Journal of Experimental Nanoscience
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t716100757

Microcapsules for controlled release fabricated via layer-by-layer self-assembly of polyelectrolytes

Chaoyang Wang[a]; Shiqu Ye[a]; Qilong Sun[a]; Chengyi He[a]; Weihua Ye[a]; Xinxing Liu[a]; Zhen Tong[a]
[a] Research Institute of Materials Science, South China University of Technology, Guangzhou 510640, China

To cite this Article Wang, Chaoyang , Ye, Shiqu , Sun, Qilong , He, Chengyi , Ye, Weihua , Liu, Xinxing and Tong, Zhen(2008) 'Microcapsules for controlled release fabricated via layer-by-layer self-assembly of polyelectrolytes', Journal of Experimental Nanoscience, 3: 2, 133 — 145
To link to this Article: DOI: 10.1080/08927020802036005
URL: http://dx.doi.org/10.1080/08927020802036005

Journal of Experimental Nanoscience
Vol. 3, No. 2, June 2008, 133–145

Taylor & Francis
Taylor & Francis Group

Microcapsules for controlled release fabricated via layer-by-layer self-assembly of polyelectrolytes

Chaoyang Wang, Shiqu Ye, Qilong Sun, Chengyi He, Weihua Ye, Xinxing Liu and Zhen Tong*

Research Institute of Materials Science, South China University of Technology, Guangzhou 510640, China

(*Received 30 June 2007; final version received 5 March 2008*)

Layer-by-layer (LbL) self-assembly of oppositely charged polyelectrolytes on different templates by alternating deposition is a versatile method enabling the construction of ultrathin multilayer films with tunable thickness, composition, and functions. The principal driving force for the LbL self-assembly is dominantly the electrostatic attraction between the polyelectrolyte components accompanied with other associative interactions, such as hydrogen-bonding, hydrophobic interaction, charge-transfer and so on. The LbL self-assembly technique has been a powerful tool for micro/nano-encapsulation. Our strategy is to fabricate the nano-multilayer wall for micro- and sub-microcapsules by the LbL of polyelectrolytes, particularly natural polymers chitosan (CHI) and alginate (ALG) for drug controlled release. Our recent work following this strategy is reviewed in the present article. After determining the charge density threshold for the LbL assembly, we immobilised enzymes of urease and superoxide dismutase on polystyrene nanoparticles through the LbL and found the decrease in enzyme bioactivity but an increase in their storage stability. We successfully fabricated the nanocapsules from natural polysaccharides of CHI and ALG multilayers by the LbL for drug release. The LbL self-assembly of CHI and ALG was used directly on indomethacin (IDM) microcrystals to reduce the release rate. We observed that increasing deposition temperature would produce a more perfect multilayer film with higher thickness and reduced the release rate efficiently. Water soluble protein insulin was spontaneously loaded into the LbL CHI/ALG microcapsules due to the electrostatic attraction and a two-temperature loading procedure was suggested to increase the loading capacity and to reduce the release rate. The LbL multilayers have been used to encapsulate the drug-loading microparticles made from solvent evaporation or adsorption with porous $CaCO_3$ microparticles to enhance the loading capacity and suppress the initial burst. Increasing the layer number, raising the deposition temperature, and cross-linking the neighboring layers were confirmed to slow down the enzymatic desorption of polyelectrolyte multilayer films and the release rate of encapsulated drug effectively.

Keywords: layer-by-layer self-assembly; microcapsules; controlled release; nano-multilayer walls

*Corresponding author. Email: mcztong@scut.edu.cn

ISSN 1745–8080 print/ISSN 1745–8099 online

DOI: 10.1080/08927020802036005
http://www.informaworld.com

1. Introduction

In 1966, Iler [1] described the formation of layers of the charged particles by adsorption from solution. Films comprising alumina fibrils, (positively charged, 5–6 nm in diameter), and silica colloids (negatively charged, 100 nm diameter) were prepared on hydrophilic glass surfaces with thicknesses ranging from 50 to 500 nm. Following the pioneering work of Decher in the early 1990s, surface modification via the layer-by-layer (LbL) solution deposition technique gained momentum, and was expanded to include sequential assembly of oppositely charged polymers (or biopolymers) and nanoparticles to produce polyelectrolyte multilayer thin films as a coating [2,3]. The stepwise growth of the multilayer film is driven by ion pair formation between oppositely charged polyelectrolyte segments, stabilising the evolving amorphous nanocomposite structures. Ion pairing is largely athermal, driven by increased entropy from the release of salt counter ions originally neutralising the polyelectrolytes in solution [4]. In the last decade, LbL self-assembly technique was developed as a powerful method for the nano- and micro-encapsulation [4–6], where polyelectrolyte multilayer films were elaborated on various particles through alternating deposition of oppositely charged polyelectrolytes due to their electrostatic attraction accompanied with other associative interactions, such as hydrogen-bonding, hydrophobic interaction, charge-transfer and so on [7–12].

