Enhanced Resistance of Polyelectrolyte Multilayer Microcapsules to Pepsin Erosion and Release Properties of Encapsulated Indomethacin

Chaoyang Wang, Shiqu Ye, Lin Dai, Xinxing Liu, and Zhen Tong*

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

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Polyelectrolyte multilayer films were prepared through layer-by-layer (LbL) self-assembly using polysaccharide sodium alginate (ALG) and chitosan (CHI). After incubation in an enzyme pepsin solution, the multilayer film was partially destroyed as detected by the decrease in fluorescent intensity because of the enzymatic degradation of CHI. The enzymatic desorption was also observed from the microcapsule wall made of the ALG/CHI multilayer film directly deposited on indomethacin (IDM) microcrystals through LbL self-assembly. After pepsin erosion, the IDM release from the microcapsules monitored by UV absorbance was obviously accelerated because of desorption. 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. Increasing the layer number and raising the deposition temperature effectively slowed down the enzymatic desorption and release rate. Especially, increasing deposition temperature was more effective because of producing a more perfect structure in the ALG/CHI multilayer film. Cross-linking the neighboring layers of ALG and CHI with 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide in the ALG/CHI multilayer film significantly reduced the enzymatic desorption and release rate. 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.

Introduction

Efficient microencapsulation of active ingredients, such as drugs, proteins, vitamins, flavors, gas bubbles, and even living cells, is becoming increasingly important for a wide variety of applications from functional foods to drug delivery for biomedical applications.[1−4] In the past decade, layer-by-layer (LbL) self-assembly technique has been developed as a powerful method for nano- and microencapsulation,[5,6] where polyelectrolyte multilayer films were elaborated on various particles through alternating deposition of oppositely charged polyelectrolytes mainly driven by the electrostatic attraction.[7−12] Coverage with polyelectrolyte multilayer films on drug microcrystals can effectively control the diffusion rate of drug from the interior to outside. Thus, the LbL multilayer microcapsules have been intensively demonstrated for use in drug-controlled release. For example, Qiu et al. reported a prolonged release of ibuprofen microcrystals of 5−45 μm encapsulated directly with synthetic polyelectrolytes and polysaccharides.[13] An et al. encapsulated the same drug with human serum albumin and dimyristoylphosphatidic acid for sustained release.[14] Ai et al. found that the release rate of furosemide microcrystals of 5 μm coated with poly(diallyldimethylammonium chloride) (PDADMAC), poly(styrene sulfonate) (PSS), and gelatin was reduced 50−300 times compared to the bare furosemide when the layer number was 16.[15] Shenoy and Sukhorukov prepared the multilayer capsules of polysaccharides to prolong the release of naproxen.[16] Dai et al. found that the vitamin K_3, biotin, and insulin microcrystals could be encapsulated with poly(allylamine)/PSS or poly-L-lysine/alginate (ALG) multilayer films for sustained release.[17] Chen and Lin investigated the release property of indomethacin (IDM) encapsulated with polyelectrolyte multilayer films.[18]

Recently, we have found that simply increasing the deposition temperature from 20 °C to 60 °C efficiently reduces the release rate of the encapsulated IDM microcrystal owing to the increase in thickness and perfectness of the chitosan (CHI) and ALG multilayer film.[19] We proposed a new combination method for sustained drug release as adsorption by porous $CaCO_3$ microparticles and encapsulation of the drug-loaded microparticles with polyelectrolyte multilayer films formed by LbL self-assembly.[20]

At the same time, the LbL multilayer capsules are required to provide protection to the encapsulated drug from enzymatic decomposition in human body and to reduce drug toxicity to the human organs.[21,22] Especially for targeting drugs, drug activity must remain before reaching the target tissue.[23] Up to now, most attention has been paid to developing new multilayer films and to understanding their physical and chemical properties.[5−12] For the promising application in the drug delivery field, biocompatible and nontoxic polyelectrolyte materials are the most fundamental condition. Thus, the biopolymers are the most competitive candidates. However, most of biopolymers, such as chitosan and gelatin, are biodegradable in the body, which is likely to cause damage to the multilayer microcapsules. Hence, it is necessary to investigate stability of the multilayer film microcapsules made from natural biopolymer in the body environment. The enzymatic degradation of planar multilayer films has been reported by some groups.[24−28] However, there are few reports concerning the effect of enzymatic degradation of multilayer film microcapsules on their controlled release properties up to now.[29]

Thus, on the basis of our successful adjustment to the release rate by changing deposition temperature for the ALG/CHI

* To whom correspondence should be addressed. Tel.: +86-20-87112886. Fax: +86-20-87110273. E-mail: mcztong@scut.edu.cn.

