Drug Development and Industrial Pharmacy, 2010; 36(4): 482–489

informa
healthcare

ORIGINAL ARTICLE

Preparation and characterization of novel sinomenine microcapsules for oral controlled drug delivery

Dengshui Shi[1], Richard Greever[2] and Youfang Chen[3]

[1]Design and Research Institute of Energy Engineering, Zhejiang University, Hangzhou, China, [2]The Korea Foundation for Advanced Studies, Seoul, South Korea and [3]Institute of Applied Chemistry, Department of Chemistry, Zhejiang University, Hangzhou, China

Abstract

Background: Developing a sustained release drug to cure arthritis is needed. Sinomenine (SIN) is abstracted from sinomenium acutum and widely used in the treatment of various rheumatism and arrhythmia with few side effects. The primary aim of this study is to develop SIN microcapsules with polyelectrolyte multilayers for controlled drug release. *Method*: SIN microcrystals were encapsulated with chitosan, gelatin, and alginate by layer-by-layer technique, such as $(gelatin/alginate)_4$ and $(chitosan/alginate)_6$. The size distribution, zeta-potential, stability, and morphology of the microcapsules were characterized by a particle size analyzer, zetasizer, ultraviolet spectroscopy, and transmission electron microscope, respectively. The in vitro controlled release pattern of SIN was studied using a diffusion cell assembly at physiological pH of 6.8 or 1.4. *Results*: Light stability of these microcapsules was improved after microencapsulation. Compared with release rate of the SIN microcapsules coated by the poly(dimethyldiallyl ammonium chloride)/alginate and gelatin/alginate multilayers, release rate of the SIN microcapsules coated with chitosan/alginate multilayers was fast. Release rate progressively decreased with the increase of chitosan/alginate bilayer number and the decrease of pH value of release medium. *Conclusion*: These novel SIN microcapsules may be developed into oral controlled drug delivery for rheumatism and arthritis.

Key words: *Controlled release; drug delivery; layer-by-layer; microencapsulation; sinomenine*

Introduction

Rheumatoid arthritis is a systemic, chronic inflammatory disease prevalent in about 1% of the western population. Two-thirds of patients are female. Rheumatoid arthritis affects joints, which leads to pain, joint destruction, and disability. Major goals of treatment are to relieve pain, reduce inflammation, slow down or stop joint damage, prevent disability, and preserve or improve the person's sense of well-being and ability to function.

Many drugs are very effective anti-inflammatory agents with analgesic properties, such as ibuprofen, ketoprofen, and indomethacin. When taken orally for chronic inflammation and pain, adverse effects often occur, that is, general serious gastrointestinal reaction (even stomach perforation), central nervous system symptoms, liver function damage, inhibition of hematopoeitic system, and allergic reactions. Inconvenience in use is also a problem, because more time of taking medicine and long period of treatment are needed. Furthermore, most drugs have low bioavailability and a short half-life, while also having an initial burst effect and high peak plasma concentrations. Therefore, developing a sustained release drug to cure arthritis is needed.

Microencapsulation of drug particles is a useful technique applied in controlled delivery. In past research, a large variety of drug delivery systems were developed to serve this purpose, that is, liposomes, micelles, emulsions, and polymer micro/nanoparticles. Several papers have been published on novel controlled release systems on cures to include ibuprofen pellets[1], chitosan (CHI) microspheres loaded with indomethacin[2], ketoprofen-loaded lipid nanoparticles[3], sinomenine (SIN) gel[4], SIN

Address for correspondence: Dr. Youfang Chen, Ph.D., Institute of Applied Chemistry, Department of Chemistry, Zhejiang University, Hangzhou, China. E-mail: yfchen@zju.edu.cn

(Received 1 Apr 2009; accepted 9 Aug 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040903262003

hydrochloride oral osmotic pump-controlled release tablets[5], and SIN liposome[6]. Thus, it is highly recommended to develop a new sustained release drug to cure arthritis.

However, problems still occur with the sustained release preparations listed herein after: First, many arthritis drugs have been synthesized with organic reagents, to include ibuprofen, indomethacin, and ketoprofen, exerting cytotoxicity by inhibiting DNA synthesis and cell replication. From a pharmaceutical point of view, some side effects are difficult to resolve. Thus, a better solution is to develop sustained release drugs, cure for arthritis, extracted from herbs that have been used for thousands of years. Second, some organic solvents were used in the preparation of SIN preparations[4–6]. Finally, thickness of the capsule shell cannot be adjusted in nanoscale; therefore release rate cannot be controlled precisely. In a word, to develop a controllable drug release system with good properties will be of utmost importance and a challenge.

