Poly(L-histidine)-Chitosan/Alginate Complex Microcapsule as a Novel Drug Delivery Agent

Ai-Zheng Chen,[1,2] Mei-Ying Chen,[1] Shi-Bin Wang,[1,2] Xiao-Nan Huang,[1] Yuan-Gang Liu,[1,2] Zong-Xiang Chen[1]

[1]*College of Chemical Engineering, Huaqiao University, Xiamen 361021, China*
[2]*Institute of Biomaterials and Tissue Engineering, Huaqiao University, Xiamen 361021, China*

Received 18 April 2011; accepted 27 July 2011
DOI 10.1002/app.35371
Published online 23 November 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Novel poly(L-histidine)-chitosan/alginate complex microcapsules were prepared from biodegradable polymers poly(L-histidine) (PLHis) in the presence of chitosan at acetate buffer solution pH 4.6. Microcapsules obtained are spherical and well-dispersed with a smooth surface and a narrow size distribution. The microcapsules can encapsulate the protein model drug hemoglobin (Hb) efficiently. The results show that the complex microcapsules with low, medium, or high molecular weight of chitosan (0.05%, w/v), the highest encapsulation efficiencies obtained are 91.3%, 85.9%, and 94.2% with loading efficiencies of 47.8%, 44.3%, and 39.7%, respectively. The release profiles indicate that Hb-loaded microcapsules conform to first-order release kinetic in whole procedure, and 84.8%, 71.4%, and 87.3% of Hb were released during 72-h incubation in PBS pH6.8 for microcapsules with low, medium, and high molecular weight chitosan (0.05%, w/v), respectively. The results also indicate that particle size and drug loading efficiency have a significant influence on the release profile and encapsulation efficiency. Our results reveal that the PLHis-chitosan/alginate complex microcapsules are able to encapsulate and release Hb and are potential carriers for protein drugs.
 J Appl Polym Sci 124: 3728–3736, 2012

Key words: poly(amino acid); controlled release; microcapsules; chitosan; alginate

INTRODUCTION

Polymeric biomaterials have been extensively investigated for developing biodegradable drug delivery vehicles.[1–3] Poly(amino acid)s, due to their biocompatibility and complete biodegradability, make them ideal candidates for applications in human life.[4,5] These polymers typically contain one type of monomer and exhibit a great variety of different molecule size distributions, which may bring various but unique characteristics for biomedical applications. Poly-L-lysine are frequently utilized as cationic polymers in gene delivery investigations,[6] immunoisolation and transplantation of living cells.[7] The alginate-poly-L-lysine microcapsules have been successfully employed to induce prolonged normoglycemia in small diabetic animal models over several days to months.[8] Derivatives of polyglutamic acid used as drug carrier systems were investigated.[9] Recently, modification of well-established biomaterials, such as poly(lactic acid) (PLA), by blending of polymers with block copolymers[10] is becoming an important strategy to improve the compatibility of the microcapsules. Poly-L-arginine that are blended with chitosan have been reported to be potential drug carriers for protein drugs,[11,12] and poly-L-ornithine-coated alginate microcapsules[13] are also exhibited high loading efficiency of protein drugs. Even though, the high price of poly(amino acid) homopolymers limits the application in biomedical field, however, the market for poly(amino acid)s is expected to expand rapidly during the next decades.

PLHis, a commercially available biodegradable polymer, has many imidazole groups in the side chains with a pKa value around 6.0, and is soluble in dilute acids(below pH 5.8).[14] PLHis is easily degraded in biological fluids and produces degraded products with nutritional function and pharmacological efficacy.[15] PLHis complexes, such as PLHis with aminoethyl groups and PLHis-carbohydrate conjugate, have been synthesized as pH-sensitive carriers to promote delivery efficiency to mammalian cells.[16–18] Our previous research on PLHis-coated alginate microcapsule has been demonstrated to have potential application for protein drugs.[19] In this

Additional Supporting Information may be found in the online version of this article.

Correspondence to: S.B. Wang (sbwang@hqu.edu.cn).

Contract grant sponsor: National High Technology Research and Development Program 863; contract grant number: 2006AA02A118.

Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 31170939.

Contract grant sponsor: Natural Science Foundation of Fujian Province; contract grant numbers: 2009J0125, 2010J05027, 2011J01223.

work, the combination of natural and synthetic polymers allows us to synthesize novel complex microcapsules as simple drug carriers for controlled release. The obtained microcapsules are expected to be used as a biodegradable and therapeutic drug carrier, namely, after releasing the drug, the PLHis coating would be degraded as L-histidine, which would have a therapeutic effect for the body.

EXPERIMENTAL PART

Materials

Sodium alginate with a viscosity of 250 cps (2% solution at 25°C, w/v), chitosan (CS) with 75–85% of deacetylation (LMWCS: low MW 50 kDa, MMWCS: medium MW 100 kDa, and HMWCS: high MW 150 kDa), and PLHis hydrochloride (M_w 39,200 Da) were purchased from Sigma Chemical (USA). Bovine erythrocyte hemoglobin (Hb) with a molecular weight of 64,500 Da, was obtained from Shanghai Lizhu Dongfeng Biotechnology (China). Phosphate buffer saline (PBS) solution pH6.8 was prepared from sodium phosphate dodecahydrate aqueous solution and hydrochloric acid. All other chemicals were of analytical purity.

