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Fabrication of novel magnetic nanoparticles-coated P(styrene-itaconic acid-divinylbenzene) microspheres☆

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ABSTRACT

In this paper, P(styrene-itaconic acid-divinylbenzene) microspheres (P(St-IA-DVB) microspheres) based on styrene (St), itaconic acid (IA) and divinylbenzene (DVB) were prepared via water-in-oil emulsions method (W/O) in the presence of emulsifiers with the size of 5–10 μm. The magnetic nanoparticles (i.e. Fe_3O_4) coated tightly on the surface of P(St-IA-DVB) microspheres were prepared in water with a continuous stirring. The morphology of blank microspheres and magnetic nanoparticles-coated microspheres was investigated in this work. *In vitro* drug release behavior was studied using doxorubicin as a model drug, and these magnetic nanoparticles-coated P(St-IA-DVB) (MNPSID) microspheres might have great potential application in magnetically targeted and thermal therapy.

1. Introduction

Polymer microspheres have many unique features, among them, small, tailored for particular request particle size characterized by uniform size distribution are the most frequently called. Microspheres based on biodegradable polymers have been widely used as drug delivery vehicles because of their sustained drug action on the lesion, high capability to cross various physiological barriers, reduced systemic side effects, facilitated extravasation into the tumor, controlled and targeted delivery of the drugs (Freiberg & Zhu, 2004; Kawaguchi, 2000; Kokai et al., 2010; Rokhade, Patil, & Aminabhavi, 2007; Williams et al., 2006; Wolska & Bryjak, 2009; Yang, Chen, Huang, Bai, & Yang, 2009; Zhao, Cheng, Huang, & Ying, 2008; Zhao et al., 2009; Zhou, Gu, Su, & Ma, 2007). In recent years, magnetic polymer microspheres have been extensively investigated and widely used in various fields, such as cell separation, protein purification, especially in tumor targeting therapy (Alvisi, Poon, & Jans, 2006; Choi et al., 2010; Jordan, Scholz, & Maier Hauff, 2001; Müller, 2009; Nilsson, Tarli, Viti, & Neri, 2000; Wajant, Gerspach, & Pfizenmaier, 2005). Composite microspheres coated by magnetic nanoparticles could be prepared by mechanical and chemical methods. It including high-speed-stirred mixer or a high-shear mill, had been used for the preparation of microspheres coating magnetic nanoparticles, and suspension polymerization or emulsion polymerization for preparing polymer microspheres (Bai, Yang, Li, Huang, & Huang, 2006; Chen, Serizawa, & Akashi, 1999; Chung & Lee, 2008; Guo, Bai, & Sun, 2003; Guo, Zhang, Zhang, Zhang, & Zhang, 2009; He, Ge, Wang, Zhang, 2006; Hua & Yu, 2009; Jin, Fu, Huang, Xiao, & Zou, 2003; Mouaziz, Larsson, & Sherrington, 2004; Quaroni & Chumanov, 1999; Sun, Wang, Wang, & Jiang, 2007; Wang & Pan, 2000; Wang & Pan, 2001; Zhang et al., 2003). Subsequently, in our present work, we prepared the P(St-IA-DVB) chemical-crosslinked microspheres by emulsion polymerization method, and Fe_3O_4 nanoparticles coated on the surface of the polymer microspheres by high-speed-stirred mixer (Fan, Neoh, Kang, Shuter, & Wang, 2007; Gan et al., 2011; Wei et al., 2010; Yang, Peng, Wen, & Li, 2010; Zhang, Jiang, Li, Sun, & Yang, 2010; Zhu, Yuan, & Liao, 2008).

