



(Protamine/dextran sulfate)₆ microcapsules templated on biocompatible calcium carbonate microspheres

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ABSTRACT

Poly (sodium 4-styrenesulfonate) (PSS) was used to synthesize biocompatible inorganic calcium carbonate (CaCO₃) microspheres. The influence of different functional group unit molar ratios of [PSS]/[Ca²⁺] to the morphology and crystal phases of CaCO₃ microspheres was studied by high scan electrode microscope (HSEM) and X-ray diffraction. These synthesized microspheres were used as the template of microcapsules. Protamine (PRM) and dextran sulfate (DXS) were self-assembled on the surface of the obtained microspheres alternatively for six layers. After the CaCO₃ was dissolved the microcapsules were obtained, which were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and TGA. This kind of biocompatible microcapsules could be used as micro-containers to load drugs and micro-reactors to perform chemical reactions.

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1. Introduction

Recently polyelectrolyte microcapsules have been introduced as a novel type of nanoengineered multifunctional material [1–3]. Permeability as a function of the wall materials of the microcapsules has received considerable attention due to an increased interest in biotechnology, medicine, catalysis, environment, food, etc., especially in the field of drug delivery [4–10]. Today, microcapsule systems have the highest potential in the pharmaceutical industry since many different requirements have to be fulfilled to deliver a drug at the right moment, in the right place, and at an adequate concentration.

Polyelectrolyte microcapsules are produced by stepwise adsorption of oppositely charged polymers onto the surface of colloidal particles followed by core dissolution, which is called LbL (Layer by Layer) technique [11–16]. Using this approach, a variety of materials, including charged and uncharged species, have been successfully assembled into nanoscale multilayered structures. The most used polymers are commercially available synthetic polyelectrolytes (PEs) such as PSS, PAH, PDADMAC and shells based on other synthetic polyelectrolytes such as poly(ethyleneimine) or Nafion have been synthesized. Particularly, some naturally occurring polyelectrolytes, such as polysaccharides are eye-catching. Recently, biocompatible polyelectrolytes of

carrageenan [17], dextran sulfate (DXS) [18], chitosan/chitosan sulfate [19], sodium alginate, and carboxymethyl cellulose [20] were reported.

The sacrificing of the encapsulated template is a key step for capsule fabrication. Several templates have been used for the capsule preparation, such as weakly cross-linked melamine–formaldehyde lattices [21], silica particles [22,23], polystyrene lattices [24] and biological templates [25], etc. Many of them have been commonly used for fundamental studies of multilayer capsules formation, because they can be commercially obtained in relatively large quantities with rather narrow size distribution and reasonable cost from many sources. However, the dissolution of many polymer cores such as melamine–formaldehyde and polystyrene requires strong acids or organic solvents, and in some cases template materials cannot be completely removed from the capsules because of the interaction between templates and capsule walls [26]. And the same time, incomplete elimination of melamine–formaldehyde oligomers formed during dissolution has strongly limited the use of these cores [27]. The search for properly decomposable colloidal particles appropriate for polyelectrolyte shell assembly leads to the exploration of other templates, such as poly-lactic/glycolic acid (PLGA) particles [28], biological cells [29], alginate–agarose particles [30] and carbonate (MnCO₃, CaCO₃, CdCO₃) particles [31]. Among these cores only carbonate particles can be decomposed under rather mild conditions.

The present study focuses on the microcapsules based on protamine (PRM) and dextran sulfate as the counter charge polyelectrolytes and biocompatible calcium carbonate as the template.

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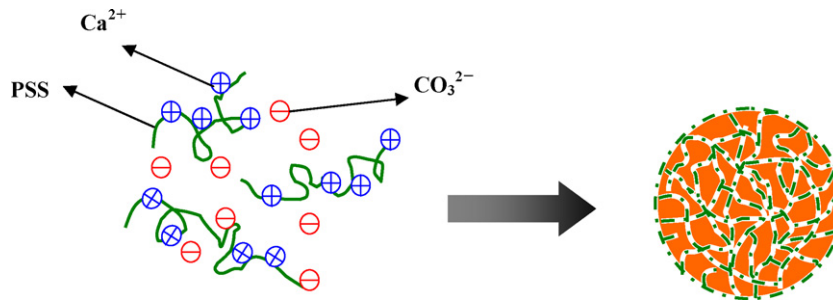


Fig. 1. Schematic illustration of synthesis of polymer doped CaCO_3 particles.

