Fabrication of Nanospheres and Vesicles as Drug Carriers by Self-Assembly of Alginate

DATASET · SEPTEMBER 2015

CITATIONS
2

READS
24

6 AUTHORS, INCLUDING:

Li-Hui Jia
Peking University
20 PUBLICATIONS **149** CITATIONS
SEE PROFILE

Bocheng Yin
University of Pittsburgh
6 PUBLICATIONS **56** CITATIONS
SEE PROFILE

Si-Xue Cheng
Wuhan University
186 PUBLICATIONS **5,156** CITATIONS
SEE PROFILE

Fabrication of Nanospheres and Vesicles as Drug Carriers by Self-Assembly of Alginate

Cui-Yun Yu,[†,‡] Li-Hui Jia,[†] Bo-Cheng Yin,[†] Xian-Zheng Zhang,[†] Si-Xue Cheng,[†,*] and Ren-Xi Zhuo[†]

Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, Wuhan 430072, People's Republic of China and, Department of Pharmacy, School of Life Sciences and Technology, University of South China, Hengyang, 421001, People's Republic of China

Received: July 21, 2008

A new method was developed to fabricate nanospheres and vesicles as drug carriers. The drug-loaded nanospheres and vesicles were prepared by self-assembly of alginate in aqueous media containing Ca^{2+} and CO_3^{2-} ions under very mild conditions. The preparation method did not involve any organic solvent and surfactant and could offer good control over the morphology and the size of self-assemblies. Through adjusting the preparation conditions, nanosized drug-delivery systems with different shapes, that is, nanospheres and vesicles, could be obtained. The morphologies of the drug-delivery systems were observed by transmission electron microscopy (TEM). 5-Fluorouracil (5-FU), an anticancer drug, was encapsulated in the nanospheres and vesicles, and in vitro drug release behavior was investigated. The effect of drug-loading content on the release was studied. The release of 5-FU could be effectively sustained from both drug-loaded nanospheres and vesicles because the presence of $CaCO_3$ in the nanospheres/vesicles could reduce the permeability of the entrapped drug for the alginate-based self-assemblies.

1. Introduction

Controlled drug-delivery technology offers numerous advantages compared to conventional therapeutic systems, by prolonging duration time, reducing side effects, retaining drug bioactivity, and thus improving the therapeutic efficiency. For antineoplastic drugs, such as doxorubicin and 5-fluorouracil, with major drawbacks including the acute toxicity to normal tissue and the inherent multidrug resistance effect, controlled drug delivery technology is of special importance.[1−3]

Among various dosage forms, nanosized and microsized drug delivery systems have been extensively investigated because of their advantages including injectable property, which can avoid the inconvenient surgical insertion[3,4] and the possibility to achieve passive targeting when their sizes are in particular ranges.[5]

Micelles and vesicles are two classes of most commonly studied nanosized drug carriers.[1,6−8] For polymeric micelles and vesicles, most commonly, they are prepared by the self-assembly of amphiphilic copolymers in selective solvents.[1,2,4,8] However, the preparation suffers from some limitations. Of particular concern is the use of organic solvents. The existence of organic solvents may lead to loss or reduction in bioactivity of some drugs. Moreover, many organic solvents are toxic and a low-level exposure to residual organic solvents may lead to lasting toxic effects.[9] Liposomes are another important class of drug delivery systems prepared by self-assembly of lipids.[10] However, it is well-known that classical liposomal drug delivery systems suffer from instability in vivo. Besides, the preparation of liposomes also involves the use of organic solvents.[11]

Biodegradable natural polymers as drug carriers have many advantages including good biocompatibility, nontoxicity, and adjustable controlled-release property.[12] Through electrostatic interactions, nanoparticulate polyelectrolyte complexes, such as alginate/chitosan, could be fabricated.[13−15] However, these polyelectrolyte complexes are generally used to deliver protein and peptide drugs,[10,11] and it is difficult to encapsulate anticancer drugs with low molecular weights in the nanoparticulate polyelectrolyte complexes through physical entrapment.[15]

So, the purpose of current study is to develop new methods to fabricate nanosized drug delivery systems for anticancer drug delivery. In this investigation, a new facile method was developed to fabricate nanospheres and vesicles, that is, self-assembly of alginate in aqueous media containing Ca^{2+} and CO_3^{2-} ions. The preparation method did not involve any organic solvent and surfactant and could offer good control over the morphology and the size of the nanospheres and vesicles. 5-Fluorouracil was encapsulated in the nanospheres and vesicles, and the in vitro drug release study showed the drug release could be effectively sustained from both drug-loaded nanospheres and vesicles.