Efficient micro-encapsulation of active ingredients, such as drugs, proteins, vitamins, flavours, gas bubbles, even living cells, is becoming increasingly important for a wide variety of applications from functional foods to drug delivery in biomedical applications [13–16]. Nano- and micro-encapsulation via LbL self-assembly has potential applications in biochemistry, pharmaceutics, controlled release, cosmetic, and catalyst [4–6]. The first approach directly used proteins, for instance, as the depositing species to prepare bioactive core-shell particles [17–19]. The second approach involved direct coverage of drug microcrystals, such as ibuprofen (IBU) [20,21], furosemide [22], vitamin K_3 [23], insulin [23], dexamethasone [24,25], and indomethacin [26–28] with polyelectrolyte multilayer films for prolonged release. The third approach demonstrated fabricating hollow microcapsules with polyelectrolyte multilayer walls by removing the template cores and loading drugs, enzymes, and proteins into the capsules for delivery [29–32]. The fourth approach was to encapsulate the drug-loaded nano- or microparticles with polyelectrolyte multilayer films to suppress the initial burst [33,34].

2. Charge density threshold for LbL self-assembly

As the starting point, we investigated the charge density effect on the film growth and permeability to small molecules taking the merit of high sensitivity of fluorescence labels. Polyelectrolyte multilayer films were prepared via the LbL self-assembly using poly(diallydimethylammonium chloride) (PDADMAC) and pyrene labelled polyanions of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and *N*,*N*-dimethylacrylamide copolymers with different AMPS mole fraction $F_{AMPS} = 0.20$–0.999. Multilayer growth with deposition from polyelectrolyte solutions was monitored by fluorescence intensity and film thickness (Figure 1), showing a charge density (represented by F_{AMPS}) threshold between 0.574 (ADPy-30) and 0.711 (ADPy-45) for our polyanion, below which the multilayer cannot be formed due to desorption in the following depositions. For the fully charged polyanion, thickness of the multilayer film increased with increasing NaCl

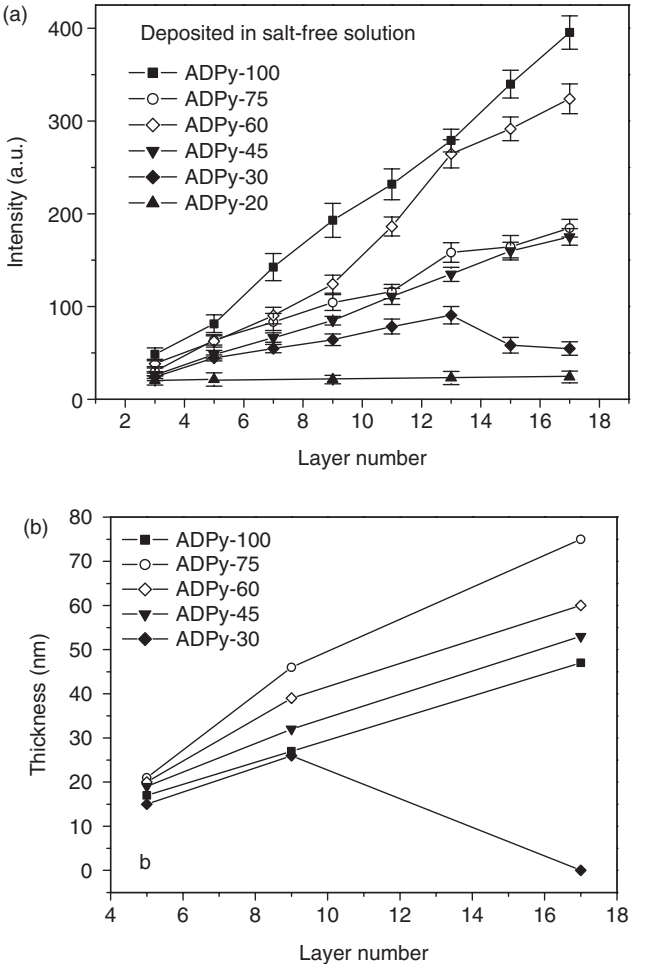

Figure 1. Fluorescence intensity (a) and thickness (b) of multilayer films made of indicated ADPy with different AMPS mole fraction and PDADMAC as a function of the layer number.

concentration in the deposition solution; while for other polyanions with lower F_{AMPS}, little growth in multilayer films was found when the polyelectrolyte solutions contained NaCl of 0.02 mol L^{-1} or higher. The quenching rate of nitromethane to the pyrene label in the multilayer film was adopted to detect the permeability of these films. Decreasing the charge density, increasing the salt concentration in deposition solutions, and reducing the layer number accelerated the quenching. The former two factors are due to the looser structure in the multilayer films, while the last factor is mainly due to the reduction of multilayer barrier capacity.

3. Bioactive nanoparticles fabricated by LbL

A novel core-shell colloid with multilayer for biocatalysis was elaborated by the LbL assembly technique. Urease was adsorbed in alternation with the oppositely charged

polyelectrolytes onto polystyrene (PS) colloid nanoparticles as either polycation or polyanion switched by the solution pH. Microelectrophoresis, transmission electron microscopy (TEM), and UV–Vis spectrum absorbance were employed to monitor the regular and stepwise growth of the multilayer films with the enzyme and counterpart polyelectrolytes. The colloid nanoparticles coated with negatively charged urease were found to be more stable than those coated with positively charged urease. The catalytic activity of the urease immobilised on the PS nanoparticles, having higher storage stability, was 23.67% of that for the free urease in aqueous solution. Addition of 0.05 M NaCl increased the activity of the immobilised urease by 65%. Coverage of synthetic polyelectrolyte layers on the urease layer reduced the activity of the immobilised urease. Therefore, by adding salts or covering with polyelectrolytes, we have achieved an enhancement or restraint of the bioactivity of immobilised enzyme, indicating a novel method for biotechnology.