Doxorubicin (DOX), an anthracycline, is an effective anticancer agent with great antitumor activity against solid tumors and used for the treatment of a number of carcinomas, such as bladder, breast, and gastric cancers (Tan, Lin, & Wang, 2005; Liu et al., 2001; Sun et al., 2009). However, its therapeutic potential has been restricted by its dose-limited cardiotoxicity and by the resistance of the tumor cells after some time of treatment. Its acute toxicities including myelosuppression, loss of hair, nausea, vomiting, mucositis, and local tissue necrosis when it leaks into the extravascular space at the site of injection (Cai, Thati, Bagby, Diab, & Davies, 2010; Kang, Cheon, & Song, 2006; Lin et al., 2011). In order to achieve better therapeutic effects and reduce the side-effect of doxorubicin, change formulation of doxorubicin became more and more important for its tumor therapy application. In our initial work, Fe_3O_4

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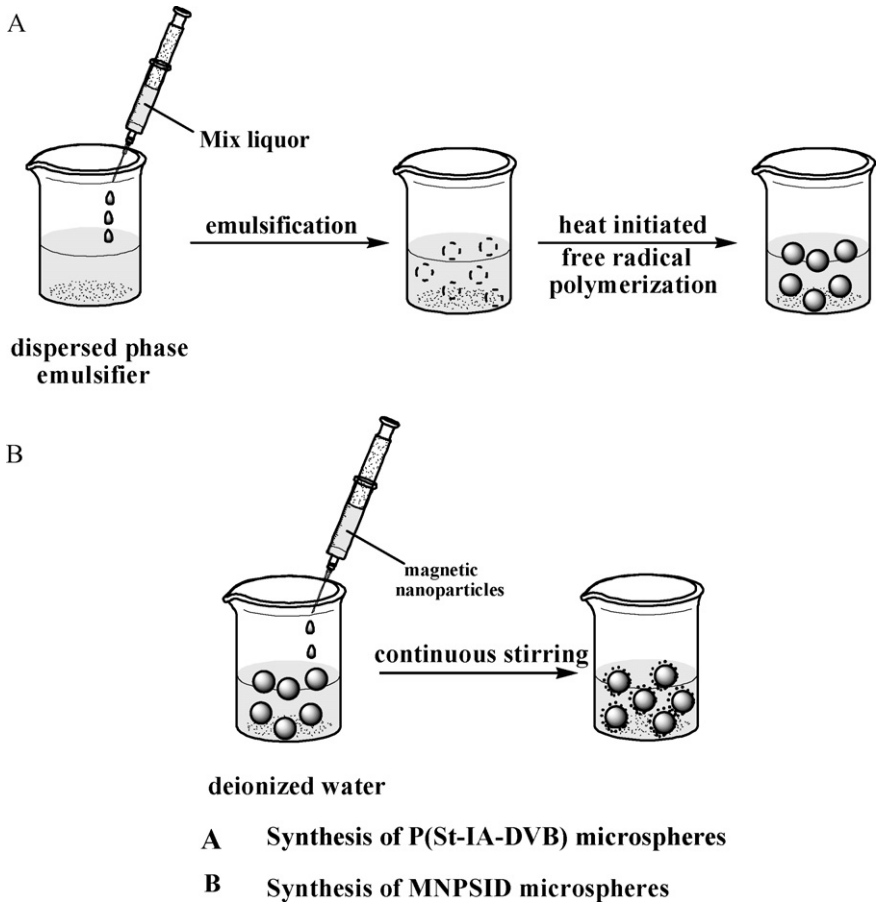


Scheme 1. Schematic illustration of synthesis of P(St-IA-DVB) microspheres and MNPSID microspheres, respectively.

nanoparticles coated P(St-IA-DVB) microspheres were synthesized and the doxorubicin–MNPSID microspheres were prepared by pH-induced precipitation method. Doxorubicin release study was also investigated in detail, which has shown more potential application in tumor targeting therapy (Lee, Park, Kim, & Byun, 2007; Mrkvan et al., 2005; Tinkov et al., 2010; Ying, Cui, Yu, & Du, 2011).

2. Materials and methods

2.1. Materials

Styrene (St) and divinylbenzene (DVB) washed with 10% NaOH aqueous solution to remove the inhibitors. *N*,*N*′-Methylene-bis-acrylamide (BIS), itaconic acid (IA), ammonium persulfate (98%) (APS) were all analytic grades, and purchased from Aldrich Company, USA. Doxorubicin chloride (doxorubicin, DOX) was purchased from Zhejiang Hisun Pharmaceutical Company (China). Fe_3O_4 magnetic nanoparticles were synthesized in our lab. All the other reagents were also analytic grade and used as received.