2. Experimental Section

2.1. Materials. Sodium alginate (viscosity, 0.02 Pa·s in 1% aqueous solution at 20 °C) and sodium bicarbonate ($NaHCO_3$) were supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Calcium hydroxide ($Ca(OH)_2$) was provided by Kermel Chemical Reagent Co. Ltd. (Tianjing, China). 5-Fluorouracil (5-FU) was purchased from Sigma. All other reagents were of analytical grade and used as received.

2.2. Preparation of Drug-Loaded Nanospheres and Vesicles. To prepare drug-loaded nanospheres, sodium alginate (0.10 g) was added in 5 mL of distilled water under stirring and then immersed in a thermostatic water bath at 55 °C for 0.5 h. $Ca(OH)_2$ solution (0.02 M, 1 mL) was added to the system dropwise, and stirred for additional 1 h at 55 °C. After that, $NaHCO_3$ solution (0.03 M, 3 mL) was added to the system

* To whom correspondence should be addressed. Fax: 86-27-68754509. E-mail: chengsixue@hotmail.com, chengsx@whu.edu.cn.
† Wuhan University.
‡ University of South China.

10.1021/jp806540z CCC: $40.75
Published on Web 10/04/2008

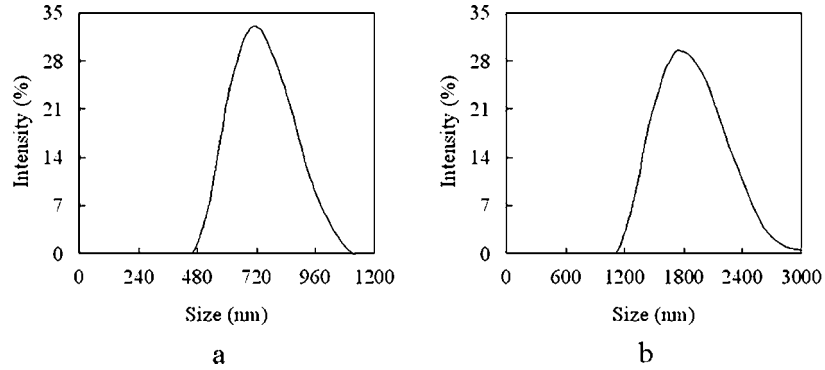


Figure 1. Size distributions of (a) drug-loaded nanospheres (sample 1), and (b) drug-loaded vesicles (sample 3).

dropwise, and stirred for additional 3 h at 55 °C. Then 5-FU (0.025 g to obtain sample 1 and 0.050 g to obtain sample 2) was added. The system was kept stirring at 55 °C for 24 h, and then cooled rapidly in a water bath of 20 °C for 0.5 h. Finally, the mixture was put into a dialysis bag and dialyzed against 500 mL of distilled water at 20 °C for 24 h to obtain the precipitated drug-loaded nanospheres.

To prepare drug-loaded vesicles, the volumes of $Ca(OH)_2$ solution (0.02 M, 2 mL) and $NaHCO_3$ solution (0.03 M, 2 mL) used were different from that for the preparation of drug-loaded nanospheres. Other conditions were the same as that for the preparation of drug-loaded nanospheres. The 5-FU feeding amounts were 0.025 g for sample 3 and 0.050 g for sample 4, respectively.