Sinomenium acutum has been used in herbal Chinese medicine for thousands of years to treat various rheumatic diseases[7,8]. SIN is an active alkaloid, extracted from stems of sinomenium acutum Rehd. et Wils. Recent studies show that SIN possesses potent anti-inflammatory, analgesic, and immunoinhibitory pharmacological effects, which provide the treatment of rheumatoid arthritis[9]. Presently, SIN is widely used in the treatment of various rheumatism and arrhythmia with few side effects.[4–6] Many clinical therapeutical trials confirmed the effectiveness of SIN in treating rheumatoid arthritis with the efficacy as high as 90%[10].

Novel vesicles (polyelectrolyte capsules) were confirmed as a promising drug delivery for encapsulation a few years ago[11]. These vesicles were fabricated with oppositely charged polyelectrolytes, using layer-by-layer (LBL) self-assembly, for instance, drug[12] and dye[13]. Compared with the other encapsulation methods, the LBL technique applied in encapsulation of SIN crystals has many advantages: (i) hazardous procedures are avoided during fabrication of the capsule wall; (ii) film thickness and the diameter of capsules can be tuned within an accuracy of a few nanometers, and the release rate of drug capsules can also be adjusted effectively[12]; (iii) SIN is sensitive to light and heat and can easily be oxidized and decomposed; (iv) it is also not stable in an aqueous solution, affected by its pH value[9]. Polyelectrolyte shell surrounding SIN crystals will protect and make them more stable after microencapsulation; (v) SIN has toxicity to stomach and allergic reactions[14] and may result in a rash because of the production of beta-imidazolyle thylamine[9]. SIN capsules coated with polyelectrolyte multilayers (PEMs) minimize adverse side effects without the expense of drug efficiency; (vi) after microencapsulation of SIN crystals, the poor oral bioavailability of SIN would be markedly elevated. Its short half-life of approximately 4 hours[15] is prolonged; and (vii) plasma concentration of SIN drug is comparatively unstable and more times of taking medicine are needed, which markedly lowers patient compliance. All these problems will be significantly improved after coating with PEMs.

In our previous research, we successfully prepared indomethacin microcapsules with poly(styrene sulfonate)/poly(dimethyldiallyl ammonium chloride) (PSS/PDDA) multilayers[16] and gelatin/alginate (GEL/ALG) multiplayer[17]. The primary aim of this study is to develop SIN microcapsules with PEMs for controlled drug release. In this article, we report in detail the preparation and characterization of the promising SIN microcapsules and the important factors during the preparation process.

Materials and methods

Materials

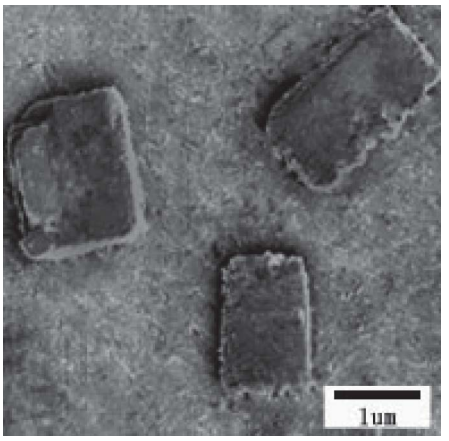
CHI (M.W. 2.5×10^5, deacetylation degree >85%; Fluka, St. Louis, MO, USA), GEL (type A, M.W. 5×10^5–5×10^{10}; Sigma, St. Louis, MO, USA), PDDA (M.W. 2×10^5; Aldrich, St. Louis, MO, USA), and ALG (M.W. 7×10^4; Sigma-Aldrich, St. Louis, MO, USA) were used in LBL assembly. Solutions of 2 mg/mL CHI, GEL, PDDA, and ALG aqueous solutions were selected for LBL assembly and prepared in ultrapure water (resistance greater than or equal to 18 MΩ/cm). CHI was dissolved by glutamic acid. All other chemicals were of analytical grade.

SIN (purity >98%) was purchased from the National Institute of Pharmaceutical and Biological Products (Beijing, China). Its chemical name is morphina-6-one, 7, 8-didehydro-4-hydroxy-3, 7-dimethoxy-17-methyl (9α, 13α, 14α). Figure 1a shows a scanning electron microscope (SEM) image (SLR10N; FEI, Eindhoven, the Netherlands) of the SIN crystals. The molecular structure of SIN is shown in Figure 1b. SIN is poorly soluble in water and its solubility is 0.46 mg/mL in water at 37°C.

