Self-Assembly of Human Serum Albumin (HSA) and L-α-Dimyristoylphosphatidic Acid (DMPA) Microcapsules for Controlled Drug Release

Zhihua An,[a] Gang Lu,[a] Helmuth Möhwald,[b] and Junbai Li*[a]

Abstract: Human serum albumin (HSA) and L-α-dimyristoylphosphatidic acid (DMPA) were applied as a pair to encapsulate ibuprofen microcrystals by means of a technique based on the layer-by-layer (LbL) assembly of oppositely charged species, for the purpose of controlling drug release. The successful adsorption of HSA and DMPA multilayers onto ibuprofen crystals was confirmed by optical microscopy. The drug release process, in a solution of pH 7.4, was monitored by optical microscopy and UV spectroscopy. The results revealed that the rate of release of ibuprofen from HSA/DMPA microcapsules decreased as the capsule wall thickness and drug crystal size increased, indicating that the permeability of the microcapsules can be controlled by simply varying the number of HSA/DMPA deposition cycles.

Keywords: controlled release · lipids · microcapsules · proteins · self-assembly

Introduction

Controlled-release drugs have many advantages over conventional dosage forms, such as minimizing deleterious side effects, prolonging the duration of activity, protecting sensitive drugs from enzymatic or acidic degradation, taste masking, and targeted release. Thus, controlled-release technologies are of interest to the pharmaceutical industry in the development of modern medications.[1] Many techniques have been developed during the past few decades,[2] for example, encapsulation, which is regarded as a versatile and feasible approach. Various microstructures such as liposomes, microgels, microemulsions and polymer micelles have been widely employed as drug-delivery systems.[3] The specific requirements in this field make microencapsulation increasingly attractive and competitive.

A recently developed layer-by-layer (LbL) self-assembly technique was employed to fabricate nanoporous polyelectrolyte microcapsules, by which oppositely charged polyelectrolytes were adsorbed onto various colloid templates, followed by removal of the template cores.[4] The permeability of polyelectrolyte microcapsules can be adjusted by controlling the number of layers as well as the environmental conditions.[5] The potential use of such microcapsules as drug carriers for controlled drug release has already been considered.[6] The presence of a lipid layer on the microcapsule wall provides the possibility to incorporate specific components, such as targets, for the purpose of drug-targeted release.

In this work, HSA and DMPA were chosen as a pair to fabricate multilayers onto drug crystals. HSA is a globular protein of known crystal structure (Figure 1a). It is abundant in human blood, where it is responsible for the transport of metabolites around the body.[7] DMPA (Figure 1b), is the important lipid component of biological membranes. Complex films of proteins and lipids have unique biological properties, which makes them of significant interest as a model for biological membranes. Such films can be constructed at a planar or curved interface by using self-assembly techniques.[8] With such a lipid/protein pair, the incorporation of membrane-specific components, such as receptor channels, into the films for the purpose of molecular recognition can be considered.

Ibuprofen is an acidic, nonsteroidal, anti-inflammatory drug (Figure 1c). It has limited solubility in solutions of low pH (<7), but is readily soluble in high pH (>7) solutions.[9] Thus, the deposition of protein and lipid on ibuprofen crystals under conditions of pH <7, and the release of ibuprofen from the capsules performed in a simulated intestinal fluid at pH 7.4, enabled us to demonstrate that the protein/lipid multilayer could be used to encapsulate drugs that are suitable for sustained release.

[a] Dr. Z. An, Dr. G. Lu, Prof. Dr. J. Li
International Joint Lab
Key Laboratory of Colloid and Interface Science
The Center for Molecular Sciences, Institute of Chemistry
Chinese Academy of Sciences, Beijing 100080 (China)
Fax: (+86) 108-261-2629
E-mail: jbli@iccas.ac.cn

[b] Prof. Dr. H. Möhwald
Max-Planck-Institute of Colloids and Interfaces
Am Mühlenberg 2, 14476 Golm/Potsdam (Germany)

