Applied Surface Science 265 (2013) 101–107

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Electrostatic self-assembly: An innovative approach to fabricate novel-structured magnetic liposomes

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ARTICLE INFO

Article history:
Received 14 July 2012
Received in revised form 23 October 2012
Accepted 23 October 2012
Available online 12 November 2012

Keywords:
Electrostatic self-assembly
Magnetic liposomes
Novel-structured
Content of magnetic nanoparticles
In vitro drug release

ABSTRACT

Electrostatic self-assembly was applied to fabricate novel-structured magnetic liposomes. According to the charge characteristics of the magnetic nanoparticles and the drug-loaded liposomes, magnetic liposomes were fabricated by alternately assembling the suitable polyelectrolytes and magnetic nanoparticles onto the drug-loaded liposomes. TEM photograph provided direct evidence for successful fabrication of the novel-structured magnetic liposomes. It was also found that electrostatic self-assembly is a universal approach to prepare novel-structured magnetic liposomes with tunable size. The reversed phase high performance liquid chromatography (RP-HPLC) method was established to determine the content of the drug in the magnetic liposomes. The content of the magnetic nanoparticles in the magnetic liposomes was determined by UV spectrophotometry, which proved that the content of magnetic nanoparticles in novel-structured magnetic liposomes was higher than in traditional-structured magnetic liposomes. In vitro drug release from the magnetic liposomes was carried out, and fitting of the release curve using Curve Expert software indicated that the in vitro drug release of the magnetic liposomes was in accordance with the First-order equation.

1. Introduction

Magnetic liposomes are a novel kind of drug delivery carrier in magnetic drug targeting system. Drug can make oriented movement and aggregation to target tissue by inducing of external magnetic fields. In addition, magnetic liposomes can be further used for magnetic hyperthermia (with exposure of the target tissue to alternating current magnetic fields), which has been found to be a promising therapeutic approach to cancer treatment [1,2]. At present, widely investigations of magnetic liposomes have been developed by the researchers all over the world. For example, thin-film dispersion method [3,4] and reverse-phase evaporation method [5] has been used to prepare magnetic liposomes.

It is obvious that effect of magnetic targeting is up to magnetic strength of the external magnetic fields and magnetic response of the magnetic liposomes. To enhance magnetite content of the magnetic liposomes is a feasible and effective way to increase magnetic response. Magnetic liposomes prepared by all the present approaches have the same structure that magnetic nanoparticles and hydrophilic drug are both encapsulated in the aqueous core of the liposomes (the structure diagram was shown in Fig. 1a). However, there is little magnetic nanoparticles packaged into the liposomes because that the inner space of the liposomes is limited. Furthermore, during the formation of liposomes vesicles, the magnetic nanoparticles are difficult to be encapsulated uniformly into the liposomes for their big density and tendency of sedimentation. The problem may be resolved if drug were packaged in the inner of liposomes, while the magnetic nanoparticles were coated onto the surface of liposomes. Our research interest is to design and prepare the novel-structured magnetic liposomes (the structure diagram was shown in Fig. 1b) with higher magnetite content and narrow size-distribution, which will have potential application in magnetic targeting therapy.

Electrostatic self-assembly is based on the spontaneous control of materials through electrostatic forces with no external intervention, which has been found to be a useful and versatile technique for fabrication of novel composite particles [6,7]. Researches on the controlled fabrication of core-shell composite microspheres, based on electrostatic self-assembly, have been reported [8–11]. This process entails the stepwise adsorption of charged polyelectrolyte and oppositely charged nanoparticles onto core particles, exploiting primarily electrostatic interactions for layer buildup. This technique possesses the advantage of tunable size, chemical composition and properties of the core-shell particles, which provides a potential approach to prepare various types of core-shell particles with novel morphology and composition. The keypoint of this technology is selecting suitable polyelectrolyte interlayer, which is determined by the charge qualities of core and shell

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http://dx.doi.org/10.1016/j.apsusc.2012.10.131

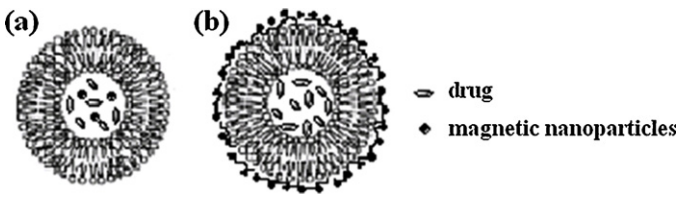

Fig. 1. Structure diagrams of the (a) traditional-structured magnetic liposomes and (b) novel-structured magnetic liposomes.

materials. The novel-structured magnetic liposomes should be prepared by electrostatic self-assembly considering that liposomes have surface charge. The novelty of this study is design of the magnetic liposomes with novel structure, and pioneering preparation of the magnetic liposomes through electrostatic self-assembly. In the present study, we provide a detailed procedure for fabrication of the novel-structured magnetic liposomes and their relevant characterizations and few representative results.