Methods

Preparation of PLHis-chitosan/alginate complex microcapsule

Sodium alginate was dissolved in distilled water and then filtered through 0.80, 0.45, and 0.22 μm membrane filter-paper in sequence. The alginate beads were formed via a high-voltage electrostatic droplet generator (SHL) by extruding 4 mL 2.0%(w/v) sterile alginate solution through a flat needle into a beaker containing 200 mL 1.5% (w/v) calcium chloride, at a rate of 5 mL/h and a electric voltage of 6.3kv.[19] Alginate beads were isolated with standard sieves and rinsed quickly twice with 5 mL distilled water to remove calcium ions on the surface. The beads were then transferred into the blends of PLHis and chitosan under shaking and allowed to react for 10 min, and separated.

Preparation of Hb-loaded PLHis-chitosan/alginate complex microcapsule

Hb was selected as protein model and dissolved in distilled water. Beads from 4 mL sterile alginate were incubated in 20 mL Hb aqueous solution at 4°C for about 24 h. Then, Hb-loaded beads were separated with standard sieves and rinsed twice with 5 mL distilled water quickly to remove Hb absorbed on the surface of the beads. Hb-loaded beads were then transferred into the blends of PLHis and chitosan under shaking for 10 min. Finally, the Hb-loaded microcapsules were separated from the reaction solutions. Microcapsules treated with PLHis (0.05%, w/v) and LMWCS (0.05%, w/v) abbreviates as PLHis/LMWCS 50/50, microcapsules treated with PLHis (0.05%, w/v) and LMWCS (0.10%, w/v) abbreviates as PLHis/LMWCS 33.3/66.7 ... and so on.

Characterization of microcapsules

The shape and surface of Hb-free and Hb-loaded microcapsules were characterized by optical microscopy (PM20, OLYMPUS). Particle sizes of microcapsules were determined by a micrometer (Olympus), and the number of particles was 50. FTIR spectra of Hb-loaded microcapsules and the components in different process were also measured on a FTIR Perkin–Elmer 1720 (Perkin–Elmer, USA) in the transmission mode with the wavenumber ranged from 4,000 cm^{-1} to 400 cm^{-1}. KBr pellets were prepared by gently mixing sample powder with KBr.

Determination of drug loading and encapsulation efficiency

Loading efficiency was determined by measuring the amount of Hb remained in the drug-loading solution. Encapsulation efficiency was determined by measuring the amount of Hb unloaded in the reaction medium where the microcapsules were synthesized. Hb concentration was monitored using a UV-visible spectrophotometer at 405 nm. Each microcapsule sample was from 4 mL sterile alginate (54.6 mg of the dried alginate beads, a mean value from three independent experiments). The drug loading and encapsulation efficiency were calculated from the following two formulas:

$$\text{Loading efficiency} = \frac{W_1}{W_2} \times 100\% \quad (1)$$

$$\text{Encapsulation efficiency} = \frac{W_3 - W_4}{W_3} \times 100\% \quad (2)$$

where W_1 is the amount of Hb entrapped in the microcapsules, W_2 is the gross weight of the Hb-loaded microcapsules, W_3 is the initial amount of Hb loaded, and W_4 is the amount of Hb in the reaction medium (unloaded). W_1 was the difference between W_3 and W_4.

Release profile *in vitro*

Hb-loaded microcapsules were placed in a shaker immersed in PBS at a rotational speed of 50rpm, at 37 ± 0.5°C in a water bath. At designed time points (0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24 h.....), 5 mL of

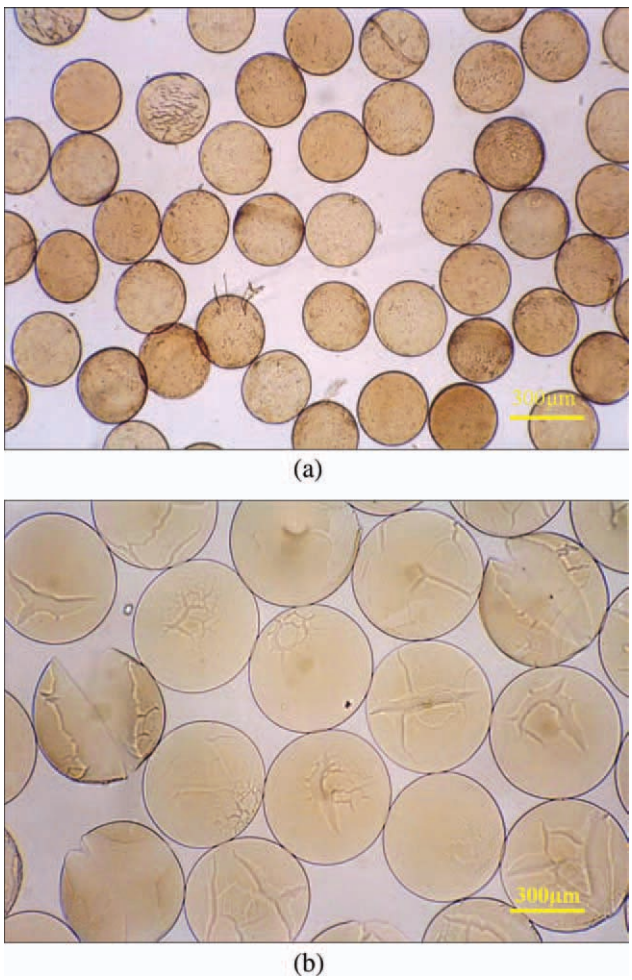

Figure 1 Morphology of Hb-loaded alginate beads in different loading solutions (a) Alginate beads incubating in distilled-water and (b) Alginate beads incubating in PBS pH6.8. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