DOI: 10.1002/chem.200400090

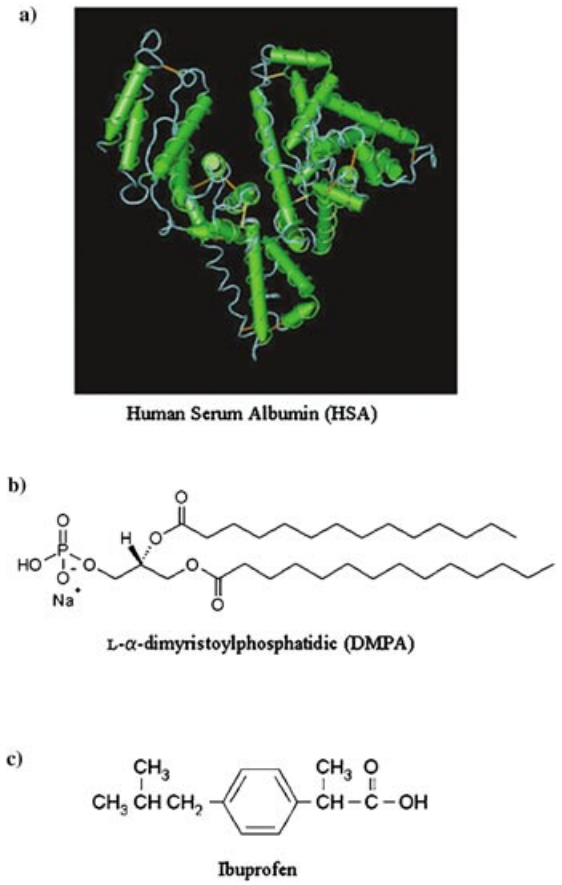


Figure 1. a) Three-dimensional structure of the HSA molecule. b) Chemical structure of L-α-dimyristoylphosphatidic acid (DMPA). c) Chemical structure of ibuprofen.

Results and Discussion

The surface of an ibuprofen crystal is negatively charged. HSA is positively charged under conditions of pH 3.8 (lower than its isoelectric point of pH 4.8). For this reason, HSA was used as the first layer to coat the ibuprofen crystals.[10] The crystals were encapsulated with HSA (mixed with fluorescein isothiocyanate (FITC)-labeled HSA) and the successful adsorption of the first layer was confirmed by using optical microscopy. Figure 2a shows microscope images of the ibuprofen crystal surface covered with positively charged HSA. The negatively charged DMPA solution (containing fluorescently labeled nitrobenzoxadiazole dipalmitoyl phosphatidylcholine (NBD-DPPC)) was then added to the drug crystal suspension and the processes repeated, resulting in the formation of multilayers. The number of layers was determined by the number of deposition cycles. Figure 2b displays the microscope images of ibuprofen crystals encapsulated by five layer pairs, in which DMPA forms the outermost layer. The fluorescent rings of lipids reveal the successful encapsulation of ibuprofen crystals by HSA/DMPA multilayers.

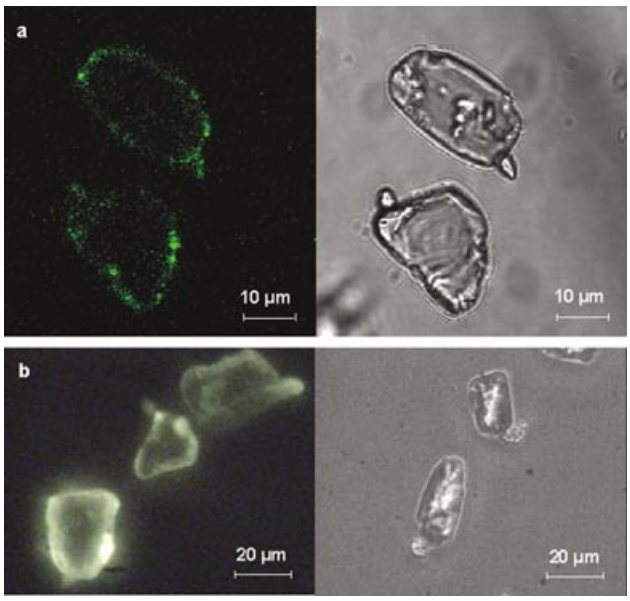


Figure 2. Microscope images of encapsulated ibuprofen crystals a) after adsorption of HSA mixed with 20% FITC-HSA as the first layer, and b) after adsorption of five layer pairs, in which DMPA mixed with 5% NBD-DPPC was the outermost layer. Left, fluorescence image; right, transmission image.

