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Characteristics, phase behavior and control release for copolymer–liposome with both pH and temperature sensitivities

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ABSTRACT

A pH- and thermo-sensitive berberine hydrochloride liposome modified by the poly(*N*-isopropylacrylamide-*co*-methacrylic acid-*co*-octadecyl acrylate) was synthesized. The morphology and size were determined by atomic force microscope (AFM) and dynamic light scattering (DLS). The phase transition temperature (T_{pm}) and phase transition pH (pH*) of the copolymer–liposome were obtained by differential scanning calorimetry (DSC). The results indicate that the size of copolymer–liposome depends on the mass ratios of the copolymer to soya bean lecithin (SPC) and the solution pH value, and the size can reach a maximum value at phase transition pH (pH*). The T_{pm} and pH* were affected by the mass ratios of the copolymer to SPC and properties of the copolymer. The release of berberine hydrochloride (BH) from the copolymer–liposome was investigated and the maximum release of BH was caused at T_{pm} and pH* of the copolymer–liposome. The results illustrate that BH released from the liposome is due to the phase transition of the copolymer–liposome, where both T_{pm} and pH* were acted as switches of the phase transition, and both spacial and temporal releases can be carried out by adjusting the temperature, pH value and release time.

1. Introduction

Since liposome was first proposed as a potential drug carrier, many attentions have been paid to design stimuli-sensitive liposome [1], such as pH [2,3], electricity [4], temperature [5–8], magnet [9–11] or light [12,13] sensitive liposome. Among all these liposomes, pH-sensitive liposome has attracted many researchers' attentions. As the pH of endosoma of the cancerous cell is lower than that of extracellular fluids, this feature has been exploited in the design of systems that facilitate the delivery of active compounds to the cytoplasm *via* a pH-dependent membrane-disruptive and/or fusogenic action [14]. The most widely studied pH-sensitive liposome is generally composed of mildly acidic amphiphiles and unsaturated phosphatidylethanolamines. One such liposome is composed of dioleoylphosphatidylethanolamine (DOPE) [15,16] and cholesteryl hemisuccinate (SHEMS), and it confers stability to the bilayer phase at neutral pH [17–19]. However, the potential application of this liposome as drug carriers *in vivo* is hampered by their relatively poor stability in the presence of serum [20–22]. To improve it, researchers have attempted to modify the surfaces of liposome in order to give the liposome some specific functions. The method by using polymers to modify the surfaces of liposome has been studied as a way to increase the sensitivity and stability of liposome. The addition of polymers can also exhibit prolonged circulation times *in vivo* and accumulate in tumors [23]. Temperature sensitive poly(*N*-isopropylacrylamide) (PNIPAM) has been used to modify liposome in many studies [24–26], whose Lower Critical Solution Temperature (LCST) was very close to the human physiological temperature. The phase transition temperature of the liposome can be varied by changing either the hydrophilic or hydrophobic monomers of the polymer [27]. Kim et al. [6,28] had prepared liposome coated with copolymers of NIPAM and acrylic acid, and it showed that drug release from the liposome was dependent on the LCST of the copolymer. Hydrophobically modified NIPAM copolymer containing methacrylic acid (MAA) as a pH-sensitive moiety was shown to trigger the content release of liposome under acidic conditions [29,30]. The properties of the copolymer–liposome are affected by the copolymers covered on the surfaces of the copolymer–liposome [31].

Berberine hydrochloride (BH, Fig. 1) is an isoquinoline alkaloid. It is widely used as an antibacterial and antifungal drug in pharmaceutical products. BH can enhance the phagocytosis of the leucocytes and endothelial system, and it can be employed in oncotherapy. Besides, BH itself has strong fluorescence, which can be used to study the structure of liposomal membrane and drug release without adding other fluorescer. In recent years, some scientists have found that the effect of BH on the inhibition to cancer cell *in vivo* is not obvious than that *in vitro*, because of an intestinal

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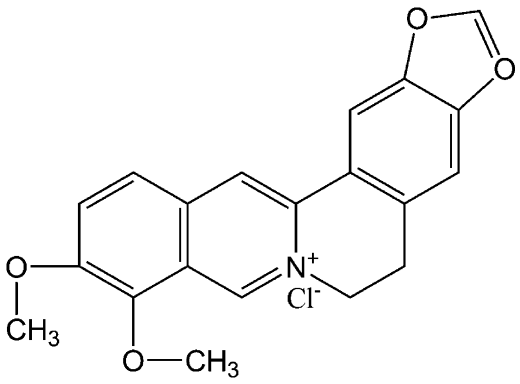


Fig. 1. Structure of the BH.

absorption [32]. And BH can cause cacoethic actions in intravenous injection. Based on the defects of the BH *in vivo*, liposome is used to entrap BH and to develop the functions of BH fully.

Drug release controlled by pH or temperature was studied in many researchers' works [33]. Our purpose is to find an optimal drug release by controlling the phase transition pH and phase transition temperature of the copolymer–liposome. A pH- and thermo-sensitive liposome modified by poly(*N*-isopropylacrylamide-*co*-methacrylic acid-*co*-octadecyl acrylate) (P(NIPAM-*co*-MAA-*co*-ODA)) was prepared. The structure of the copolymer and copolymer–liposome are shown in Fig. 2. The phase behaviors, morphology and sizes of copolymer–liposome were measured by differential scanning calorimetry (DSC), atomic force microscope (AFM) and Dynamic Lighting Scattering (DLS). The effects of BH on the phase transitions and size of the copolymer–liposome were discussed. And the pH- and temperature-stimuli release of BH from the copolymer–liposome was investigated.