We firstly prepared PSS doped calcium carbonate microparticles with a narrow size distribution ranging from 3 μm to 5 μm by a simple and reproducible procedure. These particles are biocompatible, inexpensive, and easy to produce and decompose by complexation with disodium ethylenediaminetetraacetate dehydrate (EDTA) at neutral pH. The protamine (PRM) and dextran sulfate were assembled on the surface of the calcium carbonate particles. These natural polyelectrolytes are biocompatible and nonhazardous, so they are suitable for the preparation of microcapsules which encapsulated bioactive materials such as enzyme. Subsequent core dissolution by complexation with EDTA resulted in formation of polyelectrolyte microcapsules.

2. Experimental

2.1. Materials

Poly (sodium 4-styrenesulfonate) (PSS) ($M_w \sim 70$ kDa) was purchased from Aldrich. Calcium chloride dehydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), sodium carbonate (Na_2CO_3) and disodium ethylenediaminetetraacetate dehydrate (EDTA) were all purchased from Shanghai Chemical Reagent Limited Corporation. PRM and DXS were purchased from Sigma. The water used in our experiment was prepared in a three-

stage Millipore Milli-Q Plus 185 purification system and had a receptivity higher than 18 $M\Omega$.

2.2. Synthesis of calcium carbonate particles

Firstly, PSS was mixed with calcium chloride solution in a beaker under magnetic agitation (600 rpm), and the different ratios (the functional group unit molar ratio of $[\text{PSS}]/[\text{Ca}^{2+}]$) are 1/3, 1/8, 1/17 and 1/33, respectively. Then an equal molar sodium carbonate in another beaker was rapidly poured into a flask together with calcium chloride solution contained PSS at 30 °C. After the reaction, the precipitated calcium carbonate particles were collected and washed by pure water for three times.

2.3. Fabrication of PRM/DXS multilayers on calcium carbonate template

The pH values of PRM and DXS solutions were adjusted to 6.5 with 0.1 M HCl or NaOH to avoid the severe aggregation raised by low charge density of the PEs [22]. Two counter charged PEs of PRM/DXS were assembled on the core template alternatively in 0.5 M NaCl solution for 10 min followed by three washings in water. The excess polyelectrolytes were removed by centrifugation