2.2. Preparation of blank P(St-IA-DVB) microspheres

The inverse-phase suspension polymerization method was employed to prepare microspheres, and it was shown in Scheme 1. The reaction was performed in a 250 ml three-neck flask fitted with a mechanical stirrer. The continuous phase comprised of 100 ml of castor oil and 10 ml of span 80. A determined amount of IA, St, DVB and BIS were dissolved completely in DMSO, and the organic phase was added dropwisely into the flask, with 70 °C heating using an oil bath. Then the initiator of APS was added dropwisely with syringe carefully. The reaction proceeded for 8 h with continuous stirring. The resulting microspheres were separated by centrifugation. The centrifuged microspheres were washed firstly with diethyl ether, followed by deionized water at least three times. After 3 times purification, the microspheres were lyophilized. In this paper, three kinds of P(St-IA-DVB) microspheres were prepared, and the preparing parameters were listed in Table 1.

2.3. Preparation of magnetic nanoparticles coated P(St-IA-DVB) microspheres (MNPSID microspheres)

10 mg of blank microspheres were dispersed in 50 ml deionized water with continuous stirring. 50 μl of Fe_3O_4 nanoparticles were added dropwisely in 2 ml deionized water with ultrasonic dispersion. After that they were blended and dispersed completely in the water. The reaction kept up for 4 h, and the product was washed with excess deionized water three times and centrifuged. The procedure of MNPSID microspheres was demonstrated in Scheme 1. The obtained MNPSID microspheres were freeze dried, and then kept in 4 °C before use.

2.4. Preparation of doxorubicin-loaded MNPSID microspheres

DOX–MNPSID microspheres were prepared by pH-induced precipitation method. Briefly, 2 mg of MNPSID microspheres prepared in the previous step was placed into an EP tube, and 0.2 ml of special PBS (10×, pH = 7.4) was added into this solution with stirring. And then 0.4 ml of doxorubicin aqueous solution (5 mg/ml) was added dropwisely into the above solution. Twenty minutes later, doxorubicin-loaded MNPSID microspheres were obtained. After

Table 1
Composition of the prepared MNPSID microspheres and their DOX encapsulation efficiency and drug loading.

Sample	St:DVB:IA	Fe_3O_4	Encapsulation efficiency (%)	Drug loading (%)
S-1	40:30:30	50 μl	78.6 ± 3.6	52.7 ± 4.2
S-2	50:25:25	50 μl	71.4 ± 3.8	50.9 ± 4.0
S-3	60:20:20	50 μl	67.2 ± 3.9	49.8 ± 4.3

Calculated from Eq. DL % = [amount of drug/(amount of polymer + drug)] × 100. EE % = [experimental drug loading/nominal drug loading] × 100.

that, drug-loaded microspheres were freeze dried and kept in 4 °C before use.

2.5. Fourier transforms infrared (FTIR) analysis

FTIR (KBr) spectra of magnetic nanoparticles, blank P(St-IA-DVB) microspheres and MNPSID microspheres were recorded on NICOLET 200SXV spectrophotometer (Nicolet).

2.6. Crystallographic assay

X-ray diffraction spectrometry was obtained by using X-ray diffractometer (DX-2000, DanDong Fangyuan Instrument Company, China) using Cu Ka radiation.

2.7. Scanning electron microscopy (SEM)

SEM was employed to investigate morphology of blank P(St-IA-DVB) microspheres and MNPSID microspheres. The microspheres were dispersed in deionized water, respectively. And they were frozen in liquid nitrogen and lyophilized for 72 h. Then, the microspheres were sputtered with gold before observation. In this study, morphology of the prepared particles was examined on JEOL SEM (JSM-5900LV, JEOL, and Japan).

