

Full Length Research Paper

Synthesis and characterization of porous hollow silica nanoparticles using ZnSe core as template for drug delivery application

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In this contribution, porous hollow silica nanoparticles using inorganic nano sized ZnSe as template were prepared. The hydrothermal method was used to synthesize pure ZnSe nanospheres material. The ZnSe/SiO₂ core-shell nanocomposites were prepared using a simple sol-gel method successfully. The hollow silica nanostructures were achieved by selective removal of the ZnSe core. The morphology, structure and composition of the product were determined using powder X-ray diffraction (XRD), emission scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FT-IR). The results demonstrated clearly that the pure ZnSe nanoparticles are in a spherical form with the average size of 30 nm and correspond with zinc blend structure. The porous hollow silica nanoparticles obtained were exploited as drug carrier to investigate *in vitro* release behavior of cefalexin in simulated body fluid (SBF). UV-Visible spectrometry was carried out to determine the amount of cefalexin entrapped in the carrier. Cefalexin release profile from porous hollow silica nanoparticles followed a three stage pattern and indicated a delayed release effect.

Key words: Hollow silica nanoparticles, core-shell, ZnSe, drug delivery, transmission electron microscopy (TEM).

INTRODUCTION

During the past decade, there have been widespread research efforts to develop architecture and fabrication of core-shell composite materials and hollow spheres of nanometer to micrometer size with special physical and chemical properties. They have demanding applications in pharmaceuticals, biology, optics, catalysts and drug delivery (Li et al., 2007; Ge et al., 2009; Le et al., 2007; Chen et al., 2004; Hofmeister et al., 2002; Wang et al., 2008).

As one of the most important II to VI group semiconductors, ZnSe with a room temperature bulk bandgap of 2.7 eV, is a good candidate for short wavelength lasers and other photoelectronic devices, such as blue - green laser diodes and turnable mid-IR

laser sources (Wang et al., 2008; Jiao et al., 2007; Jana et al., 2008; Jiang et al., 2005; Yang et al., 2009; Cheng and Chen, 2009). Due to the fact that SiO₂ coating semiconductor nanocrystals have an interesting field of study, they are used as bioconjugation and excellent luminescent probes (Li et al., 2009; Han et al., 2007).

The controlled release of drugs from an inert matrix has become increasingly important for oral, implantable and transferal therapeutic systems due to the advantages of safety, effectiveness and patient accessibility. Several research groups have studied the drug adsorption and release properties of mesoporous silica materials. Among a variety of nanoparticle-based drug delivery systems, mesoporous silica nanoparticles have several advantageous features for use in the delivery of both water soluble and insoluble drugs. These materials have large surface areas and porous interiors that can be used as reservoirs for storing the drug. The pore size and environment can also be modified to selectively store different

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molecules, while the size and shape of the nanoparticles can be tuned to enhance cellular uptake process. Furthermore, robust inorganic materials do not swell in organic solvents and are stable at varying pH conditions. An ideal mesoporous carrier with a high specific surface area, large pore volume and appropriate pore size (larger than the kinetic diameter of the drug) would be beneficial toward increasing the adsorption capacity (Parambadath et al., 2011; Shi et al., 2011; Yang et al., 2010; Zhu et al., 2011; Li et al., 2010; Shokri et al., 2011).

Inorganic hollow particles have interested consideration due to their excellent properties, such as low density, specific surface area, thermal insulation and permeability (Song et al., 2006; Nomura et al., 2008). Several methods have been used to prepare hollow particles, such as a polymer bead template method (Im et al., 2005), an inorganic template method (Fuji et al., 2007), a sol-gel method used for surfactant stabilized emulsions (Fujiwara et al., 2004), spray drying (Roy et al., 2005), a spray precipitation method (Nagamine et al., 2007) and spray pyrolysis (Chung et al., 2004). Recently, bacterial cells have been used as a template to fabricate hollow structures (Zhou et al., 2007a, b; Zhang et al., 2008; Nomura et al., 2010).

