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Morphology-controlled hollow nanospheres of functionalized dextran by self-assembly in aqueous solution

Xiepeng Lin, Wenxia Gao*, Xiaobo Huang, Panlong Zang, Jiuxi Chen, Huayue Wu*

College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325027, China

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ABSTRACT

Dextran propionate tryptophanate ester (DPT) was synthesized by two-step esterification to self-assemble into regular hollow nanospheres during a dialysis process. Hollow nanospheres self-assembled in the dimethylacetamide (DMAc) exchange against polymer solution, with a size in the range of 80–500 nm outside diameter and 15–110 nm shell thickness. The organic solvent had a great influence on the morphology and the size of these nanospheres. No hollow nanospheres but only nanoparticles with no cavum could self-assemble in furfuryl alcohol with the range of 57–257 nm, in DMSO with the range of 61–292 nm, in N-methyl-2-pyrrolidone with the range of 82–348 nm and in DMF with the range of 262–670 nm. The stability of these hollow nanospheres was also influenced by storage time. They were relatively stable in 2 months in deionized water stored at 25 °C or 37 °C, which can be verified by SEM and pH value variation.

1. Introduction

Hollow nano- and microspheres of functional materials have gained considerable interest because of their potential applications in controlled drug delivery, artificial cells, catalyst and as electronic materials (Huang, Meng, & Wan, 2006; Li, Liu, Kang, Neoh, & Yang, 2008; Liebert, Hornig, Hesse, & Heinze, 2005). In the biomedical field, nano- and microparticle-forming materials need to be nontoxic, biocompatible, and should have high disease site selectivity. Thus, they are commonly prepared on the basis of biopolymers, such as polysaccharides or partial derivative of glucans with bulky hydrophobic substituents (Butsele, Jerome, & Jerome, 2007; Lemarchand, Gref, Lesieur, Hommel, & Vacher, 2005).

Various methods have been used to prepare hollow spheres. Template synthesis based on core–shell structure has been well developed to achieve hollow spheres with varied shell composition. Hard cores such as silica colloids or polystyrene latexes are commonly used as templates for a variety of materials, such as polymeric, inorganic, biological, metallic materials, being coated forming the core–shell structures, which are usually assisted by layer-by-layer deposition (Tolue, Moghbeli, & Ghafelebashi, 2009; Wong & Richtering, 2008). Apart from templates assistant, preparation of hollow spheres usually involves self-assembly of block copolymers (Mazloumi et al., 2009; Wu, Li, & Feng, 2007). Specific molecular chain is required for aiming hollow spheres with defined morphology and stability. The amphiphilic character of these polymers is also required to guaranty the self-assembly of regular nanospheres.

In this paper, we propose a general self-assembly approach using new functionalized dextran to prepare hollow nanospheres. Dextran represents a valuable biopolymer that is widespread in medicine and pharmacy, such as blood plasma substitute and coating material to prevent protein opsonization (Mehvar, 2000; Purama, Goswami, Khan, & Goyal, 2009). However, the presence of a large amount of unmodified hydroxyl groups of dextran tends to re-form the supermolecular interactions yielding aggregation, even collapse of the nanoparticles. Therefore, the chemical modification of the majority of hydroxyl groups in dextran is necessary (Hornig & Heinze, 2007).

In this paper, functionalized dextran derivatives were synthesized by two-step esterification using propionic anhydride and L-tryptophan. Dextran propionate tryptophanate ester (DPT) was able to form nanospheres or hollow nanospheres by a controlled and slow exchange of different organic solvents against the polymer solution applying dialysis. The influence of organic solvents on nanospheres' morphology and the stability of these nanoparticles were also investigated in this paper.

2. Experimental

2.1. Materials

All solvents and reagents were commercially available and analytical-reagent-grade. L-Tryptophan, N,N′-carbonyldiimidazole

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* Corresponding author. Tel.: +86 577 88368280; fax: +86 577 88368280.
E-mail addresses: wenxiag@wzu.edu.cn (W. Gao), huayuewu@wzu.edu.cn (H. Wu).