Novel enzyme multilayer films on the surfaces of polystyrene (PS) colloid particles were fabricated by LbL self-assembly. Superoxide dismutase (SOD) was adsorbed on the PS particles as either polycation or polyanion switched by adjusting pH alternatingly with the oppositely charged polyelectrolytes. Zeta-potential and TEM results indicated the regular and stepwise growth of the multilayer structure. The amount of the immobilised SOD was estimated from the difference in SOD bioactivity of the supernatant after adsorption and SOD solution before adsorption by using the pyrogallol oxidation method. The immobilisation amount of SOD was 12 and 51 IU when adsorbed in pH = 8.0 as a polyanion and in pH = 4.3 as a polycation, respectively. However, the relative activity of the former was 23.4% while that of the latter was 2.9%, compared to that of free SOD in aqueous solution. Anionic SOD was found to form more regular and smooth layers on the PS particle surface and cationic SOD to aggregate. By adjusting pH of the adsorption solution we can optimise the assembled status and bioactivity of particle-immobilised enzyme.

4. Biocompatible nanocapsules fabricated by LbL

Polysaccharide multilayer nanocapsules were fabricated in aqueous media by LbL self-assembly of chitosan (CHI) and alginate (ALG) on monodisperse polystyrene (PS) nanoparticles with a diameter of 180 nm as the template, followed by removal of the templates through exposed to THF (Figure 2). The pH and added salt concentration in the polyelectrolyte deposition solutions were optimised to ensure the alternating deposition. Consequently, the most suitable pH values were found to be 6.0–8.0 for ALG and 3.5 for CHI. The NaCl concentration in the adsorption solutions was 0.5 M, which led to an average thickness of about 13 nm for five bilayers of CHI/ALG shell-wall. ζ-potential indicated the stepwise and alternating adsorption of CHI and ALG to form multilayer film on the PS nanoparticles. The characteristic bands of PS residue almost disappeared in the IR spectrum of the nanocapsule after dipped in THF, confirming thorough removal of PS templates. TEM, scanning electron microscope (SEM), and atomic force microscopy (AFM) were utilised to observe the nanocapsules of ~225 nm in diameter. A hydrophilic drug model, acridine hydrochloride (AH), was chosen to investigate the loading and release properties of the nanocapsules. The positively charged AH spontaneously deposited into the capsule due to the electrostatic interaction with the negatively charged

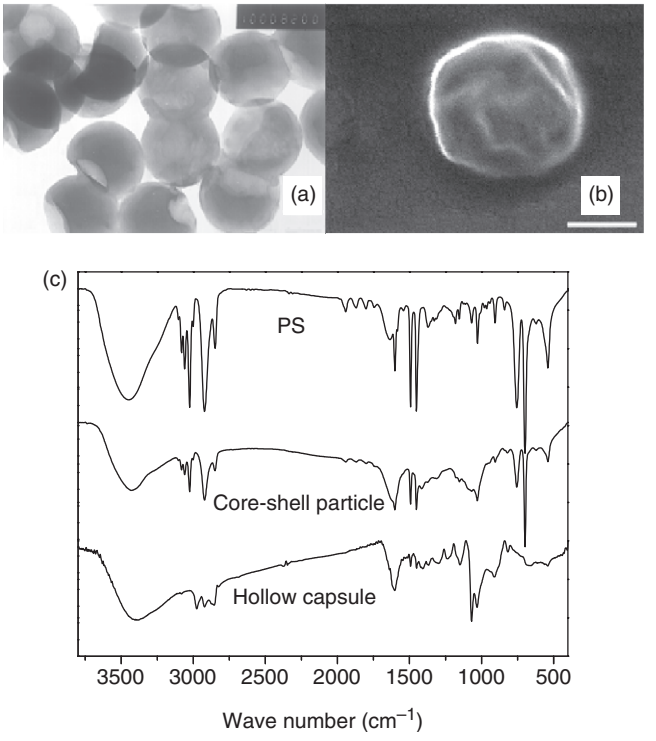

Figure 2. TEM (a) and SEM (b) images of hollow nanocapsules, the scale bars correspond to 100 nm. (c) IR spectra of PS nanoparticles, core-shell particles with five bilayers of CHI/ALG and the hollow nanocapsules. The characteristic bands for PS disappeared from the capsule spectrum.

styrene sulfonate residues from the PS template inside the capsule (Figure 3). The rate of AH release became slightly slower when the capsule wall was cross-linked with glutaraldehyde, but the accumulative released amount for the crosslinked capsule was obviously reduced. These nanocapsules made from nature polysaccharides have a potential application in controlled drug release.

5. Direct microcapsulation of drug microcrystals by LbL

Indomethacin (IDM) microcrystals sized 5–10 μm were directly encapsulated with natural polysaccharides CHI and ALG through the LbL self-assembly. Due to partial dissolution of IDM in the deposition solution, the retention of the IDM microcrystals gradually decreased with increasing deposition times and became 47.7% as 10 layers of polysaccharides formed. The release rate of the IDM from the microcapsules was monitored with UV absorbance. The half release time $t_{1/2}$ of IDM in the microcapsule increased with the layer number and the initial burst was reduced after encapsulation. It was found that added NaCl concentration even up to 0.5 M did not affect the release rate, while increasing the release temperature remarkably speeded up the release process. The prolonged release of the encapsulated IDM was still observed when the aqueous release solution contain 20 vol% ethanol. It was very interesting that increasing deposition

