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Development of core-shell microcapsules by a novel supercritical CO_2 process

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Abstract 5-fluorouracil-SiO_2-poly(*L*-lactide) (5-Fu-SiO_2-PLLA) microcapsules were prepared in a novel process of solution-enhanced dispersion by supercritical CO_2 (SEDS). The SiO_2 nanoparticles were loaded with 5-Fu by adsorption at the first place, then the 5-Fu-SiO_2 nanoparticles were coated with PLLA by a modified SEDS process. The resulted microcapsules were characterized by scanning electron microscope (SEM), laser diffraction particle size analyzer, Fourier transform infrared spectrometer (FTIR) and thermogravimeter-differential scanning calorimeter (TG-DSC). The drug load, encapsulation efficiency and drug release profiles were also determined. The resulted microcapsules exhibited a rather spherical shape, smooth surface, and a narrow particle size distribution with a mean particle size of 536 nm. The drug load and encapsulation efficiency of the samples were 0.18% and 80.53%, respectively, 25.05% of 5-Fu was released in the first half hour, then drug released in a sustained process, which was much slower than that of without coated by PLLA. The results indicated that the modified SEDS process could be used to produce drug-polymer microcapsules with a core-shell structure, high encapsulation efficiency and sustained drug release effect.

1 Introduction

Microencapsulation of pharmaceutical compounds by biodegradable polymer is of great interest in the drug controlled release system [1–3]. Pareta et al. [4] reported the preparation of polymer-coated starch/bovine serum albumin microspheres by using a novel process, which incorporates co-axial electrohydrodynamic forming; the further study by Farook et al. [5] provided the in-situ preparation of liquid-filled polymer-shell microspheres, which is ideal for the preparation of stable liquid drug or biomedicine filled microspheres in a one-step operation.

Supercritical CO_2 processes have revealed great potential to form microparticles of polymers and pharmaceutical compounds due to its mild critical conditions (Tc = 304.1 K, Pc = 7.38 MPa), non-toxicity, non-flammability, and lower price [6–9].

The process of rapid expansion of supercritical solutions (RESS) had been widely utilized to form drug-loaded polymer particles. In a RESS process, the coating polymers were dissolved in supercritical CO_2, the drugs particles were dissolved together or suspended in the said fluid, and then de-pressurized through a nozzle to cause a substantial lowering of the solvent power of CO_2 leading to very high super-saturation of solute, precipitation, nucleation and particle growth, consequently generating microparticles with a polymer coating on the surface [10–12].

However, generally most of the polymers were insoluble in supercritical CO_2 at temperatures below 80°C [13]; Moreover, usually the operating pressure of above 20 MPa was not economically.

To overcome the above limits, the supercritical CO_2 was employed as an antisolvent (SAS process) to precipitate the drug and/or polymer from their organic solution, namely a variety of antisolvent processes such as gas antisolvent (GAS) [14], supercritical antisolvent (SAS) [15], precipitation with a compressed antisolvent (PCA) [16], aerosol solvent extraction systems (ASES) [17], solution enhanced dispersion by supercritical fluids (SEDS) [18] and

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supercritical antisolvent with enhanced mass transfer (SAS-EM) [19].

In a SAS process, generally the drug and the polymer were dissolved in organic solvents, and then contacted with supercritical CO_2. Consequently the drug and polymer are co-precipitated due to a higher super-saturation produced by the mutual diffusion of organic solvent into supercritical CO_2 and vice versa when an organic liquid solution comes into contact with supercritical CO_2. However, the SAS co-precipitation process requires both the drug and the polymer are to be soluble in suitable solvents. This creates a challenge for the drugs which are insoluble in organic. Moreover, except when the two solutes have good compatibility, similar thermodynamic properties and undergo similar precipitation pathways, the co-precipitation of drug and polymer may also lead to a phase separation due to the interface problem, the resulting loosely combination of drug and polymer generates a low encapsulation efficiency and no sustained release effect of the drug [20, 21].