In this work, we reported a method for preparation of hollow silica nanospheres with porous shell structure by the sol-gel method and by using inorganic ZnSe nanoparticles as a template. The template of ZnSe nanospheres was prepared by hydrothermal method. Subsequently, the surfaces of ZnSe nanospheres were coated with SiO₂ by a simple and low cost method. The hollow silica nanospheres were obtained via the selective removal of ZnSe cores. At last, the porous hollow silica nanoparticles obtained were exploited as drug carrier to investigate *in vitro* release behavior of cefalexin in simulated body fluid.

EXPERIMENTAL

All reagents were of analytical grade and were purchased from Merck Company. Reagents were used without any further purification.

In order to obtain a pure phase of monodispersed ZnSe nanospherical particles as a template for hollow SiO₂ nanostructures, the synthesize conditions, such as the amount of the reducing agent, the effects of different sources of Zn and Se, type of the surfactant, the reaction temperature and time have been optimized (Mollaamin et al., 2011). In this work, we report only the conditions which have been obtained in a pure phase of monodispersed ZnSe nanospherical particles.

Synthesis of ZnSe nanosphere particles

5 mmol ZnCl₂ was added to 20 ml of deionized water and was sonicated (VGT-1860QTD, 42 kHz, 150 W) for 15 min. Then, this solution was added slowly to 20 ml of an aqueous solution of cetyl trimethylammonium bromide (CTAB) surfactant under sonication (molar ratio of ZnCl₂: CTAB equal to 1). To this solution, 5 mmol Na₂SeO₃·5H₂O and 5 ml N₂H₄·H₂O (80%) were added and

other crystalline phase was found in the XRD pattern. sonicated for 3 h. The mixture was transferred into an autoclave, sealed and kept at 160°C for 8 h. After cooling the system to room temperature, the product was separated by centrifugation, washed with absolute ethanol and deionized water for several times, and then dried under vacuum at 60°C for 10 h.

Synthesis of ZnSe/SiO₂ core-shell nanocomposites and hollow silica nanostructures

In a typical synthesis, about 0.1 g of ZnSe particles and 0.035 g of CTAB were added to 50 ml of absolute ethanol and were sonicated for 20 min using an ultrasound cleaner. Afterwards 10 ml NH₄OH and 50 μl tetraethyl orthosilicate (TEOS) (molar ratio of ZnSe:TEOS:CTAB equal to 6:2:1) were added to the mixture and were sonicated for 4 h. Then, the product was separated by centrifugation, washed with absolute ethanol and deionized water for several times, at last dried under vacuum at 60°C for 4 h.

In order to reach ZnSe and obtain hollow silica shell, the ZnSe/SiO₂ core-shell nanocomposites were added into HNO₃ (1 M) and kept for two weeks. The resulting product was separated by centrifugation, washed with deionized water for several times, and then dried under vacuum at 60°C for 6 h.

Drug loading and release

The loading of the drug was accomplished by the soaking of hollow silica samples in cefalexin aqueous solution with a certain concentration.

In a typical loading procedure, hollow silica powders were soaked in 50 ml solution of 120 ppm of cefalexin (weight ratio of cefalexin:SiO₂ = 2:1). The suspension was stirred vigorously for 48 h. The drug-loaded nanoparticles were centrifuged and washed with acetone three times, and then dried under vacuum at 40°C for 3 h. The amount of drug-loaded on the nanoparticles was investigated measuring the adsorption intensity of the cefalexin remained in the solution at 270 nm by means of UV-Vis spectroscopy (Shimadzu-2550).

In vitro release of cefalexin was performed by soaking the drug-loaded nanoparticles powder in simulated body fluid (SBF), with continuously stirring at 37°C in a thermostatic water bath. The release ratio of cefalexin from the drug-loaded nanoparticles was measured by examining the concentrations of cefalexin in SBF at different time intervals by means of UV-Vis spectroscopy.