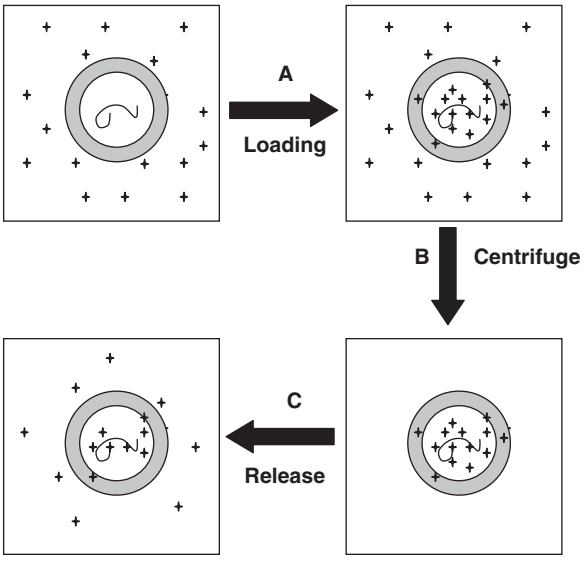

Figure 3. Schematic representation for the loading and release of AH by the nanocapsules. The positively charged AH penetrates through the capsule wall and spontaneously deposits into the capsule with the negatively charged PS co-polymer with SS units inside (step A). The loaded AH cannot be completely released from the capsules duo to the electrostatic attraction between AH and the PS co-polymers (step C).

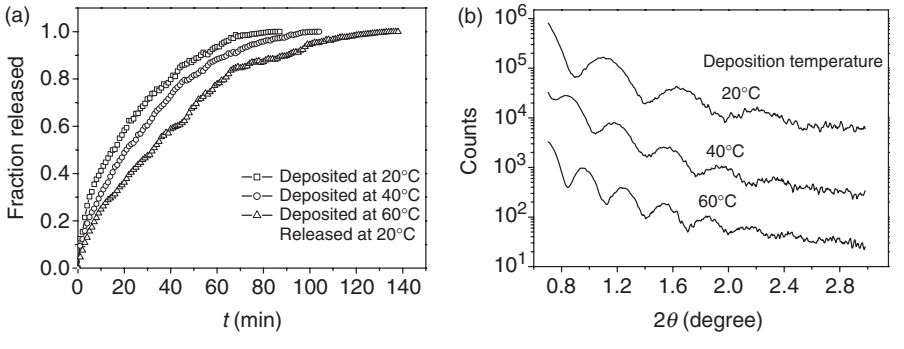

Figure 4. (a) Release profiles of IDM from $(ALG/CHI)_5$ microcapsules in pH 7.4 buffer at 20°C with indicated deposition temperatures; (b) SAXS profiles of the five-bilayer ALG/CHI films deposited on quartz slide at indicated temperatures to determine the film thickness. The data were vertically shifted to avoid overlapping.

temperature from 20°C to 60°C reduced the release rate efficiently, owing to the increase in multilayer thickness and formation of a more perfect multilayer film (Figure 4). The small-angle X-ray scattering (SAXS) profiles indicated that the thickness for the multilayer films deposited at 20°C, 40°C, and 60°C, was 16.3, 24.2, and 32.1 nm, respectively, with increased perfectness. This finding provides a simple method to control the permeability of the LbL assembled multilayer films.

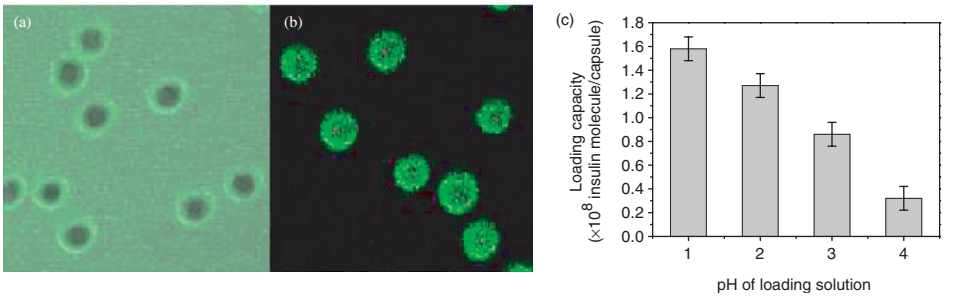

Figure 5. CLSM photos showing the FITC-insulin loaded into the ALG/CHI microcapsules at pH = 8.0 with negatively charged insulin (a) and pH = 3.0 with positively charged insulin after rinse (b); (c) the loading capacity of the ALG/CHI microcapsules as a function of pH of the insulin bulk solution.

6. Loading and release of insulin for polysaccharide multiplayer microcapsules

Polysaccharide multilayer microcapsules were fabricated in aqueous media by the LbL self-assembly of CHI and sodium ALG on melamine formaldehyde (MF) microparticles of 2.1 μm diameter as templates, followed by removal of the templates by dissolution at low pH. The loading process was observed with the confocal laser scattering microscope (CLSM) using fluorescence labelled insulin. Insulin was spontaneously loaded into the ALG/CHI microcapsules at pH below its isoelectric point of 5.5, where insulin was positively charged and the loading capacity increased with pH decreasing from 4.0 to 1.0 (Figure 5) [31]. The reason for this spontaneous loading was the electrostatic attraction between positive insulin and negatively charged complex of ALG/MF residues inside the microcapsule, which was formed during the MF particle dissolution. A novel two-temperature loading procedure was proposed as loading at 20°C for the first hour and at a higher loading temperature for the second hour. The loading insulin at 20°C for 1 h was sufficient to reach the loading equilibrium; the second hour loading at a high temperature produced a more perfect multilayer shell and reduced the loss of loaded insulin during the rinse. This procedure was very significant so that increasing the second loading temperature from 20°C to 60°C not only increased the insulin loading capacity, but also slowed down its release rate (Figure 6). The release rate of insulin at pH 7.4 was found much faster than that at pH 1.4 due to the negative charge on the insulin. Cross-linking the ALG in the microcapsule shell with calcium ions (Ca^{2+}) or re-sealing the microcapsules with additional layers also remarkably decreased the insulin release rate. The results provide a simple method to control the loading and release of charged water-soluble molecules with the polysaccharide microcapsules.