To overcome the disadvantages of SAS co-precipitation, an alternative SAS process for encapsulation of drug particles with polymer was developed by suspending the drug particles in polymer organic solution and then spraying into supercritical CO_2 by high-pressure pump [21–23]. However, during the delivery of particles suspension into supercritical CO_2 vessel, the one-way valve of pump and the delivery system were easily to be damaged or obstructed caused by the particles.

Motivated by the above-mentioned limitations, in this study, a novel stainless steel cylinder container with a piston, which can work like an injector was designed to be employed in the particles suspension delivery system. SiO_2 nanoparticles loaded with 5-Fu by adsorption was selected as drug particles model. By using the above modified SEDS process (SEDS process is a modified version of SAS process, in which the liquid solution and supercritical fluid are sprayed together using a specially designed coaxial nozzle [20]), we attempted to encapsulate the drug particles model with PLLA, and use SEM, laser diffraction particle size analyzer, FTIR, TG-DSC and UV spectrum to characterize the products.

2 Experimental part

2.1 Materials

PLLA, with molecular weight of 100 kDa, was purchased from Department of Medical Polymer Shandong Institute (Jinan, China). 5-Fu (99% EP/USP/BP) was purchased from Advanced Technology & Industrial Co., Ltd. (Hong Kong). SiO_2 nanoparticles with a mean size of 265 nm were kindly provided by the Technical Institute of Physics and Chemistry of the Chinese Academy of Sciences (Beijing, China). CO_2 with the purity of 99.9% was supplied by Hong Kong Specialty Gases Co., Ltd. (Hong Kong). All other compounds were of analytical purity.

2.2 Methods

2.2.1 Preparation of 5-Fu-SiO_2-PLLA microcapsules

An amount of SiO_2 nanoparticles were immerged into a centrifuge tube which contained 5-Fu solution in ethanol for 24 h, then the 5-Fu loaded SiO_2 were collected after centrifugal process. The concentrations of 5-Fu solution in ethanol before and after adsorption were measured by UV spectrometry at 265 nm (Lambda 18 spectrometer of Perkin Elmer, USA) to determine the drug load of 5-Fu-SiO_2. The resulting 5-Fu-SiO_2 was dispersed into the 0.5% (wt/v) PLLA solution in dichloromethane (DCM) by ultrasonic method to form a suspension.

Figure 1 showed the schematic diagram of apparatus for particles coating by SEDS process, which consists of three major components: a CO_2 supply system, a particles suspension delivery system and a high pressure vessel with a volume of 1000 ml. Specially, in the particles suspension delivery system, an 'injector' was made of a stainless steel cylinder divided into two chambers by a piston having a O-ring seal. The particles suspension was placed into the front chamber and the rear chamber was filled by water which was pressurized by an HPLC pump. The 'injector' was connected to the high pressure vessel through a stainless

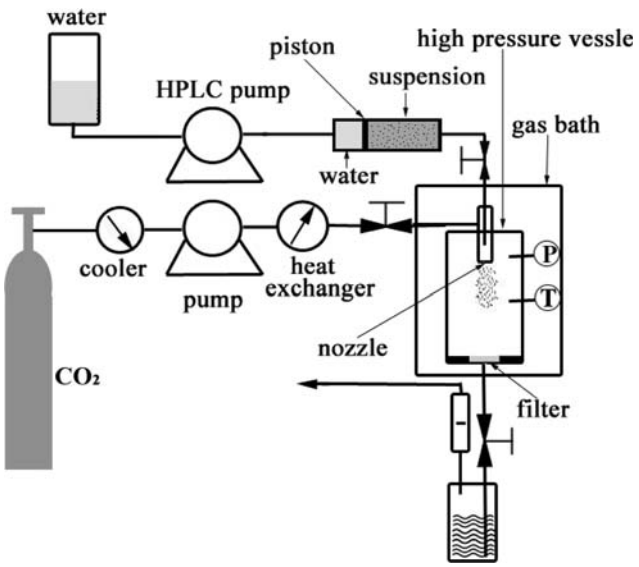

Fig. 1 Schematic diagram of apparatus for particles coating by SEDS process

steel coaxial nozzle. The SiO_2-PLLA microcapsules without 5-Fu were also prepared in this process.