Characterization

The crystal phase and particle size of the synthesized products were characterized by X-ray diffraction (XRD) using FK60-04 with Cu Kα radiation (λ = 1.54 Å), and with instrumental setting of 35 kV and 20 mA. The morphology of the nanostructures was observed by emission scanning electron microscopy (SEM, Philips-XLφ30) and transmission electron microscopy (TEM, Philips-CM120). Fourier transform infrared (FT-IR) spectra were recorded on a Shimadzu-840S spectrophotometer using KBr pellet.

RESULTS AND DISCUSSION

The XRD patterns of pure ZnSe nanoparticles (b) and ZnSe/SiO₂ core-shell nanocomposites (a) are shown in Figure 1. All peaks can be well indexed to cubic zinc blend (JCPDS, No. 37-1463 Card, a = 5.66882(8) Å). No

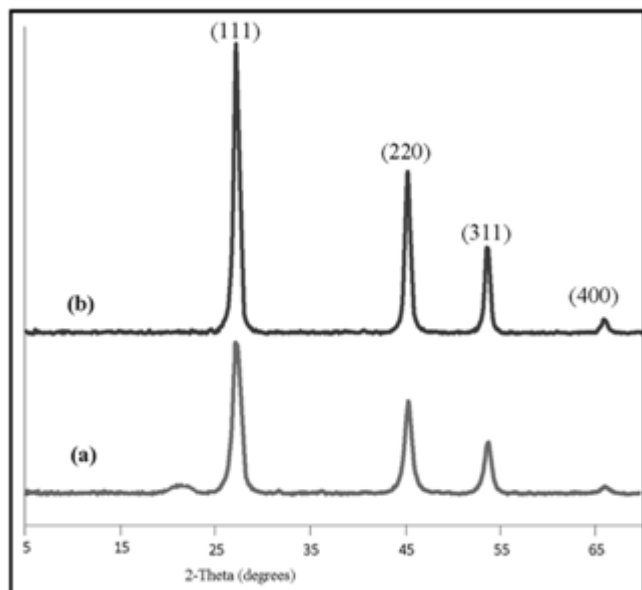


Figure 1. XRD patterns: (a) ZnSe/SiO₂ core-shell nanocomposites and (b) bare ZnSe nanoparticles.

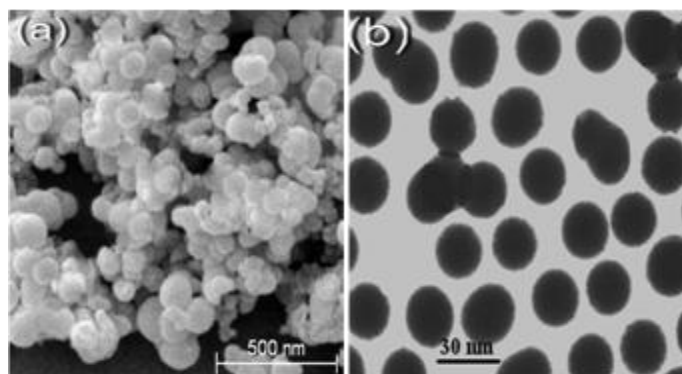


Figure 2. (a) SEM and (b) TEM images of ZnSe nanospheres.

The lattice constant obtained from XRD data was $a = 5.649 \text{ \AA}$. This result indicated that the lattice parameter of the nanoparticles is smaller than those of the bulk crystalline ZnSe ($a = 5.66882(8) \text{ \AA}$). The result also shows that in most reported nanoparticles, the lattice constant often decreases with decreasing the particle size (Jorgensen et al., 2008; Qi et al., 2002; Zhang et al., 2002). In Figure 1a, a board new peak appeared at diffraction degree about 22° , which presents the SiO₂ amorphous state in the product. Also, the peaks of ZnSe/SiO₂ core-shell nanocomposites (Figure 1a) are a little broader with less intensity than those of the pure ZnSe nanoparticles (Figure 1b), which is probably due to the presence of SiO₂ in an amorphous state around the ZnSe nanoparticles.