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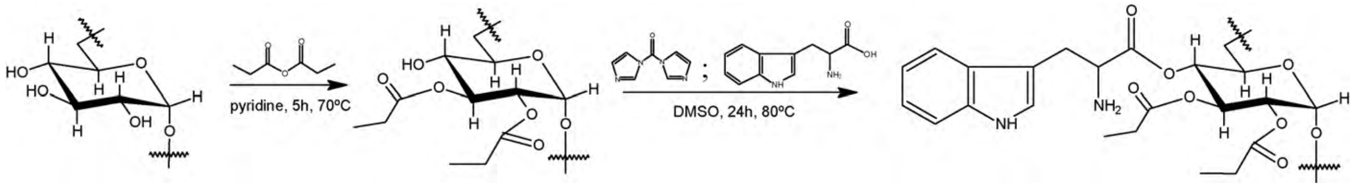

Scheme 1. Synthesis procedures of dextran propionate (1) and dextran propionate tryptophanate (DPT) (2).

(CDI) and dextran (Mw 33666 g mol^{-1}) were obtained from Shanghai Huamao Pharmaceutical Co., Ltd. All chemicals were directly used without purification. Dialysis bag was obtained from Xinhui Zeao Science and Technology Co., Ltd.

2.2. Measurements

NMR spectra were acquired using a 300-Bruker spectrometer 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR and reported as parts per million (ppm) from the internal standard TMS. FTIR spectra were recorded on a Bruker EQUINOX 55 spectrometer with the KBr-technique. Scanning electronic microscopy (SEM) images were taken on a Nova NanoSEM 200 scanning electron microscope.

2.3. Synthesis of functionalized dextran (Scheme 1)

2.3.1. Synthesis of dextran propionate (1)

The grafted copolymers of dextran propionate were prepared according to the reported method (Liebert et al., 2005). To a solution of 2 g dextran in 20 mL DMSO, 3.2 mL propionic anhydride, 10 mL pyridine and 90.62 μL triethylamine were added. The mixture was allowed to react at 80 °C under stirring for 5 h, and then precipitated in 300 mL isopropanol, washed twice with ethanol and dried to get dextran propionate. Yield: 1.71 g (50.3%); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.4–3.5 (H-AGU), 2.3 (CH_2-propionate), 1.0 ppm (CH_3-propionate); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 173.6 (O–CO), 98.5 (C1), 73.4-68.3 (C2–C5), 63.8 (C6), 27.1 (CH_2-propionate), 9.2 ppm (CH_3-propionate); FTIR (KBr): 3430 (OH), 2927 (C–H), 1750 (C=O Ester), 1155 (C–O), 1026 cm^{-1} (C–O).

2.3.2. Preparation of dextran propionate tryptophanate (DPT) (2)

To a solution of 0.90 g dextran propionate in 10 mL DMSO, 1.38 g L-tryptophan and 2.19 g CDI were added. The mixture was allowed to react at 90 °C under stirring for 24 h. The product was precipitated in 200 mL water, washed twice with 50 mL water and then dried to get dextran propionate tryptophanate. Yield: 0.75 g (49.4%); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 10.8 (NH), 7.8, 7.5, 7.3, 7.0, 6.6 (H-indole), 5.4-3.5 (H-AGU), 2.3 (CH_2-propionate), 2.1 (NH_2), 1.0 ppm (CH_3-propionate); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 173.6 (O–CO), 167.2 (O–CO-tryptophanate), 136.1, 127.3, 123.5, 120.9, 118.5, 118.8, 111.3, 109.2 (C-indole), 77.0–68.3 (C-AGU), 55.7 (CH-tryptophanate), 30.4 (CH_2-propionate), 9.2 ppm (CH_3-propionate); FTIR (KBr): 3394 (OH, NH), 2927 (C-H), 1750 (C=O Ester), 1657 (C=O Ester), 1091 (C–O), 1017 cm^{-1} (C–O).

2.4. Nanoparticle preparation

Preparation of the nanoparticles was carried out by a dialysis process. DPT (40 mg) was dissolved in 10 mL DMAc and then dialyzed against deionized water for 7 days. For SEM images, the dialyzate was centrifugated by high-speed frozen centrifuge and placed on a mica surface. The system was lyophilized for 6 h and sputtered with gold.

2.5. Degradation properties

The physical and chemical stability of these hollow nanospheres during the storage in deionized water had been studied in this paper according to the method as previous report (Masson, Maurin, Devissaguet, & Fessi, 1996). The nanosphere integrity was evaluated by the study of visual appearance and particle sizes of the carriers, characterized by SEM. The pH of the suspension was additionally analyzed to evaluate the nanospheres degradation.