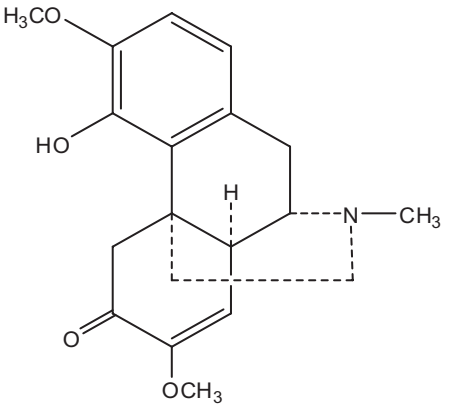
Methods

Encapsulation method

Before coating, SIN powder was finely ground into microcrystals by using a mortar and a pestle (before coating) and then dispersed in ultrapure water. After a 10-minute ultrasonication process, they were left standing for several hours. The encapsulation of SIN crystals was accomplished by the alternate adsorption of oppositely charged polyelectrolytes using the centrifugation



(a)



(b)

Figure 1. (a) SEM images of bare SIN crystals. (b) Molecular structure of SIN.

technique as previously reported[16]. The deposition temperature was controlled as 20°C, 40°C, and 60°C.

In each encapsulation, polycation was used as the first coating layers and ALG was used as the outermost layers. Capsules made of four pairs of gelatin/alginate bilayers were denoted as $(GEL/ALG)_2$, six pairs of chitosan/alginate bilayers were denoted as $(CHI/ALG)_6$.

Measurement of particle size

The average particle size and size distribution of bare drug crystals and coated drug capsules were determined by laser diffraction (LA-300; HORIBA Jobin Yvon, Edison, NJ, USA). The particle solution (1.5 mL) was pipetted into a cuvette. Data were collected at room temperature. All particle solutions were sonicated for several minutes to disperse aggregated drug particles before the data were collected.

Measurement of zeta potentials

Zeta potential of coated drug capsules was analyzed after deposition of every layer with a zetasizer (440sx; Beckman, Fullerton, CA, USA). Particle solution (1.5 mL) was pipetted into a cuvette. Data were collected at room temperature. All particle solutions were sonicated for several minutes to break aggregated drug particles before the data were collected. All experiments were performed in triplicate ($n = 3$).

Loading efficiency and encapsulation efficiency

SIN capsules (3 mg) in a dialyzer were immersed in ethanol for 24 hours. The solution was passed through a 0.22-μm membrane filter (Millipore, Billerica, MA, USA) and then diluted with 0.2% NaOH aqueous solution. Finally, the drug content was assayed by measuring UV absorbance at 264 nm. Experiments were performed in triplicate ($n = 3$) and loading efficiency and encapsulation efficiency were calculated using the following equations:

$$\text{Loading efficiency} = \frac{\text{ART concentration (mg/mL)} \times \text{solution volume (mL)}}{\text{Weight of SIN capsules (mg)}} \times 100\%$$

$$\text{Encapsulation efficiency} = \frac{\text{calculated SIN weight in capsules}}{\text{theoretical SIN weight in capsules}} \times 100\%.$$

Light stability

Stability experiments were performed to evaluate the stability of SIN capsules coated with $(CHI/ALG)_6$ multilayers in natural light at room temperature. After 1, 4, 6, 8, and 24 hours, bare SIN crystals and SIN capsules were dissolved in ethanol; the quantity of SIN was measured in a quartz cuvette utilizing the UV method at 264 nm.

Surface morphology

Bare SIN microcrystals were observed by an SEM (SLR10N, FEI). A transmission electron microscope (TEM, JEM-1230; JEOL, Tokyo, Japan) was used to image coated drug capsules. Drug capsules were placed on a copper grid. After the copper grid was dried, the image was taken at ambient temperature (20–25°C).

In vitro dissolution study

In vitro drug release studies were carried out in a dialyzer obtained about 3 mg bare SIN crystals or SIN capsules, coated by $(CHI/ALG)_{2,4,6}$, $(PDDA/ALG)_4$, or $(GEL/ALG)_4$. Two different pH solutions were used as the release medium: pH 1.4 solution (a simulated gastric fluid without enzyme, prepared by diluting a concentrated

HCl solution) and pH 6.8 solution (a simulated intestinal fluid, prepared with 0.02 M phosphate buffer, PBS). Drug dissolution was conducted in a shaking bath, with temperature and a shaking rate of 37 ± 0.5°C and 50 rpm, respectively. At different sampling times (0, 0.5, 1.5, 2, 3, 4 hours, etc.), 5-mL samples were removed. The medium was removed and replaced with a fresh solution after sampling. The quantity of SIN that had dissolved and diffused into the receptor medium was conducted in a quartz cuvette utilizing the UV method at 264 nm. All release experiments were performed in triplicate ($n = 3$).