2.3. Determination of Drug-Loading Content and Encapsulation Efficiency. After the preparation of drug-loaded nanospheres or vesicles, the aqueous phase was collected, and the drug content in the aqueous phase was determined as the drug loss. The drug concentration was determined by an UV−vis spectrophotometer (PerkinElmer Lambda Bio 40). The drug loading content and encapsulation efficiency were calculated as follows.

Drug-loading content = (drug recovered in nanospheres or vesicles/nanospheres or vesicles recovered) × 100%

Encapsulation efficiency = (drug fed−drug loss)/drug fed × 100%

2.4. Characterizations of Drug-Loaded Nanospheres and Vesicles. The size and size distribution of micelles were measured by using a Zetasizer Nano ZS (Malvern Instruments). Before measurement, the stock solution was diluted with distilled water (1:4) to obtain a proper concentration for size determination.

The morphology of drug-loaded nanospheres before drug release was visualized by a FEI Quanta 200 environment scanning electron microscope. The water-soaked sample was directly observed by ESEM mode, and the dried sample was sputter coated with gold and then observed by SEM mode.

The morphologies of drug-loaded nanospheres and vesicles before drug release and after drug release for 15 days were observed on by a JEOL JEM-100CXII transmission electron microscope (TEM). Before visualization, a droplet of suspension containing nanospheres or vesicles was placed on copper grid with Formvar film and dried.

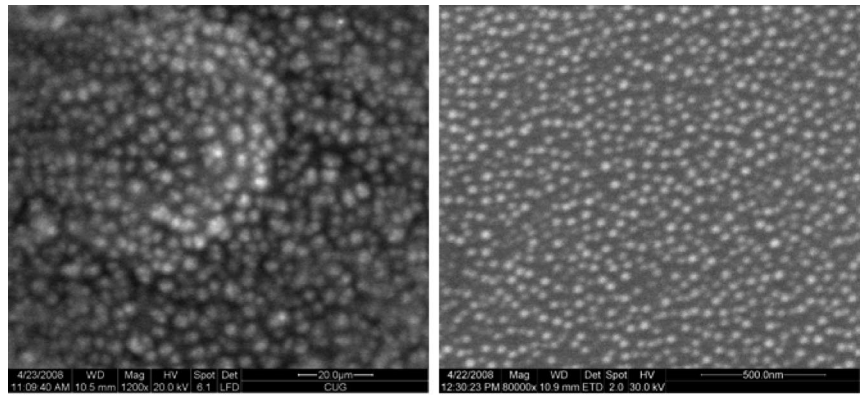
2.5. In Vitro Drug Release Study. Drug-loaded nanospheres or vesicles were put in a dialysis bag, then immersed in 150 mL of Tris-HCl buffer solution (pH 7.4, 0.02 M), and shaken in a shaking water bath at 37 °C. At predetermined intervals, 150 mL of the buffer solution was taken out and replaced by fresh buffer solution. The drug concentration was determined by measuring the absorbance at 267 nm in a UV−vis spectrophotometer (PerkinElmer Lambda Bio 40).

3. Results and Discussion

3.1. Preparation and Characterizations of Drug-Loaded Nanospheres and Vesicles. In the current study, we used natural polymer, alginate, to prepare the self-assembled aggregates in aqueous solutions. As an ionic polysaccharide, alginate has the ability to bind divalent cations, such as Ca^{2+} or Sr^{2+}. Alginate is a copolymer composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) units. Divalent cations can induce interchain association with G units, leading to the formation of junction zones. This is generally accepted as the egg-box model.[16,17] In this investigation, the concentrations of Ca^{2+} for the preparation of self-assemblies are relatively low to avoid the complete gelation and formation of bulk gels. During the preparation, with the addition of $Ca(OH)_2$ solution into the alginate solution, Ca^{2+} ions are involved in coordinating with G units in alginate chains. Once $NaHCO_3$ solution is added into the above mixture, free carbonate ions (CO_3^{2-}) tend to deprive the coordinated calcium cations due to the electrostatic interaction. However, it is very interesting to note that the precipitation of $CaCO_3$ does not take place because the concentrations of both Ca^{2+} and CO_3^{2-} ions are low, and the alginate acts as an efficient stabilizer and prevents the precipitation of $CaCO_3$.[18] Through adjusting the concentration of Ca^{2+}, self-assemblies with different morphologies could be obtained.