samples were withdrawn for further quantification of the released Hb and replaced by 5 mL of fresh buffer to maintain 200 mL of incubation volume. Hb concentration was evaluated at 405 nm in a UV-visible spectrophotometer and the cumulative release of Hb was calculated.

Short-term stability

The stability of PLHis-chitosan/alginate microcapsule was tested in an "explosion assay" by introducing a high osmotic swelling pressure.[19] Microcapsules were suspended in a physiological sodium chloride solution (0.15 mol L^{-1}) for 15 min, and then transferred to distilled water, which led to a considerably osmotic swelling pressure inside the microcapsules. The fraction of intact microcapsules in the water was monitored by microscopy at 2, 5, 10, 20, 40, and 60 min.

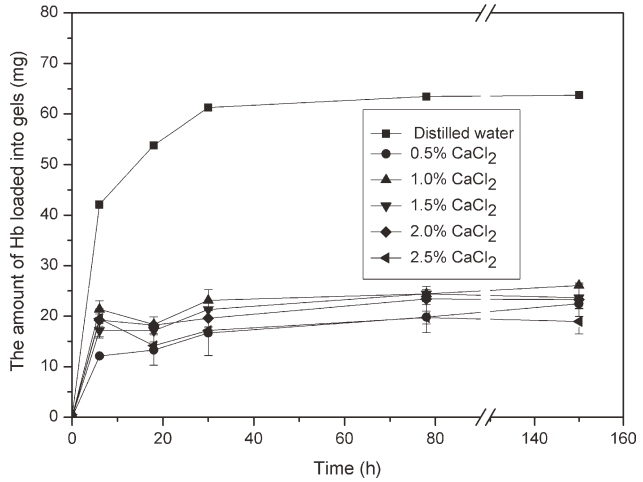

Figure 2 Loading efficiency in the presence of different concentration of calcium ions.

RESULTS

Loading of protein drug in microcapsules

Alginate beads were prepared via a high voltage electrostatic droplet generator. Alginate concentration, electric voltage, push speed of syringe, distance between needle tip and gelling solution, have dramatic effect on the size distribution of alginate beads. With appropriate experiment parameters, beads ranged from 100 to 1000 μm in diameter can be obtained.[18]

Figure 1 displays the effect of solvent for Hb in the drug-loading process. Hb was dissolved in twice-distilled water and PBS pH6.8, respectively. Alginate beads from 4 mL sterile alginate with similar diameter range were suspending independently in the Hb solutions. When alginate beads were incubated in PBS pH6.8, there was an increasing amount of Hb loaded in the beads. However, as shown in Figure 1, alginate beads were swollen and the strength was degreased, which would affect the stability of microcapsules. The phenomenon is consistent with the limitation that alginate beads are instable in a phosphate buffer above pH5.0.[20,21] It may result from the displacement of calcium ions from alginate beads in phosphate buffer saline.

Figure 2 shows the effect of Ca^{2+} in the drug-loading process. It is quite clear that less Hb was loaded

TABLE I
The Hb Loading of Alginate Beads with Various Diameter Ranges

Diameter of gel beads (μm)	Amount of Hb loaded (mg)
250–300	26.93 ± 0.00
450–600	25.35 ± 0.81
650–750	14.41 ± 0.81

Initial amount of Hb to be loaded was 100 mg.