Results and discussion

Size distribution of SIN microcapsules

Size distribution of bare SIN crystals and SIN capsules coated with $(CHI/ALG)_6$ multilayers are presented in Figure 2. Average sizes of bare SIN crystals and coated SIN capsules were 1.860 (SD, $\sigma = 320$ μm) and 1.872 μm ($\sigma = 0.333$ μm), respectively. The similar data suggested that the size of SIN crystals only had little change after encapsulation. Ai et al. reported that a PDDA/PSS bilayer and a PSS layer had a thickness of 2 and 0.7 nm, respectively[12]. Nabok et al. found that thickness per poly(allylamine hydrochloride)/PSS bilayer was from 0.58 to 1.26 nm[18]. Their reports suggested that the thickness of PEMs was very minor, compared with the size of bare drug crystals. The data of size distribution also can account for the reason that SIN capsules did not aggregate during encapsulation.

Zeta potentials of SIN microcapsules

In this study, CHI and ALG were directly coated on SIN crystals via LBL self-assembly procedure as described in 'Methods'. Surface electrical potentials for coated SIN capsules at each adsorption step are shown in Figure 3. Zeta potential is around +33 mV for the first CHI layer, and zeta potential is about −32 mV for the second ALG layer. After the second layer, zeta potential is +30 mV for the other outermost CHI (odd) layer and −30 mV for the outermost CHI (even) layer. Overall, obvious alternating and similar zeta potential in the surface charge indicate successful alternating deposition of CHI and ALG directly on the SIN crystals, without aggregation and precipitation. It may result in that SIN capsules repel each other and disperse homogeneously in a solution or blood.

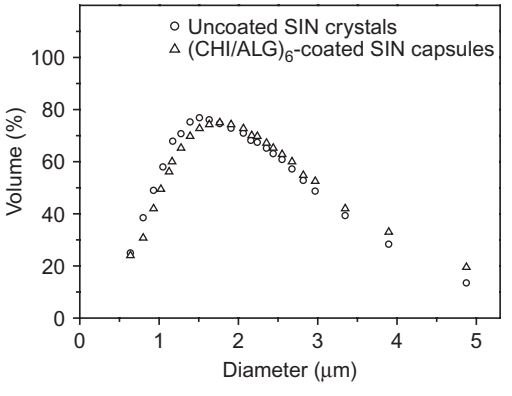


Figure 2. Size distribution of bare SIN crystals and SIN microcapsules that were coated with $(CHI/ALG)_6$ multilayers at 20°C.

Loading efficiency and encapsulation efficiency

Loading efficiency of coated SIN microcapsules varied with the number of PEMs. SIN microcapsules coated with $(CHI/ALG)_4$ multilayers had a loading efficiency of 96% (Table 1), which was a little higher than that of the microcapsules coated with $(CHI/ALG)_6$ multilayers (92%).

Loading efficiency of drug capsules prepared by other encapsulation methods was much lower than that of the LBL technique. For example, vitamin A capsules were encapsulated by coacervation[19]; in addition, a magnetic nanoparticle drug carrier was chemically

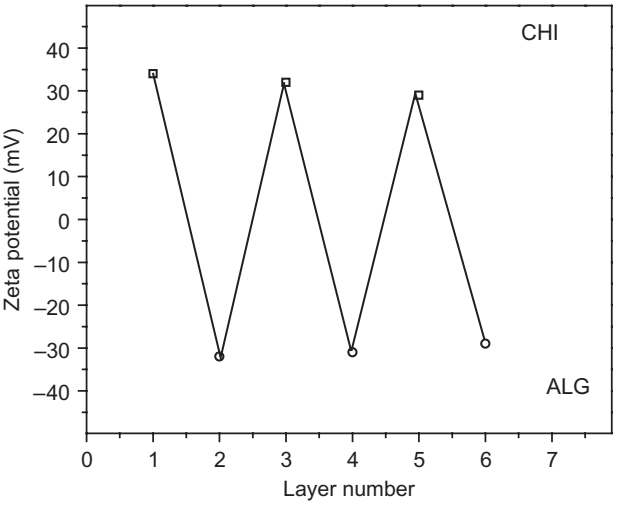


Figure 3. Zeta potential of coated SIN capsules versus the number of adsorption steps for shell compositions of $(CHI/ALG)_6$ multilayers that were deposited at 20°C.

