

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231645796>

# Calcium Carbonate/Carboxymethyl Chitosan Hybrid Microspheres and Nanospheres for Drug Delivery

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY C · OCTOBER 2010

Impact Factor: 4.77 · DOI: 10.1021/jp105906p

---

CITATIONS

60

READS

36

7 AUTHORS, INCLUDING:



Feng Li

25 PUBLICATIONS 349 CITATIONS

[SEE PROFILE](#)



Si-Xue Cheng

Wuhan University

186 PUBLICATIONS 5,159 CITATIONS

[SEE PROFILE](#)

# Calcium Carbonate/Carboxymethyl Chitosan Hybrid Microspheres and Nanospheres for Drug Delivery

Jun Wang, Ji-Si Chen, Jing-Yi Zong, Dong Zhao, Feng Li, Ren-Xi Zhuo, and Si-Xue Cheng\*

Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, Wuhan 430072, People's Republic of China

Received: June 26, 2010; Revised Manuscript Received: September 30, 2010

Calcium carbonate/carboxymethyl chitosan ( $\text{CaCO}_3/\text{CMC}$ ) hybrid microspheres and nanospheres were prepared by the precipitation of calcium carbonate in an aqueous solution containing CMC. Through adjusting the preparation conditions, the size of  $\text{CaCO}_3/\text{CMC}$  hybrid particles could be easily controlled at micro- to nanometer ranges with relatively narrow size distributions. The obtained microspheres and nanospheres were characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, thermogravimetric analysis, and differential scanning calorimetry. The size and size distribution were measured by a particle size analyzer. Doxorubicin hydrochloride (DOX·HCl), a water-soluble anticancer drug, was loaded in the hybrid microspheres and nanospheres with a high encapsulation efficiency. The in vitro drug release showed that the release of DOX·HCl from the microspheres and nanospheres could be effectively sustained.

## 1. Introduction

Drug delivery systems offer numerous advantages compared with conventional formulations, such as improved efficacy, reduced toxicity, reduced frequency of doses, and convenience. Of the different drug delivery systems reported, drug-loaded nanoparticles and microparticles attained importance because of their injectable property, the possibility to achieve passive targeting when their sizes are in particular ranges. For example, microspheres with diameters of 1–5  $\mu\text{m}$  would be ideal for passive targeting of professional antigen-presenting cells.<sup>1</sup> Drug carriers with diameters less than 600 nm may be taken up selectively by tumor tissues because tumor vasculature is hyperpermeable.<sup>2</sup> In addition, polymeric micro- and nanoparticles are of especial interest for oral drug delivery because their small size and large surface area favor their absorption compared to larger carriers. Especially polymeric nanoparticles have the ability to transport across the gastrointestinal tract barrier through cellular uptake.<sup>3</sup>

Most commonly, the widely adopted methodologies for fabricating synthetic polymer based microspheres and nanospheres for drug delivery involve the use of toxic organic solvents,<sup>4,5</sup> which is of particular concern because a low-level exposure to residual toxic organic solvents may lead to lasting toxic effects. Compared with the polymer-based microspheres and nanospheres, inorganic/polymer hybrid particles have unique advantages as drug carriers, including the mild preparation conditions which do not involve any organic solvent and surfactant, and other favorable properties.<sup>6,7</sup> Thus they have attracted increasing attention in recent years. Among them, calcium carbonate/polymer hybrid microspheres have gained much importance due to ideal biocompatibility and biodegradability, which are of critical importance for the clinical application. In addition, calcium carbonate ( $\text{CaCO}_3$ ) is pH-sensitive and the drug release could be triggered by the extracellular acid environment in solid tumor tissues and lysosomes inside cancer

cells.<sup>8</sup> The large specific surface area and the capability to load various drugs also make  $\text{CaCO}_3$  an ideal candidate as a drug carrier.<sup>9–11</sup> To the end of modifying the drug release property and controlling the particle size, biodegradable polymers such as carboxymethyl cellulose<sup>6,12</sup> were utilized to form hybrid particles with  $\text{CaCO}_3$ . As far as we know, most studies carried out in this field were limited to microsized drug delivery systems.

