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Effect factors of micelle preparation for a pH-sensitive copolymer containing zwitterionic sulfobetaines

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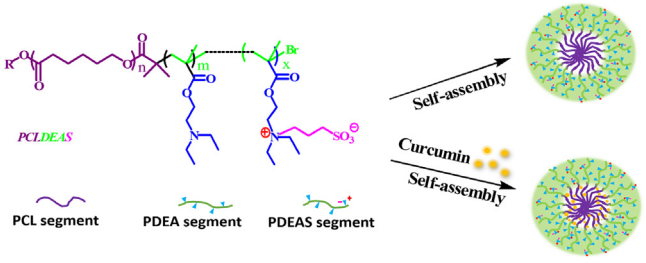
HIGHLIGHTS

- We discussed the influence of preparation factors using solvent evaporation method.
- The micelle prepared using a pH-sensitive copolymer containing zwitterionic sulfobetaines.
- The preparation factors may influence the micellization process.
- The sizes and zeta-potential of the micelles controlled by preparation parameters.
- The size further influences the drug loading/drug release of micelles.

GRAPHICAL ABSTRACT



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ABSTRACT

Polymeric micelles have wide applications for the delivery of water-insoluble drugs. Solvent evaporation method is commonly used to prepare polymer micelles, although the influential factors of micellization process of this method are insufficiently clear. In the work, a pH-sensitive copolymer containing sulfobetaines, poly(ε-caprolactone)-b-poly(N,N-diethylaminoethylmethacrylate)-r-poly(N-(3-sulfopropyl)-N-methacryloxyethy-N,N-diethylammoniumbetaine) (PCLDEAS), was selected as the material for micelle preparation by solvent evaporation method to investigate the influence of preparation factors on the micelle sizes/size distributions and zeta-potentials. The preparation variables included copolymer concentration, the organic solvent/water phase ratio, the micelle concentration and the compositions of aqueous phase. The influence of these factors further on drug loading/releasing was also studied. In conclusion, to control the preparation factors of solvent evaporation method may be employed to control the properties of the micelles including sizes, drug loading and drug release.

1. Introduction

In the past few years, considerable interests have been attracted by the drug delivery system, especially by polymeric micelles due to their unique core–shell structures through self-assembly in aqueous media [1]. Polymeric micelles seem to be one of the most advantageous vehicles for the delivery of water-insoluble drugs [2], a variety of drugs with diverse characteristics, including genes and proteins. All these drugs can be incorporated into the cores of polymeric micelles. Compared to surfactant micelles, polymeric micelles are generally more stable, with a remarkably lowered critical micelle concentration (CMC), and have a slower rate of dissociation, allowing retention of loaded drugs for a longer period of time and, eventually, achieving higher accumulation of a drug at the target site [3]. However, traditional polymeric micelles exhibit

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some disadvantages such as short of tumor-targeting abilities, lack of multiple functionalities and a burst release of drug due to the limitation in the number of building blocks. In order to overcome this limitation, the so-called smart drug delivery systems are designed to react on certain stimulus like pH [4], temperature [5], redox potential [6], enzymes, light, and ultrasound [7] for the triggered release [8]. Among them, pH-sensitive micelles are more attractive due to the fact that in tumors the pH can be significantly more acidic (~6.5–5.0) than systemic pH (7.4) caused by poor vasculature and consequent anaerobic conditions prevailing in the malignant cells. Polymeric micelles with pH-sensitive modality can register such pH-gradients and, as a response, can facilitate the release of the payload near the target compartments either by destabilization of the micelles themselves or by decomposition of the pH-sensitive linking units that connect the drug to the carriers [9].

Some of pH-sensitive destabilization micelles are achieved by the protonation of weak alkalis such as amine, pyridine and imidazole groups. These micelles are commonly formed by pH-sensitive polymers such as poly(N,N-diethyl amino ethylmeth acrylate) (PDEA) [10], poly(4- or 2-vinylpyridine) (PVP) [11], poly(ε-amino ester) (PbAE) [12] and poly(L-histidine) (pHis) [13]. Among them, PDEA-based micelles have great potential as an acid triggering antitumor vehicle for its pK_a is 7.2, which is sensitve to pH difference between the extra celluar matrix (ECM) of tumors (pH = 5.0) and normal tissue/blood (pH = 7.4). Tertiary amine groups in PDEA based polymers are protonated when they are exposed at slightly acidic condition. Thus, PDEA is a good candidate as pH-sensitive switch because of the protonation of PDEA in micelles around tumors. Furthermore, PDEA is beneficial for cellular internalization due to the electrostatic interaction with negatively charged cell membranes [14]. However, PDEA was insoluble at pH above its pK_a (7.2), and the charge property of PDEA can also evoke cytotoxicity. Therefore, PDEA is often combined with more hydrophilic polymers to form stable micelles and decrease its toxicity. PEG, a biocompatible polymer, is widely used as the shells of micelles for it can stretch in aqueous media to form a hydrophilic brush corona for micelles to prolong systemic circulation [15]. Nevertheless, PEG is also susceptible to oxidation damage and unfavorable for cellular uptake because of its shielding effect [16]. Thus, zwitterionic polymers have received growing attentions because they have the advantages of excellent biocompatibility and non-bioadhesive property and overcome the disadvantages of PEG [17]. Based on such characteristics, we have developed pH-sensitive copolymers composed of poly(ε-caprolactone)-b-poly(N,N-diethylaminoethyl methacrylate)-r-poly(N-(3-sulfopropyl)-N-methacryloxyethy-N,N-diethylammoniumbetaine) (PCLDEAS), where the core composed of PCL would be as a drug container, the shell PDEA as a pH-sensitive switch, and the SBMA as a hydrophilic protective layer. The PCLDEAS micelles presented excellent blood compatibility, protein-resistance property, capability for drug loading and so on [18].