Table 1. Loading efficiency and encapsulation efficiency of SIN microcapsules that were coated with $(CHI/ALG)_{4,6}$ multilayers at 20°C.

SIN capsules	$(CHI/ALG)_4$	$(CHI/ALG)_6$
Loading efficiency (%)	96	92
Encapsulation efficiency (%)	93	90

bound with doxorubicin and encapsulated by CHI-grafted copolymer[20]. Ai et al.[12] prepared furosemide microcapsules coated with $(PSS/PDDA)_2$ $(PSS/GEL)_4$ multilayers and reported their results similar to ours; They found that much less 'ballast' material was used in the encapsulation procedure compared to other polymer-based delivery systems.

Encapsulation efficiency of coated SIN microcapsules also differed with number of PEMs. The encapsulation efficiency of SIN microcapsules, coated with $(CHI/ALG)_4$ multilayers, was 93%, at the same time that of SIN microcapsules, coated with $(CHI/ALG)_6$ multilayers, was 90%.

Light stability of SIN capsules

To understand the effect of encapsulation of PEMs on the stability of SIN crystals, light stability of SIN capsules was measured at different times. As shown in Figure 4, after 2, 4, 8, and 24 hours exposure to natural light, the content of bare SIN crystals decreased gradually; at the same time, the content of SIN in $(CHI/ALG)_6$ polyelectrolyte shell nearly remained constant. Therefore $(CHI/ALG)_6$ multilayers surrounding SIN crystals appeared to enhance their light stability. The microencapsulation of SIN crystals with PEMs protected the drug from light and produced a stable oral capsule formulation.

Effect of PEM type on release properties of SIN microcapsules

As the deposition had been demonstrated and quantified, the problem arose whether deposited substances could be released afterwards, which is an important question for practical applications of drug delivery. In this work, SIN capsules were prepared from 2 mg/mL CHI and 2 mg/mL ALG aqueous solution. Because of the large imbalance of drug concentration between the capsule interior and the bulk, release rates were very quick at the initial stage and then became slower. Eventually, a plateau in the release profile indicated that the drug core dissolved completely. Figure 5 shows the release profiles of SIN microcapsules coated with different PEMs in PBS (pH 6.8). The release profile of bare SIN microcrystals is also presented in this plot for comparison. As can be seen from Figure 4, a compositional dependence of release rate is observed. For the SIN microcapsules coated with $(CHI/ALG)_4$ multilayers, the release is fast. The half-release times ($t_{1/2}$) of these microcapsules increase slightly from about 0.8 hour of the bare SIN microcrystals to 1.5 hours of the encapsulated crystals, indicating that the $(CHI/ALG)_4$ multilayers are highly permeable to SIN. For the SIN microcapsules coated with $(PDDA/ALG)_4$ and $(GEL/ALG)_4$ multilayers, the release rates are much slower. The half-release times increase substantially up to approximately 2.6 and 6.6 hours for the SIN microcapsules coated with $(PDDA/ALG)_4$ and $(GEL/ALG)_4$ multilayers, respectively, which is three and eight times as long as that of bare SIN crystals. This implies that the PDDA/ALG and GEL/ALG multilayers are much less permeable to SIN.

Ai et al.[12] reported that the thicknesses of different PEMs were evidently varied through quartz crystal microbalance study. Tieke et al.'s theory also can explain the results presented in Figure 4[21]. Through analyzing the dependence of flux and water content in the permeate on the charge density of the polyelectrolyte

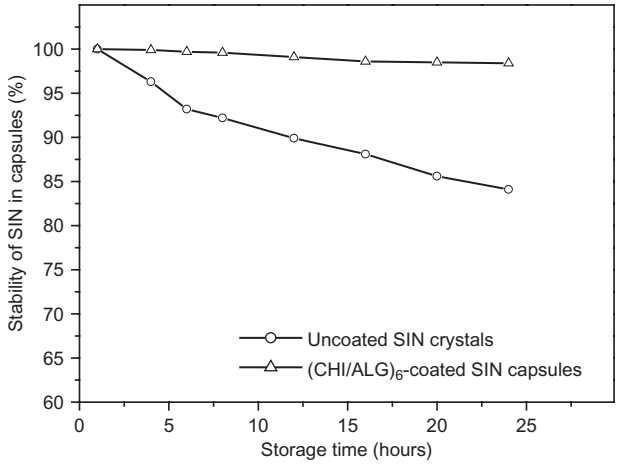


Figure 4. Stability of SIN in microcapsules in natural light at room temperature. These microcapsules were coated with $(CHI/ALG)_6$ multilayers at 20°C.