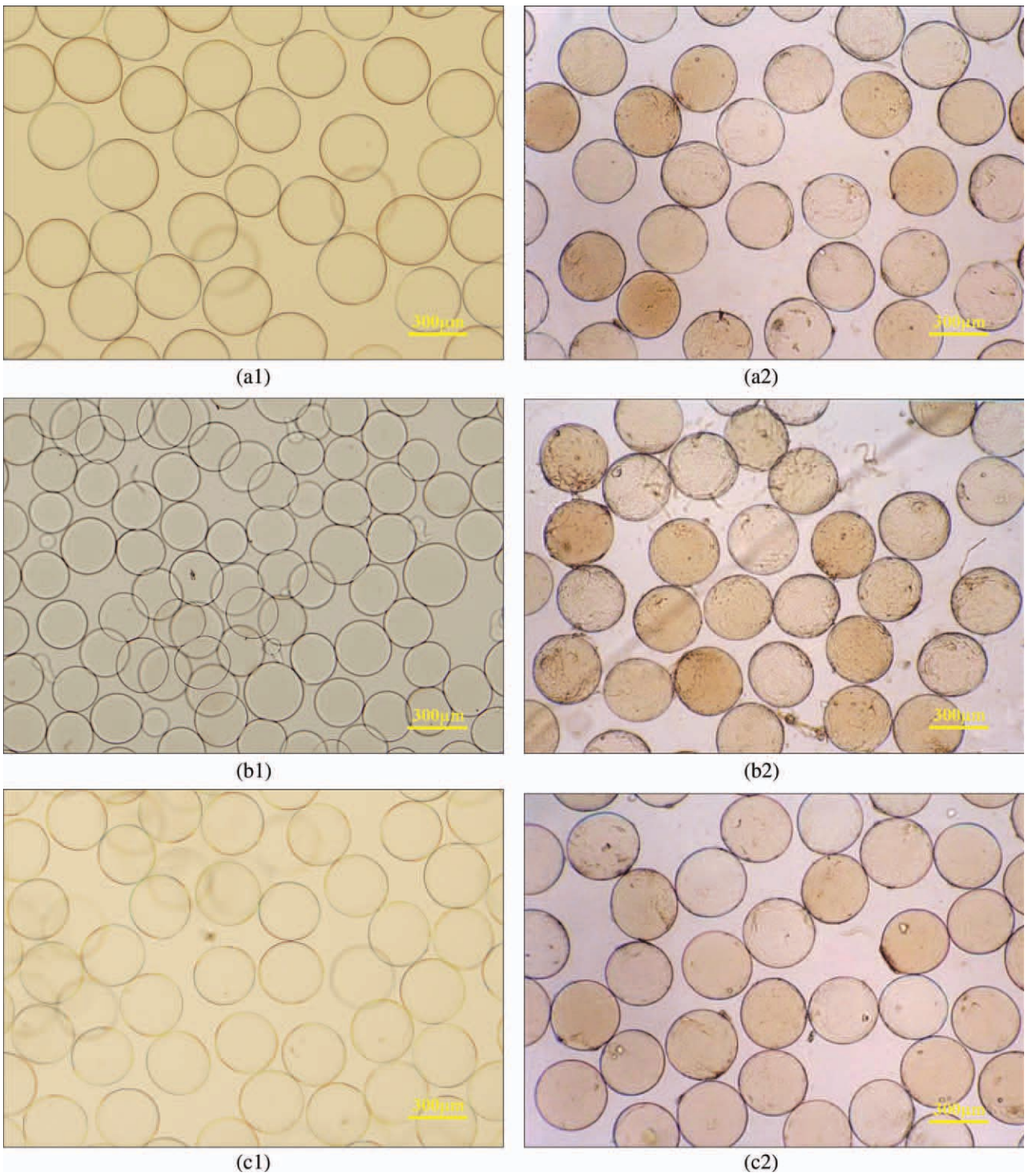

Figure 3 Microscope photos of unloaded and Hb-loaded complex microcapsules. a1. Unloaded microcapsules of PLHis/LMWCS 50/50; a2. Hb-loaded microcapsules of PLHis/LMWCS 50/50; b1. Unloaded microcapsules of PLHis/MMWCS 50/50; b2. Hb-loaded microcapsules of PLHis/MMWCS 50/50; c1. Unloaded microcapsules of PLHis/HMWCS 50/50 and c2. Hb-loaded microcapsules of PLHis/HMWCS 50/50. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

into the alginate beads when the calcium ions were presented in the loading solutions, this indicates that the presence of calcium ions would greatly reduce the diffusion of Hb into the alginate beads, and most of the Hb were adsorbed onto instead of adsorbed into the beads. For this reason, the specific surface area became important for the amount of Hb loading, the beads with a smaller particle size would

TABLE II
The Average Diameters of Gel Beads and Complex Microcapsules

Chitosan M_w	Diameters of alginate beads (μm)	Diameters of microcapsules (μm)
Low	255.0 ± 9.6	275.1 ± 12.6
	279.8 ± 6.7	283.0 ± 8.2
	279.8 ± 9.0	290.3 ± 11.0
Medium	256.9 ± 9.0	271.6 ± 10.9
	276.9 ± 6.3	290.1 ± 11.7
	279.6 ± 7.2	288.6 ± 4.7
High	251.6 ± 10.2	270.6 ± 13.1
	278.8 ± 5.9	304.8 ± 9.6
	283.2 ± 7.0	289.4 ± 9.9

Concentrations of PLHis and chitosan both were 0.05% (w/v).

have a higher Hb loading due to the larger specific surface area, and this was also supported by the results as shown in Table I.

Characterization of microcapsules

The morphology and size distribution of the microcapsules subjected to different treatments are shown in Figure 3 and Table II, respectively. The microcapsules are spherical and smooth-surfaced with narrow size distribution. The morphology of Hb-loaded is similar to that of Hb-free microcapsules. Therefore, the loading of the Hb does not change the morphology of the microcapsules significantly. However, as shown in Table II, after coating with PLHis and chitosan, the resulting microcapsules have a larger particle size than alginate beads.