2.8. In vitro release behavior of microspheres

The drug loading and encapsulation efficiency of the DOX–MNPSID microspheres were determined by a subtraction method. Briefly, 0.5 ml deionized water containing 2 mg DOX-microspheres were centrifuged through a filter with a molecular mass cut off 10 kDa. While the free doxorubicin could pass through the filter, the doxorubicin-encapsulated microspheres could not pass through the filter. The unincorporated doxorubicin was quantified using reverse-phase High Performance Liquid Chromatography (RP-HPLC) with a C_{18} column (4.6 mm × 150 mm 5 μm, Sunfire). The drug loading (*DL*) and entrapment efficiency (*EE*) of the microspheres were calculated according to Eqs. (1) and (2):

$$DL\ \% = \frac{\text{amount of drug}}{\text{amount of polymer} + \text{drug}} \times 100 \quad (1)$$

$$EE\ \% = \frac{\text{experimental drug loading}}{\text{nominal drug loading}} \times 100 \quad (2)$$

In vitro release behavior of doxorubicin from DOX–MNPSID microspheres was studied using the modified-dialysis method, which was shown as following: 2 mg of drug-loaded microspheres were transferred into an EP tube, and 0.5 ml of free doxorubicin solution in water (0.25 mg/ml) was used as control. Then dialyzed against 25 ml phosphate buffer at pH 7.4 both containing Tween20 (0.5%) at 37 °C with gentle shaking. A total of 25 ml of the surrounding dialysis medium was removed at predetermined time points for analysis, and 25 ml of fresh buffer at the relevant pH was added to the dialysis medium. The released drug was quantified using reverse-phase High Performance Liquid Chromatography (RP-HPLC) with a C_{18} column (4.6 mm × 150 mm 5 μm, Sunfire). Each experiment was repeated 3 times, and the results were expressed as: mean value ± SD.

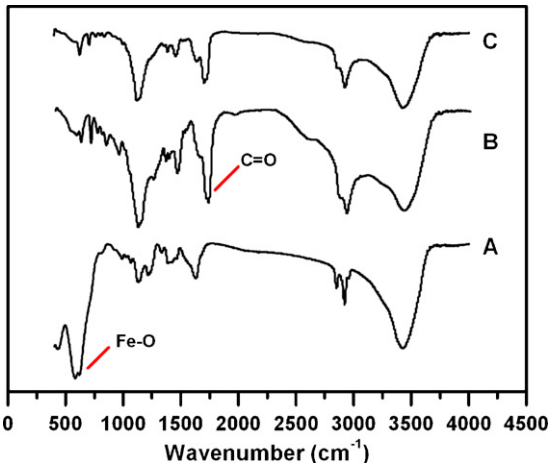


Fig. 1. FTIR spectra of (A) Fe_3O_4 nanoparticles; (B) blank P(St-IA-DVB) microspheres; (C) MNPSID microspheres.

3. Results and discussion

3.1. Characterization of microspheres

The blank P(St-IA-DVB) microspheres were synthesized by inverse-phase suspension polymerization method and MNPSID microspheres were synthesized by co-precipitation method, respectively. Table 1 summarized the preparation parameters of blank P(St-IA-DVB) microspheres and its drug loading and encapsulation efficiency.

FT-IR spectra of Fe_3O_4 nanoparticles, blank P(St-IA-DVB) microspheres and MNPSID microspheres were shown in Fig. 1. The FT-IR spectrum of the mixture is not the same with the pure one, because bands' vibration peaks were overlapped. In general, the absorption bands of 582 cm^{-1} were attributed to Fe–O stretching peak from spectrum A of Fe_3O_4 nanoparticles. The peak at 1730 cm^{-1} belonged to the C=O block from blank P(St-IA-DVB) microspheres. These peaks were all appeared in the spectrum C, which is contributed to the magnetic nanoparticles were well-coated on the surface of blank P(St-IA-DVB) microspheres.

The crystallographic structure of Fe_3O_4 nanoparticles, blank P(St-IA-DVB) microspheres, and MNPSID microspheres were determined by XRD. As presented in Fig. 2, the semi-crystalline exhibited a reflection peak at about 2.5247 and a relatively weak reflection at 2.9615, which were assigned to two different crystal forms.