Figure 2 shows the SEM and TEM images of the ZnSe

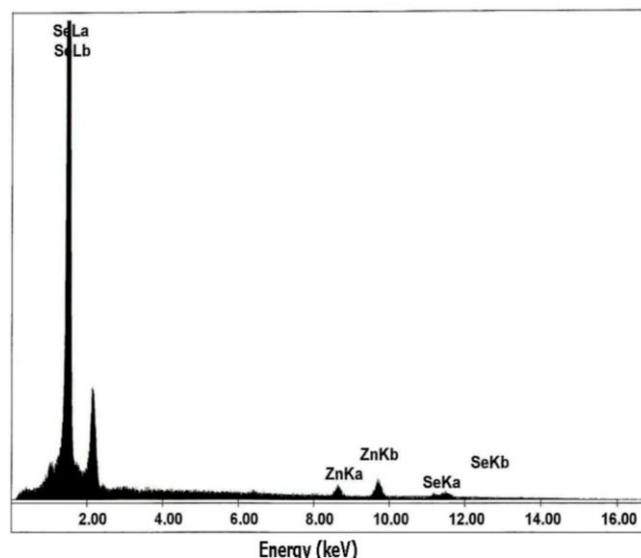


Figure 3. EDX spectrum of ZnSe nanospheres.

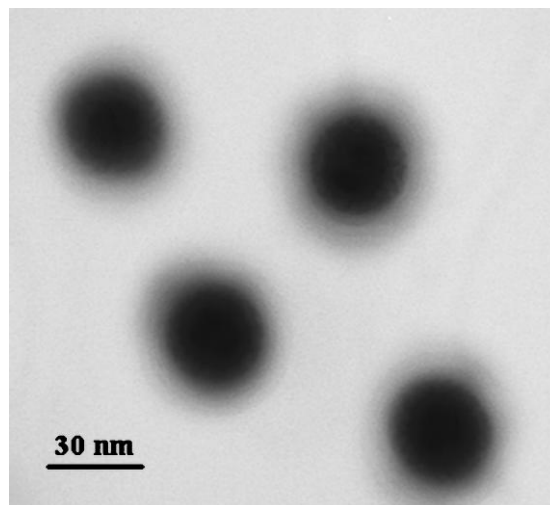


Figure 4. TEM image of ZnSe/SiO₂ core-shell nanocomposites.

nanoparticles. These images clearly demonstrate that the products are spherical with average diameter of 30 nm. The energy dispersive X-ray (EDX) analysis of prepared sample confirms that the product consists of Zn and Se, and the Zn and Se elemental ratio is 1:1 (Figure 3). Also, the SiO₂ shell around of ZnSe core is clearly obvious in the TEM image of ZnSe/SiO₂ core-shell nanocomposites (Figure 4). It is clear that ZnSe/SiO₂ core-shell nanocomposites are larger than the ZnSe nanoparticles without shell. The thickness of SiO₂ shell is about 10 nm, which can be controlled by changing the amount of ZnSe to TEOS ratio (Li et al., 2009; Han et al., 2007). The elemental analysis of ZnSe/SiO₂ core-shell nanocomposites confirms the presence of Si in the product

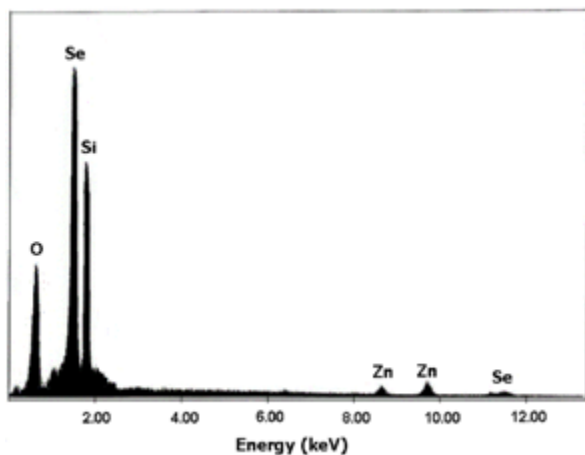


Figure 5. EDX spectrum of ZnSe/SiO₂ core-shell nanocomposites.