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multilayer film microcapsules directly on IDM microcrystals, we intended to develop some new methods to enhance the resistance to enzymatic erosion of pepsin for the multilayer film microcapsules. IDM is a nonsteroidal anti-inflammatory drug and has been widely used in treatment of soft tissue problems associated with trauma, osteoarthritis, and rheumatoid arthritis. The high incidence and severe side effects, which are dose-related and are associated with long-term administration, have limited its use. This has led to the search for a new delivery system, which can overcome the side effects by controlling its release. In this work, however, IDM was taken as a model drug only because of its low solubility in aqueous solution during LbL assembly of CHI and ALG alternately deposited on IDM microcrystals directly. These polysaccharides were chosen for their biocompatibility and are suitable for medicine applications. Pepsin has been chosen because it is one of the main enzymes in the gastric juice, to which the orally administered drug must be subjected. Fluorescent-labeled polyelectrolytes were used to make the multilayer film desorption detectable. Release rate of model drug IDM from the ALG/CHI multilayer film microcapsules was adopted as a measure to appraise the stability enhancement to the enzymatic erosion.

Experimental Section

Materials. Chitosan (CHI, molecular weight (MW) 15 000, Fluka), pepsin (Sigma), and polyethyleneimine (PEI, MW 25 000, Aldrich) were used as received. Alginate (ALG, Kimitsu Chemical Industries Co., Japan) was dialyzed and freeze-dried before use and its MW was 120 000 as determined by gel permeation chromatography (GPC) with a Waters apparatus using 0.1 mol/L of Na_2SO_4 aqueous solution as the elution and narrowly distributed poly(ethylene oxide) as the standard. Fluorescein isothiocynate (FITC, Amresco, U.S.) was used as received and fluorescent-labeled ALG (FITC-ALG) was synthesized as described in our previous paper.[30] Rhodamine B was purchased from Beijing Chemical Plant and was used as received. Indomethacin (IDM, MW 357.8, Sigma) was gently ground into powder of micrometer with a pestle and mortar. 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC) and *N*-hydrosulfosuccinimide (sulfo-NHS) were purchased from Sigma. Highly purified water was produced by deionization and filtration with a Millipore purification apparatus (resistivity > 18.2 MΩ cm). Other chemicals were all analytical reagents and were used as received.

Fabrication of Multilayers on Quartz Slide. To detect the multilayer desorption induced by pepsin enzymatic degradation by monitoring fluorescence intensity, multilayer films of FITC-ALG/CHI were prepared on a flat quartz slide as the substrate. Prior to use, the quartz slide was cleaned with the "piranha" solution (98% H_2SO_4 and 30% H_2O_2 mixture of 7:3 in volume; *caution: piranha should be handled with extreme care and only a small volume should be prepared at one time*) at 80 °C for 1 h and then was dipped in the H_2O/30% H_2O_2/29% NH_3 (5:1:1 in volume) mixture at 70 °C for 1 h accompanied with plentiful pure water rinse after each treatment.[31] The concentration of aqueous CHI and FITC-ALG deposition solutions was 1 g/L with 0.5 M NaCl, and CHI solution contained 0.3 vol % of acetic acid for dissolution. The concentration of PEI aqueous solution, used for the first layer, was 1 g/L without NaCl. Polyelectrolytes were alternately deposited on the quartz slide to form flat FITC-ALG/CHI multilayer films by dipping the slide into a deposition solution for 20 min and by rinsing with pure water three times for one layer. The resulting planar films of $(FITC\text{-}ALG/CHI)_5$ deposited at 20 °C, $(FITC\text{-}ALG/CHI)_{10}$ deposited at 20 °C, and $(FITC\text{-}ALG/CHI)_5$ deposited at 60 °C were referred to as F-L10T20, F-L20T20, and F-L10T60, respectively (where L is layer number and T is deposition temperature).