Careful examination of representative microspheres, including blank P(St-IA-DVB) and MNPSID microspheres of each formulation using SEM microscopy revealed more detail information regarding the external morphological feature. The surface morphology of microspheres was presented in Fig. 3. On comparing the SEM photographs of Fig. 3(A) and (B), it seems that there is no significant difference in the size and uniformity of the microspheres among these three composite microspheres. According to Fig. 3(B), we could clear see that Fe_3O_4 nanoparticles were well-coated

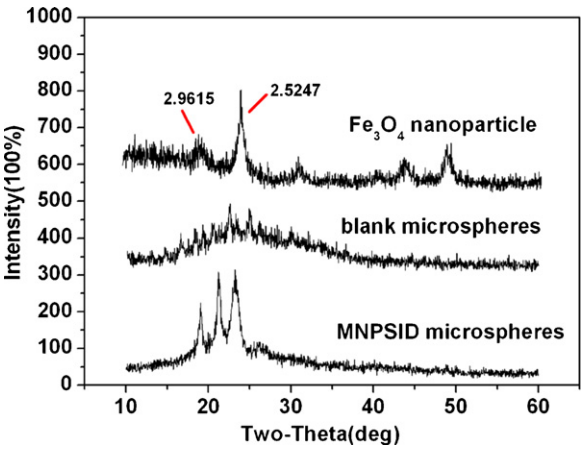


Fig. 2. X-ray powder diffraction pattern of Fe_3O_4 nanoparticles, blank P(St-IA-DVB) microspheres, MNPSID microspheres, respectively.

on the surface of P(St-IA-DVB) microspheres, and appeared the favourable uniformity. Meanwhile, we could see that the muddy liquid became limpid very soon after 30 s, which was shown in Scheme 2. The reason might be due to the fact that Fe_3O_4 nanoparticles and P(St-IA-DVB) microspheres have the stronger mutual magnetic attraction.

DOX–MNPSID microspheres were prepared by pH-induced precipitation method. Fig. 4 shows the release profiles of free doxorubicin and DOX–MNPSID microspheres in PBS (pH 7.4, 37 °C). The encapsulation efficiency and drug loading of doxorubicin were 78.6% and 52.7%, respectively. From Fig. 4, in comparison to free doxorubicin, a typical two-phase-release profile of DOX–MNPSID microspheres was observed. That is, a relatively rapid release in the first stage followed by a sustained and slow release over a prolonged time up to 168 h. As can be seen in Fig. 4, about 70.6% of loaded drug released from the microspheres after incubation at pH 7.4 for 24 h. On the contrary of control experiment, free diffusion of doxorubicin only through the dialysis membrane was showed that about 100% of the doxorubicin put inside the membrane released through the membrane at pH 7.4 for 8 h. Generally, doxorubicin release rate from MNPSID microspheres might mainly determined by the structure of the microspheres where the doxorubicin is