Figure 4 shows the FTIR spectra of Hb, Hb-loaded alginate beads, and Hb-loaded PLHis-Chitosan/alginate microcapsules. The major peaks of 1655 cm^{-1} and 1407 cm^{-1} of Hb spectrum, 1655 cm^{-1} and 1408 cm^{-1} of Hb-loaded alginate beads spectrum, 1655 cm^{-1} and 1409 cm^{-1} of PLHis-Chitosan/alginate microcapsules spectrum are the characteristic peaks

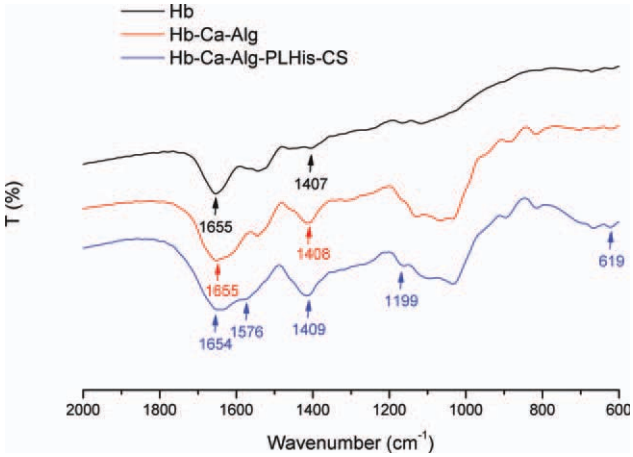

Figure 4 FTIR spectra of Hb, Hb-Ca-Alg beads and Hb-Ca-Alg-PLHis-CS microcapsules. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of amide group in Hb, it is found that there is no significant difference, which indicates that protein structure is stable during the process; after coating with PLHis and Chitosan, new peaks of 1576 cm^{-1}, 1199 cm^{-1}, and 619 cm^{-1} are observed in the FTIR spectrum of PLHis-Chitosan/alginate microcapsules, the peaks of 1576 cm^{-1}, 1199 cm^{-1} are mainly attributed to the amide group of chitosan, and comparing with the FTIR spectrum of PLHis (shows in Supporting Information), it can be confirmed that the peak of 619 cm^{-1} is generated by the component of PLHis in microcapsules. This reveals that the alginate beads are successfully covered by the PLHis and chitosan mixture.

Drug loading and encapsulation efficiency of complex microcapsules under different conditions

To optimize drug loading conditions, the loading and encapsulation efficiency were measured at different conditions. As shown in Table III, the higher

TABLE III
Loading and Encapsulation Efficiency of Microcapsules Subjected to Different Treatments

Formulation	Amount of Hb loaded (mg)	Loss in procedure (mg)	Loading efficiency (%)	Encapsulation efficiency (%)
PLHis/LMWCS 50/50	25.92	6.16	26.6	76.3
	27.80	5.99	28.5	78.5
	38.83	3.48	39.3	91.0
	54.87	4.79	47.8	91.3
PLHis/MMWCS 50/50	20.59	6.18	20.9	70.0
	26.93	5.65	28.0	79.0
	38.16	6.37	36.8	83.3
	50.55	7.12	44.3	85.9
PLHis/HMWCS 50/50	25.34	3.78	28.3	85.1
	29.74	3.42	32.5	88.5
	38.21	2.21	39.7	94.2
	49.46	3.61	45.6	92.7

TABLE IV
Loading and Encapsulation Efficiency of MCSPLHis50 Microcapsules

A. Microcapsules from alginate beads with various diameter ranges

Bead size (μm)	Amount of Hb loaded (mg)	Loss in procedure (mg)	Loading efficiency (%)	Encapsulation efficiency (%)
250–300	26.93 ± 0.00	6.84 ± 1.68	26.9 ± 1.7	74.6 ± 6.2
450–600	25.35 ± 0.81	6.74 ± 0.79	25.4 ± 1.7	73.5 ± 4.0
650–750	14.41 ± 0.81	3.43 ± 0.21	16.8 ± 0.8	76.2 ± 0.0

B. Microcapsules from various polymer concentrations

Formulation (w/v, %)			Amount of Hb loaded	Loss in procedure	Loading efficiency	Encapsulation efficiency
Total	PLHis	CS	(mg)	(mg)	(%)	(%)
0.20	0.05	0.15	27.14 ± 1.53	4.31 ± 0.91	29.5 ± 0.6	84.2 ± 2.4
0.30	0.05	0.25	23.19 ± 2.44	4.13 ± 0.34	25.8 ± 2.8	82.0 ± 3.4
0.40	0.05	0.35	20.81 ± 1.94	4.31 ± 0.11	23.2 ± 2.0	79.2 ± 1.4

the initial amount of drug encapsulated, the higher the drug loading and encapsulation efficiency for microcapsules. The highest encapsulation efficiencies obtained were 91.3%, 85.9%, and 94.2% with loading efficiencies of 47.8%, 44.3%, and 39.7% for PLHis/LMWCS 50/50, PLHis/MMWCS 50/50 and PLHis/HMWCS 50/50 microcapsules, respectively. High Hb loss was observed for PLHis/LMWCS 50/50 and PLHis/MMWCS 50/50 during their preparations. Table IV shows the drug loading and encapsulation efficiency of three classes of microcapsules under different conditions. The variations in loading and encapsulation efficiencies for microcapsules are similar as described in Table III. Interestingly, the loss for PLHis/MMWCS 50/50 microcapsules decreases with a less amount of Hb encapsulated in the microcapsules. However, an increasing polymer concentration decreases the loss of Hb and a higher encapsulation efficiency was obtained (Table IV).