3. Results and discussion

3.1. Hollow nanospheres morphologies

The resulting DPT hollow nanospheres were confirmed by SEM, as shown in Fig. 1. Hollow nanospheres (80–500 nm in average diameter) were obtained at a high formation yield. Moreover, some broken nanospheres were also observed (Fig. 1), which indicated that the nanospheres were hollow and their shells were about 15–110 nm. Moreover, both the inside and outside of those hollow microspheres were smooth. The formation of hollow nanoparticles in water resulted from self-assembling of regular nanospheres containing hydrophobic domains inside the core and a more hydrophilic shell. The amphiphilic character of these polymers guarantied the self-assembling of regular nanospheres. This nanoparticle's material prepared on the basis of dextran via two-step esterification using biocompatible propionate and tryptophanate moieties, led to highly functionalized derivatives with an adjustable hydrophilic–hydrophobic balance and adjustable solubility (Scheme 1) (Qiu, Feng, Wu, Zhang, & Zhuo, 2009; Wang, Zhang, Wang, Wang, & Yang, 2007; Wei, Cheng, Hou, & Sun, 2008). Less polar microdomains in the core of these hollow nanospheres can be used for the encapsulation of hydrophobic drugs.

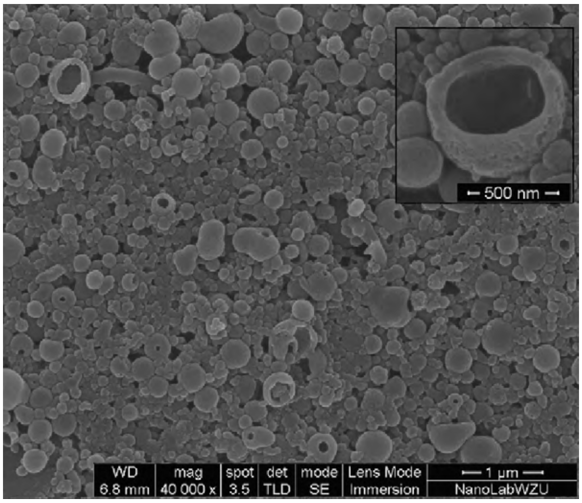

Fig. 1. SEM images of DPT hollow nanospheres prepared by dialysis of DMAc against water.

However, the ability to self-assemble nanoparticles did not result from a particular substituent but from a certain balance of functional groups with an inherent difference in polarity. The difference in the introduced functional groups and their substitution degree would result in different size and morphology of nanoparticles. Therefore, only self-assembling nanoparticles not hollow nanospheres could be obtained by slow exchange of water against DMAc using multifunctionalized dextran bearing furoyl-, pyroglutamyl-, propyl-, and acetyl moieties in varying proportions (Hornig & Heinze, 2007).

3.2. Effect of solvents on nanosphere forming

Besides the effect of particular substituent of dextran, organic solvent also had a great influence on the size and morphology of the nanospheres, since the formation of the nanoparticles during dialysis is based upon the slow exchange of the organic solvent against deionized water (Wang et al., 2007). These effects were also investigated in our work. Kinds of organic solvent with different dipole moment and viscosity including DMF (0.82 cp), DMAc (0.92 cp), N-methyl-2-pyrrolidone (1.65 cp), DMSO (2.24 cp), furfuryl alcohol (4.62 cp) and 1,2-dihydroxypropane (43.0 cp) were studied. These six organic solvents with the viscosity from 0.82 cp to 43.0 cp were selected for investigation. SEM images of DPT nanospheres prepared by 2 days' dialysis of organic solvents against water, as shown in Fig. 2.

Nanoparticles of DPT prepared in furfuryl alcohol were in the range of 57–257 nm, 61–292 nm in DMSO, 82–348 nm in N-methyl-2-pyrrolidone, 103–349 nm in DMAc, and 262–670 nm in DMF. Round nanospheres with smooth surface were obtained in each of these organic solvents. Therefore, these solvents were appropriate solvents for nanoparticle preparation of dextran derivatives. In addition, DMSO was less toxic than other five organic solvents, thus, seemed to be more suitable for biological applications.

