_2$MA polymer is 0.065 mg/mL. When lg*C* is greater than 0.5, all pyrene molecules were surrounded by polymer micelle and completely located in a non-polar environment, the I_1/I_3 ratio became independent with polymer concentration.

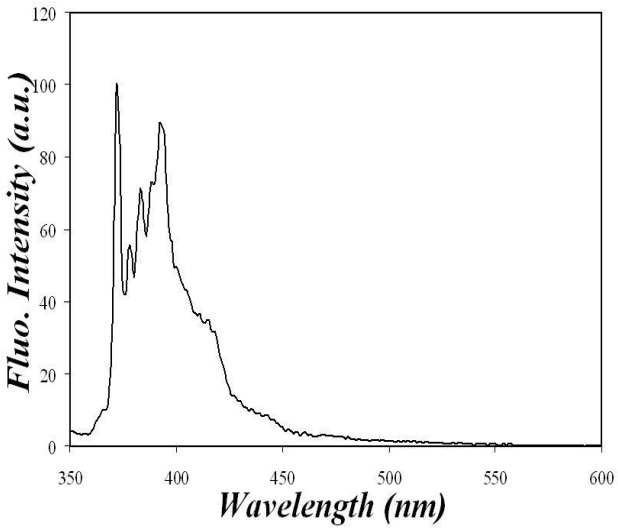

Figure 3. Steay-state fluorescence spectrum of pyrene in PMM aqueous solution with a polymer concentration of 0.085 mg/mL.

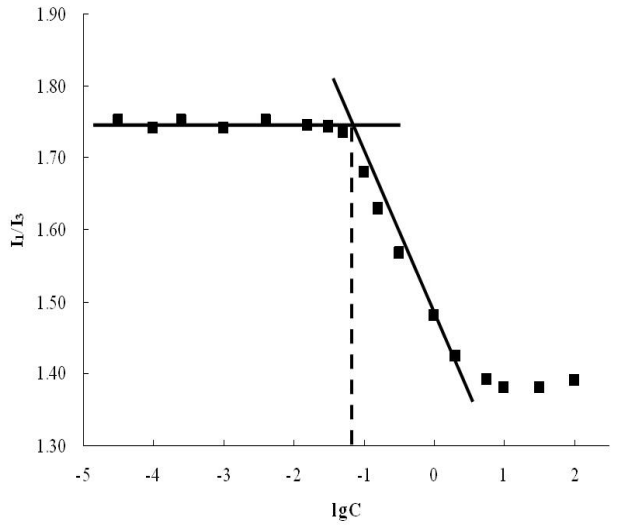

Figure 4. Relationship between I_1/I_3 ratio and logarithm of polymer micelle concentration.

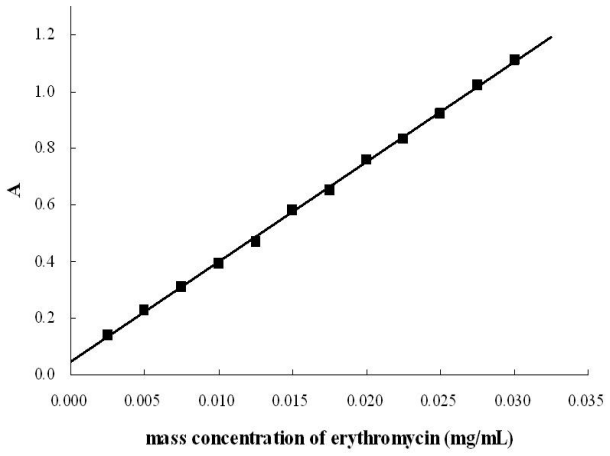

Figure 5. Standard curve of erythromycin.