The release of ibuprofen crystals from HSA/DMPA microcapsules was induced by adding a buffer solution of pH 7.4 to the microcapsule dispersion and was recorded as a function of time (Figure 3a). It can be seen that, initially, the individual microcrystals were well separated; however, with the addition of a buffer solution of pH 7.4, they began to

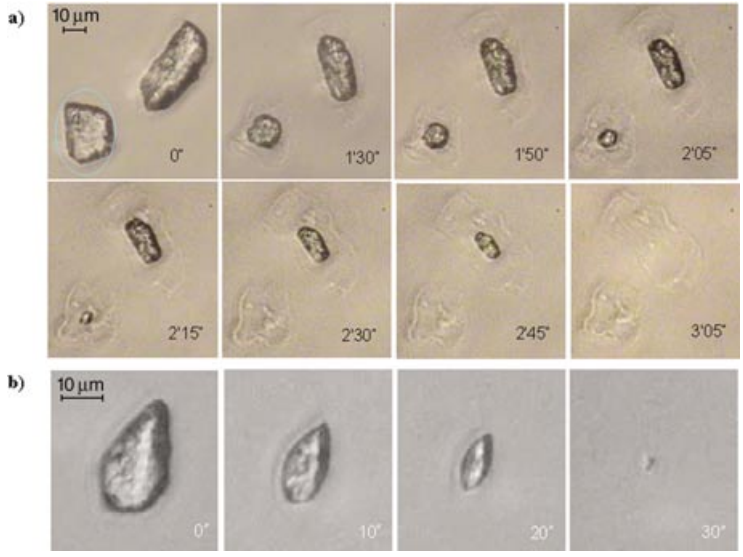


Figure 3. a) The dynamic release of ibuprofen from HSA/DMPA microcapsules. b) Dissolution process of bare ibuprofen crystals. The experiments were performed in a buffer solution of pH 7.4.

dissolve and shrink, making the surrounding HSA/DMPA shells more visible. The HSA/DMPA shells maintained the shape of the crystals throughout the release process until the dissolution of ibuprofen was complete. It is clear that the ibuprofen molecules diffuse out of the capsule through the capsule wall, rather than being released on breaking a capsule, indicating that the HSA/DMPA microcapsules are permeable to small molecules. The shapes of the hollow capsules remain basically the same as those of the original crystals, demonstrating that the capsules are very flexible and have high elastic strength.

As a control experiment, the process of dissolution of bare ibuprofen crystals of similar size was also recorded (Figure 3b). This showed that, following the addition of a buffer solution of pH 7.4, the bare crystals dissolved faster than the encapsulated crystals. Comparison of an encapsulated crystal (blue circle in Figure 3a), with a bare crystal of similar size (approximately 20 μm), revealed that the complete dissolution of the bare crystal took only 30 s, whereas the encapsulated crystal required 2.5 min. This clearly demonstrates that HSA/DMPA microcapsules act as a barrier, prolonging the release time of the ibuprofen crystal.

To obtain more quantitative release data, the release profiles were measured on ensembles by using UV spectroscopy (Figure 4a). These results show that, whereas the bare crystal was partially dissolved after 25 s, coating it with five and ten layer pairs increased the dissolution half-time to 50 and 75 s, respectively. This indicates that the HSA/DMPA capsules can substantially sustain the release of the encapsulated substances. The drug release time decreases as the number of coating layers increases. These times may not be sufficient for specific pharmaceutical applications; however, it has been shown that by reducing the pH, the release times can be increased by up to 3 orders of magnitude.[6]

The rate of drug release also depends on the size of the ibuprofen crystals. The half-times of release for the 15 μm and 36 μm ibuprofen crystals from ten layer pairs of HSA/DMPA capsules were 75 and 130 s, respectively (Figure 4b). This indicates that the release rate of the ibuprofen crystals from the capsules decreases with increasing ibuprofen crystal size. The faster release for smaller crystals is due to their higher surface area to volume ratio; the smaller crystals have a much greater surface area in contact with the buffer solution and, thus, they dissolve more quickly.In fact, the release of the encapsulated ibuprofen crystals takes place by two simultaneous processes; the dissolution of the ibuprofen crystals and the diffusion of the ibuprofen molecules out of the capsules. Thus, the wall thickness of the HSA/DMPA microcapsules, that is, the number of HSA/DMPA layers, will also influence the release rate. This can be quantified by calculating the permeability coefficient (P) by using Equation (1),[11]

$$P = \frac{(\Delta c_t/\Delta t)Vd}{S(c_0 - ct)} \quad (1)$$

in which P ($cm^2 s^{-1}$) is the permeability coefficient of the capsule membrane, S is the effective surface area of the dispersed capsules, d (cm) is the thickness of the capsule wall,