Nevertheless, a comparison of the solvents showed that nanoparticles prepared in furfuryl alcohol and DMSO were much smaller than those in DMAc or in DMF. There was a tendency to smaller size of nanoparticles with the increase in viscosity of the organic solvent. This phenomenon showed that the penetration of DPT into water slowed down with the increase of solvent's viscosity, thus slowing the process of self-assembly. It led to smaller particle size of nanoparticles with more uniform morphology. For example, the viscosity of DMSO was 2.24 cp and that of furfuryl alcohol was 4.62 cp, much greater than that of water, which leads to relatively low DPT infiltration into water. So the self-assembled microspheres were relatively small.

However, it was not favorable for the preparation of smaller nanoparticles using solvent with much greater viscosity. We also select 1,2-dihydroxypropane with the viscosity of 43 cp as the organic solvent for self-assembly. Self-assembled nanoparticles were obtained after 14 days' dialysis since the penetration rate was very slow. The size distribution of nanoparticles was not uniform, as shown in Fig. 1. Most of the nanoparticle size distributed in the range of 71–153 nm, and there were also some relatively large nanoparticles more than 540 nm. It may be the further interaction by the role of hydrogen bonding, which led to the self-assembled nanoparticles gathered together, during relatively long time of dialysis since the penetration rate was very slow.

Besides the difference in particle size, organic solvents also have an influence on nanospheres' morphology (Yu & Eisenberg, 1997; Zhang et al., 2004). Interestingly, self-assembling hollow nanospheres could be obtained by slow exchange of DMAc against water. While no hollow nanospheres could be obtained using other five organic solvents against water. It seemed that DMAc was more suitable for preparing hollow nanospheres. The influence factors were still under study.

3.3. Study on stability of nanosphere

The physical and chemical stability of these hollow nanospheres during storage in aqueous media had been studied in this paper. The nanosphere integrity was evaluated by the study of visual appearance and particle sizes of the carriers. The pH of the suspension was additionally analyzed to evaluate the nanospheres' degradation. The mean particle diameter and morphology were determined by Scanning electronic microscopy (SEM). The pH value was measured using a Mettier delta 320 pH meter.

Fig. 3(a) presents the morphology variation of the hollow nanospheres stored in deionized water at 25 °C for 30 days. It indicated that the hollow nanospheres (86–445 nm in average diameter) maintained good shape, and the cavity still retained. The microspheres here demonstrated more satisfactory morphologies. This stable type of hollow particles was because of chemical modification of the majority of hydroxyl groups, which avoided the establishment of a hydrogen-bond system. And it also avoided the large amount of unmodified hydroxyl groups to re-form the supermolecular interactions for polysaccharides yielding aggregation and, consequently, collapse of the nanoparticles (Geze, Aous, Baussanne, Defaye, & Wouessidjewe, 2002).

Fig. 3(b) presents parts of the hollow nanospheres, which were no longer spherical after 60 days' storage in deionized water at 25 °C. The collapse of nanosize particles may be caused by the presence of hydroxyl groups tending to re-form the supermolecular interactions. And the hydroxyl groups included some unmodified hydroxyl groups of functionalized dextran and the free hydroxyl groups after ester hydrolysis (Hornig & Heinze, 2007). Therefore, the hydrogen-bond-based superstructure of the backbone usually resulted in metastable nanostructures, which led to more supermolecular interaction with time going on (Yul'chibayeva, Muratov, Yul'chibayev, & Usmanov, 1982). When the storage of hollow nanospheres in deionized water at 25 °C was long enough to 4 months, the morphology of hollow nanospheres completely collapsed into a linear, rod-like and other irregular shape with heterogeneous particles size, as shown in Fig. 3(c).

This phenomenon showed that the degradation of DPT in aqueous medium may follow two main mechanisms: a surface-erosin and an erosin in the bulk (Belbella, Vauthier, Fessi, & Devissaguet, 1996). At the very beginning of storage, there was little degradation of the polymer. This surface-erosin, involving the diffusion of the aqueous medium in the matrix but no bulk hydrolysis attacking the whole polymeric matrix, insured the good shape of hollow nanospheres during the first 30 days' storage. In the advanced stage, a large number of free hydroxyl groups occurred for the formation of pores and invasion of DPT by water resulting in an acceleration of ester hydrolysis. This led to more strong supermolecular interaction. When after 4 months' storage, there were some chains with different molecular weight generated by scission of initial DPT chains, which led to a significant mass loss of the matrix and then the final collapse of the polymer (Grizzi, Garreau, Li, & Vert, 1995). The high polydispersity of polymer molecular weight resulted in heterogeneous particles size such as a linear, rod-like and other irregular shape in the end after aggregation, as shown in Fig. 3(c).