2. Experimental

2.1. Materials

Chloroform, methanol, ferrous sulfate hepta-hydrate ($FeSO_4{\cdot}7H_2O$), ferric chloride hexa-hydrate ($FeCl_3{\cdot}6H_2O$), sodium hydroxide, sodium chloride, sodium acetate, glacial acetic acid and oil of vitriol were purchased from Xi'an Chemical Regent Factory (Xi'an, Shaanxi, China). Physiological saline was purchased form Jingxi Shuanghe Pharmaceuticals Co. Ltd. (Xi'an, Shaanxi, China). Hydroxylamine hydrochloride was purchased from Dengfeng Chemical Co. Ltd. (Tianjin, China). 1,10-Phenanthroline was purchased from Fuchen Chemical Co. Ltd. (Tianjin, China). All these chemicals except methanol were of analytical grade and were used as received. Methanol used was of HPLC grade and purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Egg phosphatidylcholine (EPC) was supplied by Xi'an Libang Pharmaceuticals Co. Ltd. (Xi'an, Shaanxi, China). Cholesterol was purchased from Aoboxing Biotechnology Company Co. Ltd. (Beijing, China). Doxorubicin hydrochloride was purchased from Meiji Pharmaceutical Ltd (Shantou, Guangdong, China). Doxorubicin hydrochloride reference substance was purchased from Shanghai Shunbo Bioengineering Co. Ltd. (Shanghai, China). Poly (sodium 4-styrenesulonate) (PSS, $M_w \sim 70{,}000$) and Poly (diallyldimethylammonium chloride) (PDADMAC, $M_w = 100{,}000$–$200{,}000$) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). PSS powder was dissolved in distilled water to prepare aqueous solution of 0.5 mg ml^{-1} (in 0.5 M NaCl). PDADMAC were diluted with distilled water to concentration of 1 mg ml^{-1} (in 0.5 M NaCl). The pH values of the PDADMAC solution and PSS solution used in this work are 5.88 and 5.86, respectively. All of these strong polyelectrolytes were dissolved in distilled water without any adjustment of pH. The distilled water was prepared from deionized water by distilling it twice.

2.2. Preparation of magnetic nanoparticles

Magnetic nanoparticles were prepared by chemical co-precipitation [12]. Ferrous sulfate hepta-hydrate and ferric chloride hexa-hydrate with molar ratio of 2:3 were dissolved in 300 ml distilled water under mechanical stirring in a three-necked, round-bottomed flask. When the mixture was heated to 30 °C in a water bath, sodium hydroxide solution (3 M) was injected into the flask to adjust the pH to 13. The temperature of the solution was kept at 30 °C for 30 min, and then heated at 80 °C for 30 min. After it was cooled down to ambient temperature, the obtained aqueous ferro-fluid contained magnetic nanoparticles was repeatedly washed with distilled water, and was diluted to be solid content of 2%.

2.3. Preparation of drug-loaded liposomes

Egg phosphatidylcholine (EPC) and cholesterol were chosen as the liposomes composition, and doxorubicin hydrochloride was chosen as model drug. EPC and cholesterol were dissolved in methanol/chloroform (1:2, v/v) mixed solvent to prepare 0.1 g ml^{-1} EPC solution and 0.1 g ml^{-1} cholesterol solution, respectively. Doxorubicin hydrochloride was dissolved in physiological saline to prepare 0.2 mg ml^{-1} solution. 10 ml EPC solution and 3 ml cholesterol solution were mixed into a round bottom flask. The organic phase was evaporated under vacuum in a rotary evaporator. A thin lipid film was formed following the evaporation of the solvents. To remove the traces of the solvents completely, the round bottom flask containing thin lipid film was put under high vacuum for one more hour. 50 ml doxorubicin hydrochloride solution was added to the round bottom flask, and mixed by vortex mixer for 20 min for hydration.

The drug-loaded liposomes thus formed are large in size (>1 μm). Preparation of the liposomes with smaller size was done by sequential extrusion through 400 nm and 200 nm polycarbonate membranes (three times for each of the membranes) using an extruder (NLI Ltd., CAN). Unencapsulated doxorubicin hydrochloride was removed from the liposomes using sephadex G50 minicolumn by centrifugation. Herein, a self-made sephadex G50 minicolumn was used, which is prepared by filling the barrel of a 5 ml disposable plastic syringe with swollen sephadex G50 [13–15]. Liposomes suspension was slowly injected into the sephadex G50 minicolumn. Then the minicolumn was centrifuged at 4000 × *g* for 5 min and washed twice with physiological saline to separate drug-loaded liposomes from free doxorubicin hydrochloride.

2.4. Preparation of magnetic liposomes

Suitable polyelectrolyte interlayer can be selected according to the charge quality of the drug-loaded liposomes and the magnetic nanoparticles. The magnetic liposomes were fabricated by alternatively adsorbing polyelectrolyte and magnetic nanoparticles onto the surface of drug-loaded liposomes.

Adsorptions of polyelectrolyte and magnetic nanoparticles onto the drug-loaded liposomes were carried out according to the following procedure details. The polyelectrolyte was adsorbed by dropping PDADMAC or PSS solution to drug-loaded liposomes suspension, occasionally stirring. After adsorbing 20 min, excess polyelectrolyte was removed by four repeated centrifugation/water wash/redispersion cycles. In a similar fashion, the magnetic nanoparticles were adsorbed by dropping the aqueous ferro-fluid to the drug-loaded liposomes suspension. Thereafter, the liposomes suspension was centrifuged at 500 × *g* for 10 min to remove excess unadsorbed magnetic nanoparticles.

2.5. Zeta potential and particle size analyses

Zeta potential and particle size of the liposomes and the magnetic nanoparticles were measured using a Zetasizer NanoZS (Malvern Instrument Ltd., UK) after the samples were diluted with air-equilibrated distilled water. Zeta potential was automatically calculated from the electrophoretic mobility of particles using the Smoluchowski relationship [16]. Size distribution and mean particle sizes of the samples were determined using the dynamic light scattering.