2. Materials and methods

2.1. Materials

N-Isopropylacrylamide (NIPAM) (Shanghai Wujing Chemical Science and Technology) was dispersed in heptane, solubilized by acetone addition, and then allowed to recrystallize at −20 °C prior to use. Methacrylic acid (MAA) (Shanghai Lingfeng Chemical Reagent) was distilled under reduced pressure to remove inhibitors at 55 °C before used. Azobisisobutyronitrile (AIBN) (Shanghai Lingfeng Chemical Reagent) was recrystallized in ethanol and dried under vacuum. Octadecyl acrylate (ODA) (Beijing East Yakeli Chemical Science and Technology), soya bean lecithin (SPC) (purity 97%, Shanghai Gengben Biological Technology) and berberine hydrochloride (BH, Dongbei Pharmaceutical Factory) were used as received. 1,4-dioxane, diethyl ether, tetrahydrofuran, chloroform, absolute methanol (Shanghai Lingfeng Chemical Reagent) were of analytical reagent grades and used as received.

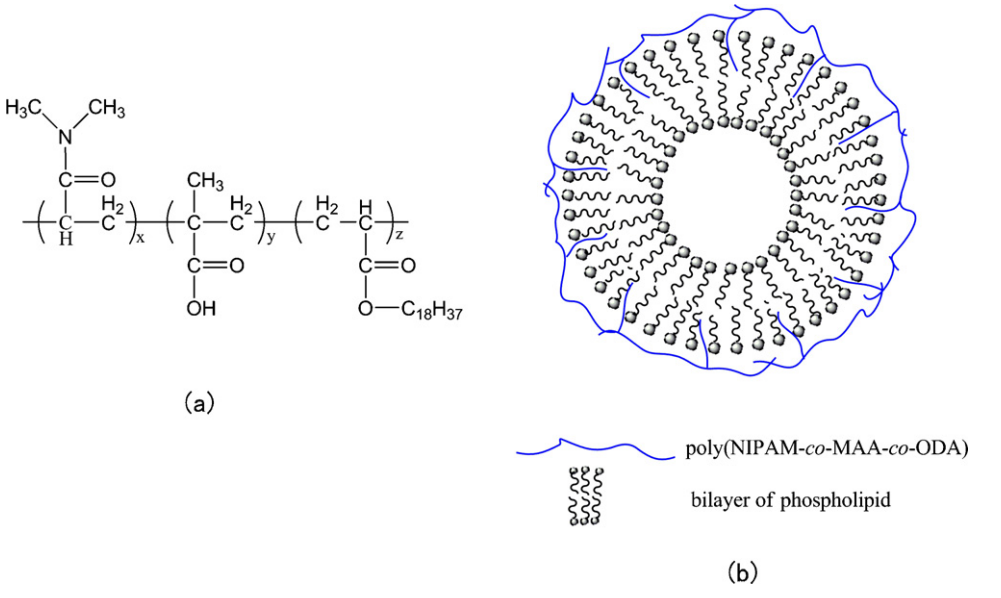
2.2. Synthesis of the P(NIPAM-co-MAA-co-ODA)

P(NIPAM-*co*-MAA-*co*-ODA) was prepared by the previous method [29]. P(NIPAM$_{95}$-*co*-MAA$_4$-*co*-ODA) was synthesized as following steps. Monomers (NIPAM: 3.1788 g, MAA: 0.1045 g, and ODA: 0.0965 g) of the molar ratio 95:4:1 and the initiator (AIBN: 0.0048 g) were dissolved in an appropriate amount of distilled 1,4-dioxane. The mixture was first degassed by bubbling N_2 for 15 min, and then was refluxed at 69 °C for 15 h under N_2 atmosphere. The polyelectrolyte was recovered by precipitation in diethyl ether, resolubilized in tetrahydrofuran, reprecipitated, and washed extensively with diethyl ether. After repeated above operations three times, the product was dried under vacuum for 5 days.

2.3. Synthesis of copolymer–liposome

The liposome modified by the copolymer was prepared by membrane evaporation method [15], defined as copolymer–liposome. In detail, SPC and the copolymer were dissolved in a mixture of chloroform and methanol (volume ratio was 2:1). The solution was evaporated at 43 °C by rotatory evaporator, after which the lipid membrane was gained.

The lipid membrane was then dissolved in PBS solutions with different pH and stirred for one hour at room temperature. The concentration of the lipid was 1 mg/ml. The copolymer–liposome solution defined as blank copolymer–liposome (BK–liposome) was formed. The other BK–liposomes with different mass ratios of SPC to copolymer were prepared using aforementioned method.



(a) (b)

Fig. 2. Structure of the copolymer ((a) *x*, mass ratio of NIPAM; *y*, mass ratio of MAA; *z*, mass ratio of ODA) and schematic diagram of copolymer–liposome (b).