In this study, we prepared calcium carbonate/carboxymethyl chitosan ( $\text{CaCO}_3/\text{CMC}$ ) hybrid microspheres and nanospheres by the precipitation of calcium carbonate in the aqueous solution containing CMC. CMC is a biocompatible and biodegradable derivative of chitosan, with improved water solubility.<sup>13,14</sup> Compared with drug carriers based on synthetic polymers, the current systems are exceedingly suitable for applications in biomedical fields since both  $\text{CaCO}_3$  and CMC have good biocompatibility and biodegradability property. In addition, the porous inner structure endows the hybrid micro/nanospheres with the capability for loading both hydrophilic and hydrophobic drugs.<sup>8,11</sup>

Through adjusting the preparation conditions, the size of the  $\text{CaCO}_3/\text{CMC}$  hybrid particles could be easily controlled at micro- to nanometer ranges with relatively narrow size distributions. The preparation of  $\text{CaCO}_3/\text{CMC}$  hybrid particles did not involve any organic solvent and could offer good control over the morphology of particles with relatively narrow size distributions.

Doxorubicin hydrochloride (DOX·HCl), a water-soluble anticancer drug, was encapsulated in the hybrid micro/nanospheres. As we know, free doxorubicin is most commonly administrated through intravenous injection since oral bioavailability of doxorubicin is low because it is eliminated by the first-pass extraction of the cytochrome P450-dependent metabolic process and the overexpression of the multidrug efflux pump transporter P-glycoprotein (P-gp), which is rich in the intestine, liver, and kidney. Through drug delivery strategies such as encapsulating in polymer nanospheres, the bioavailability of doxorubicin could be enhanced and the drug concentration

\* Corresponding author, chengsixue@hotmail.com or chengsixue@whu.edu.cn.

in plasma could significantly increase after oral administration.<sup>15</sup> Because of the nanoporous structure of the hybrid micro/nanospheres prepared by this method, the drug could be loaded into the hybrid micro/nanospheres by capillary force.<sup>6</sup> In addition, the negatively charged CMC could provide attractive forces for positively charged DOX. Since many anticancer drugs are positively charged at physiological conditions, the hybrid micro/nanospheres prepared in this study could encapsulate water-soluble drugs with high encapsulation efficiency and could effectively sustain the drug release. In addition, the hydrophilic CMC on the micro/nanospheres surfaces could improve the dispersion stability of the particles in water and endow the particles with long circulation property *in vivo*.

## 2. Experimental Section

**2.1. Materials.** Chitosan ( $M_w = 100000\text{--}300000 \text{ g/mol}$ ) was purchased from Acros. Doxorubicin hydrochloride was provided by Zhejiang Hisun Pharmaceutical Co., Ltd. (China). Monochloroacetic acid was supplied by Sinopharm Chemical Reagent Co., Ltd. (China). All other reagents were of analytical grade and used as received.

**2.2. Synthesis of Carboxymethyl Chitosan.** Carboxymethyl chitosan (CMC) was synthesized according to a literature procedure.<sup>13</sup> A 6.8 g portion of sodium hydroxide was dissolved in 10 mL of water, and 40 mL of 2-propanol was added to the solution. Then 5 g of chitosan was dispersed into the solution and stirred at 50 °C for 1 h. After that, 7.5 g of monochloroacetic acid in 10 mL of 2-propanol was added to the mixture dropwise and the reaction was carried out at 50 °C for 4 h. The resulting solution was filtered, washed with 80% alcohol until the filtrate was neutral, and then dried in an oven to obtain CMC. The substitution degree of CMC was determined to be 89% by potentiometric titration.<sup>14</sup>

**2.3. Preparation of Hybrid Microspheres and Nanospheres.** Fifty milligrams of CMC was dissolved in 10 mL of deionized water at room temperature, then 2.5 mL of Na<sub>2</sub>CO<sub>3</sub> (0.5 M) was added and stirred for 0.5 h. After that, 2.5 mL of CaCl<sub>2</sub> (0.5 M) was added to the mixtures dropwise and stirred for 12 h. The precipitate was washed by water several times and then dried in an oven to obtain CaCO<sub>3</sub>/CMC hybrid microspheres.

A 42 mg portion of CMC was dissolved in 20 mL of deionized water at room temperature, then 2 mL of Na<sub>2</sub>CO<sub>3</sub> (0.02 M) was added and stirred for 0.5 h. After that, 2 mL of CaCl<sub>2</sub> (0.02 M) was added to the mixtures dropwise and stirred for 5 h. The precipitate was centrifuged, washed with water several times, and dried in an oven to obtain CaCO<sub>3</sub>/CMC hybrid nanospheres.