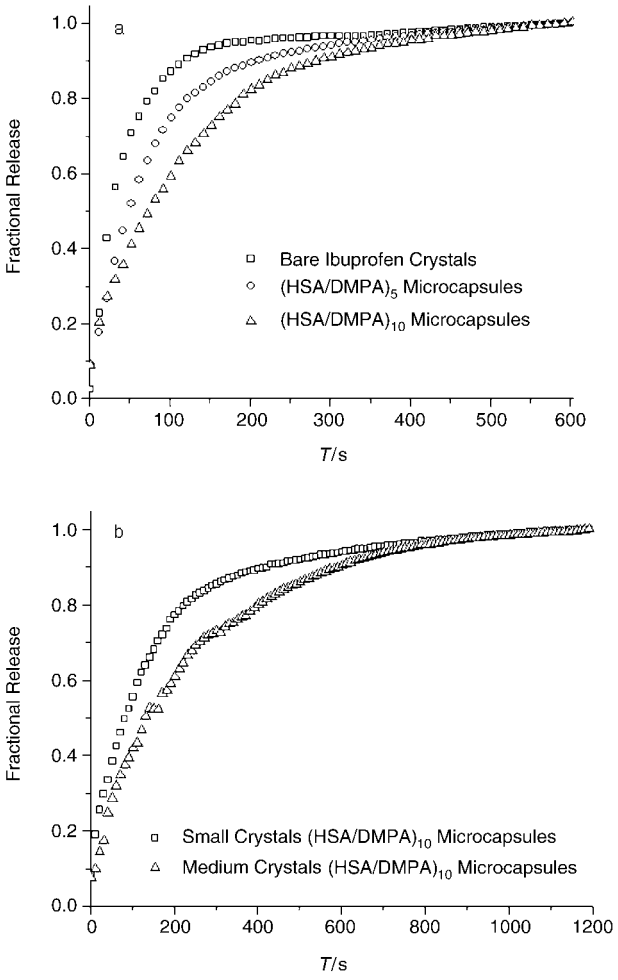


Figure 4. Release profiles of ibuprofen from HSA/DMPA capsules, performed in a buffer solution of pH 7.4. a) 0, 5, or 10 layer pairs encapsulating small crystal cores, b) 10 layer pairs encapsulating small (15 μm) or medium (36 μm) crystal cores.

V is the volume of the bulk solution, $\Delta c_t/\Delta t$ is the concentration change per unit time, which can be evaluated from the derivative of the fractional release curve, and c_t and c_0 are the drug concentrations in the bulk solution and within the capsules, respectively. We assumed that 1) the microcapsules were cubic, with a mean size of $15 \times 15 \times 15\ \mu m^3$, 2) c_0 was equal to the saturation solubility of ibuprofen in the buffer solution, and 3) the average thickness of an HSA/DMPA capsule was 10 nm (as confirmed by the single-particle light scattering experiment[12]). By using Equation (1) we calculated the permeability coefficients for the capsules with five ($(HSA/DMPA)_5$) and ten ($(HSA/DMPA)_{10}$) layer pairs to be 3.7×10^{-10} and $2.0 \times 10^{-10}\ cm^2 s^{-1}$, respectively. These values are indeed a factor of 10 lower than those for polyelectrolyte capsules with the same number of layers ($(PSS/PAH)_5$, $7.6 \times 10^{-9}\ cm^2 s^{-1}$; PSS = polystyrenesulfonate, PAH = poly(allylamine hydrochloride)).[13] Furthermore, the permeability decreases with the increase in capsule thickness. This result is consistent with that obtained from measuring the half-time of drug release.

Conclusion

HSA and DMPA can be applied successfully as a pair to encapsulate ibuprofen microcrystals by using a method based on the LbL assembly technique. In a simulated intestinal fluid, the rate of release of the ibuprofen crystals from the HSA/DMPA capsules can be clearly delayed. The permeability of the microcapsules can be controlled by simply varying the number of HSA/DMPA deposition cycles. The retardation effect can be increased by several orders of magnitude by decreasing the pH of the experimental conditions. The bilayer structure formed on the HSA surface may provide a means to incorporate membrane-specific components, such as channels and receptors, to improve targeted drug delivery and recognition.