Encapsulation and Release of IDM Microcrystal. Polysaccharides ALG and CHI were directly deposited on the IDM microcrystal of about 5 μm using layer-by-layer (LbL) self-assembly in aqueous solutions as described previously.[19] The first layer was deposited by adding 1 mL of ALG solution (1 g/L, 0.5 M NaCl) into a dispersed solution of 100 mg IDM microcrystal at pH 4.0 adjusted by acetic acid. The mixture was incubated for 15 min under gentle shaking. The excess ALG was removed by two refining circles of centrifugation (5000 rpm, 5 min)/washing/redisperse in water. The following CHI layer was deposited in the same procedure with 1 mL of CHI solution (1 g/L, 0.5 M NaCl, 0.3 vol % acetic acid). Alternate ALG and CHI layers were deposited successively in the identical way until the desired number of layers was achieved. To prevent the IDM from dissolving during the LbL process, the rinse water used in this work was saturated with IDM as suggested by Qiu et al.[13] The IDM-loaded microcapsule was slightly rinsed with water before release experiment to eliminate the IDM residue adsorbed on the surface. The resulting IDM microcapsules with shells of $(ALG/CHI)_5$ deposited at 20 °C, $(ALG/CHI)_{10}$ deposited at 20 °C, and $(ALG/CHI)_5$ deposited at 60 °C were referred to as M-L10T20, M-L20T20, and M-L10T60, respectively.

The released amount of IDM from the microcapsule was in situ monitored by UV absorbance at 320 nm.[19] The bare IDM microcrystal and IDM microcapsules were immersed in pH 7.4 phosphates buffer (1.36 g KH_2PO_4 dissolved in 79 mL of 0.1 M NaOH solution, diluted to 200 mL with purified water) in a 3 mL quartz cuvette. UV absorbance was measured as a function of dipping time and was converted to the IDM concentration in the cuvette with a calibration curve. All the release experiments were carried out at 37 °C and pH 7.4.

Enzymatic Desorption of Multilayer Films. A mutilayer-coated quartz slide was dipped in a quartz cuvette containing pepsin solution of 0.5 g/L for a required time and then was air-dried prior to fluorescence intensity measurement. The fluorescence emission of the dipping solution in the quartz cuvette after removing the slide was also measured. For easy comparison, a blank solution without pepsin was used in the same procedure. The pH value of the pepsin solution was adjusted to 1.4 with acetate/sodium acetate buffer. The incubation temperature was 37 °C.

After encapsulation with the ALG/CHI multilayer, the IDM microcapsule was immersed in the pepsin solution of 0.5 g/L for a specified time at pH 1.4 and 37 °C and was rinsed following two repeat refining circles of centrifugation (5000 rpm, 5 min)/washing/redispersal with water. The pepsin solution for treatment was saturated with IDM to prevent drug leakage from the microcapsules. The IDM microcapsule after the pepsin solution treatment was allowed to release at pH 7.4 and 37 °C.

Chemical Treatments of Multilayer Films. Film F-L10T20 and microcapsule M-L10T20 were cross-linked through two methods: one was to cross-link CHI in the film by glutaraldehyde (GA), and another was to cross-link CHI and ALG together by EDC. For GA cross-linking, F-L10T20 and M-L10T20 were incubated in 2 wt % of GA aqueous solution for 30 min and then were rinsed with water. Cross-linked F-L10T20 and M-L10T20 with GA were referred to as F-L10T20GA and M-L10T20GA, respectively. EDC cross-linking was conducted as following: 0.4 M EDC and 0.1 M sulfo-NHS with 0.15 M NaCl at pH 4.5 were prepared. The flat quartz slide and IDM microcapsule with the ALG/CHI multilayer film were treated in the mixture of equal volume EDC and NHS aqueous solutions for 12 h at 4 °C, respectively. After EDC cross-linking, the slide and IDM microcapsule were rinsed. Cross-linked F-L10T20 and M-L10T20 with EDC were referred to as F-L10T20EDC and M-L10T20EDC, respectively.

Characterization. Fluorescence intensity and UV absorbance were measured with Hitachi F-4500 and UV-3010 spectrometers, respectively. The multilayer film morphology was observed with a Seiko SII atomic force microscope (AFM) in the tapping mode at room temperature. The confocal micrograph was taken with an MRC 600 (Bio-Rad) confocal laser scanning microscopy (CLSM) with a 60× water immersion objective with a numerical aperture of 1.4. The microcapsule was visualized by rhodamine B probing, and the excitation wavelength was 488 nm as previously described.[15,19]