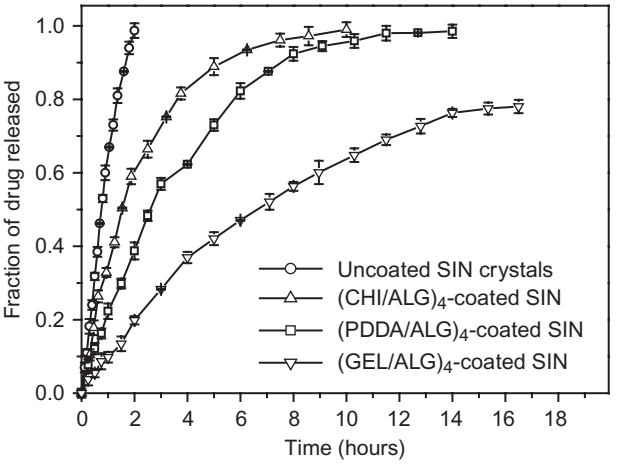


Figure 5. Release profiles of SIN from the microcapsules in pH 6.8 PBS at 37°C. These microcapsules were coated with $(CHI/ALG)_4$, $(PDDA/ALG)_4$, or $(GEL/ALG)_4$, respectively, at 20°C (error bar representing SDs, $n = 3$).

complex, they reported that cross-linking density and the mesh size of PEMs were controlled by the charge density of the polyelectrolyte complex; furthermore, the charge density of different polyelectrolyte complex varied mainly with the change of the chemical structure of the polyelectrolytes.

Effect of number of PEMs on release properties of SIN microcapsules

$(CHI/ALG)_{2,4,6}$ nanoshell indicated different number of CHI/ALG bilayers. Release profiles of these SIN capsules in PBS were plotted in Figure 6. As shown in this figure, bare SIN microcrystals were completely released after 2 hours. Total release times were 7, 10, and 13 hours for two-, four-, and six-layer CHI/ALG coating, respectively. After encapsulation, the total release times were 3.5, 5.0, and 6.5 times longer, compared to the bare SIN crystals. It was observed that release rate progressively decreased as CHI/ALG bilayer number increased. These results agreed with our previous experimental results, which discussed indomethacin microcapsules fabricated by $(PSS/PDDA)_{2,4,6}$ multilayers and by $(GEL/ALG)_{2,4,6}$ multilayers[17].

Past research reported that the thickness of polymeric nanoshell increased regularly with consecutive adsorption of polycations and polyanions onto colloidal templates[12]. Varying number of PEMs may easily change thickness of polyelectrolyte shell with nanometer precision; thereupon it may further change the release rate of encapsulated substances. Their findings may explain our result presented in Figure 5.

Effect of deposition temperature on release properties of SIN microcapsules

In terms of effective methods, such as polyelectrolyte type, bilayer number, ionic strength, and so on, temperature is another important experimental parameter to control the growth rate of LBL self-assembled multilayers. Shi et al. studied the thickness of PDDA $(PSS/PDDA)_2$ + $(PDDA/45\text{-nm silica})_{5-14}$ with a quartz crystal microbalance; they found that the thickness of the silica layer increased with the deposition temperature[22]. Tan et al. reported that the thickness of PAH/PSS multilayers on a flat substrate increased, when the deposition temperature increased[23]. Their findings suggested a simple way to tune the structure and properties of PEMs. Thus, in this work, the possibility of adjusting the release property of SIN microcapsule shell through the deposition temperature was studied.

The release profile of the IDM microcrystal coated with $(CHI/ALG)_4$ multilayers and deposited at different temperatures is presented in Figure 7. The release temperature is kept at 37°C. The half-release times ($t_{1/2}$) were 1.5, 2.3, and 3.6 hours for the capsule multilayers deposited at 20°C, 40°C, and 60°C, respectively. This result indicates that the $t_{1/2}$ can be prolonged to 2.4 times so long as the deposition temperature is simply raised from 20°C to 60°C. The release time also increases from 10.1 to 14.2 hours when the deposition temperature increases from 20°C to 60°C. Therefore, the release rate of the LBL assembled multilayer capsules can be evidently reduced with the increase of the deposition temperature. The main reason for the above-marked change in the release rate results from raising the deposition temperature: the CHI/ALG multilayer on the SIN microcrystal becomes thicker. Additionally, the film may become more compact through conformational rearrangement.