The degradation was further investigated by the pH variation during storage. Fig. 4 presents the pH variation of the hollow nanospheres in deionized water stored at 25 °C or 37 °C. The initial pH of the suspension stored at 25 °C was 6.95. While the pH decreased to a final value of 4.87 after 17 weeks' storage. At 37 °C, there was a great variation of pH value in the former 4 weeks, compared with that at 25 °C. Temperature seemed to promote the acidification of the medium. Although there was a great variation of pH value in the former 4 weeks rather than the later 17 weeks' storage, the hollow nanospheres still maintained good shape as shown in Fig. 3(a). Since there were many free DPT moleculars which

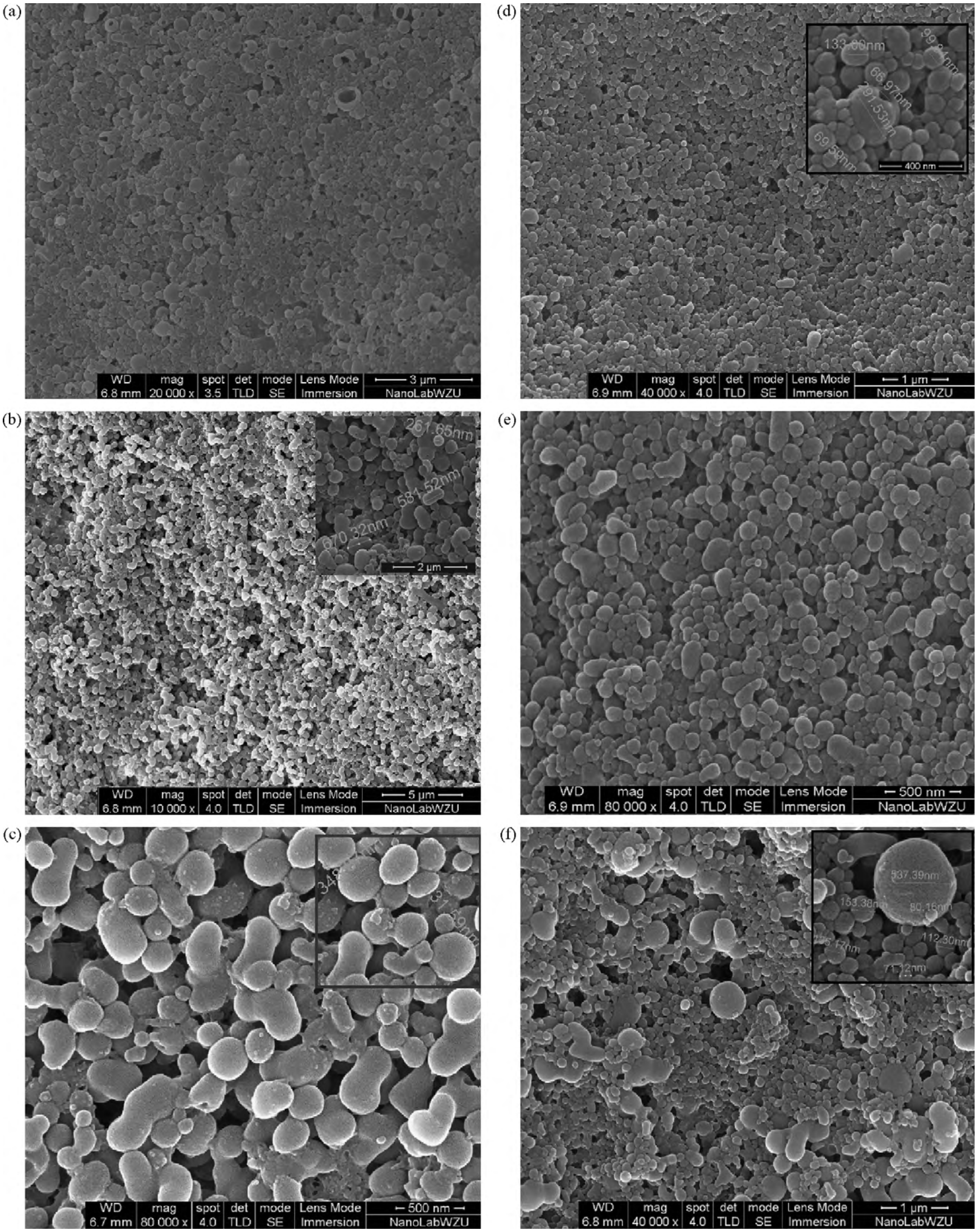

Fig. 2. SEM images of DPT nanospheres prepared by dialysis of (a) DMAc against water, (b) DMF against water, (c) N-methyl-2-pyrrolidone against water, (d) DMSO against water, (e) furfuryl alcohol against water and (f) 1,2-dihydroxypropane against water.