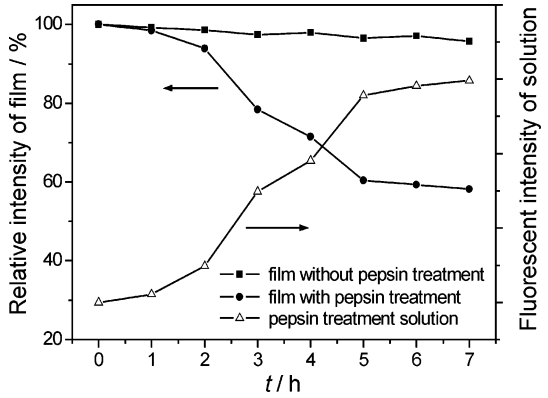

Figure 1. The relative fluorescence intensity of F-L10T20 film as a function of treatment time with or without pepsin and the fluorescent intensity of the corresponding pepsin solution.

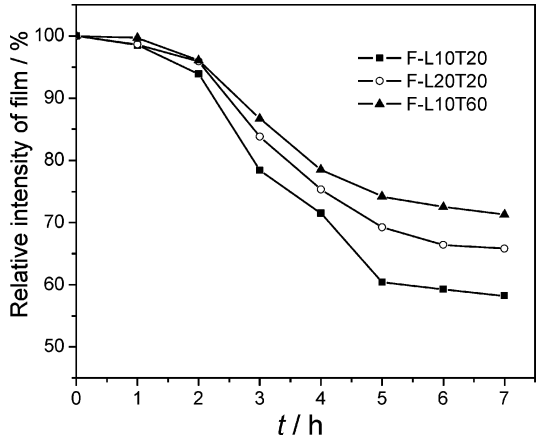

Figure 2. The fluorescent intensity of films F-L10T20, F-L20T20, and F-L10T60 as a function of pepsin treatment time. F-L10T20: $(FITC\text{-}ALG/CHI)_5$ deposited at 20 °C; F-L20T20: $(FITC\text{-}ALG/CHI)_{10}$ deposited at 20 °C; F-L10T60: $(FITC\text{-}ALG/CHI)_5$ deposited at 60 °C.

Results and Discussion

Enzymatic Desorption of Planar Multilayer Films. The FITC-ALG/CHI multilayer film was deposited on a quartz slide through LbL assembly to demonstrate the enzymatic desorption induced by the pepsin erosion. The electrostatic attraction was the dominant driving force for the oppositely charged ALG and CHI adsorbed alternately on the surface of quartz slide, and the first PEI layer was adopted to ensure the following adsorption. Increase in fluorescent intensity with the layer number confirmed the formation of polyelectrolyte multilayer films.[31]

Chitosan has been found to be enzymatically degradable to produce short chains.[32,33] A broad spectra of enzymes, including cellulase, protease, pepsin, lipase, and so forth, have been used for CHI degradation.[34] For example, Muraki et al. reported that D-glucocosamine oligosaccharides with polymerization degree of 6−8 were obtained from CHI when degraded by cellulase at pH 5.6 and 50 °C for 6 h.[35] For the LbL multilayer film, Serizawa et al. found that the CHI/dextran multilayer was desorbed in the presence of chitosanase, which was a special enzyme for CHI.[24] Considering the fact that pepsin is abundant in the gastric juice, we have chosen it to investigate the enzymatic desorption of ALG/CHI multilayer films, which will change the release rate of the IDM microcrystal encapsulated in ALG/CHI multilayer film microcapsules.

With the degradation of CHI in film F-L10T20, FITC-ALG was partially dissolved into the pepsin solution, leading to a decrease in fluorescence intensity of the film. Figure 1 demonstrates this intensity change of F-L10T20 against the pepsin treatment time *t*. To simulate the gastric juice environment, the pepsin solution was fixed at pH 1.4 and 37 °C. The film without pepsin treatment was stable and maintained its emission intensity during 7 h incubation in water. In contrast, the fluorescence intensity of the film decreased over a period of 7 h incubation in pepsin solution with a corresponding increase in the fluorescence intensity of the incubation solution. This intensity decrease was caused by the dissolution of FITC-ALG from the multilayer film because of the degradation of its counterpart CHI. The desorption of the FITC-ALG/CHI multilayer film was slow within the first 1 h and then accelerated and finally reached 60% of its original intensity after 5 h incubation in the pepsin solution. The results from Figure 1 obviously indicate the gradual desorption of the ALG/CHI multilayer film because of the chitosan degradation by pepsin.