Release profile *in vitro*

The microcapsules exhibit sustained release for proteins. As shown in Figure 5(a,b), the percentage of cumulative Hb release reaches 85.0%, 81.0%, and 87.3% from microcapsules of PLHis/LMWCS 50/50, PLHis/MMWCS 50/50, and PLHis/HMWCS 50/50 at 76, 96, and 72 h without burst effect, respectively. PLHis/MMWCS 50/50 microcapsules have the slowest release rate after the 20 h, which have the highest release rate during the first 16 h. When increasing chitosan concentrations, as shown in Figure 5(b), a retarded drug release effect in the first 18 h for microcapsules from blends of PLHis (0.05%, w/v) and chitosan (0.1%, w/v) is resulted.

Figure 5(c,d) show the release profiles of microcapsules with various loading efficiencies. All the microcapsules were treated with PLHis (0.05%, w/v) and chitosan (0.05%, w/v). Microcapsules with drug loading efficiency between 30 and 40% show high initial burst release during the first 16 h, followed by a slower release. Microcapsules with drug loading between 40 and 50% have the slowest release rate with the lowest burst effect. Interestingly, different release profiles are observed for PLHis/LMWCS 50/50 and PLHis/MMWCS 50/50 microcapsules with various drug loading efficiencies. For PLHis/LMWCS 50/50 microcapsules, 73.6%, 79.8%, and 77.1% Hb were released at 64 h with loading efficiencies of 28.5%, 39.3%, and 47.8%, respectively, whereas 87.5%, 71.8%, and 63.6% of Hb was released from PLHis/MMWCS 50/50 at 64 h, giving loading efficiencies of 20.9%, 36.8%, and 44.3%, respectively. The results reveal that with increasing in drug loading efficiency, microcapsules exhibit a lower percentage of cumulative release of Hb.

The polymer concentration and the alginate beads diameter also have dramatic effect on the Hb release profile. Figure 5(e) shows the release profiles of microcapsules from various polymer concentrations, the release rate decreases when the concentration of chitosan is increased. As shown in Figure 5(f), microcapsules with a bigger size have a slower release rate in the initial phase and a higher one in the latter phase, while the opposite phenomenon is observed in the microcapsules with a smaller size, and this is probably due to the balance between specific surface area and diffusion path length of microcapsules.

Short-term stability

Stability of microcapsules is of interest when evaluating their possible utility as drug carriers. Figure 6 shows the fractions of intact microcapsules in the