were adsorpted both inside and outside the cavity of these hollow nanospheres for their good adsorption capacity, these free DPT moleculars were more easily hydrolyzed than those DPT moleculars well ordered arrangement in those self-assembled nanoparticles (Liu, Chen, & Park, 2005). Therefore, there was a great variation of pH value in the former 4 week mainly because of the hydrolysis of free DPT moleculars rather than nanoparticles. At the very beginning of storage, there was little degradation of the polymer. This surface-erosin, involving the diffusion of the aqueous medium in the matrix but no bulk hydrolysis attacking the whole polymeric matrix, insured the good shape of hollow nanospheres during the first 30 days' storage.

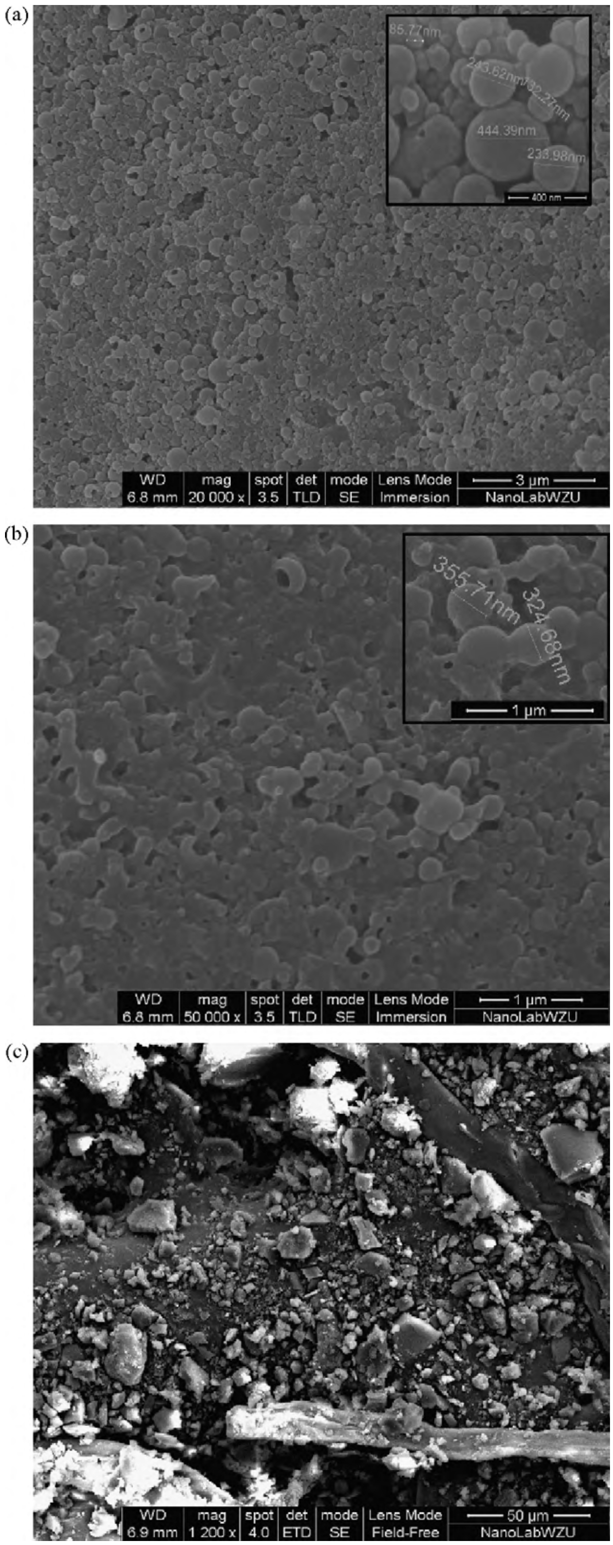

Fig. 3. SEM images of DPT hollow nanospheres stored in deionized water at 25 °C for 30 days (a), 60 days (b), and 120 days (c).