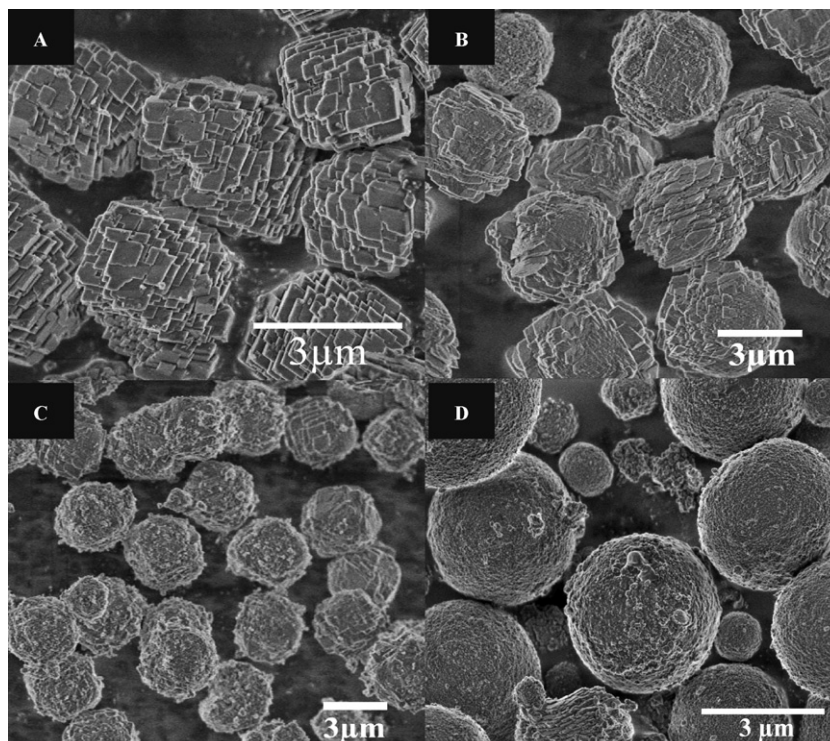


Fig. 2. HSEM images of CaCO_3 particles with different molar ratios (the functional group unit molar ratio) of $[\text{PSS}]/[\text{Ca}^{2+}]$: 1/3 (A), 1/8 (B), 1/17 (C) and 1/33 (D).

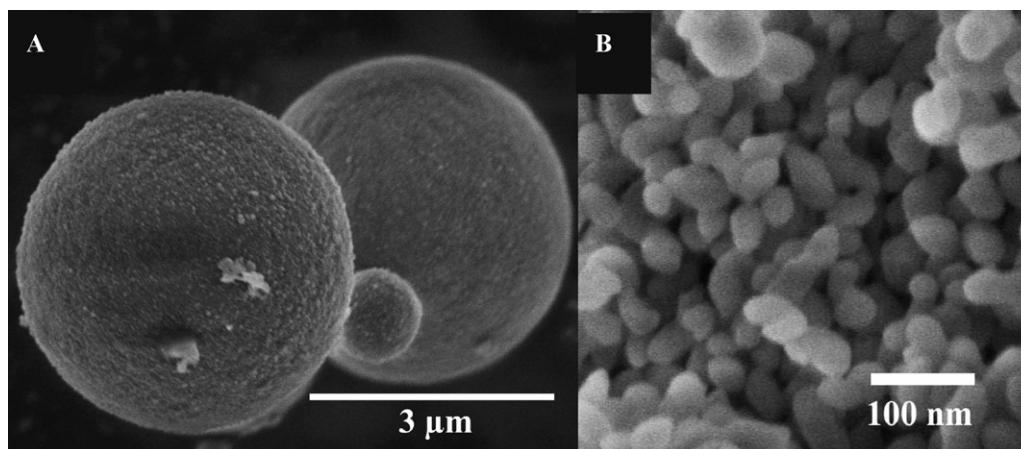


Fig. 3. HSEM images of CaCO_3 particles with the molar ratio (the functional group unit molar ratio) of $[\text{PSS}]/[\text{Ca}^{2+}]$: 1/33 (A) and HSEM of the surface (B).

at $3000 \times g$ for 3 min. After assembled four bilayers, the microcapsules were obtained by dissolving calcium carbonate template in 0.02 M EDTA solution for 30 min under shaking, and then the capsules were washed with water three times. The calcium carbonate template can be dissolved completely for incubating in EDTA solution for three times.

2.4. Characteristics

Scanning electron microscopy (SEM, JSM-6360L; HSEM, HITACHI S-4800) was used to characterize the morphology of calcium carbonate particles and microcapsules. Transmission electron microscopy (TEM, JEOL, JEM-100CX) was used to characterize the microcapsules. The surface potential of the colloid was characterized by Malvern Zetasizer 3000HS. XRD studies were performed using a D/max-rA (Rigaku, Japan) and the beam was provided by $\text{Cu K}\alpha$ ($\lambda = 1.5405 \text{ \AA}$). Pellets were prepared by grinding the solids with potassium bromide, which were used for IR spectroscopy analysis (Thermo, Nicolet 6700).