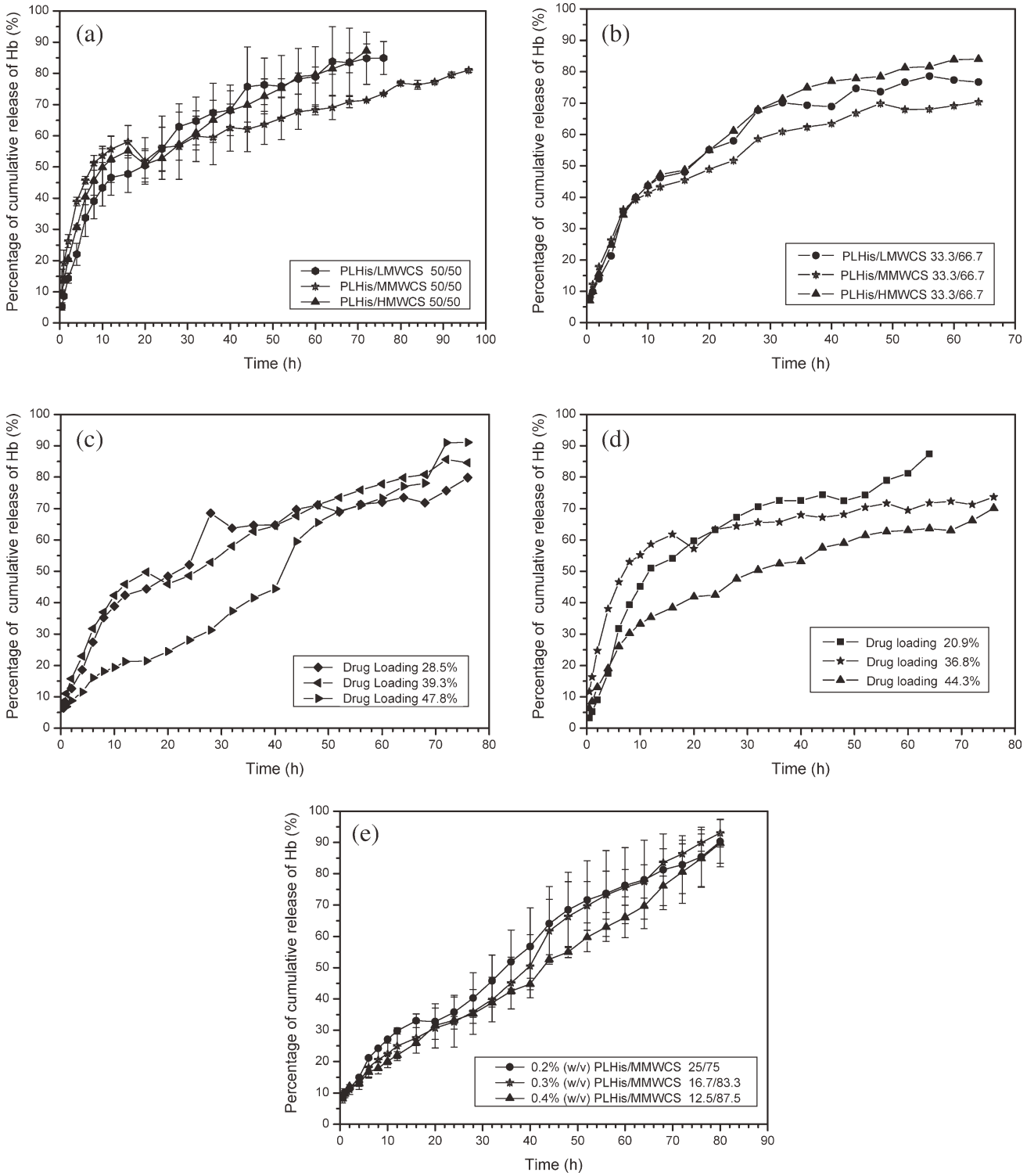

Figure 5 Release profiles of Hb from various microcapsules (a) with different molecular weight of chitosan in the ratio of 50/50 to PLHis, (b) with different molecular weight of chitosan in the ratio of 33.3/66.7 to PLHis, (c) PLHis/LMWCS 50/50 microcapsules with different drug loading, (d) PLHis/MMWCS 50/50 microcapsules with different drug loading, (e) PLHis/MMWCS microcapsules with different polymer concentrations and ratios, and (f) PLHis/MMWCS microcapsules from alginate beads with various diameter ranges.

end of the test. It is observed that microcapsules from PLHis and chitosan are more stable. The fraction of intact microcapsules of PLHis/LMWCS 50/50, PLHis/MMWCS 50/50 and PLHis/HMWCS 50/50 after test periods of 60 min is 95.8%, 84.8%, and 95.6%, respectively.

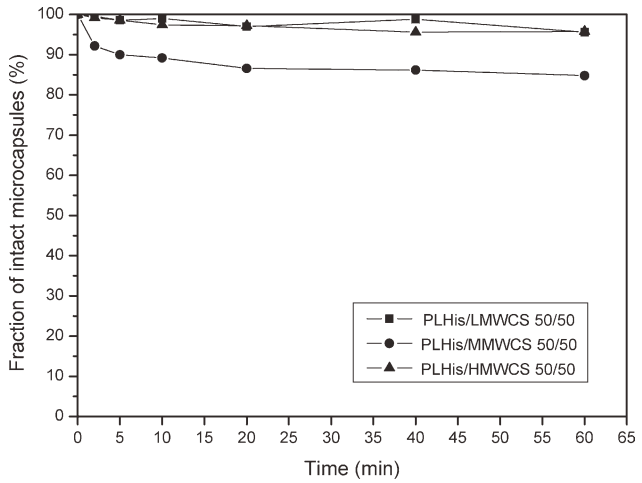

Figure 6 Short-term stability of microcapsules with different molecular weight of chitosan in the ratio of 50/50 to PLHis.

DISCUSSION

In recent years, the study of controlled release of drugs, especially for peptide and protein drugs, from polymeric devices, have drawn much attention. It has been shown that many drugs need to be administered at varying rates, such as those used at the beginning of wound treatment; an initial burst provides immediate relief followed by prolonged release to promote gradual healing.[22] Therefore, alternative drug delivery systems are essential. Chitosan is known for its biocompatibility for a long time and it has been employed in various medical applications such as topical ocular application, implantation, or injection.[23] Moreover, chitosan is abundant in nature with its production of low cost, generally obtained by alkaline deacetylation of chitin from crustaceans, such as shrimps. In this work, new PLHis-chitosan/alginate complex microcapsules have been obtained by selecting blends of PLHis and chitosan as polycationic polymers. To study the release kinetic of complex, the mathematic models as shown in Table V were applied to fit the release data. From Table V, the curve fitting analysis conducted on the data suggested that the release profiles of microcapsules conform to first-order release kinetic well in whole procedure with relatively high correlation coefficients and small standard deviations.

The *in vitro* release profiles show that these polymeric systems are capable of sustained release for the drug, approaching 84.8%, 71.4%, and 87.3% cumulative release of Hb from PLHis/LMWCS 50/50, PLHis/MMWCS 50/50, and PLHis/HMWCS 50/50 microcapsules at 72 h. The remaining loaded Hb could be released at later stages, and the complete drug release could be achieved by degradation of the network. Hb-loaded microcapsules were observed with an initial burst release with 5.3%, 14.0%, 9.4% cumulative release of Hb in the first 30 min, and 14.4%, 26.3%, 20.5% in the first 2 h from PLHis/LMWCS 50/50, PLHis/MMWCS 50/50 and PLHis/HMWCS 50/50 microcapsules, respectively.

Comparing with our previous studies on calcium alginate beads, chitosan coated alginate microcapsules[24,25] and PLHis coated alginate microcapsules,[19] microcapsules from blends of PLHis with chitosan prolong sustained release, this probably due to the more dense membrane structure created by the mixture of PLHis and chitosan. Similar phenomena were also observed in the studies on alginate/poly-L-arginine-chitosan complex microcapsules.[12,24,25]

Drug loading and particle size could also bring different release profiles for microcapsules. Nonuniform drug loading is difficult to achieve in real systems. Lu et al. proposed using multilaminate systems to produce matrix systems with nonuniform drug distributions to improve sustained release.[26] We attained different drug loading efficiency via choosing proper experimental conditions.