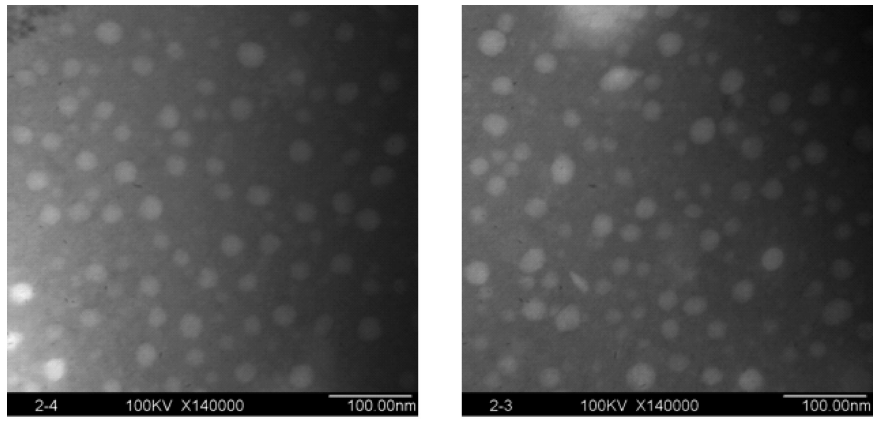
As we know, self-assembled aggregates could be formed by amphiphilic copolymers in selective solvents, and the aggregates of multimorphologies of could be obtained for copolymers with particular structures under certain self-assembling conditions, such as in the presence of acid or salt with different concentrations.[19] In this study, nanospheres could be obtained at a low concentration of Ca^{2+} ions and vesicles could be obtained at a high concentration of Ca^{2+} ions. The ion-induced morphological change is related to the Ca^{2+} binding to deprotonated carboxyl groups of alginate in the basic aqueous solution.[19] The presence of coordinated Ca^{2+} reduces the electrostatic repulsion between the $-COO^-$ groups, and the alginate chains in the Ca^{2+} rich domain become more condense and form the inner part of the nanospheres or the central part of the vesicular membranes. According to previous studies, nucleation of inorganic compounds, such as calcium phosphate, could be used to prepare mineralized cross-linked poly(acrylic acid-*b*-isoprene) micelles.[20] In our study, the addition of CO_3^{2-} ions could further stabilize the structures formed.

The size distributions of nanospheres and vesicles in aqueous solutions are shown in Figure 1. Both nanospheres and vesicles exhibit unimodal size distributions, indicating our fabrication method could offer good control over the size of the self-assemblies.



a b

Figure 2. (a) ESEM image of water-soaked drug-loaded nanospheres (sample 1) and (b) SEM image of dried drug-loaded nanospheres (sample 1).



a b

Figure 3. TEM images of nanospheres (sample 1) (a) before drug release, and (b) after drug release for 15 days.

The ESEM image of the water-soaked nanospheres and the SEM image of dried nanospheres are shown in parts a and b of Figure 2, respectively. Both water-soaked and dried nanospheres exhibit a regular spherical shape. The size of water-soaked nanospheres from ESEM is in good agreement with the value measured by the particle sizer (part a of Figure 1). The dried nanospheres have a much smaller size as compared with wet nanospheres. (The scale bar of ESEM image in part a of Figure 2 is 20 μm and the scale bar of SEM image in part b of Figure 2 is 500 nm.) Obviously, the nanospheres we prepared have bibulous ability. Once the water is removed, the nanospheres would shrink rapidly. The TEM image of nanospheres before drug release is depicted in part a of Figure 3, in good agreement with SEM observation, the size of dried nanospheres is less than 50 nm. After drug release for 15 days, the nanospheres (part b of Figure 3) do not show obvious morphological change as compared with the nanospheres before drug release (part a of Figure 3), indicating that the nanosphere drug delivery system is thermodynamically stable during the drug release process.