7. Combination of solvent evaporation and LbL for drug-loaded capsules

The initial burst release of drug from microparticles remains an unsolved problem. Here, we deposited polysaccharides on drug-loaded microspheres using the LbL self-assembly to produce core-shell microparticles for sustained drug release [34]. The ibuprofen (IBU)-loaded poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) microparticles were

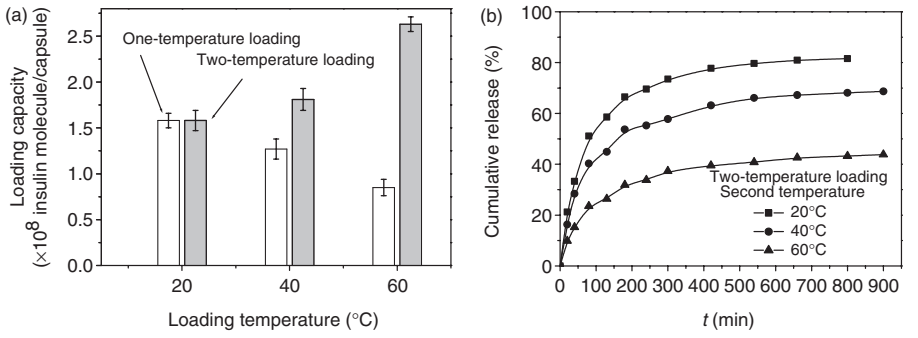

Figure 6. (a) The loading capacity of the ALG/CHI microcapsules as a function of loading temperature at pH 10. The white columns represent the one-temperature loading and the gray columns represent two-temperature loading at the shown second loading temperature; (b) release profiles of insulin from $(ALG/CHI)_5$ microcapsules loaded with two-temperature loading at indicated second loading temperature.

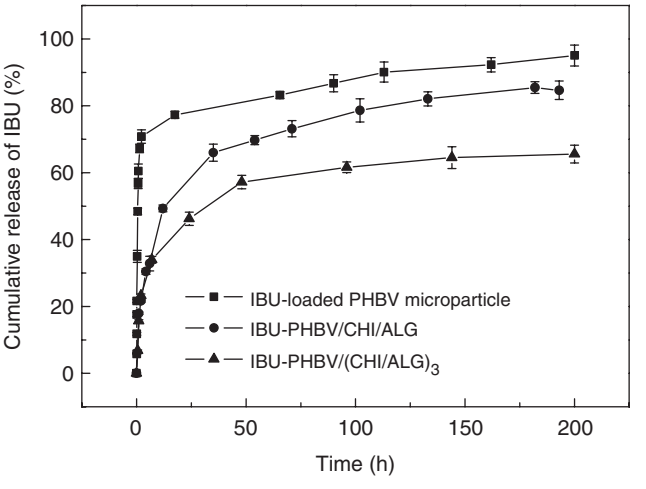

Figure 7. Release profiles of the IBU-loaded PHBV microparticle coated with multilayer films in pH 7.4 PBS at 37°C.

fabricated by conventional solvent evaporation. The processing parameters, such as pH of water phase, drug/polymer ratio, polymer type, and emulsifier concentration, were optimised according to the encapsulation efficiency and drug loading as pH = 4.0, drug/polymer ratio = 10/50 wt%, hydroxyvalerate (HV) in PHBV = 6 wt%, and PVA concentration = 1 w/v%. The multilayer shells of CHI/sodium ALG and were formed on the IBU-loaded PHBV microparticles using the LbL self-assembly. The *in vitro* release experiments revealed that, as for the microparticles with three CHI/ALG bilayers, the initial burst release of IBU from the microparticles was significantly suppressed and the half release time was prolonged from 1 h for the bare microparticles without coverage to 62 h for the microparticles with three CHI/ALG bilayers (Figure 7). The compact CHI/ALG multilayer film was observed with an AFM due to the matched distance of

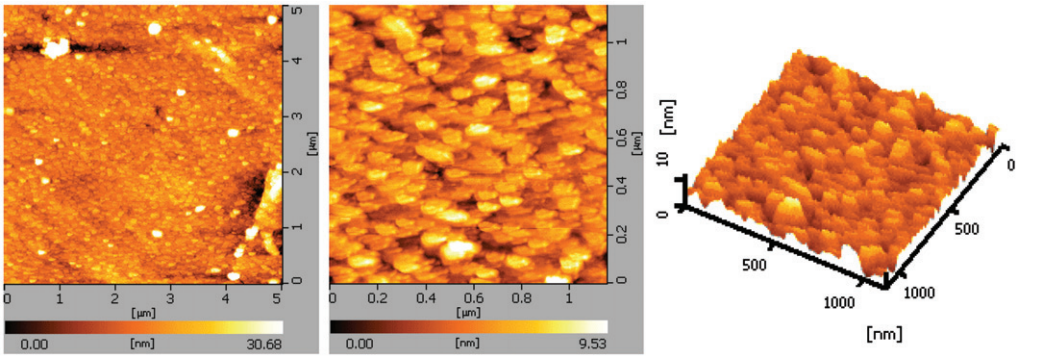

Figure 8. AFM images of the $(CHI/ALG)_3$ film on quartz slide.

charges along the CHI chain and those along the ALG chains (Figure 8). The present combination for encapsulating drug-loaded microparticles demonstrates an effective way to prolong the drug release with reduced initial burst.