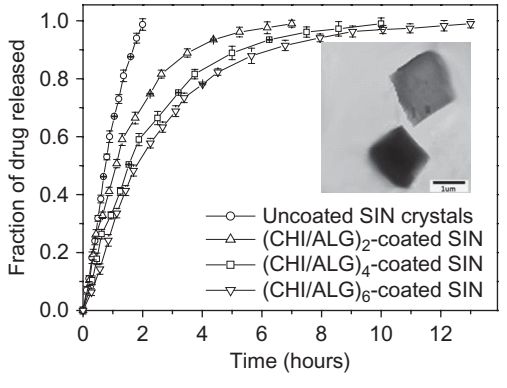


Figure 6. Release profiles of SIN from the microcapsules in pH 6.8 PBS at 37°C (error bar for SDs, n = 3). These microcapsules were coated with $(CHI/ALG)_{2,4,6}$ multilayers at 20°C. The inset shows a TEM image of SIN capsules coated with $(CHI/ALG)_6$ multilayers. The capsules were very similar in shape to the SEM image of bare crystals in Figure 1a.

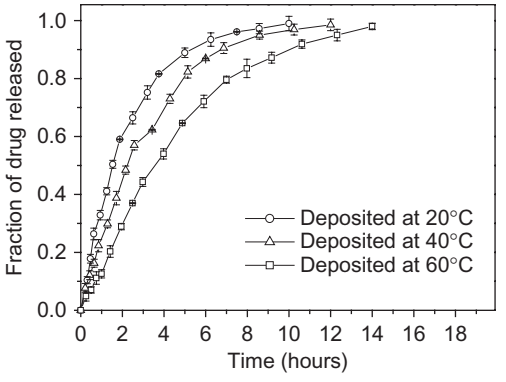


Figure 7. Release profiles of SIN from the microcapsules in PBS (pH 6.8) at 37°C (error bar representing SDs, n = 3). These microcapsules were coated with $(CHI/ALG)_4$ multilayers at 20°C, 40°C, or 60°C, respectively.

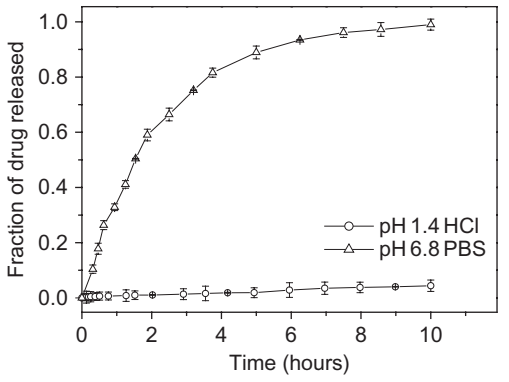


Figure 8. Release profiles of SIN from the microcapsules at pH 1.4 HCl and pH 6.8 PBS solutions at 37°C (error bar for SDs, $n = 3$). These microcapsules were coated with $(CHI/ALG)_4$ multilayers at 20°C.

Effect of pH value of release medium on release properties of SIN microcapsules

Figure 8 depicts the release profiles of SIN from microcapsules coated with $(CHI/ALG)_4$ multilayer at pH 1.4 and 6.8. At pH 1.4, the drug total release time of the four-layer coating was much longer compared to that at pH 6.8. Accordingly, the coated drug is released more slowly in the simulated gastric fluid (i.e., pH 1.4) compared to simulated intestinal fluid without enzyme (i.e., pH 6.8). The four-layer coating is thick enough to provide slow release in a gastric environment (pH 1.4). This result agreed with our previous report about the release property of indomethacin microcapsules coated with $(PSS/PDDA)_4$ multilayers[16].

In comparison with other SIN delivery systems, including SIN gel[4], SIN hydrochloride oral osmotic pump-controlled release tablets[5], and SIN liposome[6], the method described in this article has some main advantages as follows: first, the polyelectrolyte monolayers can be easily used to adjust layer thickness and diameter of the microcapsules; second, capsules with diameters that are less than 1 μm may be applied to intravenous injection; last, much less polymer material is needed in the encapsulation procedure and it also provides good results.

Conclusion

In this study, sustained drug release was achieved by SIN microcrystals, encapsulated with CHI/ALG, PDDA/ALG, and GEL/ALG multilayer shells. SIN microcrystals become more stable against light after encapsulation. It was proved that polyelctrolyte type and the number of PEMs can effectively control release properties of SIN microcapsules. $(CHI/ALG)_4$ coating provided very slow release in gastric environment. Our results suggest that optimization of the capsule wall thickness and composition is expected to yield a new-type oral drug delivery for controlled drug release.