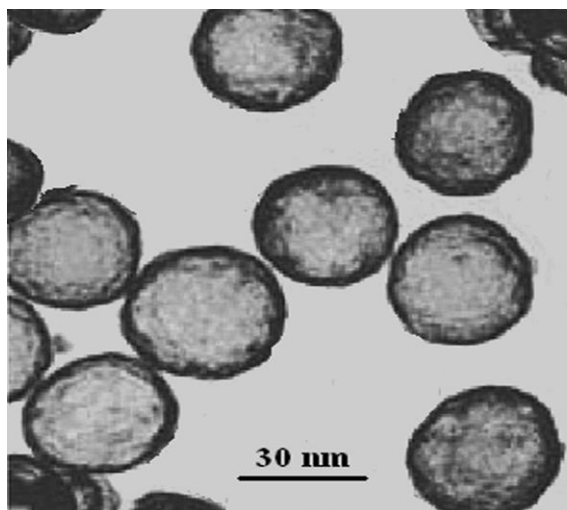


Figure 6. TEM image of hollow silica nanostructures.

product (Figure 5).

TEM image at Figure 6 shows the hollow silica shells. The size of the spherical silica nanoparticles is around 40 nm in diameter with the wall thickness of about 10 nm. The shape and size of hollow silica can be controlled by employing different nanosized ZnSe templates. The SiO₂ shells are obtained by removing of the ZnSe templates from ZnSe/SiO₂ core-shell particles by etching in HNO₃ aqueous solution.

The FT-IR spectra of pure ZnSe nanoparticles, ZnSe/SiO₂ core-shell nanocomposites and hollow silica nanostructures are as shown in Figure 7a to c, respectively. The vibrational peaks in the range of 3350 to 3450 cm⁻¹ and 1600 to 1650 cm⁻¹ can be attributed to the stretching and bending vibrations of structural hydroxyl

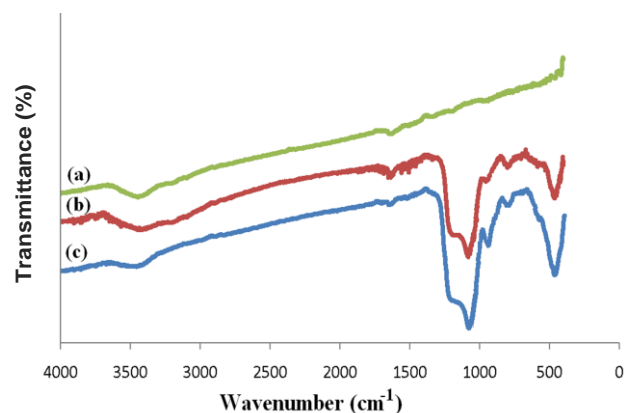


Figure 7. FT-IR spectra of (a) bare ZnSe nanoparticles, (b) ZnSe/SiO₂ core-shell nanocomposites and (c) hollow SiO₂ nanostructures.

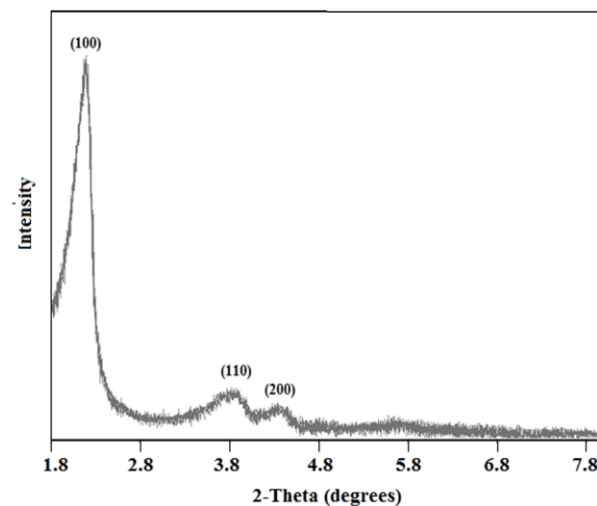


Figure 8. Low angle XRD pattern of silica nanoshells.