In the running of the experiment [20], the CO_2 fed from a CO_2 cylinder was cooled down to around 0°C by a cooler in order to ensure the liquefaction of the gas and also to prevent cavitations. Then a high pressure meter pump was used to deliver liquefied CO_2 to the high pressure vessel. After leaving the pump head, the liquefied CO_2 was preheated to desired operating temperature by using a heat exchanger. The high pressure vessel was incubated in a gas bath to keep the temperature constant during the experiment. When the desired pressure of the high pressure vessel was reached, a steady flow of CO_2 was maintained and the system pressure was controlled by adjusting a downstream valve and monitored by a pressure gauge to keep the pressure constant. When the desired pressure and temperature were stabilized, the 5-Fu-SiO_2 suspension in PLLA DCM solution was placed into the front chamber of the 'injector' and then injected through a stainless steel coaxial nozzle into precipitation vessel by the pressure of water pressurized by an HPLC pump (P3000, Knauer, Germany). When the spraying was finished, fresh CO_2 was used continually to wash the products to remove the residual organic solvent for about 30 min. During the process of washing, the system operating conditions were maintained as described before. After washing, the high pressure vessel was slowly depressurized and the products were collected for characterization.

2.2.2 *Surface morphology and particle size distribution characterization*

The surface morphological examination of the samples was performed using a scanning electron microscope (JEOL, JSM–6490, Japan). The particle size and particle size distribution of the samples were analyzed using a laser diffraction particle size analyzer in liquid module (LS 13320, Beckman Coulter, USA).

2.2.3 *FTIR analysis*

FTIR spectrum for the various samples in different process were obtained on a FTIR Perkin Elmer 1720 (Perkin Elmer, USA) in the transmission mode with the wavenumber ranged from 4,000 cm^{-1} to 400 cm^{-1}. KBr pellets were prepared by gently mixing sample powder with KBr.

2.2.4 *TG-DSC measurement*

TG-DSC measurements of the samples were performed in a Netzsch STA 449C (Netzsch Instruments, Burlington, Germany) at a heating rate of 10°C/min over a temperature range of 30 to 600°C.

2.2.5 *Determination of drug load and encapsulation efficiency*

Based on the TG curve of 5-Fu-SiO_2-PLLA and the drug load of 5-Fu-SiO_2 measured by UV Spectrometry at 265 nm, the drug load of 5-Fu-SiO_2-PLLA were calculated. In measuring the encapsulation efficiency, approximately 50 mg sample of 5-Fu-SiO_2-PLLA microcapsules, accurately weighed, was suspended in 10 ml of ethanol which was a good solvent for 5-Fu but a poor solvent for PLLA to wash off the unencapsulated 5-Fu, the amount of 5-Fu in the washing medium was also determined by UV spectrometry at 265 nm. The drug load and encapsulation efficiency were calculated by Eqs. 1 and 2 respectively.

$$\text{Drug load} = W_1/W_2 \times 100\% \quad (1)$$

$$\text{Encapsulationefficiency} = W_3/W_1 \times 100\% \quad (2)$$

Where W_1 is the weight of 5-Fu in the microcapsules, W_2 is the gross weight of microcapsules, W_3 is the weight of 5-Fu in the microcapsules after washing off the unencapsulated 5-Fu.

2.2.6 *Drug release studies*

An approximately 100 mg sample was suspended in 1 ml ethanol and placed in a pre-treated dialysis bag, then the bag was put into a tube contained 9 ml pH 6.8 PBS, and incubated in a shaking water-bath at 37°C at 60 rpm. 3 ml solution was periodically removed and the amount of 5-Fu was analyzed by UV spectrometry at 265 nm. In order to maintain the origin PBS volume of 10 ml, 3 ml of fresh PBS was periodically added. Drug release profiles were calculated in terms of cumulative release percentage of 5-Fu (%, w/w) with incubation time. Each experiment was carried out in triplicate.