Acknowledgments

The financial support to this work of the Natural Science Fund of Zhejiang Province (No: Y4090100, Y20770) is gratefully acknowledged.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Costa FO, Sousa JJS, Pais AACC, Formosinho SJ. (2003). Comparison of dissolution profiles of ibuprofen pellets. J Control Release, 89:199–212.
2. Aggarwal A, Kaur S, Tiwary AK, Gupta S. (2001). Chitosan microspheres prepared by an aqueous process: Release of indomethacin. J Microencapsul, 18:819–23.
3. Puglia C, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, et al. (2008). Lipid nanoparticles for prolonged topical delivery: An in vitro and in vivo investigation. Int J Pharm, 357:295–304.
4. Zhang XZ, Pan XG, Luo SD, Xue CB, Cai HY. (2003). Studies on the preparation and quality standards for sinomenine gel. China Pharm, 14:275–7.
5. Wei S, He Z, Wang L. (2003). Preparation of sinomenine hydrochloride oral osmotic pump-controlled release tablets and the factor influencing the drug release rate. J Shenyang Pharm Univ, 20:165–9.
6. Ma TS, Li G, Song SC, Liu ZH, Zhu LC. (2007). Study on preparation techniques of sinomenine hydrochloride liposome. Zhongguo Yaoshi, 3:209–11.
7. Liu JH, Li WD, Teng HL, Lin ZB. (2005). Immunopharmacological action of sinomenine, an alkaloid isolated from Sinomenium acutum, and its mechanism of action in treating rheumatoid arthritis. Yao Xue Xue Bao, 40:127–31.
8. Liu ZQ, Chan K, Zhou H, Jiang ZH, Wong YF, Xu HX, et al. (2005). The pharmacokinetics and tissue distribution of sinomenine in rats and its protein binding ability in vitro. Life Sci, 77:3197–209.
9. Chinese Pharmacopoeia Commission. (2005). Pharmacopoeia of People's Republic of China. Beijing, China: Chemical Industry Press.
10. Yi DD, Cai HS, Luo SD, Wang F. (2002). Effect of sinomenine gel on adjuvant arthritis in rats. Chin Hosp Pharm J, 22:465–8.
11. Caruso F, Caruso RA, Mohwald H. (1998). Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating. Science, 282:1111–4.
12. Ai H, Jones SA, de Villiers MM, Lvov YM. (2003). Nano-encapsulation of furosemide microcrystals for controlled drug release. J Control Release, 86:59–68.
13. Antipov AA, Sukhorukov GB, Donath E, Mohwald H. (2001). Sustained release properties of polyelectrolyte multilayer capsules. J Phys Chem B, 105:2281–4.
14. Liu Q, Zhou LL, Li R. (1997). Research overview of sinomenine. Zhong Cao Yao, 28:247–9.

15. Wang AL, Li ZB, Yuan M, Yu ACH, Zhu X, Tso MOM. (2007). Sinomenine inhibits activation of rat retinal microglia induced by advanced glycation end products. Int J Immunopharmacol, 7:1552–8.
16. Chen Y, Lin X. (2005). Studies on the drug release properties of nano-encapsulated indomethacin microparticles. J Microencapsul, 221:47–55.
17. Chen YF, Lin XF. (2007). Controlled release of the indomethacin microencapsulation based on layer-by-layer assembly by polyelectrolyte multilayers. Chin J Biomed Eng, 161:8–13.
18. Nabok AV, Hassan AK, Ray AK. (1999). Optical and electrical characterisation of polyelectrolyte self-assembled thin films. Mater Sci Eng C, 8–9:505–8.
19. Junyaprasert VB, Mitrevej A, Sinchaipanid N, Boonme P, Wurster DE. (2001). Effect of process variables on the microencapsulation of vitamin a palmitate by gelatin-acacia coacervation. Drug Dev Ind Pharm, 27:561–6.
20. Yuan Q, Venkatasubramanian R, Hein S, Misra RDK. (2008). A stimulus-responsive magnetic nanoparticle drug carrier: Magnetite encapsulated by chitosan-grafted-copolymer. Acta Biomater, 4:1024–37.
21. Tieke L, van Ackern F, Krasemann L, Toutianoush A. (2001). Ultrathin self-assembled polyelectrolyte multilayer membranes. Eur Phys J E, 5:29–39.
22. Shi JS, Hua F, Cui TH, Lvov YM. (2003). Temperature effect on layer-by-layer self-assembly of linear polyions and silica multilayers. Chem Lett, 32:316–7.
23. Tan HL, McMurdo MJ, Pan GQ, Van Patten PG. (2003). Temperature dependence of polyelectrolyte multilayer assembly. Langmuir, 19:9311–4.