Part a of Figure 4 shows the TEM image of a vesicle before drug release. The vesicle bears a well-defined spherical shape. The layer structure of the vesicle could be seen clearly, and the thickness of the wall is around 90 nm from the TEM image. The morphology of a vesicle after drug release for 15 days (part b of Figure 4) is almost the same as that before drug release, implying the vesicle drug delivery system can keep its shape during the drug release process in the aqueous solution.

3.2. Drug Loading and In Vitro Release. Generally, polysaccharides have strong affinity with water and the polysaccharide matrices are highly hydrolyzed. The drugs with low molecular weights encapsulated in the polysaccharides can be diffused out quickly and easily, leading to a low encapsulation efficiency and a fast drug release rate.

According to previous literatures, the permeability of calcium phosphate mineralized shell cross-linked polymeric micelles could be greatly reduced approximately 50 or 100% respectively for hybrid nanostructures enclosed within 10 or 20 nm thick calcium phosphate layers.[20] In our study, during the preparation of drug-loaded nanospheres and vesicles, 5-FU is added to the system at 55 °C and then the system is dialyzed at 20 °C to form drug-loaded self-assemblies. Because of the different solubilities of 5-FU at different temperatures, 5-FU would precipitate from the supersaturating solution when the temperature decreases to 20 °C from the initial elevated temperature of 55 °C. The precipitated 5-FU could be adsorbed inside the self-assemblies. With the presence of $CaCO_3$ in the alginate-based nanospheres/vesicles, the drug could be effectively encapsulated in the highly hydrolyzed nanospheres/vesicles with encapsulation efficiencies higher than 15% for all samples as listed in Table 1.

As can be seen in Table 1, vesicles can be yielded with higher drug encapsulation efficiencies compared with nanospheres. As we know, vesicles can entrap hydrophilic molecules within the vesicle lumen and also integrate hydrophobic molecules within

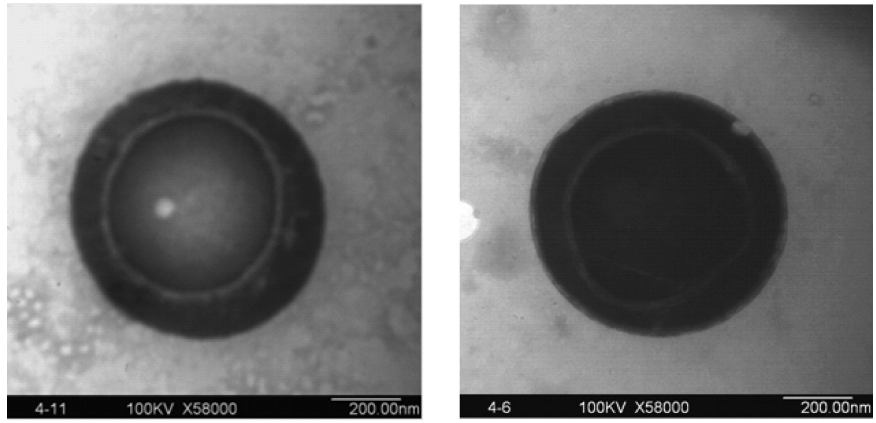


Figure 4. TEM images of vesicles (sample 3) (a) before drug release, and (b) after drug release for 15 days.

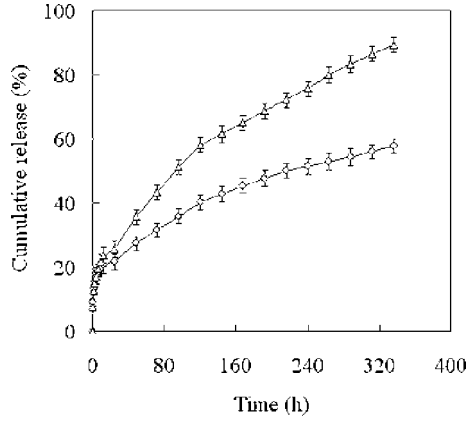


Figure 5. 5-FU release from nanospheres. (Δ - sample 1, ○ - sample 2)