8. Combination of adsorption by porous $CaCO_3$ microparticle and encapsulation by polyelectrolyte multilayer films for sustained drug delivery

Combination of adsorption by porous $CaCO_3$ microparticles and encapsulation by polyelectrolyte multilayers via the LbL self-assembly was proposed for sustained drug release [33]. First, porous calcium carbonate microparticles with an average diameter of 5 μm were prepared for loading IBU as a model drug. Adsorption of IBU into the pores was characterised by ultraviolet, infrared, thermogravimetric analysis, BET experiment, and X-ray diffraction. The adsorbed IBU amount Γ was 45.1 mg g^{-1} for one-time adsorption and increased with increasing adsorption times. Finally, multilayer films of protamine sulfate (PRO) and sodium poly(styrene sulfonate) (PSS) were formed on the IBU-loaded $CaCO_3$ microparticles by the LbL self-assembly. Amorphous IBU loaded in the pores of the $CaCO_3$ microparticles had a rapider release in the gastric fluid and a slower release in the intestinal fluid, compared with the bare IBU microcrystals. Polyelectrolyte multilayer assembled on the drug-loaded particles by the LbL reduced the release rate in the both fluids (Figure 9). The porous inorganic particles are useful to increase loading capacity and the polyelectrolyte multilayer films coated on the particle assuage the initial burst release.

9. Enhanced resistance of polyelectrolyte multilayer microcapsules to pepsin erosion and release properties of encapsulated indomethacin

The ALG and CHI multilayer films deposited directly on IDM microcrystals through the LbL self-assembly were partially destroyed after incubation in an enzyme pepsin solution due to the enzymatic degradation of CHI. After pepsin erosion, the IDM release from the microcapsules monitored by UV absorbance was obviously accelerated due to desorption (Figure 10). In order to enhance the stability of the ALG/CHI multilayer film to the enzymatic erosion, some physical and chemical methods were established to increase film thickness or to cross-link the polysaccharides within the film [28]. Increasing the

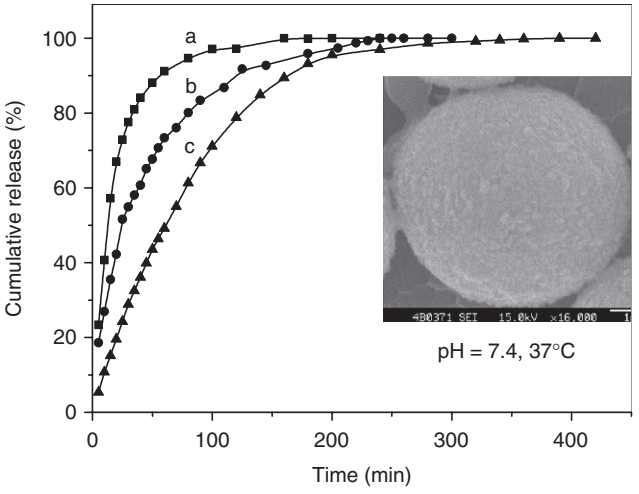

Figure 9. Release profiles of (a) bare IBU microcrystals, (b) IBU-loaded $CaCO_3$ microparticles and (c) IBU-loaded microcapsules with 5 bilayers of PRO/PSS in simulated intestinal fluid (pH 7.4) at 37°C. The insert is SEM image of the IBU-loaded microcapsules coated with five bilayers of PRO/PSS.

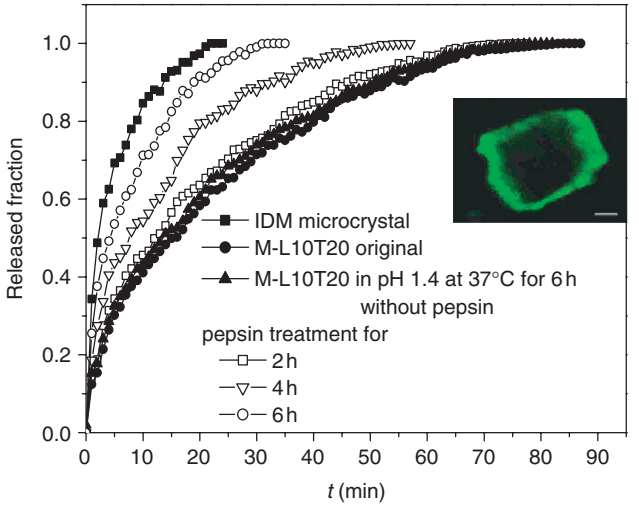

Figure 10. IDM release profiles from microcapsule M-L10T20 after pepsin treatment for indicated times compared with that from the same M-L10T20 after immersed in an acid solution of pH 1.4 at 37°C for 6 h without pepsin. The inset is CLSM image of M-L10T20 microcapsule and the scale bar is 2 μm. The IDM microcapsules with shells of $(ALG/CHI)_5$ deposited at 20°C was referred to as M-L10T20.