Experimental Section

Materials: Ibuprofen powder (crystals) was purchased from TCI (Japan). Fluorescein isothiocyanate conjugated human serum albumin (FITC-HSA), human serum albumin (HSA, lyophilized powder protein ca. 95% by biuret 66500 Da), fluorescently labeled nitrobenzoxadiazole dipalmitoyl phosphatidylcholine (NBD-DPPC), and L-α-dimyristoylphosphatidic acid (DMPA) were purchased from Sigma. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus purification system and had a resistivity greater than 18.2 MΩ cm.

An HSA solution was prepared by directly dissolving the weighed amount of HSA powder in a buffer solution of pH 3.8. The aqueous DMPA solution was prepared as follows: DMPA was dissolved in a solution of chloroform/methanol (1:1). The solvent was evaporated in a rotavap at 30 °C, and water was added up to a final lipid concentration of 0.025 mg mL^{-1}. The aqueous lipid solution was then sonicated for 5 min.

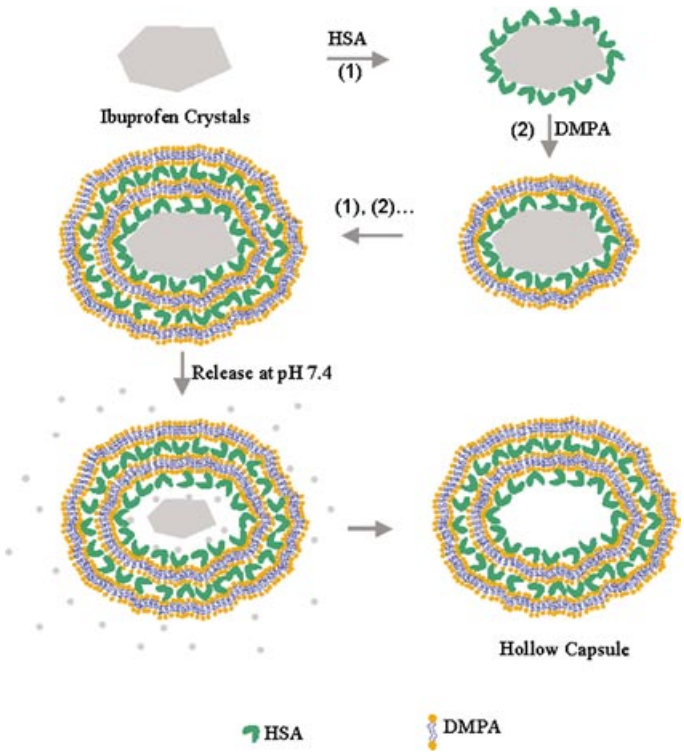
The ibuprofen powder as received is composed of polydispersed white crystals. This was finely ground with an agate mortar and the ground crystals were fractionated by sedimentation into three fractions. The mean crystal size and size distribution were estimated by measuring more than 100 crystals, according to the method proposed by Jolivet and co-workers.[14] The mean crystal size D, standard deviation $\sigma(D)$, and the polydispersity index p of the fractions are listed in Table 1.

Table 1. Characterization of the fractionated ibuprofen crystals.

Fraction	D [μm^{-1}]	$\sigma(D)$	p
first	60	9.0	0.15
second	36	5.5	0.15
third	15	3.0	0.20

Encapsulation: HSA/DMPA multilayer encapsulation of ibuprofen crystals was accomplished by the consecutive adsorption of oppositely charged HSA and DMPA using the centrifugation technique. In each experiment, ibuprofen crystals (50 mg) were dispersed in 2 mL of HSA solution (1 mg mL^{-1}, pH 3.8). The HSA was left to adsorb for 2 h, with occasional shaking. The dispersion was then centrifuged at room temperature. The supernatant was removed carefully and the crystals were redispersed in pure water. The centrifugation and resuspension procedure was repeated three times to remove the unadsorbed HSA from the solution before the next lipid solution was added. The above process was repeated until the desired number of coating layers was reached (Scheme 1).

Release experiments: The process of drug release was performed in a simulated intestinal fluid (pH 7.4) prepared with phosphate buffer (0.02 M). The release measurements were performed on an Agilent-8453 UV/Visible spectrophotometer. The release of ibuprofen from protein and lipid multilayer capsules was conducted in a 1-cm quartz cuvette with constant magnetic stirring, and was monitored by using UV spectroscopy at a wavelength of 260 nm. All release measurements were conducted at room temperature.