3 Results and discussion

3.1 Surface morphology and particle size distribution

In the SEDS process, the 5-Fu-SiO_2 particle suspension in PLLA solution and supercritical CO_2 were sprayed together through the coaxial nozzle into the high-pressure vessel. The supercritical CO_2 was used as both anti-solvent for its chemical properties and 'spray enhancer' by mechanical effect. When the solution drop contacted with supercritical CO_2, since the DCM was highly soluble with supercritical CO_2, a very fast mutual diffusion into and out of the drop occurred, and vice versa when the supercritical CO_2 contacted with the dropt. Then the PLLA solution in the drop

approached a spontaneous super-saturation due to the extraction of DCM from the drop, it generated the precipitation of PLLA on the surface of particles. Moreover, the 5-Fu-SiO_2 acted as crystal nucleus, it led to the easier precipitation of PLLA and coating on 5-Fu-SiO_2.

Figure 2a, b showed the surface morphology of SiO_2 and SiO_2-PLLA, respectively. It was found that after SEDS process, the particles were successfully coated by PLLA, and thus the successful coating SiO_2 nanoparticles with PLLA provided the feasibility of coating drug-loaded SiO_2 nanoparticles with PLLA as composite microcapsules. Figure 2c, d showed the surface morphology of 5-Fu-SiO_2 and 5-Fu-SiO_2-PLLA, respectively, the results indicated that there was no significant difference between the surface morphology of (a) SiO_2 and (c) 5-Fu-SiO_2, neither was that of between (b) SiO_2-PLLA and (d) 5-Fu-SiO_2-PLLA, which was coated by PLLA. However, the coated particles within the PLLA film were partially aggregated and some agglomeration took place. In this process, the 5-Fu-SiO_2-PLLA microcapsules with a matrix structure were formed with the 5-Fu-SiO_2 as the host particles and the PLLA as a coating, and exhibited a core-shell structure.

Figures 3 and 4 showed the particle size distribution of 5-Fu-SiO_2 and 5-Fu-SiO_2-PLLA correspondingly, the mean particle size of 5-Fu-SiO_2 was 266 nm, after coating with PLLA, the particle mean size was increased to 536 nm, and particle size of some particles ranged from about 1 μm to 4 μm, it is probably because of the agglomeration of some 5-Fu-SiO_2-PLLA microcapsules, or the phenomenon that several 5-Fu-SiO_2 particles were coated together by PLLA due to the agglomeration of some 5-Fu-SiO_2 particles in the suspension.

3.2 FTIR analysis

FTIR analysis was an appropriate characterization to determine the chemical composition of the samples before and after the adsorption and coating process. The FTIR spectra of PLLA, 5-Fu, SiO_2, 5-Fu-SiO_2 and 5-Fu-SiO_2-PLLA were shown in Fig. 5.

In Fig. 5, the major peaks of 1759 cm^{-1} of PLLA spectrum and 1760 cm^{-1} of 5-Fu-SiO_2-PLLA were attributed to the stretching vibration from the carbonyl group of PLLA; the major peaks of 1724 cm^{-1} of 5-Fu spectrum, 1725 cm^{-1} of 5-Fu-SiO_2 spectrum and 1725 cm^{-1} of 5-Fu-SiO_2-PLLA spectrum were the characteristic peaks of carbonyl group in 5-Fu; the major peaks of 1099 cm^{-1} of SiO_2 spectrum, 1073 cm^{-1} of 5-Fu-SiO_2 spectrum and 1088 cm^{-1} of 5-Fu-SiO_2-PLLA spectrum were the Si-O-Si transmission peaks in asymmetric stretching mode of SiO_2.