**2.4. Characterizations of Hybrid Microspheres and Nanospheres.** The surface morphology of the hybrid microspheres and nanospheres was observed by a Hitachi X650 scanning electron microscope (SEM) operating at an accelerating voltage of 30 kV. The inner structure of the dried hybrid microspheres was observed by a Sirion TMP (FEI) scanning electron microscope operating at an accelerating voltage of 5 kV. To observe the inner structure of the microspheres, the microspheres were ground in liquid nitrogen. Before SEM observation, all samples were sputter-coated with gold under vacuum for 50 s with an electrocurrent of 51 mA.

The sizes and size distributions of hybrid microspheres were measured by a Winner 2000 (Jinan Winner Particle Technology Co. Ltd., China) particle sizer, and hybrid nanospheres were measured by a Nano ZS (Malvern Instruments) particle sizer.

The Fourier transform infrared (FTIR) spectra of the dried hybrid microspheres and nanospheres were obtained on a

PerkinElmer-2 spectrometer. The absorption spectra of the samples (in KBr pellets) were recorded in transmission mode and the spectra were collected over the 4000–500 cm<sup>-1</sup> wavenumber range with an OPD velocity of 0.2 cm/s.

The hybrid microspheres and nanospheres were characterized by X-ray photoelectron spectroscopy (XPS) (XSAM 800, Kratos) using a magnesium anode (1253.6 eV) as the exciting source. The binding energy spectra from 0 to 600 eV were collected with energy steps of 0.25 eV at a dwell time of 100 ms per point. The relative amounts of C, O, Ca, and N on the surfaces of the microspheres and nanospheres were determined based on the X-ray photoelectron spectra.

## 2.5. Drug Loading and Characterizations of Drug-Loaded

**Hybrid Microspheres and Nanospheres.** A 0.5 mg portion of DOX·HCl was dissolved in 5 mL of deionized water. Ten milligrams of microspheres or nanospheres was added into the solution and stirred for 12 h. For preparation of drug-loaded microspheres, the microspheres were then centrifuged and washed by deionized water two times. For preparation of drug-loaded nanospheres, the mixture was then put in the dialysis bag and dialyzed against 100 mL water for 24 h to remove the free drug.

The drug-loading content and encapsulation efficiency are calculated as follows.

$$\text{drug loading content} =$$

$$\frac{(\text{drug recovered in micro- or nanospheres})}{(\text{micro- or nanospheres recovered})} \times 100\%$$

$$\text{encapsulation efficiency} =$$

$$\frac{(\text{drug fed} - \text{drug lost})}{\text{drug fed}} \times 100\%$$

The data are given as mean ± standard deviation (SD) based on the measurements of the samples from three branches.

To evaluate the *in vitro* drug release property of microspheres, the drug-loaded microspheres (10 mg) were immersed in 8 mL of PBS solution (pH 7.4) in a centrifuge tube and shaken in a shaking water bath at 37 °C. At predetermined intervals, the sample was centrifuged and then 4 mL of solution was taken out and replaced by 4 mL of fresh PBS solution. To evaluate the *in vitro* drug release property of nanospheres, the drug-loaded nanospheres (8 mg) were put in a dialysis bag and immersed in 8 mL of PBS solution (pH 7.4) in a centrifuge tube and shaken in a shaking water bath at 37 °C. At predetermined intervals, 4 mL of solution outside the dialysis bag was taken out and replaced by 4 mL of fresh PBS solution. The drug concentration was determined by the absorbance at 485 nm in a UV-vis spectrophotometer (PerkinElmer Lambda Bio 40). The data are given as mean ± standard deviation (SD) based on the measurements of the samples from three branches.

The blank and drug-loaded hybrid microspheres and nanospheres were characterized by thermogravimetric analysis (TGA) (Netzsch STA 449C) in the temperature range of 30–1200 °C with a heating rate of 10 °C/min in N<sub>2</sub>.

Differential scanning calorimetry (DSC) was carried out with a Perkin-Elmer DSC 7 thermal analyzer. Samples were heated from 50 to 250 °C at a heating rate of 10 °C/min.

Drug-loaded microspheres were observed by a confocal laser scanning microscope (Nikon C1-si TE2000). The image was recorded using Nikon EZ-C1 FreeViewer software.