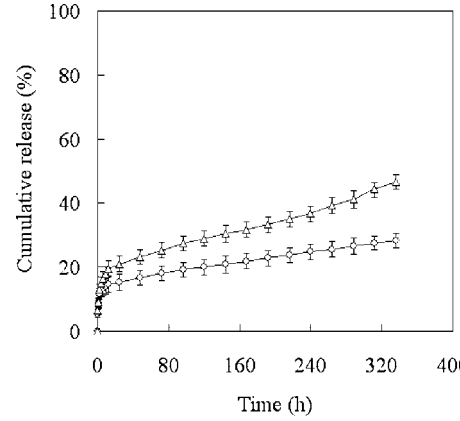


Figure 6. 5-FU release from vesicles. (Δ - sample 3, ○ - sample 4)

TABLE 1: Properties of Alginate-Based Drug Delivery Systems

sample	drug delivery system	drug loading content (wt%)	encapsulation efficiency (%)
1	nanosphere	3.4	17.0
2	nanosphere	5.7	17.1
3	vesicle	4.1	20.6
4	vesicle	9.1	27.3

the membrane core. The solubility of 5-FU in water increases obviously with increasing temperature. As a result, 5-FU can be both incorporated in the vesicle lumen because 5-FU is water soluble at 55 °C and encapsulated in the vesicle membrane due to the precipitation of 5-FU at 20 °C. This is in accordance with the fact that vesicles possess of higher encapsulation efficiencies compared with nanospheres.

In this study, we used 5-FU with low molecular weights as a model drug to evaluate the efficiency of our self-assemblies for sustained drug release. As demonstrated in Figure 5, the drug release from the nanospheres could be effectively retarded. For example, the time for 50% release, $T_{50\%}$, is 96 h for sample 1. It is well-known that due to the high water content of the polysaccharide-based release matrices, the release of low molecular weight drugs usually cannot be effectively controlled, and the release rate is very fast with an obvious burst release.[3,21] As mentioned before, on the basis of the studies of other researchers, the permeability of polymeric micelles could be greatly reduced through mineralized cross-linking using an inorganic compound.[20] In our investigation, the presence of $CaCO_3$ could reduce the permeability of the encapsulated drug for the alginate based self-assemblies. As a result, the release of 5-FU could be effectively sustained and no burst release is observed.

In addition, by comparing the two release profiles, we find that the percentage of the accumulative release from the nanospheres with a lower 5-FU loading level (sample 1) is higher as compared with the nanospheres with a higher 5-FU loading level (sample 2). For the samples with different drug loadings, an increase in the drug loading is expected to result in elevated amount of drug released because diffusion driving force of concentration gradient is enhanced. However, the percentage of the accumulative release is different from the amount of the drug released. Although sample 2 with a higher drug-loading level has a higher amount of released drug, it still shows a lower percentage of the accumulative release.

Figure 6 illustrates the release profiles of 5-FU from vesicles with different drug-loading levels. The drug release from the vesicles could be also effectively sustained and the time for 50% release, $T_{50\%}$, exceeds 200 h for both sample 3 and sample 4, which is much longer than the previously reported values for the release of low molecular weight drug such as 5-FU from polysaccharide-based drug delivery systems.[3,21] Compared with nanospheres, the releases from vesicles are slower. The main reason is that 5-FU is encapsulated in both vesicle lumen and vesicle membrane. The release of the drug is expectedly slow because the thickness of the vesicles is higher than the diameter of nanospheres. The release data are in agreement with the fact that vesicles possess a higher encapsulation efficiency compared with nanospheres. Similarly, the vesicles with a lower drug-

loading level (sample 3) have a higher percentage of the accumulative release as presented in Figure 6.