As described in the Experimental Section, we deposited the $(FITC\text{-}ALG/CHI)_5$ film on a flat quartz slide at 20 °C as F-L10T20 and increased its thickness to about 2 times using the following two methods. Film F-L20T20 was constructed as $(FITC\text{-}ALG/CHI)_{10}$ with doubled layer number at the same deposition temperature (20 °C), and the film thickness should be doubled. Film F-L10T60 was $(FITC\text{-}ALG/CHI)_5$ deposited at 60 °C, which had a similar thickness (~32 nm) of F-L20T20 as confirmed by small-angle X-ray scattering.[19] This means that both films F-L20T20 and F-L10T60 have about doubled thickness as film F-L10T20. F-L10T60 has higher thickness for every layer. The desorption of these three multilayer films in pepsin solution at pH 1.4 and 37 °C was detected with the decrease in fluorescent intensity as shown in Figure 2. We can see that the desorption rate of film F-L20T20 is slower than that of film F-L10T20 because of its doubled layer number. Film F-L10T60 desorption is even slower than F-L20T20 though both have similar thickness. This results from the fact that F-L10T60 has more perfect structure than that of F-L20T20 when deposited at higher temperature.[19]

AFM images of surface morphology of films F-L10T20, F-L20T20, and F-L10T60 before and after pepsin erosion at pH 1.4 and 37 °C for 4 h are depicted in Figure 3. The original FITC-ALG/CHI multilayer films appeared smooth. The average roughness (the root−mean−squared surface roughness calculated over the scan area using the SII software) was 8.9, 12.7, and 14.1 nm for original F-L10T20, F-L20T20, and F-L10T60, respectively. After eroding in pepsin solution, the average roughness increased up to 23.6, 18.5, and 14.3 nm for F-L10T20, F-L20T20, and F-L10T60, respectively. Fredin et al. also found that the poly(β-aminoester)/PSS multilayer film roughness increased gradually during the desorption but was not induced by the enzymatic erosion.[36] Some pits or holes appeared obviously at the surfaces. This surface morphology change reflects the gradual corrosion of the multilayer film by the enzymatic degradation of CHI. The smaller increase in roughness for F-L10T60 after pepsin treatment also manifests its perfect structure to resist the enzymatic erosion, consistent with the result in Figure 2.

Besides these physical methods, cross-linking the multilayers is expected to improve the resistance to enzymatic erosion.[27,37−39] Film F-L10T20 was cross-linked with two cross-linkers to test this concept. One was glutaraldehyde (GA) to cross-link the CHI component in the ALG/CHI multilayer film. The GA aqueous solution consists of mono- and polymeric GA, and both can react with primary amine groups.[40] Another was 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC) to cross-link

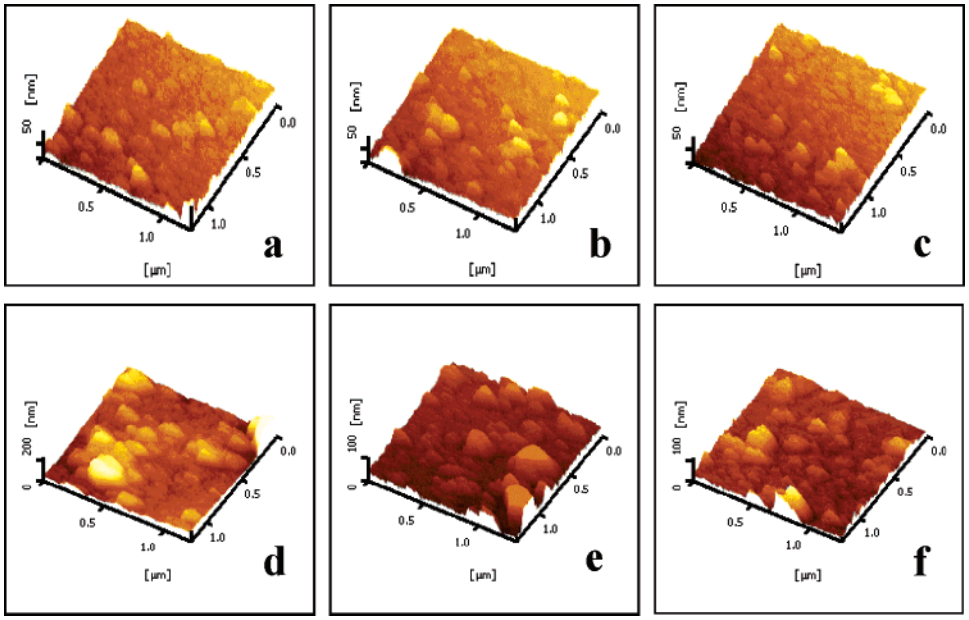

Figure 3. AFM images of films F-L10T20 (a, before; d, after), F-L20T20 (b, before; e, after), and F-L10T60 (c, before; f, after) on quartz slides before and after 4 h pepsin solution treatment.