## 3. Results and Discussion

**3.1. Preparation and Characterizations of Hybrid Microspheres and Nanospheres.** The fabrication procedure of calcium carbonate/carboxymethyl chitosan (CaCO<sub>3</sub>/CMC) hy-

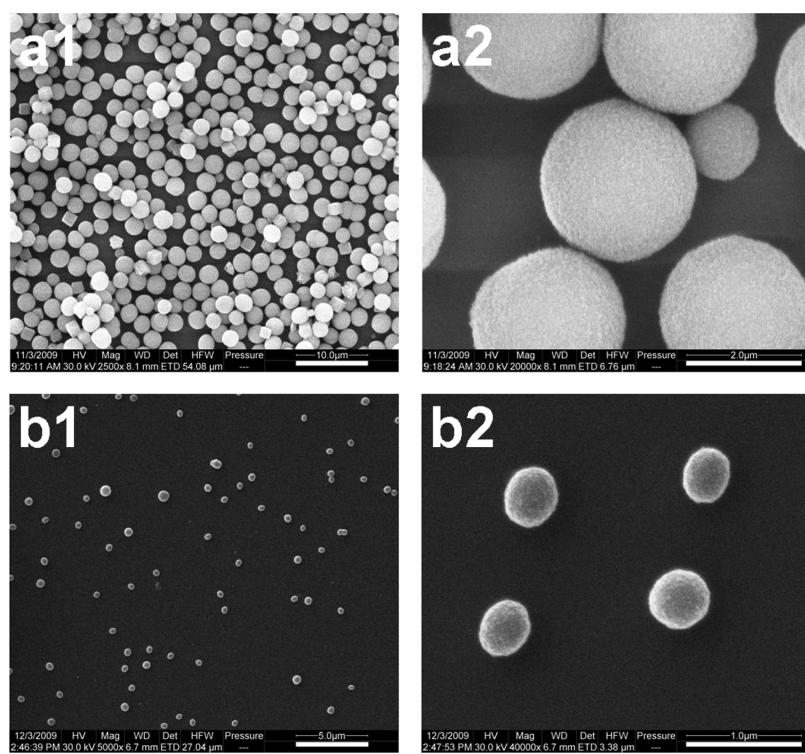
**TABLE 1: Concentrations of CMC, Na<sub>2</sub>CO<sub>3</sub>, and CaCl<sub>2</sub> for Preparation of Hybrid Micro/Nanospheres and Properties of Resultant Micro/Nanospheres**

sample	CMC concentration (g/L)	Na <sub>2</sub> CO <sub>3</sub> concentration (M)	CaCl <sub>2</sub> concentration (M)	content of CMC determined by TGA (wt %)	drug loading content (wt %)	encapsulation efficiency (%)
microspheres	5	0.5	0.5	11.2	3.2 ± 0.3	65.7 ± 6.0
nanospheres	2.1	0.02	0.02	20.3	3.2 ± 0.2	66.9 ± 3.4

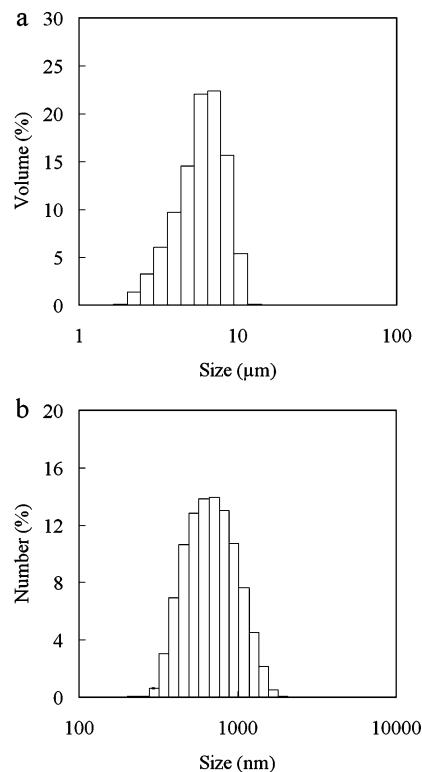
brid microspheres and nanospheres is shown in Figure S1 in the Supporting Information. During the preparation, CMC was dissolved in deionized water first, and then Na<sub>2</sub>CO<sub>3</sub> solution and CaCl<sub>2</sub> solution at particular concentrations (Table 1) were added, respectively. Previous studies have shown that polysaccharides could act as an efficient stabilizer and prevent the precipitation of inorganic compounds.<sup>16–18</sup> In this study, due to the existence of CMC, a charged polysaccharide, the precipitation of CaCO<sub>3</sub> could be effectively retarded and the crystallization and growth behavior of the CaCO<sub>3</sub> particles could be controlled. In addition, as an ionic polysaccharide, CMC had the ability to bind Ca<sup>2+</sup> cations. With the addition of CaCl<sub>2</sub> solution to the CMC solution, Ca<sup>2+</sup> ions interacted with CMC chains. As we know, CMC has two sorts of protonable groups, and a two-step dissociation of the groups occurs with an increase in pH, i.e., deprotonation of –COOH groups and then followed by the deprotonation of –NH<sub>3</sub> groups. The –COOH groups have a protonation constant ( $pK_a$ ) around 2.2–3.2 and –NH<sub>3</sub> groups have a  $pK_a$  at 6.2–7.8 depending on the deacetylation degree, substitution degree, and molecular weight of CMC.<sup>19</sup> So in the basic and neutral solutions, the CMC chains exist in the form of stretching conformation due to the repulsion between the deprotonated carboxyl groups. In this study, the presence of bound Ca<sup>2+</sup> ions reduced the electrostatic repulsion between –COO<sup>–</sup> groups in the CMC chains. Thus, the CMC chains in the Ca<sup>2+</sup>-rich domains became more condense, while the chains in the Ca<sup>2+</sup> deficient domains had the stronger electrostatic repulsion between the –COO<sup>–</sup> groups and thus had a higher