3. Results and discussion

3.1. Characterization of calcium carbonate particles

PSS as dispersant can control the morphology of calcium carbonate particles and enhance their surface electric potential during the

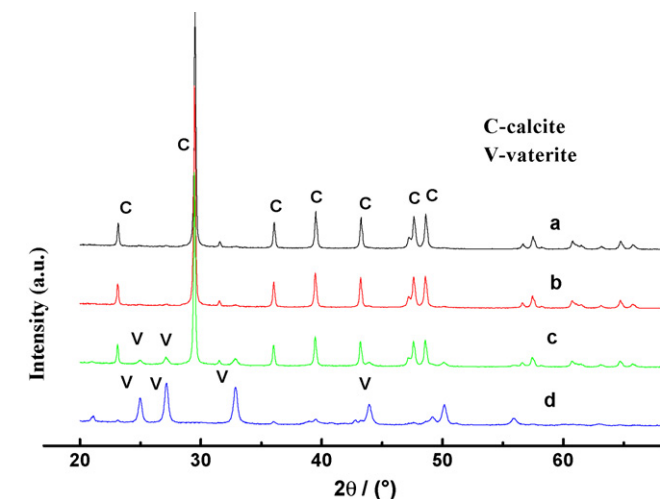


Fig. 4. XRD data of CaCO_3 particles with different molar ratios (the functional group unit molar ratio) of $[\text{PSS}]/[\text{Ca}^{2+}]$: 1/3 (a), 1/8 (b), 1/17 (c) and 1/33 (d).

growth of these particles. PSS possess functional groups on its long hydrophilic chains, which can complex with Ca^{2+} and disperse them to avoid agglomerating when calcium carbonate particles growing. The schematic illustration of synthesis of polymer doped calcium carbonate particles was showed in Fig. 1.

In the presence of PSS, the polydispersity of the calcium carbonate particles was decreased and more homogeneous calcium carbonate particles with the size around $4 \mu\text{m}$ were obtained. Fig. 2 showed the SEM images of calcium carbonate particles with different ratios (the functional group unit molar ratio of $[\text{PSS}]/[\text{Ca}^{2+}]$) are 1/3 (A), 1/8 (B), 1/17 (C) and 1/33 (D), respectively. We can see that their morphologies are different. When the molar ratio of $[\text{PSS}]/[\text{Ca}^{2+}]$ is 1/3, the particles were formed as calcite slice crystals. With the decrease of $[\text{PSS}]/[\text{Ca}^{2+}]$ ratio the diamond calcite slice crystals reduced and the sphere-like morphology was obtained showed in Fig. 3(A). From Fig. 3(B) we can see that many ellipse particles with the diameter about 50 nm fabricated calcium carbonate microspheres. Fig. 4 exhibits the experimental X-ray diffraction pattern for the four samples. The patterns showed that when the molar ratio of $[\text{PSS}]/[\text{Ca}^{2+}]$ is 1/3, calcite could be found as the main crystal phases in the resultants and no other phases. However, the ratio of vaterite increased if less PSS was added. When the ratio of $[\text{PSS}]/[\text{Ca}^{2+}]$ is 1/33, vaterite could be found as the main crystal phases.

The influence of different functional group unit molar ratios of $[\text{PSS}]/[\text{Ca}^{2+}]$ to the size and the mean of the cores was also studied. The ratios of $[\text{PSS}]/[\text{Ca}^{2+}]$ were 1/3, 1/8, 1/17, 1/33 and 0, respectively. Fig. 5 showed that the size of the cores had a decrease

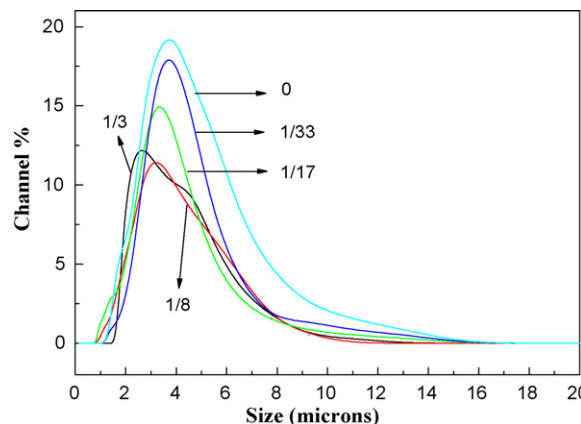


Fig. 5. Size distributions of cores synthesized by different functional group unit molar ratios of $[\text{PSS}]/[\text{Ca}^{2+}]$: 1/3, 1/8, 1/17, 1/33 and 0.