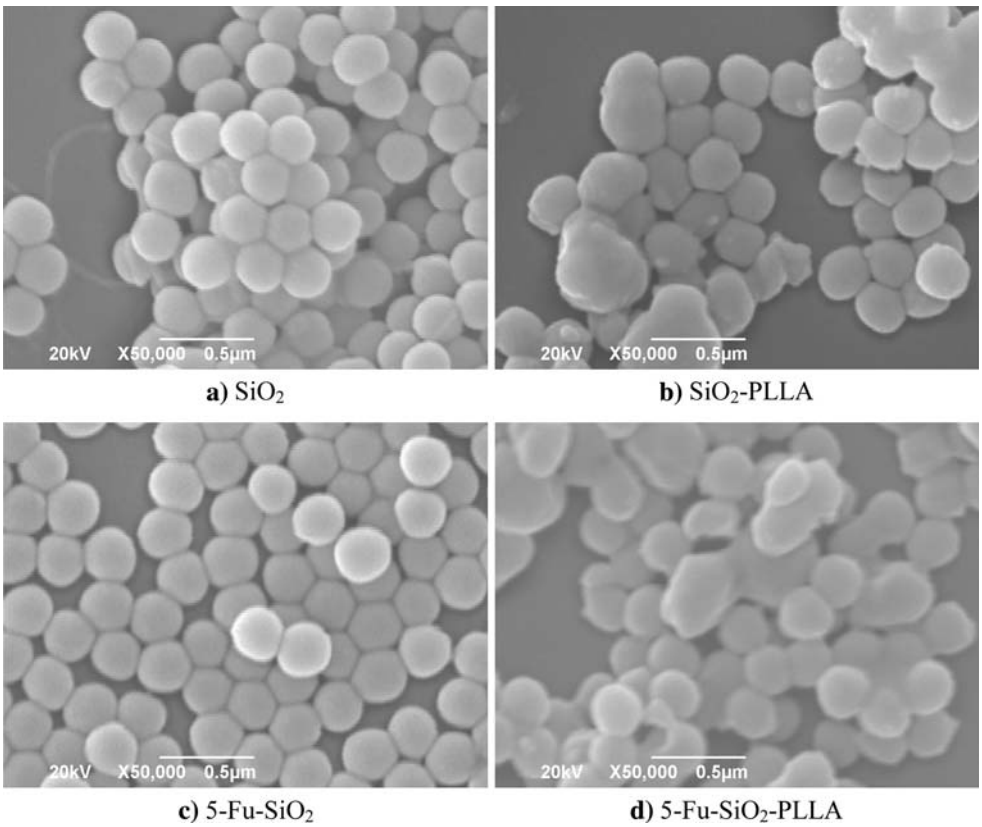

Fig. 2 SEM photographs of different samples

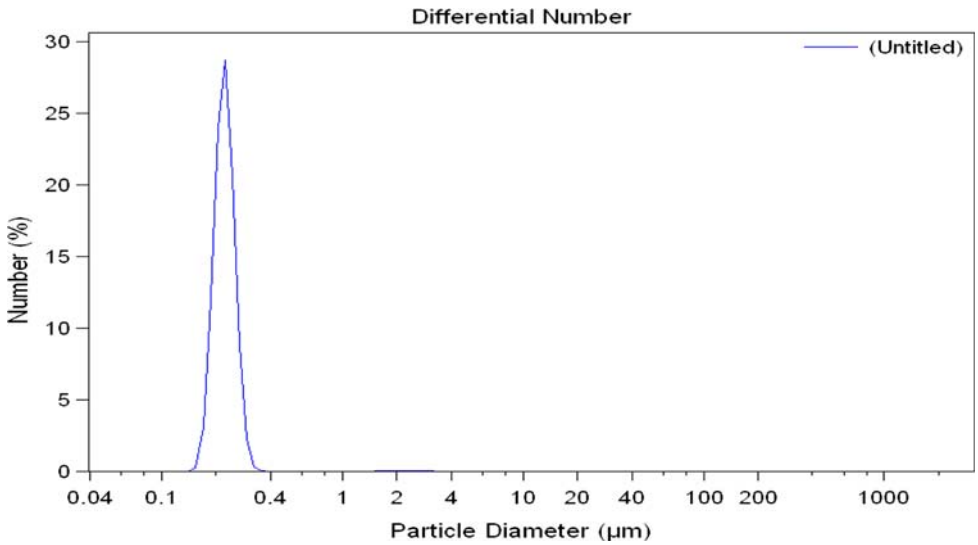

Fig. 3 Particle size distribution of 5-Fu-SiO_2

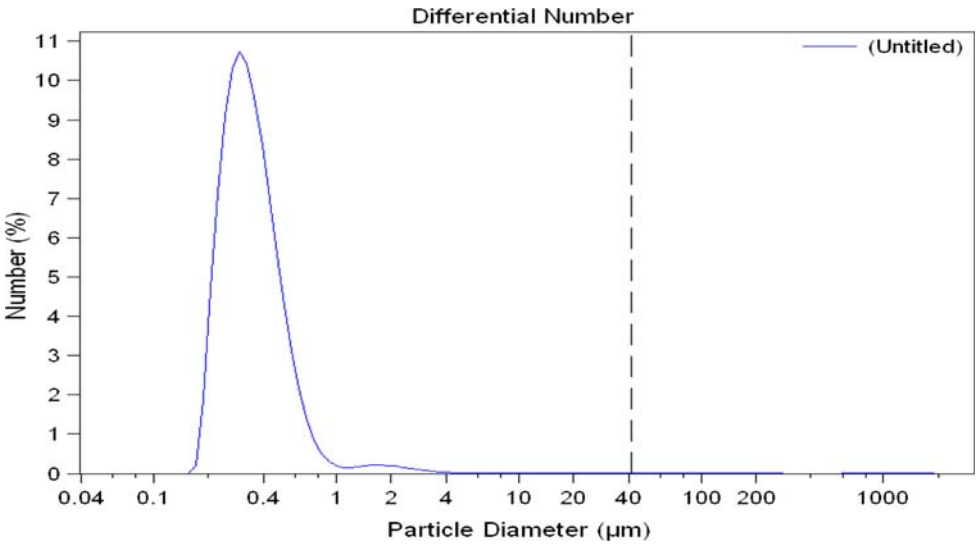

Fig. 4 Particle size distribution of 5-Fu-SiO_2-PLLA

The results indicted that the 5-Fu was successfully loaded on SiO_2, and the resulting 5-Fu-SiO_2 were also successfully coated by PLLA.

However, compared with single component, no new peak was observed from the spectrums of composites, which was indicating that there was no chemical bond combination during the processes of drug adsorption and coating of 5-Fu-SiO_2 with PLLA by SEDS process.

Consequently, the SEDS process was a typical physical process to prepare core-shell structure microcapsules by coating polymer on the surface of host particles. Furthermore, it was also favorable for drugs since any chemical interaction with the materials may cause in a change in its properties, which could change the effectiveness of the drugs.

3.3 TG-DSC measurement

TG measurement was a good method to measure the content of the components with different phase change temperature and the fusion temperature, and DSC was a tool used to measure the temperature and energy variation involved in the phase transitions for a composite.

Figures 6 and 7 showed the TG-DSC curves of PLLA and 5-Fu-SiO_2-PLLA, respectively. By comparing the curves, it can be confirmed that the content of SiO_2 in 5-Fu-SiO_2-PLLA microcapsules was 54.22%, and the peak at 250.0°C was corresponded to the phase change temperature and the fusion temperature because of 5-Fu in microcapsules, it also revealed the successful loading of 5-Fu in the microcapsules.