affinity with water molecules. As a result, CaCO<sub>3</sub>/CMC hybrid particles with lower CMC content in the inner part and high CMC content in the surface layer were obtained. CMC played an important role on the nucleation and the growth of calcium carbonate and functionalized as a stabilizing agent for colloidal particles. The negative charges on the surfaces of the hybrid particles ensured their good dispersibility and high colloidal stability in water. To form uniform particles, the concentration of CMC solution should be accurately controlled at a suitable level. If the CMC concentration is too high, particles with irregular shapes may be formed. While if the CMC concentration is too low, the particular size could not be effectively controlled by the existence of CMC. Through adjusting the concentrations of Na<sub>2</sub>CO<sub>3</sub> and CaCl<sub>2</sub> solutions, hybrid particles with different sizes could be obtained, i.e., microspheres could be obtained at higher concentrations and nanospheres could be obtained at lower concentrations. Generally, the particle size increases with increasing concentrations of Na<sub>2</sub>CO<sub>3</sub> and CaCl<sub>2</sub> solutions added in the system when keeping the CMC concentration constant. In the current study, we present the data of typical microspheres and nanospheres prepared at two different conditions as representative examples.

As shown in Figure 1, SEM images show that the microspheres exhibit a regular spherical shape with a mean size less than 5  $\mu\text{m}$  (Figure 1a). The nanospheres also have a spherical shape with a mean size about 0.4  $\mu\text{m}$  (Figure 1b). The SEM image with a high magnification shows the hybrid microspheres



**Figure 1.** SEM images of hybrid microspheres (a1, scale bar = 10  $\mu\text{m}$ ; a2, scale bar = 2  $\mu\text{m}$ ) and nanospheres (b1, scale bar = 5  $\mu\text{m}$ ; b2, scale bar = 1  $\mu\text{m}$ ).



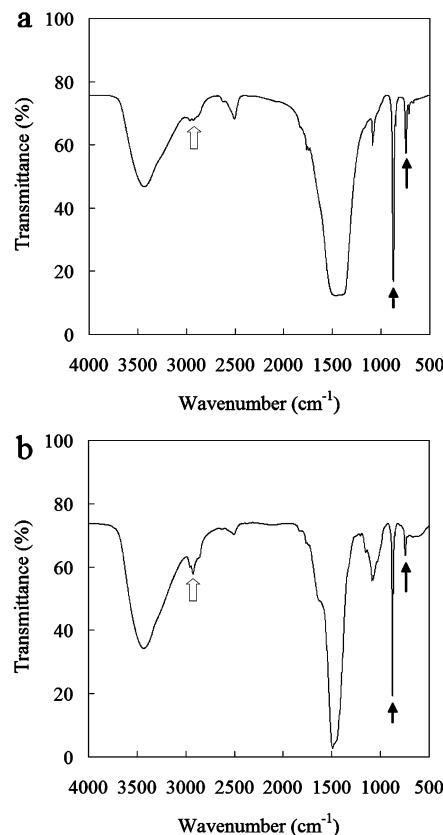
**Figure 2.** Size distributions of hybrid microspheres (a) and nanospheres (b).

have a porous surface and inner structures with a solid core construction (Figure S2 in the Supporting Information).

The size distributions of microspheres and nanospheres in aqueous solutions measured by particle size analyzers are shown in Figure 2; both microspheres and nanospheres exhibit unimodal size distributions with relatively narrow distributions. Compared with the sizes of water-soaked micro/nanospheres measured by particle size analyzers, the sizes of dried micro/nanospheres from SEM observation are smaller. This is due to that fact that the CMC-rich outer layers of the micro/nanospheres are highly hydrolyzed in water, leading to a larger size measured by the particle size analyzers based on a laser light scattering (LLS) technique. Once the water is removed, the surface layers of micro/nanospheres shrink.