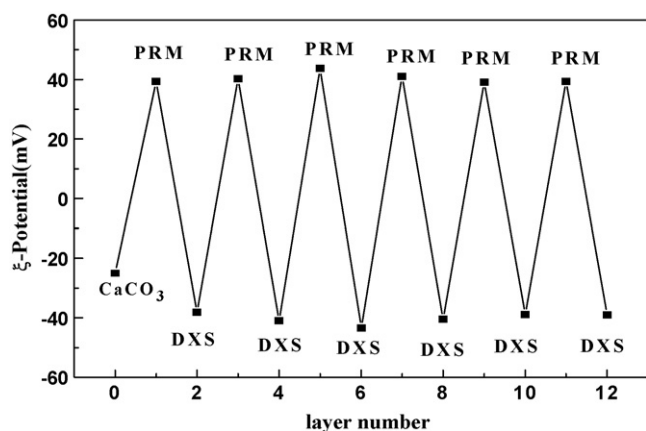


Fig. 6. ξ -Potential of (PRM/DXS)₆ microparticles as a function of PES layer number.

tendency when more PSS was added. However, the diameter of the five samples had not an obvious change. And when the ratios of [PSS]/[Ca²⁺] were 1/17 and 1/33, their distributions of size were more uniform than the other three.

3.2. Characterization of microcapsules

The PEs were assembled on the surface of calcium carbonate microspheres alternatively due to the electrostatic attraction between PRM and DXS with counter charge. Fig. 6 show the ξ -potential as a function of the polymer coating layer number for calcium carbonate particles. The particles exposed to PRM (positively charged) showed a ξ -potential of +40 mV, while to DXS (negatively charged) exhibited a value of -39 mV, which can prove that the two PEs assembled alternatively successfully.

Quantification of the PEs assembled was conducted by TGA. Fig. 7 is TGA curve of (PRM/DXS)₆/CaCO₃ recorded at a heating rate of 20 °C/min in nitrogen. Three independent weight losses in the TGA curve can be clearly identified. The first weight loss took place in the temperature range 50–200 °C, yielding a value of 1.07% which should be the result of evaporation of adsorbed and combined water molecules. The second weight loss occurred in the temperature range 250–550 °C, a typically high enough temperature to induce thermal degradation of ordinary carbon polymers. The PEs content in the composite could be estimated as 10.47%. The last weight loss was determined above 600 °C with a very fast rate, which is the result of calcium carbonate degradation.

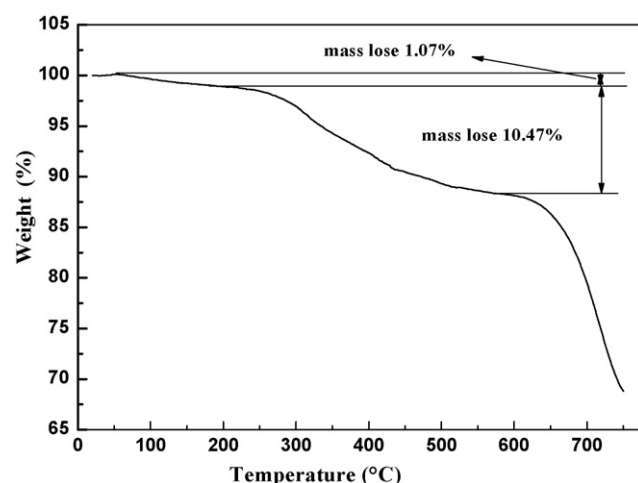


Fig. 7. TGA curve of (PRM/DXS)₆/CaCO₃ recorded at a heating rate of 20 °C/min in nitrogen.

The composites assembled six bilayers were mixed with the EDTA solution (pH value is 7.5) for a certain time to dissolve the template. And then the obtained microcapsules were washed for three times by pure water. This is a very promising way especially when pH sensitive weak PEs are utilized as the layer components in the present case. The obtained microcapsule was characterized by SEM. From Fig. 8 we can see that after removing the template a typical morphology for capsules in the dry state was observed. Creases and folds were formed due to the collapse of the capsules after evaporation of the liquid content and their size was nearly the same as the calcium carbonate template. Fig. 9 is the TEM image of microcapsules. From Fig. 9 we can see that the obtained microcapsules could avoid the rupture which maybe caused by the osmotic pressure between the inner and outer of the capsule wall during removing the template. And their shape was almost spherical. The shadows in the picture may be caused by the creases and folds.