Scheme 1. Schematic illustration of drug crystals encapsulated by HSA/DMPA multilayers, followed by drug release at pH 7.4.

Optical microscopy: The microscopic images and the release process of individual drug crystals were recorded by using an Olympus AX-70 equipped with a CCD camera.

Acknowledgements

We thank H. Zastrow for technical discussions and A. Heilig for assistance with microscopy. This work was financially supported by the National Nature Science Foundation of China (NNSFC29925307 and NNSFC90206035), as well as the collaborated project of the German Max Planck Society.

[1] K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, *Chem. Rev.* **1999**, *99*, 3181–3198.
[2] a) R. Langer, *Nature* **2001**, *293*, 58–59; b) V. P. Torchilin, V. S. Trubetskoy, *Adv. Drug Delivery Rev.* **1995**, *16*, 141–155; c) J. T. Santin, M. J. Cima, R. Langer, *Nature* **1999**, *397*, 335–338.
[3] a) N. Murthy, Y. X. Thng, S. Schuck, M. C. Xu, J. M. Fréchet, *J. Am. Chem. Soc.* **2002**, *124*, 12398–12399; b) M. Delgado, C. Spanka, L. D. Kerwin, P. Wentworth, K. D. Jandak, *Biomacromolecules* **2002**, *3*, 262–271.
[4] a) G. Decher, *Science* **1997**, *277*, 1232–1237; b) E. Donath, G. Sukhorukov, F. Caruso, S. Davis, H. Möhwald, *Angew. Chem.* **1998**, *110*, 2323–2327; *Angew. Chem. Int. Ed.* **1998**, *37*, 2201–2205.
[5] a) G. Ibarz, L. Dähne, E. Donath, H. Möhwald, *Adv. Mater.* **2001**, *13*, 1324–1327; b) A. A. Antipov, G. B. Sukhorukov, H. Möhwald, *Langmuir* **2003**, *19*, 2444–2448; c) G. B. Sukhorukov, A. A. Antipov, A. Voigt, E. Donath, H. Möhwald, *Macromol. Rapid Commun.* **2001**, *22*, 44–46.
[6] X. P. Qiu, S. Leporatti, E. Donath, H. Möhwald, *Langmuir* **2001**, *17*, 5375–5380.

[7] a) X. M. He, D. C. Carter, *Nature* **1992**, *358*, 209–214; b) D. C. Carter, X. M. He, S. H. Munson, P. D. Twigg, K. M. Gernert, M. B. Broom, T. Y. Miller, *Science* **1989**, *244*, 1195–1198; c) M. A. N. Coelho, E. P. Vieira, H. Motschmann, H. Möhwald, A. F. Thunemann, *Langmuir* **2003**, *19*, 7544–7550.
[8] a) J. B. Li, Y. Zhang, L. L. Yan, *Angew. Chem.* **2001**, *113*, 915–918; *Angew. Chem. Int. Ed.* **2001**, *40*, 891–894; b) G. Lu, H. Chen, J. Li, *Colloids Surf. A* **2003**, *215*, 25–32; c) E. J. Choi, M. D. Foster, *Langmuir* **2002**, *18*, 557–561; d) N. V. Efremova, B. Bondurant, D. F. O'Brien, D. E. Leckband, *Biochemistry* **2000**, *39*, 3441–3451; e) G. Lu, Z. H. An, J. B. Li, *Biochem. Biophys. Res. Commun.* **2004**, *315*, 224–227.
[9] A. Avdeef, C. M. Berger, C. Brownell, *Pharm. Res.* **2000**, *17*, 85–89.
[10] J. R. Olivieri, A. F. Craievich, *Eur. Biophys. J.* **1995**, *24*, 77–84.
[11] T. Jimbo, P. Ramirez, A. Tanioka, S. Mafe, N. Minoura, *J. Colloid Interface Sci.* **2000**, *225*, 447–454.
[12] Z. H. An, C. Tao, G. Lu, H. Möhwald, J. B. Li, *Chem. Mater.* **2004**, unpublished results.
[13] X. P. Qiu, E. Donath, H. Möhwald, *Macromol. Mater. Eng.* **2001**, *286*, 591–597.
[14] L. Vayssieres, C. Chaneac, E. Tronc, J. P. Jolivet, *J. Colloid Interface Sci.* **1998**, *205*, 205–212.

Received: January 27, 2004
Revised: August 3, 2004
Published online: October 13, 2004