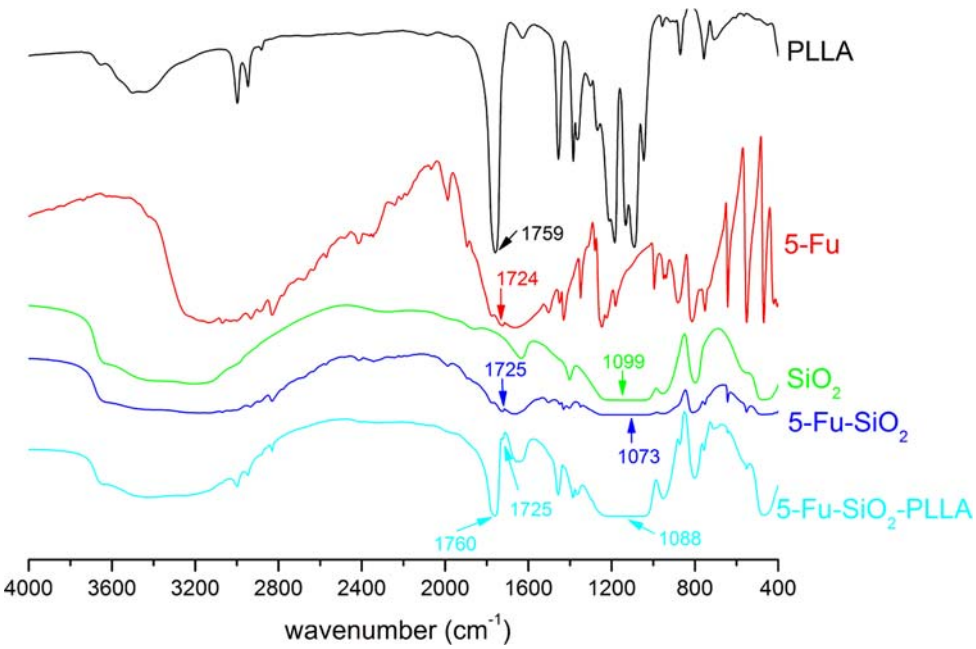

Fig. 5 FTIR spectrum of the samples in different process

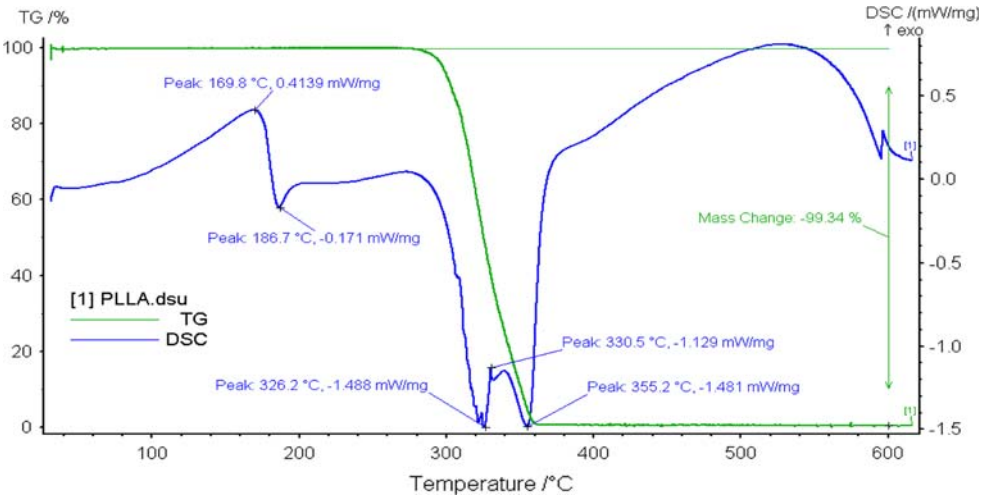

Fig. 6 TG-DSC curves of PLLA

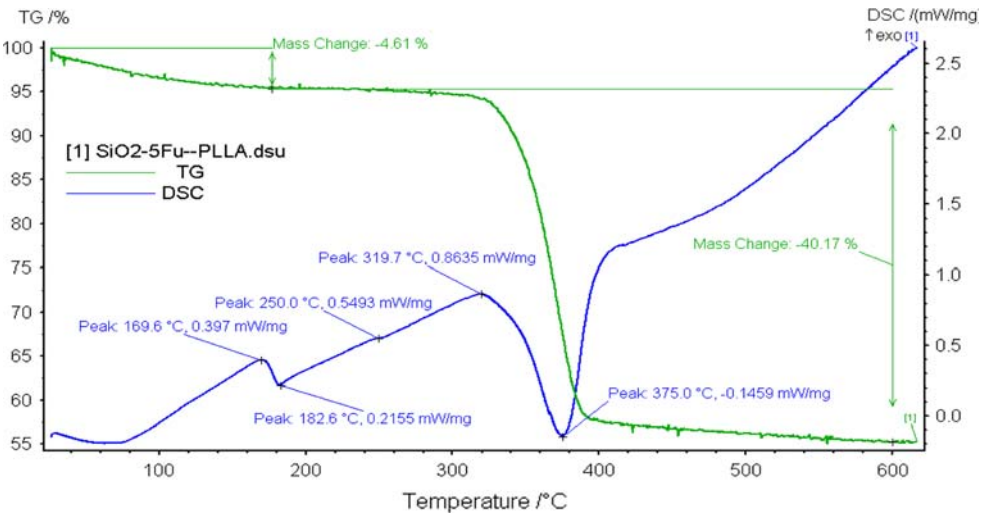

Fig. 7 TG-DSC curves of 5-Fu-SiO_2-PLLA

3.4 Drug load and encapsulation efficiency

By measuring the concentrations of 5-Fu solution in ethanol before and after adsorption, the drug load of 5-Fu-SiO_2 was calculated. After adsorption, the 5-Fu-SiO_2 with a drug load of 0.33% was obtained, and according to the SiO_2 content of 54.22% in the 5-Fu-SiO_2-PLLA, the drug load of 5-Fu-SiO_2-PLLA was calculated, the result was 0.18%.

In the measurement of encapsulation efficiency, the drug of the uncoated particles would be dissolved in ethanol, after coating PLLA on the surface of 5-Fu-SiO_2 by SEDS process. The result showed that the encapsulation efficiency was as high as 80.53%, which was much higher than that of by co-precipitation process [20].

The results indicated that drug-polymer microcapsules with high encapsulation efficiency could be produced by the modified SEDS in this study.

3.5 Drug release profiles

The drug release curves of 5-Fu-SiO_2 and 5-Fu-SiO_2-PLLA were shown in Fig. 8. It was found that 80.91% of 5-Fu was released in a burst from 5-Fu-SiO_2 in the first half hour. Then the drug release shifted to a flat stage, only 15.66% more drugs was released in the next 120 h. While after further coating with PLLA by SEDS process, only 25.05% of 5-Fu was released in the first half hour, this also demonstrated that about 25.05% of 5-Fu could be easily removed, which was close to the result of encapsulation efficiency measurement. Then the drug release was in a sustained process, 95.18% of 5-Fu was release in 120 h.