The template retained in the (PRM/DXS)₆ capsules was characterized by TGA. Fig. 10 is TGA curve of microcapsules recorded at a heating rate of 20 °C/min in nitrogen. Three apparent weight loss processes were found in the TGA curve. The first weight loss of 8.59% could be assigned to the water release (from beginning to 350 °C). The second weight loss occurred in the temperature range 250–600 °C with a very fast rate, which could be explained by the thermal decomposition of PEs. The last weight loss was determined

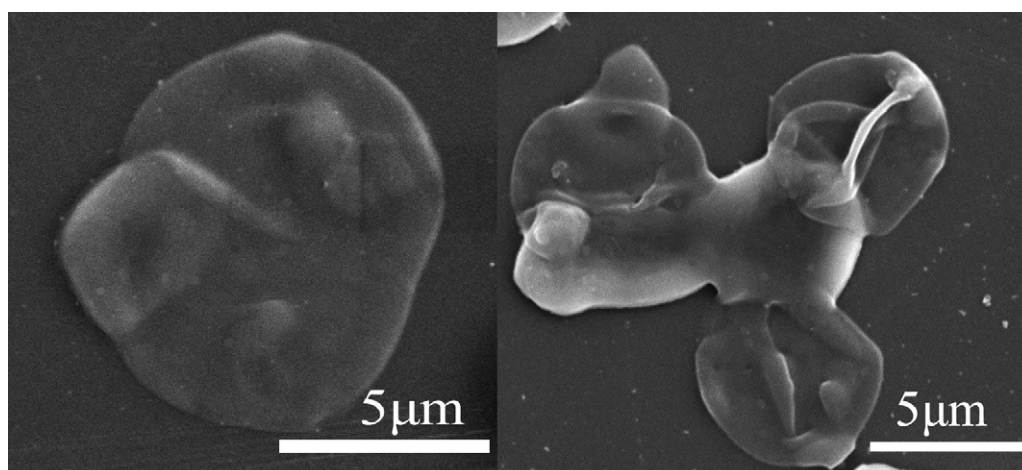


Fig. 8. The SEM images of (PRM/DXS)₆ microcapsules.

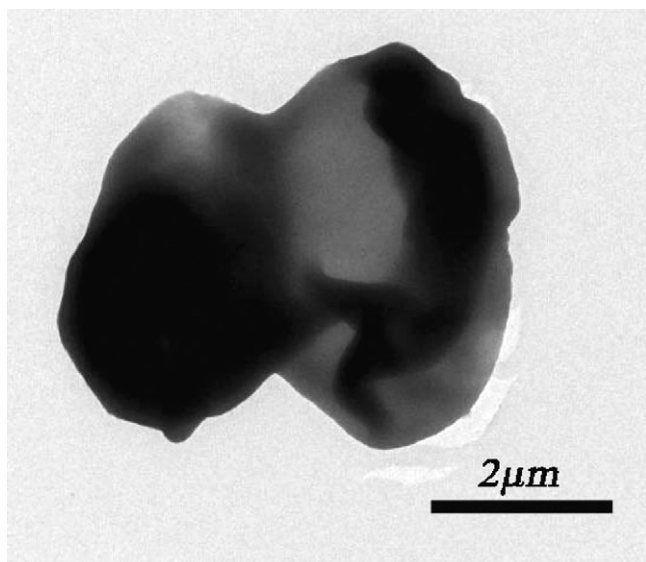


Fig. 9. The TEM images of (PRM/DXS)₆ microcapsules.

above 600 °C, which is the result of calcium carbonate degradation. It could be estimated that the PEs amount involved in the capsules was about 52.17% and the remained calcium carbonate was about 39.24%.