4. Conclusions

A new strategy to fabricate nanosized natural polymer-based drug-delivery systems with improved drug-release properties was developed. Through self-assembly of alginate in aqueous media containing Ca^{2+} and $CO3^{2-}$ ions under very mild conditions, nanosized drug-delivery systems with different shapes, that is nanospheres and vesicles, could be obtained. The morphology of the obtained self-assemblies could be easily controlled by adjusting the ion concentration. Because of the presence of $CaCO_3$ in the nanospheres/vesicles, the drug could be effectively encapsulated in the self-assemblies, and the drug release could be effectively sustained.

Acknowledgment. Financial support from National Natural Science Foundation of China (20774070) to Si-Xue Cheng is gratefully acknowledged. Financial supports from Ministry of Science and Technology of China (973 Programme 2005CB623903) and National Natural Science Foundation of China (50633020) are also appreciated.

References and Notes

(1) Yoo, H. S.; Park, T. G. *J. Controlled Release* **2001**, *70*, 63.
(2) Shuai, X. T.; Ai, H.; Nasongkl, N.; Kim, S.; Gao, J. M. *J. Controlled Release* **2004**, *98*, 415.
(3) Yu, C. Y.; Zhang, X. C.; Zhou, F. Z.; Zhang, X. Z.; Cheng, S. X.; Zhuo, R. X *Int. J. Pharm.* **2008**, *357*, 15.
(4) Yokoyama, M.; Fukushima, S.; Uehara, R.; Okamoto, K.; Kataoka, K.; Sakurai, Y.; Okano, T. *J. Controlled Release* **1998**, *50*, 79.
(5) Chawla, J. S.; Amiji, M. M. *Int. J. Pharm.* **2002**, *249*, 127.
(6) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967.
(7) Antonietti, M.; Förster, S. *Adv. Mater.* **2003**, *15*, 1323.
(8) Kishimura, A.; Koide, A.; Osada, K.; Yamasaki, Y.; Kataoka, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 6085.
(9) Lees-Haley, P. R.; Williams, C. W. *J. Clin. Psychol.* **1997**, *53*, 699.
(10) Gabizon, A.; Goren, D.; Horowitz, A. T.; Tzemach, D.; Lossos, A.; Siegal, T. *Adv. Drug Deliv. Rev.* **1997**, *24*, 337.
(11) Gu, X. B.; Schwartzc, J. L.; Pang, X. W.; Zhou, Y. F.; Siroise, D. A.; Sridhar, R. *Cancer Lett.* **2006**, *239*, 281.
(12) Stops, F.; Fell, J. T.; Collett, J. H.; Martini, L. G. *Int. J. Pharm.* **2008**, *350*, 301.
(13) Sarmento, B.; Ferreira, D.; Veiga, F.; Ribeiro, A. *Carbohydr. Polym.* **2006**, *66*, 1.
(14) Sarmento, B.; Martins, S.; Ribeiro, A.; Veiga, F.; Neufeld, R.; Ferreira, D. *Int. J. Pept. Res. Ther.* **2006**, *12*, 131.
(15) Cafaggi, S.; Russo, E.; Stefani, R.; Leardi, R.; Caviglioli, G.; Parodi, B.; Bignardi, G.; De Totero, D.; Aiello, C.; Viale, M. *J. Controlled Release* **2007**, *121*, 110.
(16) Sikorski, P.; Mo, F.; Skjåk-Bræk, G.; Stokke, B. T. *Biomacromolecules* **2007**, *8*, 2098.
(17) Li, L. B.; Fang, Y. P.; Vreeker, R.; Appelqvist, I. *Biomacromolecules* **2007**, *8*, 464.
(18) Quignard, F.; Cot, D.; Renzo, F. D.; Gérardin, C. *Prog. Solid State Chem.* **2006**, *34*, 161.
(19) Zhang, L.; Yu, K.; Eisenberg, A. *Science* **1996**, *272*, 1777.
(20) Perkin, K. K.; Turner, J. L.; Wooley, K. L.; Mann, S. *Nano Lett.* **2005**, *5*, 1457.
(21) Arıca, B.; Çalış, S. C.; Kaş, H. S.; Sargon, M. F.; Hıncal, A. A. *Int. J. Pharm.* **2002**, *242*, 267.

JP806540Z