CONCLUSIONS

These microcapsules represent a type of proteins delivery system that can be prepared in a typical physical process without tedious process and without changing the molecular structure of proteins. Microcapsule systems are good at achieving zero-order kinetic having high loading and encapsulation efficiency. From the studies, it is suggested that these polymeric microcapsules could be used as alternative carriers for peptides and proteins in

TABLE V
The Mathematical Models and Statistics Obtained from the Drug Release Profiles of Complex Microcapsules

		Zero-order release		First-order release		Higuchi's model	
Microcapsules	*N*	R	SD	R	SD	R	SD
PLHis/LMWCS 50/50	24	0.929	9.527	0.988	0.0942	0.985	4.489
PLHis/MMWCS 50/50	29	0.878	8.328	0.953	0.1209	0.941	5.858
PLHis/HMWCS 50/50	23	0.927	8.510	0.980	0.1113	0.975	5.043

Zero-order: $Mt/M\infty = kt + C$; First-order: $\ln(1-Mt/M\infty) = -kt + C$; Higuchi: $Mt/M\infty$, $kt0.5+C$; the fraction of drug released is up to *t*: time; *k*, the kinetic constant; C, constant; *N*, number of data points; R, correlation coefficient; SD, standard deviation of the fit.

biomedical application. This investigation also constitutes a basis for future entrapment and release studies for other macromolecular drug or others relevant for biomedical application.

References

1. Liu, Y.; Cui, Y. J Appl Polym Sci 2011, 120, 3613.
2. Wu, C.; Xie, J.; Branford-White, C.; Quan, J.; Zhu, L. J Appl Polym Sci 2011, 3, 1654.
3. Sullad, A. G.; Manjeshwar, L. S.; Aminabhavi, T. M. J Appl Polym Sci 2010, 117, 1361.
4. Matsumura, Y. Adv Drug Deliv Rev 2008, 60, 899.
5. Akagi, T.; Wang, X.; Uto, T.; Baba, M.; Akashi, M. Biomaterials 2007, 28, 3427.
6. Yamagata, M.; Kawano, T.; Shiba, K.; Mori, T.; Katayama, Y.; Niidome, T. Bioorg Med Chem 2007, 15, 526.
7. Maki, J.; Masuda, C.; Morikawa, S.; Morita, M.; Inubushi, T.; Matsusue, Y.; Taguchi, H.; Tooyama, I. Biomaterials 2007, 28, 434.
8. Tam, S. K.; Dusseault, J.; Polizu, S.; Menard, M.; Halle, J. P.; Yahia, L. Biomaterials 2005, 26, 6950.
9. Hoste, K.; Schacht, E.; Seymour, L. J Control Release 2000, 64, 53.
10. Cai, K. Y.; Yao, K. D.; Lin, S. B.; Yang, Z. M.; Li, X. Q.; Xie, H. Q.; Qing, T. W.; Gao, L. B. Biomaterials 2002, 23, 1153.
11. Wang, S. B.; Liu, Y. G.; Weng, L. F.; Ma, X. J. Macromol Biosci 2003, 3, 347.
12. Wang, S. B.; Chen, A. Z.; Weng, L. J.; Chen, M. Y.; Xie, X. L. Macromol Biosci 2004, 4, 27.
13. Darrabie, M. D.; Kendall, W. F., Jr.; Opara, E. C. Biomaterials 2005, 26, 6846.
14. Patchornik, A.; Berger, A.; Katchalski, E. J Am Chem Soc 1957, 79, 5227.
15. Wade, A. M.; Tucker, H. N. J Nutr Biochem 1998, 9, 308.
16. Asayama, S.; Kawakami, H.; Nagaoka, S. Polym Adv Technol 2004, 15, 439.
17. Asayama, S.; Sekine, T.; Hamaya, A.; Kawakami, H.; Nagaoka, S. Polym Adv Technol 2005, 16, 567.
18. Wang, C. Y.; Huang, L. Biochemistry 1984, 23, 4409.
19. Wang, S. B.; Xu, F. H.; He, H. S.; Weng, L. J. Macromol Biosci 2005, 5, 408.
20. Chan, L. W.; Heng, P. W. S. Biomaterials 2002, 23, 1319.
21. Ribeiro, A. J.; Silva, C.; Ferreira, D.; Veiga, F. Eur J Pharm Sci 2005, 25, 31.
22. Huang, X.; Brazel, C. S. J Control Release 2001, 73, 121.
23. Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Peppas, N. A.; Gurny, R. Eur J Pharm Biopharm 2004, 57, 19.
24. Chen, A. Z.; Wang, S. B.; Chen, M. Y.; Weng, L. J. J Biomim Biomater Tissue Eng 2009, 3, 25.
25. Wang, S. B.; Chen, A. Z.; Chen, M. Y.; Weng, L. J. Transaction-7th World Biomaterials Congress, Sydney, Australia, May17–21 2004, p 1182.
26. Lu, S. X.; Ramirez, W. F.; Anseth, K. S. AIChE J 1998, 44, 1689.