FT-IR spectroscopy analysis was used to characterize the obtained microcapsules. IR spectrums of PRM, DXS and microcapsules were showed in Fig. 11, respectively. From this figure we can see that the ν_{as} and ν_s absorption of S=O bond of sulfonic group in DXS roughly located at 1255 cm^{-1} and 1023 cm^{-1} . The ν_s absorption of C–N and N–H bonds of ammonium group in PRM roughly appeared at 1069 cm^{-1} and 3375 cm^{-1} . Compared with the spectrums of DXS and PRM, the absorption of S=O bond of sulfonic group, C–N and N–H bonds of ammonium group in PRM/DXS capsules appeared nearly at the same corresponding frequency but became weaker. Besides, the absorbance of carbonate was found at 872.5 cm^{-1} and 745 cm^{-1} and a split peak at 1439 cm^{-1} and 1490 cm^{-1} , which were the absorbance of vaterite of the remained calcium carbonate.

This kind of biocompatible microcapsules would have potentials in drug delivery and micro-reactors to perform chemical reactions, which would be introduced in our future work.

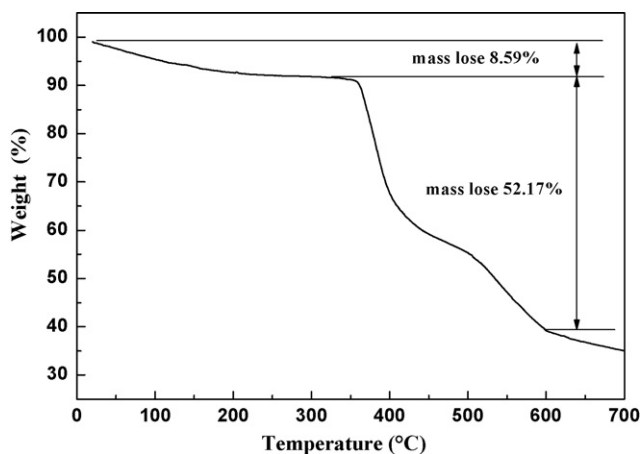


Fig. 10. TGA curve of (PRM/DXS)₆ capsules recorded at a heating rate of 20 °C/min in nitrogen.

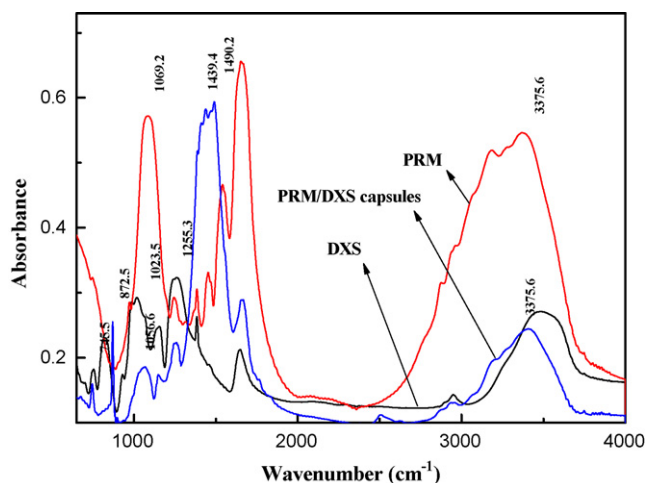


Fig. 11. FTIR spectrum of DXS, PRM and PRM/DXS capsules.

4. Conclusions

The successful fabrication of (PRM/DXS)₆ microcapsules templated on PSS doped calcium carbonate microspheres has been demonstrated. PSS could control the morphology of the calcium carbonate microspheres during their growth. When the ratio of [PSS]/[Ca²⁺] is 1/33, vaterite could be found as the main crystal phases and the sphere-like morphology was obtained. (PRM/DXS)₆ was assembled on the template by self-assembled technique. After removed the template the morphology of obtained microcapsules still kept well. It could be estimated by TGA that the PEs amount involved in the capsules was about 52.17% and the remained calcium carbonate was about 39.24%. This kind of biocompatible microcapsules could be used as micro-containers to load drugs and micro-reactors to perform chemical reactions.

Acknowledgments

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