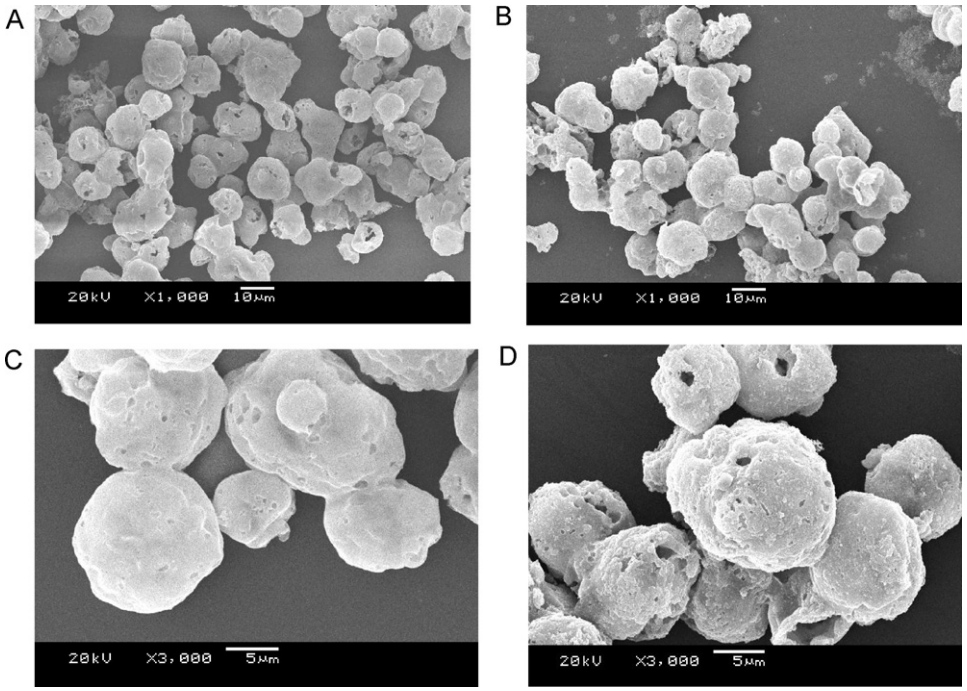
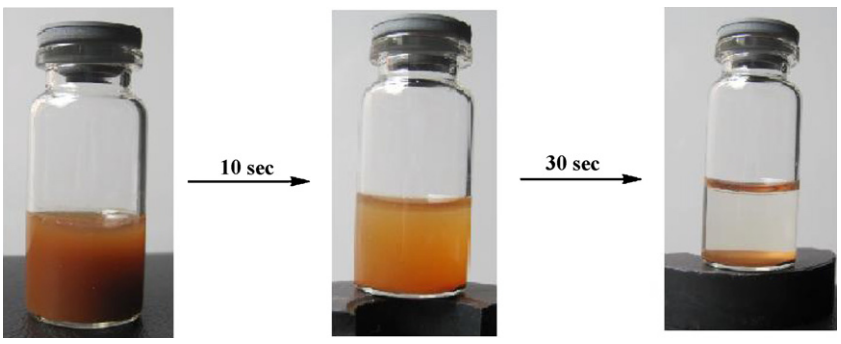


Fig. 3. SEM image of blank P(St-IA-DVB) microspheres (A, magnification: ×1000); (C, magnification: ×3000) and MNPSID microspheres (B, magnification: ×1000); (D, magnification: ×3000), respectively.



Scheme 2. Schematic illustration of MNPSID microspheres above the magnet after 10 s and 30 s.

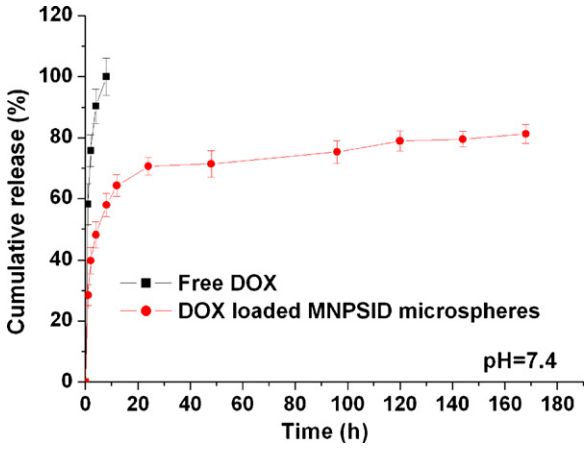


Fig. 4. Cumulative release of free DOX and DOX–MNPSID microspheres at pH 7.4.

contained and its chemical properties associated with both the microspheres and doxorubicin. The doxorubicin encapsulated in a slowly degrading MNPSID microspheres provide the opportunity for slower release effects, which is greatly depended on the structure of the microspheres where the doxorubicin is contained. In another aspect, the doxorubicin release is also diffusion controlled as it can travel through the pores formed during sphere formation. In some cases, doxorubicin containing nucleophilic groups can cause increased chain scission of the microspheres, which also increases the rate of doxorubicin expulsion. Consequently, this delay of doxorubicin release from microspheres suggested that these biodegradable magnetic nanoparticles-coated microspheres might be a promising drug-vector for targeted delivery of anticancer drugs.

4. Conclusion

In this study, magnetic nanoparticles-coated microspheres were successfully prepared. These microspheres were original, stable and mono-disperse. Simultaneously, they showed great potential application in doxorubicin delivery. In some cases, the doxorubicin loaded in these microspheres might be a novel anticancer agent, which implied the great application in magnetic targeted tumor therapy.

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