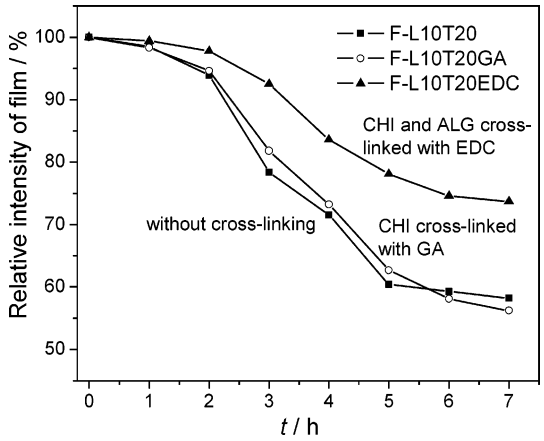

Figure 4. The fluorescent intensity of films F-L10T20, F-L10T20GA, and F-L10T20EDC as a function of pepsin treatment time.

CHI and ALG components together in the multilayer film. EDC was found to mediate the ester bond formation between the hydroxyl and carboxyl groups[41] and the peptide bond formation between the primary amine and carboxyl groups[42] to cross-link proteins and polysaccharides. In our case, the reaction occurred between hydroxyl or primary amine groups in CHI and carboxyl groups in ALG to form ester or amide groups.[41] GA and EDC cross-linking resulted in two ALG/CHI multilayer films of F-L10T20GA and F-L10T20EDC, respectively.

Figure 4 shows a decrease in fluorescence intensity of these films owing to the pepsin desorption. The desorption rate indicated by the curve slope is similar for F-L10T20 and F-L10T20GA, though the latter was cross-linked. In contrast, F-L10T20EDC cross-linked with EDC shows a much slower desorption rate, demonstrating that the EDC cross-linking can improve the ALG/CHI film resistance to the pepsin erosion more effectively than GA cross-linking. The reason is that the EDC joins CHI and ALG macromolecules together over the multilayer film, thus producing a more compact ALG/CHI multilayer film with lower mobility. When CHI is partially degraded, the CHI residues are likely still linked to the ALG chains, enhancing the film stability. GA cross-linking only occurs among the CHI macromolecules, providing little help to the film stability. After pepsin desorption for 4 h, the surface morphology of the ALG/CHI multilayer films was observed with AFM and is illustrated in Figure 5. The average roughness was 23.6, 21.5, and 13.4 nm for F-L10T20, F-L10T20GA, and F-L10T20EDC, respectively. Among them, F-L10T20EDC gives the minimal roughness, confirming its higher resistance to the pepsin erosion.

Release of Encapsulated IDM After Pepsin Erosion. To study the effect of pepsin erosion on the release of drug encapsulated by the multilayer film, IDM microcrystal of about 5 μm was directly encapsulated with polysaccharides ALG and CHI by LbL self-assembly as described previously.[19] The microcapsule formation on the IDM microcrystal by directly deposited multilayer of ALG and CHI can be viewed from CLSM image of M-L10T20 of $(ALG/CHI)_5$ as shown in the inset of Figure 6.

When administrated orally, the enzyme erosion in the stomach and intestine is ineluctable. To the authors' knowledge, the enzyme erosion effect on the drug release from the LbL assembled multilayer microcapsules was only reported by Itoh et al.[29] Therefore, on the basis of understanding enzyme erosion to the ALG/CHI multilayer films on flat quartz slide, we further studied the IDM release from the ALG/CHI multilayer microcapsules after pepsin treatment. First, we examined the incubation time effect on the IDM release at pH 7.4 and 37 °C from microcapsule M-L10T20 after pepsin treatment at pH 1.4 and 37 °C to simulate the situation of erosion in stomach and release in intestine. Because the saturation solubility of IDM at pH 7.4 is much higher than that at pH 1.4, IDM is scarcely released from the microcapsule M-L10T20 at pH 1.4.[19] Figure 6 demonstrates IDM release profiles from microcapsule M-L10T20 after pepsin corrosion compared with that from the same microcapsule but only incubated in an acetic acid solution of pH 1.4 at 37 °C for 6 h without pepsin. The release rate from the M-L10T20 only treated with acetic solution without pepsin is similar to that of the same microcapsule without any treatments. This means that the accelerated IDM release from the pepsin-treated microcapsules is induced by enzymatic erosion of the multilayer walls. With Fick's first law of diffusion, the IDM release from the ALG/CHI multilayer microcapsule