Figure 3 displays the FTIR spectra of the hybrid microspheres and nanospheres. The presence of CMC in the hybrid microspheres and nanospheres is confirmed by the peak at  $2920\text{ cm}^{-1}$ . The bands at  $875$  and  $745\text{ cm}^{-1}$  are characteristic bands of  $\text{CaCO}_3$ ; i.e., the band at  $875\text{ cm}^{-1}$  is assigned to the bending vibration of calcite and  $745\text{ cm}^{-1}$  is ascribed to the vibrational band of vaterite.<sup>19,20</sup> Compared with the hybrid microspheres (Figure 3a), hybrid nanospheres (Figure 3b) have a stronger peak at  $2920\text{ cm}^{-1}$  and weaker peaks at  $875$  and  $745\text{ cm}^{-1}$ , indicating the nanospheres have a higher content of CMC and a lower content of  $\text{CaCO}_3$  compared with the microspheres.

In the current study, the hybrid microspheres and nanospheres were characterized by XPS and the relative atomic concentrations of C, O, Ca, and N on the surface layers of the particles were determined. The XPS patterns of hybrid microspheres and nanospheres are shown in Figure 4. For hybrid microspheres and nanospheres, the C and O concentrations determined by XPS are the total concentrations from both CMC and  $\text{CaCO}_3$ , the Ca concentration is an overall contribution from  $\text{CaCO}_3$  and the  $\text{Ca}^{2+}$  ions coordinated with CMC, and the N concentration is the only contribution from CMC. Thus from the relative



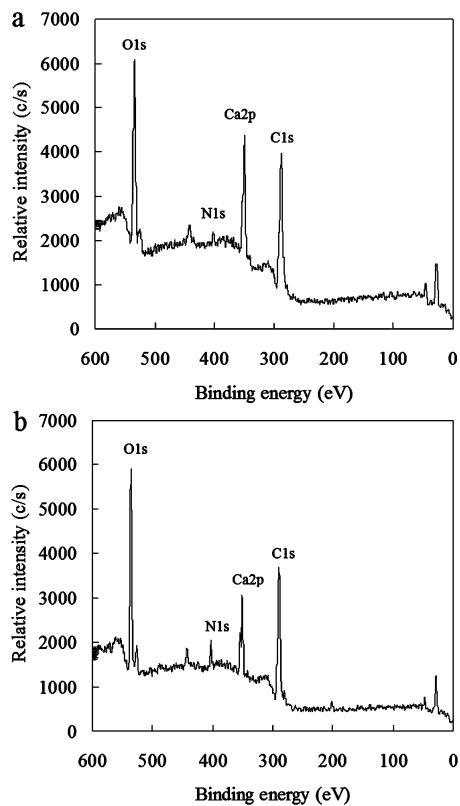
**Figure 3.** FTIR spectra of hybrid microspheres (a) and nanospheres (b).

atomic concentration of N element, we can estimate the relative content of CMC on the surface layers of the particles. The relative atomic concentrations of N are 1.7% and 4.1% for hybrid microspheres and nanospheres, respectively, implying that hybrid nanospheres have a higher CMC content on the surface.

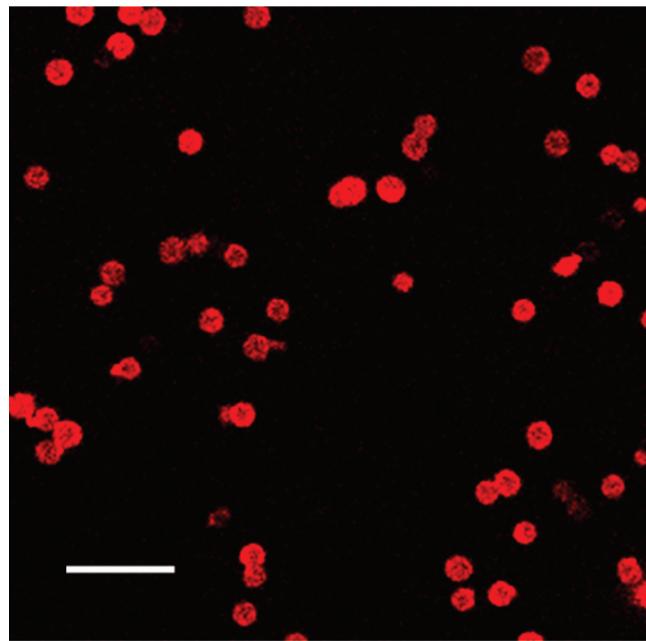
The contents of CMC in the blank hybrid micro/nanospheres determined by TGA (Figure S3 in the Supporting Information) are listed in Table 1. Being consistent with XPS characterization on the particle surfaces, the CMC content in the whole nanospheres is also higher than that in the whole microspheres.