layer number and raising the deposition temperature effectively slowed down the enzymatic desorption and release rate. Especially, increasing deposition temperature was more effective due to producing a more perfect structure in the ALG/CHI multilayer film. Cross-linking the neighboring layers of ALG and CHI with

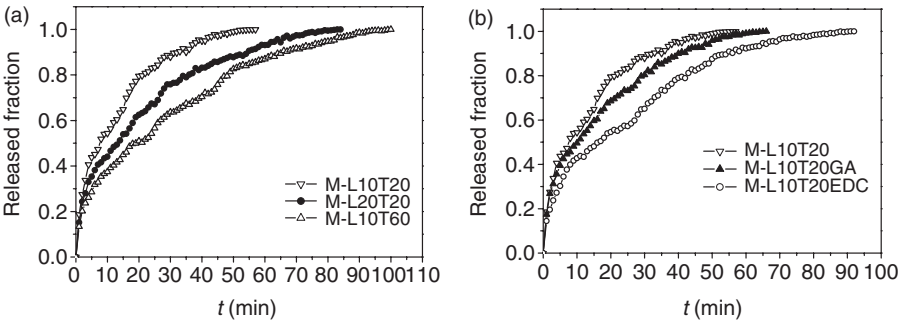

Figure 11. (a) IDM release profiles from microcapsules M-L10T20, M-L20T20, and M-L10T60 after 4 h pepsin treatment; (b) IDM release profiles from microcapsules M-L10T20, M-L10T20GA, and M-L10T20EDC after 4 h pepsin treatment. The IDM microcapsules with shells of $(ALG/CHI)_{10}$ deposited at 20°C and $(ALG/CHI)_5$ deposited at 60°C were referred to as M-L20T20 and M-L10T60, respectively. Cross-linked M-L10T20 with GA and EDC were referred to as M-L10T20GA and M-L10T20EDC, respectively.

1-ethyl-3-(3-dimethylamino-propyl)carbodiimide in the ALG/CHI multilayer film significantly reduced the enzymatic desorption and release rate (Figure 11). Therefore, increasing deposition temperature and cross-linking neighboring layers are effective methods to protect the multilayer film fabricated using LbL assembly from the enzymatic erosion and to prolong the release of the encapsulated drug.

10. Outlook

The LbL self-assembly technique has become a powerful method for micro/nano-encapsulation. Using polyelectrolyte multilayer films to coat drug can simply reduce the release rate and assuage the initial burst release, which has been demonstrated in the above examples. However, LbL is a time-consuming procedure with low loading efficiency, which is a big disadvantage for industrial applications. How to overcome this problem is one of most important topics in this area. On the other hand, the stability and metabolisability of the polyelectrolyte multilayers fabricated by the LbL in the physiological environment still requires further investigation in the near future.

Acknowledgments

The financial support to this work by the NSF of China (No: 20534020, 20574023, and 90206010) NCET-07-0306 and the Scientific and Technologic Program of Guangzhou Municipality (2007J1-C0351) is gratefully acknowledged.

References

[1] R.K. Iler, *Multilayers of colloidal particles*, J. Colloid Interface Sci. 21 (1966), p. 569.
[2] G. Decher and J.D. Hong, *Build-up of ultrathin multilayer films by a self-assembly process, I. Consecutive adsorption of anionic and cationic bipolar amphiphiles on charged surfaces*, Makromol. Chem. Macromol. Symp. 46 (1991), p. 321.