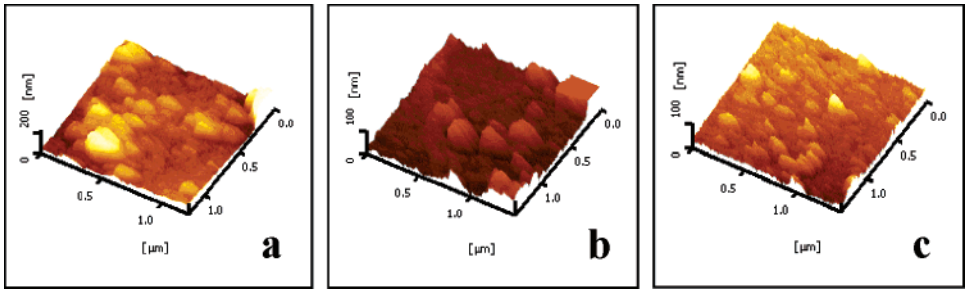

Figure 5. AFM images of films F-L10T20, F-L10T20GA, and F-L10T20EDC after 4 h pepsin treatment.

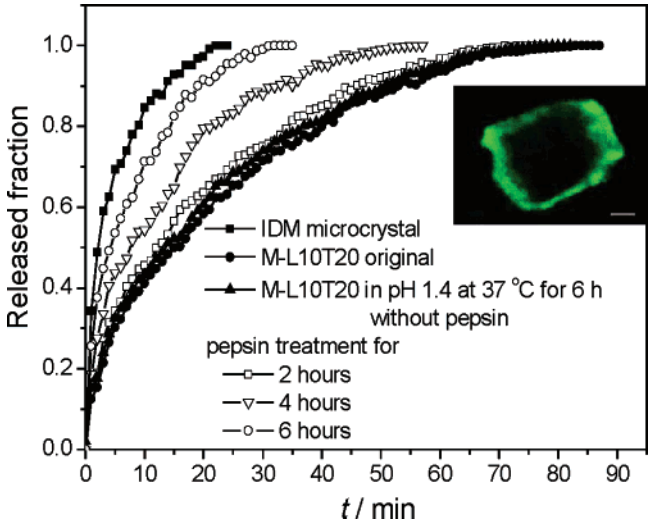

Figure 6. IDM release profiles from microcapsule M-L10T20 after pepsin treatment for indicated times compared with that from the same M-L10T20 after immersion in an acid solution of pH 1.4 at 37 °C for 6 h without pepsin. The inset is CLSM image of M-L10T20 microcapsule and the scale bar is 2 μm.

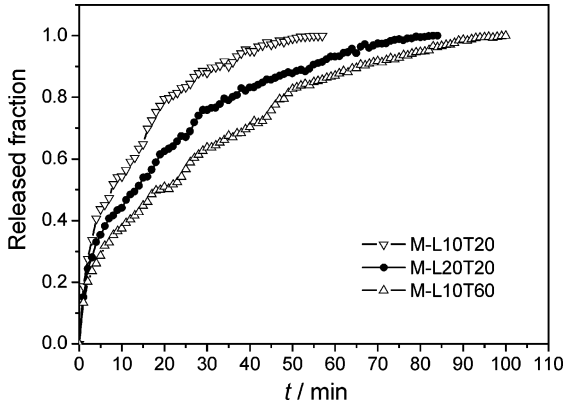

Figure 7. IDM release profiles from microcapsules M-L10T20, M-L20T20, and M-L10T60 after 4 h pepsin treatment.