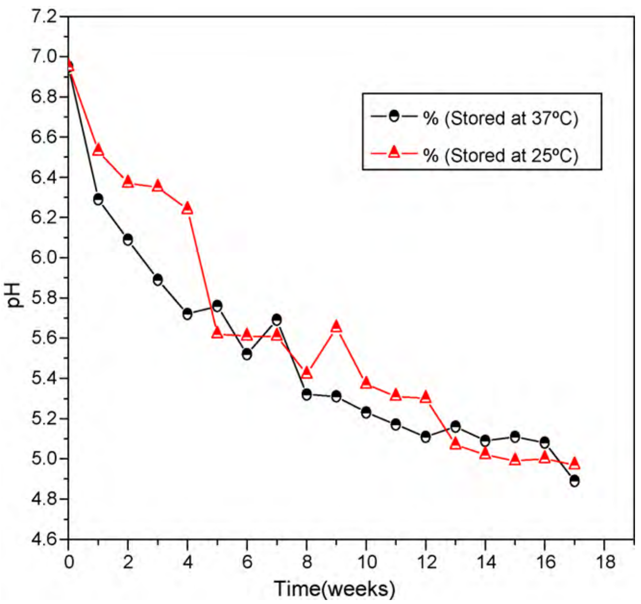

Fig. 4. Evolution of pH of DPT hollow nanospheres in deionized water, stored at 25 °C or 37 °C.

While in the later 13 weeks, slower pH variation appeared in both media stored at different temperatures. When stored more than 13 weeks, the change of medium pH value was very slow, which meant the degradation rate of polyester was very slow. Moreover, the pH value in these two media tended to be the same in the end of storage. This conclusion was consistent with the conclusion of the above-mentioned SEM images. The degradation of polyester in aqueous media generally induced an acidification of the medium. This phenomenon was also observed with poly (ε-caprolacton) (Masson et al., 1996; Shinoda et al., 2003) and poly(D,L-lactide) nanoparticles in aqueous media in the same way (Coffin & McGinity, 1992).

The investigation of the degradation showed that these hollow nanospheres were stable in 2 months in deionized water. Thus, these relatively stable hollow nanospheres were suitable for the preparation of solution formulations, such as eye drops, oral solution, injection agents and other water-soluble formulations. However, the influence of surfactants, temperature, buffer, preservative and other factors needed to be undertaken in later research in order to determine the best stability conditions and further stability of these hollow nanospheres was necessary.

4. Conclusion

Self-assembling nanospheres were prepared using dextran propionate tryptophanate ester (DPT) synthesized by two-step esterification. The slow exchange of DMAc against deionized water via dialysis process resulted in the self-assembly of regular hollow nanospheres, with a size in the range of 80–500 nm outside diameter and 15–110 nm shell thickness. It was testified that the size and morphology of the hollow nanospheres depended on the organic solvents' exchange against deionized water during dialysis. Hollow nanospheres were only obtained via dialysis in DMAc against deionized water. Nanoparticles with no cavum were prepared in furfuryl alcohol with the range of 57–257 nm, in DMSO with the range of 61–292 nm, in N-methyl-2-pyrrolidone with the range of 82–348 nm and in DMF with the range of 262–670 nm. Moreover, the stability of these hollow nanospheres was also influenced by storage time, characterized by SEM and the variation of pH value. These hollow nanospheres were relatively stable in 2 months in deionized water stored at 25 °C or 37 °C. Thus, these hollow nanospheres could be used for the encapsulation of hydrophobic drugs in controlled release or certain molecular recognition. And they may be valuable for the preparation of solution formulations, such as eye drops, oral solution, injection agents and other water-soluble formulations.

Acknowledgements

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