In the previous work, the PCLDEAS micelles were prepared by solvent evaporation method which is the common method to prepare micelles [19]. In the solvent evaporation method, the copolymers are firstly dissolved in a volatile, water miscible organic solvent which is the solvent for all blocks of copolymer. Self-assembly is then triggered by the addition of the organic phase to water or aqueous medium, which is non-solvent for the core-forming block and solvent for corona-forming block (or vice versa [20]). The organic solvent is finally removed by the evaporation. The solvent evaporation method over other micelle preparation methods bears several advantages such as fast, feasibility for scale up [21] and less chance for drug loss during dialysis in the encapsulation process [22]. However, although solvent evaporation method has been widely used to prepare micelles, the influential factors of micellization process of this method is insufficiently clear. What is more, whether the factors of micelle preparation will affect drug loading and releasing must be considered in the polymeric micelles as drug delivery systems. Thereby, in this study, we select a pH-sensitive copolymer containing zwitterionic sulfobetaines, poly(ε-caprolactone)-b-poly(N,N-diethylaminoethyl methacrylate)-r-poly(N-(3-sulfopropyl)-N-methacryloxyethy-N,N-diethylammoniumbetaine) (PCLDEAS), as a material for micelle preparation by solvent evaporation method to investigate the influence of preparation variables such as the copolymer concentration, the organic solvent/water phase ratio, the micelle concentration and the medium compositions on the micelle sizes/size distributions and zeta-potentials and to research the influence of these factors on drug loading/releasing. The sizes and size distributions of the micelles were measured by dynamic light scattering (DLS). The micelle morphologies were directly visualized under scanning electron microscopy (SEM). Moreover, the drug loading and release were also measured to confirm the preparation parameter affection on the pH-sensitive micelles containing zwitterionic sulfobetaine as a drug vehicle.

2. Materials and methods

2.1. Materials

The amphiphilic copolymer, poly(ε-caprolactone)-b-poly(N,N-diethylaminoethyl methacrylate)-r-poly(N-(3-sulfopropyl)-N-methacryloxyethy-N,N-diethylammoniumbetaine) (PCLDEAS), used as a material for micelle preparation, was synthesized via the ring opening polymerization (ROP) and followed by atom transfer radical polymerization (ATRP) method and by sulfonation. The operation process and technological details were reported in our previous work [18], only alternating the initiator pentaerythritol by laurinol in ROP of ε-caprolactone. The obtained PCLDEAS copolymer was characterized by ^{1}H NMR and element analysis methods.

All solvents and other chemicals were of analytical grade and purchased from Chengdu Kelong Chemicals Ltd. Toluene and tetrahydrofuran (THF) were purified by refluxing over sodium before use. N,N-dimethylformamide (DMF) was purified by distillation under vacuum over CaH_2 before utilization. Deionized water (18.25 MΩ, Milli-Q system Millipore, Bedford, MA) was used to prepare micelles solution.

2.2. Preparation of polymeric micelles

The PCLDEAS micelles were prepared by solvent evaporation method (see Scheme 1). Typically, the copolymer was firstly dissolved in THF (a co-solvent for the all blocks of the copolymer) to form copolymer solution. Then, this copolymer solution was added drop-wise at a certain speed into 5 mL of water with stirring. Finally, the organic solvent was removed by evaporation under stirring for 24 h followed by a rotary vacuum evaporating for 10 min. The obtained solution with a blue tint indicated that the micelles were successfully prepared.

2.3. Characterization of micelles

The sizes and zeta-potentials of the micelles were measured using a Zetasizer Nano-ZS (Malvern Instrument Ltd., UK) instrument. The instrument is equipped with a He–Ne laser beam at a wavelength of 633 nm. Our tests were performed by monitoring the scattered light intensity at a 90° scattering angle at 25 °C. All samples were filtered through membrane filters with nominal pore size of 0.45 μm (Millipore, USA) to remove dust particles before measurement. The experiments were performed at room