groups of the adsorbed water (Li et al., 2009; Wang et al., 2008). The peaks in the ranges of 1050 to 1110 cm⁻¹ and 750 to 800 cm⁻¹ in Figure 7b and c are corresponded to the asymmetric and symmetric stretching vibration modes of the Si-O-Si, respectively, and are absent in Figure 7a. Also, the peak which appeared at 440 to 480 cm⁻¹ is due to the bending vibration mode of Si-O-Si (Li et al., 2009; Wang et al., 2008). These results clearly show that silica is successfully coating the ZnSe nanoparticles.

Figure 8 shows low angle XRD pattern of silica nanoshells. It clearly indicates three peaks indexed as 100, 110 and 200 which can be associated with well ordered 2D hexagonal mesostructure (Qu et al., 2006).

Semiconductor nanocrystals with SiO₂ coating are used as bioconjugation and excellent luminescent probes. Also, the hollow nanostructures via selective removal of

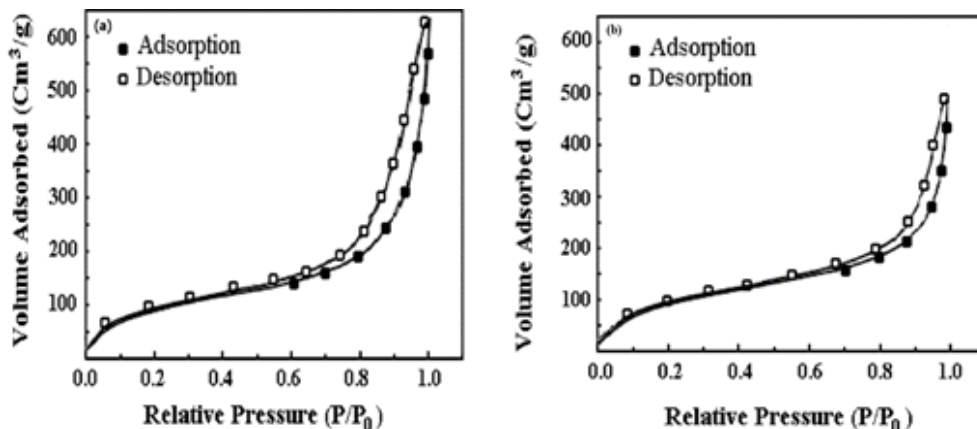


Figure 9. N₂ adsorption-desorption isotherms of the hollow silica spheres (a) before and (b) after the loading of cefalexin.

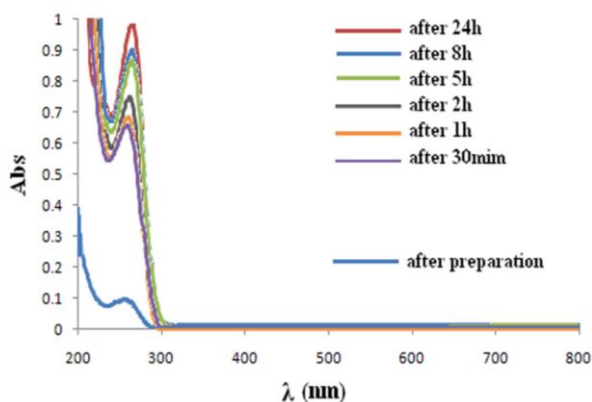


Figure 10. UV-Vis spectra of release of cefalexin in SBF at the beginning, and 30 min, 1, 2, 5, 8 and 24 h after the preparation of the suspension.