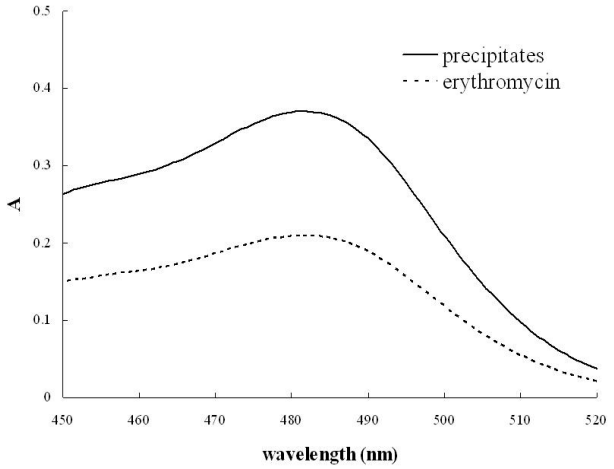

Figure 6. UV absorbance spectra of centrifugal precipitates and erythromycin.

Encapsulation efficiency and drug loading content of the polymer micelle

A standard curve for erythromycin UV spectra was obtained according to the method introduced in experimental section. Figure 5 shows that there is a linear relationship between the UV absorbance of erythromycin standard solution and mass concentration of erythromycin, the linear equation is $A = 0.0377 + 36.5C$ with $R^2 = 0.999$, suggesting that the absorbance of erythromycin is linear to concentration within this range of erythromycin concentration.

Erythromycin (6.3 g) was used to prepare erythromycin-loaded polymeric micelle according to the procedures described in experimental section. The precipitates obtained from centrifugal separation were dissolved in ethyl alcohol to prepare solutions with different concentrations, UV absorbance spectra of these solutions were acquired to compare with the spectra of erythromycin standard solutions, and the results were presented in Figure 6. Figure 6 shows that the profile and peak position of UV absorbance spectra obtained from the two solutions are same, suggesting that the precipitates obtained from centrifugal separation are erythromycin unloaded into polymeric micelle, these erythromycin can be completely separated from the erythromycin-loaded polymeric micelle by centrifuge. The mass concentration of unloaded erythromycin is determined by UV absorbance of solution.

According to eqs 1 and 2, the encapsulation efficiency and drug loading content of polymeric micelle towards erythromycin were calculated to be 76.7% and 22.4%, respectively. PEG-*b*-PMEO$_2$MA polymeric micelles are formed in aqueous solution by self-assembly, the hydrophobic MEO$_2$MA blocks associate to a core of micelle and the hydrophilic PEG blocks give a shell of micelle. Erythromycin molecules are solubilized by the hydrophobic core.

Micelle size and zeta-potential determination

The measured average particle size and zeta-potential of erythromycin-loaded and unloaded polymeric micelle are listed in Table 1.

t test was conducted to size of polymeric micelle before and after loading erythromycin, *t* was calculated to equal 4.31 and $t_{0.05,4}$ was found to be 2.215 from *t* distribution table, suggesting that the size of polymeric size significantly changes before and after drug loading.

Table 1. Average particle size and zeta-potential of micelle.

Sample	Particle size (nm)	Zeta-potential (mV)
polymeric micelle	256.6	-44.59
erythromycin-loaded mecelle	741.2	-67.91

t test was also conducted to zeta-potential of polymeric micelle before and after loading erythromycin, *t* was calculated to equal 9.76 and $t_{0.05,8}$ was found to be 1.908 from *t* distribution table, again suggesting that the zeta-potential of polymeric size significantly changes before and after drug loading.

The size of polymeric micelle increases for around 3 times after drug loading, this is because erythromycin was solubilized into the micelle and to expand the micelle. Zeta-potential became more negative after loading erythromycin, suggesting that erythromycin-loaded polymeric micelle is more stable.

Effect of erythromycin addition on encapsulation efficiency and drug loading content

Figure 7 gives the relationships between addition of erythromycin and encapsulation efficiency and loading content of micelle with addition of erythromycin equal 7%, 15%, 21%, 28%, 36%, respectively of the polymer mass.