[3] G. Decher and J. Schmitt, *Fine-tuning of the film thickness of ultrathin multilayer films composed of consequently alternating layers of anionic and cationic polyelectrolyte*, Prog. Colloid Polym. Sci. 89 (1992), p. 160.
[4] G. Decher and J.B. Schlenoff (eds.). *Multilayer Thin Films: Sequential Assembly of Nanocomposite Material*, Wiley-VHC, Weinheim, 2003.
[5] C.S. Peyratout and L. Dahne, *Tailor-made polyelectrolyte microcapsules: from multilayers to smart containers*, Angew. Chem. Int. Ed. 43 (2004), p. 3762.
[6] Z.P. Liang, C.Y. Wang, Q.L. Sun, and Z. Tong, *Novel microcapsule fabricated by LbL nano self-assembly*, Prog. Chem. 16 (2004), p. 485.
[7] G. Decher, *Fuzzy nanoassemblies: towards layered polymeric multicomposites*, Science 277 (1997), p. 1232.
[8] F. Caruso, R.A. Caruso, and H. Möhwald, *Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating*, Science 282 (1998), p. 1111.
[9] P.T. Hammond, *Recent explorations in electrostatic multilayer thin film assembly*, Curr. Opin. Colloid Interface Sci. 4 (2000), p. 430.
[10] M. Schönhoff, *Self-assembled polyelectrolyte multilayers*, Curr. Opin. Colloid Interface Sci. 8 (2003), p. 86.
[11] D.T. Haynie, L. Zhang, J.S. Rudra, W. Zhao, Y. Zhong, and N. Palath, *Polypeptide multilayer films*, Biomacromolecules 6 (2005), p. 2895.
[12] Q.L. Sun, Z. Tong, C.Y. Wang, B.Y. Ren, X.X. Liu, and F. Zeng, *Charge density threshold for LbL self-assembly and small molecule diffusion in polyelectrolyte multilayer films*, Polymer 46 (2005), p. 4958.
[13] R.P. Lanza, R. Langer, and J. Vacanti, *Principles of Tissue Engineering*, Academic Press, San Diego, 2000.
[14] S.R. Bhatia, S.F. Khattak, and S.C. Roberts, *Polyelectrolytes for cell encapsulation*, Curr. Opin. Colloid Interface Sci. 10 (2005), p. 45.
[15] C.Y. Wang, H.X. Liu, Q.X. Gao, X.X. Liu, and Z. Tong, *Facile fabrication of hybrid colloidosomes with alginate gel cores and shells of porous CaCO3 microparticles*, Chem. Phys. Chem. 8 (2007), p. 1157.
[16] C.Y. Wang, S.S. Liu, X.X. Liu, and Z. Tong, *Multilayer shell walls with versatile electron transfer properties*, Macromol. Rapid Commun. 28 (2007), p. 1167.
[17] F. Caruso and H. Möhwald, *Protein multilayer formation on colloids through a stepwise self-assembly technique*, J. Am. Chem. Soc. 121 (1999), p. 6039.
[18] C.Y. He, Z.P. Liang, C.Y. Wang, X.X. Liu, and Z. Tong, *Immobilization of superoxide dismutase by layer-by-layer assembly on surface of PS colloid particles and their bioactivity*, Chem. J. Chinese U.– Chinese 26 (2005), p. 88.
[19] Z.P. Liang, C.Y. Wang, Z. Tong, W.H. Ye, and S.Q. Ye, *Bio-catalytic nanoparticles with urease immobilized in multilayer assembled through layer-by-layer technique*, React. Funct. Polym. 63 (2005), p. 85.
[20] X. Qiu, S. Leporatti, E. Donath, and H. Möhwald, *Studies on the drug release properties of polysaccharide multilayers encapsulated ibuprofen microparticles*, Langmuir 17 (2001), p. 5375.
[21] Z. An, G. Lu, H. Möhwald, and J. Li, *Self-assembly of human serum albumin (HSA) and L-a-dimyristoylphosphatidic acid (DMPA) microcapsules for controlled drug release*, Chem. Eur. J. 10 (2004), p. 5848.
[22] H. Ai, S.A. Jones, M.M. de Villiers, and Y.M. Lvov, *Nano-encapsulation of furosemide microcrystals for controlled drug release*, J. Cont. Release 86 (2003), p. 59.
[23] Z. Dai, A. Heilig, H. Zastrow, E. Donath, and H. Möhwald, *Novel formulations of vitamins and insulin by nanoengineering of polyelectrolyte multilayers around microcrystals*, Chem. Eur. J. 10 (2004), p. 6369.

[24] N. Pargaonkar, L.M. Lvov, N. Li, J.H. Steenekamp, and M.M. de Villiers, *Controlled release of dexamethasone from microcapsules produced by polyelectrolyte layer-by-layer nanoassembly*, Pharm. Res. 22 (2005), p. 826.
[25] A.S. Zahr, M. de Villiers, and M.V. Pishko, *Encapsulation of drug nanoparticles in self-assembled macromolecular nanoshells*, Langmuir 21 (2005), p. 403.
[26] Y. Chen and X. Lin, *Studies on the drug release properties of nanoencapsulated indomethacin microparticles*, J. Microencapsul. 22 (2005), p. 47.
[27] S.Q. Ye, C.Y. Wang, X.X. Liu, and Z. Tong, *Deposition temperature effect on release rate of indomethacin microcrystals from microcapsules of layer-by-layer assembled chitosan and alginate multilayer films*, J. Cont. Release 106 (2005), p. 319.
[28] C.Y. Wang, S.Q. Ye, L. Dai, X.X. Liu, and Z. Tong, *Enhanced resistance of polyelectrolyte multilayer microcapsules to pepsin erosion and release properties of encapsulated indomethacin*, Biomacromolecules 8 (2007), p. 1739.
[29] O.P. Tiourina and G.B. Sukhorukov, *Multilayer alginate/protamine microsized capsules: encapsulation of a-chymotrypsin and controlled release study*, Int. J. Pharm. 242 (2002), p. 155.
[30] Z.W. Mao, L. Ma, C.Y. Gao, and J.C. Shen, *Preformed microcapsules for loading and sustained release of ciprofloxacin hydrochloride*, J. Cont. Release 104 (2005), p. 193.
[31] S.Q. Ye, C.Y. Wang, X.X. Liu, Z. Tong, B.Y. Ren, and F. Zeng, *New loading process and release properties of insulin from polysaccharide microcapsules fabricated through layer-by-layer assembly*, J. Cont. Release 112 (2006), p. 79.
[32] S.Q. Ye, C.Y. Wang, X.X. Liu, and Z. Tong, *Multilayer nanocapsules of polysaccharide chitosan and alginate through layer-by-layer assembly directly on PS nanoparticles for release*, J. Biomater. Sci. Polym. Ed. 16 (2005), p. 909.
[33] C.Y. Wang, C.Y. He, Z. Tong, X.X. Liu, B.Y. Ren, and F. Zeng, *Combination of adsorption by porous $CaCO_3$ microparticles and encapsulation by polyelectrolyte multilayer films for sustained drug delivery*, Int. J. Pharm. 308 (2006), p. 160.
[34] C.Y. Wang, W.H. Ye, Y. Zheng, X.X. Liu, and Z. Tong, *Fabrication of drug-loaded biodegradable microcapsules for controlled release by combination of solvent evaporation and layer-by-layer self-assembly*, Int. J. Pharm. 338 (2007), p. 165.