**3.2. Drug Loading and Properties of Drug-Loaded Hybrid Microspheres and Nanospheres.** According to previous studies as well as our SEM observation, the  $\text{CaCO}_3$  microparticles prepared in the presence of polysaccharides have a large number of nanopores, which provide a strong capability to load drugs regardless of their surface charge and hydrophilicity.<sup>6,11</sup> In our study, doxorubicin hydrochloride (DOX·HCl) was chosen as a model drug. The negatively charged CMC in the hybrid micro/nanospheres could provide additional attractive forces for the positively charged drug. As shown in Table 1, the encapsulation efficiencies of both microspheres and nanospheres are higher than 60%, indicating the drug could be readily loaded into the hybrid particles due to the high porous structure of  $\text{CaCO}_3$  and the electrostatic interaction between the deprotonated carboxyl groups of CMC in the hybrid particles and the protonated amino groups of the drug molecules.

In the current study, the drug distribution in the microspheres was observed by confocal microscopy. The image of drug-loaded microspheres is shown in Figure 5. To further study the state of the loaded drug, DSC was used to characterize the thermal property of both blank micro/nanospheres and drug-loaded micro/nanospheres. From Figure S4 (Supporting Infor-



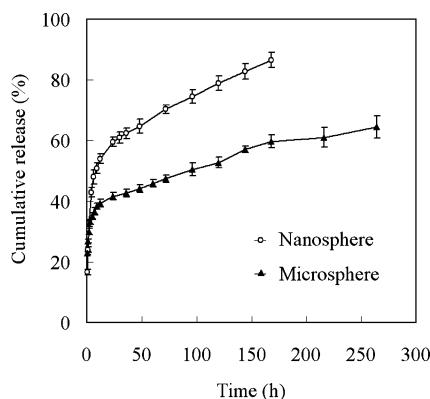
**Figure 4.** XPS spectra of hybrid microspheres with relative atomic concentrations of C 58.6%, O 33.4%, Ca 6.2%, and N 1.7% (a) and nanospheres with relative atomic concentrations of C 57.1%, O 34.4%, Ca 4.4%, and N 4.1% (b).



**Figure 5.** Confocal microscopy image of drug-loaded microspheres (scale bar = 10  $\mu\text{m}$ ).

mation), the melting peak of DOX·HCl could be observed at around 220 °C, which is in accordance with the previous literature.<sup>15</sup> The absence of a characteristic drug melting peak around this temperature for drug-loaded hybrid microspheres and nanospheres indicates that drug exists in an amorphous state in the microspheres and nanospheres.

As we know, polysaccharides are highly hydrolyzed in water. The drugs with low molecular weights encapsulated in the



**Figure 6.** In vitro drug release from hybrid microspheres and nanospheres.

polysaccharides can be diffused out quickly and easily, leading to a low encapsulation efficiency and a fast drug release rate for water-soluble drugs with low molecular weights. According to previous studies, the existence of inorganic compounds such as  $\text{CaCO}_3$  in the polysaccharide based nanospheres could reduce the permeability of the polysaccharide based drug delivery systems.<sup>17</sup> In our hybrid systems, the content of inorganic compound  $\text{CaCO}_3$  is relatively high (higher than 70 wt %). As a result, the encapsulation efficiencies of the current systems are much higher than the previously reported systems with polysaccharide as the main component.<sup>17</sup> Since most anticancer drugs are positively charged at physiological conditions,<sup>6</sup> the hybrid micro/nanospheres prepared by this method could be used as promising drug carriers for many antitumor drugs.

Figure 6 shows the release profile of DOX·HCl from the hybrid microspheres and nanospheres at 37 °C in PBS (pH 7.4). Due to the hydrophilicity of the CMC chains, the initial burst release could be observed. After 24 h, the release rate became very slow because the hybrid particles could effectively sustain the drug release. Compared with hybrid microspheres, the release from hybrid nanospheres was faster because of their higher surface/volume ratio and lower  $\text{CaCO}_3$  content.