Figure 7 shows that encapsulation efficiency of polymeric micelle to erythromycin gradually decreases with an increase in addition of erythromycin, especially when erythromycin content is greater than 28%, a significant decrease of encapsulation efficiency can be observed from Figure 7. The loading content of erythromycin increases with increasing erythromycin addition, but the increment is not significant when erythromycin addition is above 28%. To this end, an erythromycin content of 28% can be considered as an optimal drug addition.

In vitro study of erythromycin release from polymeric micelle

In order to simulate the conditions and environment of human blood, in vitro experiments for erythromycin release was conducted in a buffer solution with pH of 7.5 at 37°C. A micelle solution with 28% erythromycin addition was used for sustained-release investigation, the results are shown in Figure 8.

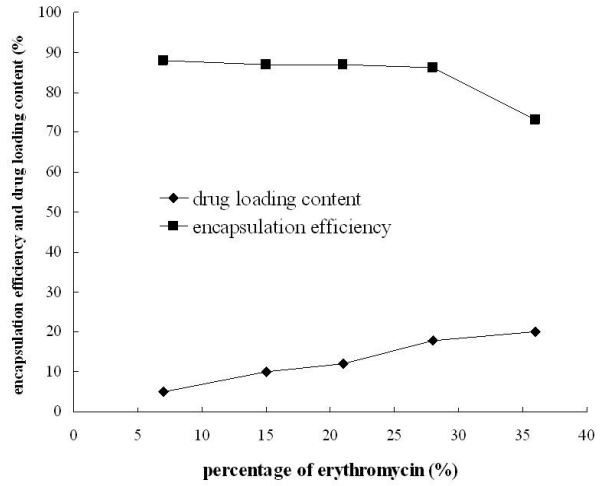

Figure 7. Effect of erythromycin addition on encapsulation efficiency and drug loading content.

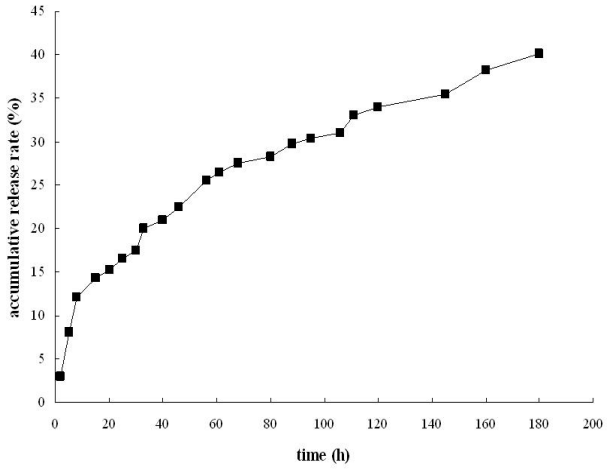

Figure 8. Erythromycin sustained-release curve.

Figure 8 illustrates the relationship between accumulative release rate of erythromycin from polymeric micelle and release time. It can be seen from Figure 8, the drug-loaded polymeric micelle release erythromycin gradually, and the accumulative release amount increases with time. The trend of drug release is smooth with no sudden change observed from the curve. Therefore, the drug-loaded micelle prepared by PEG-*b*-$PMEO_2MA$ copolymer has a good sustained-release effect. After drug administration, the drug can be stably and gradually released by polymeric micelle for a long period in human body, and the drug toxicity decreases correspondingly.

In this paper, an amphiphilic block copolymer PEG-*b*-$PMEO_2MA$ was synthesized by ATRP, erythromycin-loaded polymeric micelle was successfully prepared through dialysis. CMC of the polymer micelle was determined to equal 0.065 mg/mL by pyrene fluorescence method, suggesting that the micelle has a good ability against dilution. After erythromycin loading, the size of micelle increases to be approximately thrice the size of unloaded micelle, and zeta potential becomes more negative. When addition of erythromycin reaches 28%, encapsulation efficiency and drug loading content of the micelle are equal to 87.1% and 16.8%, respectively. In vitro study shows that the drug loaded PEG-*b*-$PMEO_2MA$ polymeric micelle is able to stably release drug for a long period.

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