Generally, the drug release of sustained delivery systems made from the slowly erodible polymers is controlled by both of polymer erosion and drug diffusion. While PLLA is a very long degradation polymer which almost is not erodible during the drug release process, and due to the small molecular weight of 5-Fu (Mw 130.08) and its relatively high solubility in the release conditions, the drug release was controlled by drug diffusion [20].

The drug release profile of 5-Fu-SiO_2-PLLA suggested that there was no burst effect and had a sustained drug release effect.

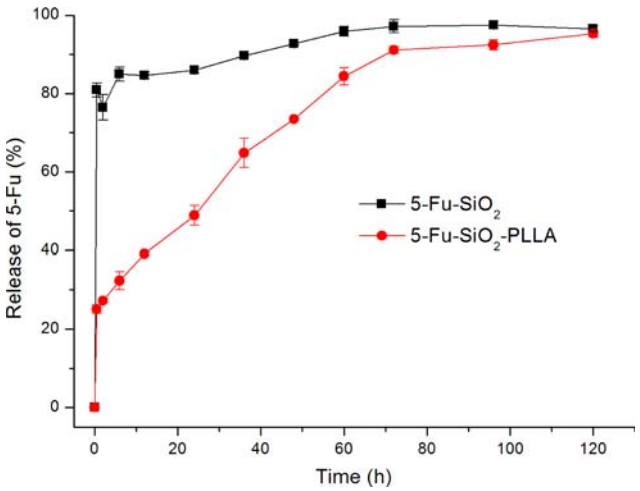

Fig. 8 Drug release curves of 5-Fu-SiO_2 and 5-Fu-SiO_2-PLLA

4 Conclusions

The 5-Fu-SiO_2-PLLA drug-polymer microcapsules with a core-shell structure were successfully prepared in a modified SEDS process, which an 'injector' was employed in the particles suspension delivery system. This study demonstrated that the modified SEDS process could be used to produce drug-polymer microcapsules with a core-shell structure, high encapsulation efficiency and sustained drug release effect.

Furthermore, since most of the proteins are insoluble in organic solvents, the application of SAS co-precipitation process was limited for protein, while the protein particles can be used as the host particles and coated with polymers by using this modified SEDS process without dissolving the protein to avoid the decreasing of protein's bioactivity.

This study suggests that the modified SEDS process is a quite promising technique to produce drug-polymer carriers for the design of drug controlled release systems.

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