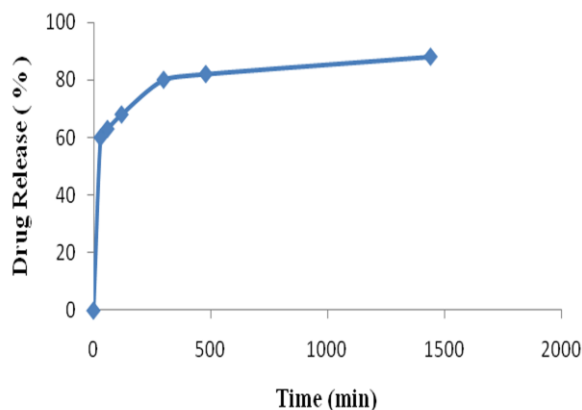


Figure 11. *In vitro* release profile of cefalexin from porous hollow silica nanospheres.

ZnSe attract the attention of many researchers in applying them in drug delivery and catalysis.

In this work, the hollow silica nanospheres obtained were employed for drug delivery application. The results of FTIR and TEM exhibited the formation of hollow spherical SiO₂. Figure 9a and b show N₂ adsorption-desorption isotherms of the hollow silica nanospheres before and after the loading of cefalexin drug, respectively. The specific surface area (BET) of the obtained shells is 882 m²/g. After the loading of the drug, the specific surface area has been obtained to be 380 m²/g. The decrease of the specific surface area of the nanoshells is due to the loading of the drug in the inner core and pores of the nanoshells. This confirms that cefalexin was adsorbed not only on external surfaces of the silica nanoparticles, but also mainly inside the pores. SiO₂-drug conjugation methods include silanization and electrostatic attraction. Cefalexin contains amino and hydroxyl groups, which can interact with silanol groups in silica via hydrogen bonding.

The drug release *in vitro* has been investigated by means of UV-Vis spectroscopy. The spectra have been taken at the beginning and 30 min, 1, 2, 5, 8, and 24 h after the preparation of the suspension (Figure 10). As seen in Figure 7, it can be found that by the passage of time, the intensity of the adsorption peaks increases. The increase of the intensity of the adsorption peaks is due to the increase of concentration of cefalexin in the simulated body fluid (SBF).

Figure 11 shows the release profile of cefalexin from the hollow carrier. The drug release has happened in three stages. At the first stage, 60% of the drug has been discharged within 30 min which is probably due to the rapid release of the drug loosely adsorbed on the surface of the nanoshells or free cefalexin molecules. However, this type of fast release is not favorable for the practical controlled release of drug. The second stage has lasted about 4½ h in which 20% of the drug released from the pores of the nanoshells and the third stage, which has

lasted 19 h, 8% of the drug released. It is related to the release of located drug inside the hole of nanoshells. Also, it is probable that slow release is pertained to the strong chemical adsorption of cefalexin molecules in the silica. The rested 12% which was maintained inside the hole of nanoshells needs much more time to release. It can be concluded that the porous hollow silica nanospheres carrier markedly delayed the release of cefalexin and can be employed in drug delivery application.

Conclusions

A novel template for preparation of hollow silica nanoparticles using ZnSe nanospheres was developed. ZnSe/SiO₂ core-shell nanocomposites were successfully synthesized by a simple chemical method. The hollow nanostructures were obtained with a diameter of 30 nm and wall thickness of approximately 10 nm via selective removal of ZnSe from ZnSe/SiO₂ core-shell nanocomposites. The ZnSe/SiO₂ core-shell nanocomposites and hollow SiO₂ nanostructures are highly favorable for nonelectronic devices, drug delivery and catalyst. The results showed that the porous hollow silica nanoparticles obtained were exploited as drug carrier to investigate *in vitro* release behavior of cefalexin in simulated body fluid. Cefalexin release profile from porous hollow silica nanoparticles followed a three stage pattern, which was explained as the drug release from surface, pore channels in the wall and inside hollow part of the hollow silica nanospheres and indicated a delayed release effect. Therefore, the porous hollow silica nanospheres can be employed in drug delivery application.

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