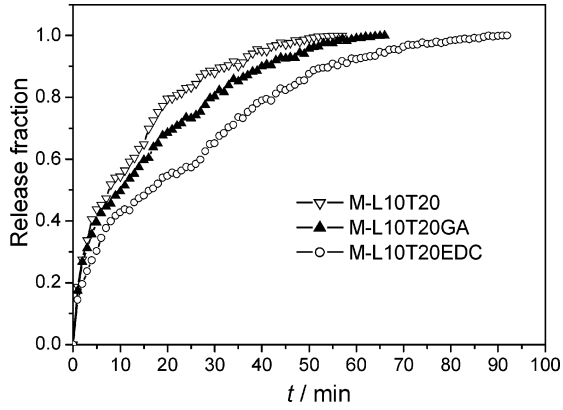

Figure 8. IDM release profiles from microcapsules M-L10T20, M-L10T20GA, and M-L10T20EDC after 4 h pepsin treatment.

has been quantitatively described.[19] By fitting the release data in Figure 6, we evaluated the diffusion coefficient D for IDM passing through the ALG/CHI multilayer film of the microcapsule accordingly. As the incubation time increased, the release rate increased gradually and the diffusion coefficient D rose from 3.5×10^{-9} to 13.3×10^{-9} cm^2/s. After pepsin erosion, the release of encapsulated IDM microcrystals was accelerated because of the increase in permeability of the ALG/CHI multilayer film covering on the drug. However, even after incubation in the simulating gastric juice for 6 h, the ALG/CHI multilayer film still maintained the barrier capability to diffusion of the encapsulated IDM when compared with the release of bare IDM microcrystals.

With the knowledge of how to enhance the resistance to pepsin erosion for the planar ALG/CHI multilayer film, we tried to improve the stability to pepsin erosion of the ALG/CHI multilayer microcapsules following a similar procedure. Enzymatic desorption of microcapsules M-L10T20 (5 bilayers deposited at 20 °C), M-L20T20 (10 bilayers deposited at 20 °C), and M-L10T60 (5 bilayers deposited at 60 °C) was conducted in a pepsin solution for 4 h. Then, IDM released from the pepsin-corroded microcapsules was monitored and is shown in Figure 7. The release rate decreases in the sequence of M-L10T20, M-L20T20, and M-L10T60, reflecting enhanced resistance to the enzymatic erosion with increasing multilayer film thickness and structural perfectness. The diffusion coefficient D from ALG/CHI microcapsule is 10.4×10^{-9} cm^2/s for M-L10T20 and decreases to 6.7×10^{-9} and 4.3×10^{-9} cm^2/s for M-L20T20 and M-L10T60, respectively. These results suggest that deposition of ALG and CHI layers through LbL self-assembly at higher temperature is a more effective and simple way to increase the resistance to enzymatic desorption for the multilayer film microcapsules.

Besides physical methods, cross-linking the multilayer wall can also improve the resistance to enzymatic erosion of the microcapsule.[27,37−39] The ALG/CHI multilayer film deposited on the IDM microcrystal was also cross-linked with GA or EDC to make microcapsules of M-L10T20GA or M-L10T20EDC. Figure 8 depicts the IDM release profiles from these microcapsules after incubation in pepsin solution for 4 h. As expected from the results of the planar multilayer film, the release rate from M-L10T20EDC is the slowest because the EDC cross-linked ALG/CHI multilayer film possesses the highest resistance to the pepsin erosion and enzyme desorption. Therefore, the cross-linking neighboring layers in the multilayer film can

endow the film a higher resistance to enzymatic desorption, prolonging release of the drug encapsulated in this film.

Conclusions

Partial desorption of the ALG/CHI multilayer film made through LbL assembly was found to be induced by the enzymatic degradation of CHI when being incubated in the pepsin solution. The same enzymatic desorption was also observed from the microcapsule wall formed with the ALG/CHI multilayer film directly deposited on IDM microcrystals using LbL self-assembly. After pepsin erosion, the IDM release from the microcapsule was obviously accelerated because of the desorption of the ALG/CHI films. To enhance the stability of the ALG/CHI multilayer films to the enzymatic desorption, some physical and chemical methods were proposed in the present work. Increasing the layer number and raising the deposition temperature effectively slowed down the enzymatic desorption and the release rate. Especially, increasing deposition temperature was more effective in enhancing resistance to the pepsin erosion because of producing a more perfect ALG/CHI multilayer film. Cross-linking neighboring layers of ALG and CHI in ALG/CHI multilayer film with EDC significantly reduced the enzymatic desorption and the drug release rate. Therefore, increasing deposition temperature and cross-linking neighboring layers will be effective methods to protect the multilayer film made by LbL assembly from enzymatic erosion and to prolong the release of the encapsulated drug.

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