#### 4. Conclusions

Through adjustments in the preparation conditions, calcium carbonate/carboxymethyl chitosan ( $\text{CaCO}_3/\text{CMC}$ ) hybrid microspheres and nanospheres of different sizes were prepared. The drug loading and release properties of hybrid microspheres and nanospheres were studied and the results showed the water-soluble DOX·HCl could be effectively loaded in the hybrid microparticles and nanospheres with a high encapsulation efficiency, and the drug release could be effectively sustained, indicating the hybrid microspheres and nanospheres were suitable for delivery of water-soluble drugs.

**Acknowledgment.** Financial support from National Natural Science Foundation of China (20774070 and 21074099) to Si-Xue Cheng is gratefully acknowledged. Financial support from the Ministry of Science and Technology of China (National Basic Research Program of China 2009CB930300) is appreciated.

**Supporting Information Available:** Figures showing schematic diagram of preparation procedure of microspheres and nanospheres, SEM images of the inner structure of a cracked microsphere, TGA curves of microspheres and nanospheres, and DSC curves of blank and drug-loaded microspheres and nanospheres. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- (1) Little, S. R.; Lynn, D. M.; Ge, Q.; Anderson, D. G.; Puram, S. V.; Chen, J.; Eisen, H. N.; Langer, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9534.
- (2) Chawla, J. S.; Amiji, M. M. *Int. J. Pharm.* **2002**, *249*, 127.
- (3) des Rieux, A.; Fievez, V.; Garinot, M.; Schneider, Y. J.; Préat, V. *J. Controlled Release* **2006**, *116*, 1.
- (4) Jain, R. A. *Biomaterials* **2000**, *21*, 2475.
- (5) Sinha, V. R.; Bansal, K.; Kaushik, R.; Kumria, R.; Trehan, A. *Int. J. Pharm.* **2004**, *278*, 1.
- (6) Peng, C.; Zhao, Q.; Gao, C. *Colloids Surf., A* **2010**, *353*, 132.
- (7) Andreeva, D. V.; Gorin, D. A.; Möhwald, H.; Sukhorukov, G. B. *Langmuir* **2007**, *23*, 9031.
- (8) Wei, W.; Ma, G. H.; Hu, G.; Yu, D.; Mcleish, T.; Su, Z. G.; Shen, Z. Y. *J. Am. Chem. Soc.* **2008**, *130*, 15808.
- (9) Ueno, Y.; Futagawa, H.; Takagi, Y.; Ueno, A.; Mizushima, Y. *J. Controlled Release* **2005**, *103*, 93.
- (10) Wang, C.; Liu, H.; Gao, Q.; Liu, X.; Tong, Z. *Carbohydr. Polym.* **2008**, *71*, 476.
- (11) Wang, C.; He, C.; Tong, Z.; Liu, X.; Ren, B.; Zeng, F. *Int. J. Pharm.* **2006**, *308*, 160.
- (12) Zhao, Q.; Han, B.; Wang, Z.; Gao, C.; Peng, C.; Shen, J. *Nanomedicine* **2007**, *3*, 63.
- (13) Liu, X. F.; Guan, Y. L.; Yang, D. Z.; Li, Z.; Yao, K. D. *J. Appl. Polym. Sci.* **2001**, *79*, 1324.
- (14) Ge, H. C.; Luo, D. K. *Carbohydr. Res.* **2005**, *340*, 1351.
- (15) Kalaria, D. R.; Sharma, G.; Beniwal, V.; RaviKumar, M. N. V. *Pharm. Res.* **2009**, *26*, 492.
- (16) Zhao, D. H.; Zhu, Y. C.; Li, F.; Ruan, Q.; Zhang, S.; Zhang, L.; Xu, F. *Mater. Res. Bull.* **2010**, *45*, 80.
- (17) Yu, C. Y.; Jia, L. H.; Yin, B. C.; Zhang, X. Z.; Cheng, S. X.; Zhuo, R. X. *J. Phys. Chem. C* **2008**, *112*, 16774.
- (18) Liang, O.; Zhao, Y.; Shen, Q.; Wang, D.; Xu, D. *J. Cryst. Growth* **2004**, *261*, 571.
- (19) Wang, L. C.; Chen, X. G.; Liu, C. S.; Li, P. W.; Zhou, Y. M. *J. Polym. Sci., Part B: Polym. Phys.* **2008**, *46*, 1419.
- (20) Naka, K.; Keum, D. K.; Tanaka, Y.; Chujo, Y. *Chem. Commun.* **2000**, 1537.

JP105906P