

Chitin/PLGA blend microspheres as a biodegradable drug delivery system: a new delivery system for protein

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Abstract

Novel chitin/PLGAs and chitin/PLA based microspheres were developed for the delivery of protein. These biodegradable microspheres were prepared by polymers blending and wet phase-inversion methods. The parameters such as selected non-solvents, temperature of water and ratio of polylactide to polyglycolide were adjusted to improve thermodynamic compatibility of individual polymer (chitin and PLGAs or chitin/PLA), which affects the hydration and degradation properties of the blend microspheres. Triphasic pattern of drug release model is observed from the release of protein from the chitin/PLGAs and chitin/PLA microspheres: the initially fast release (the first phase), the following slow release (the second phase) and the second burst release (the third phase). Formulations of the blends, which are based on the balance among the hydration rate of the chitin phase and degradation of chitin/PLA and PLGA phase, can lead to a controllable release of bovine serum albumin (BSA). In conclusion, such a chitin/PLGA 50/50 microsphere is novel and interesting, and may be used as a protein delivery system.

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1. Introduction

Biodegradable polyesters derived from lactic acid, glycolic acid and ϵ -caprolactone have gained much interest in biomedical research for the delivery of various drugs [1–6]. Among the various biodegradable polyesters, PLGA [poly(D,L-lactide-co-glycolide)] was particularly suitable to be used for the drug delivery application. PLGA polymers are already approved by the US Food and Drug Administration (FDA), such as biodegradable structures, implantable screws, pins, drug delivery devices and tissue engineering scaffolds [7,8]. PLGA can be metabolized via degradation of lactic acid by lactate dehydrogenase and via glycine metabolism. The degradation rate of a PLGA polymer can be controlled by the molar ratio of the lactic and glycolic acids in the polymer chain, the degree of crystallinity,

molecular weight and stereochemistry of the polyester [9–13].

It has been reported that the release of macromolecular proteins from the matrices of hydrophobic polyesters occurs primarily through pore diffusion (i.e. the non-crystalline region) and the release rate is usually very slow [4]. Owing to this reason, PLGA polymers have been blended with several hydrophilic polymers biocompatible to modify the hydration of the polymeric matrix [14,15]. Depending on the thermodynamic properties of the chosen polymers, different degrees of phase separation can be observed from the blended polymers, leading to the variation of their hydration, degradation and drug release behaviors. For instance, the permeability coefficients of peptide drug were found to increase monotonically with the proportion of polyvinyl alcohol (PVA) in a PLGA matrix [16]. Furthermore, block copolymers containing PLGA and poly(ethyleneglycol) [PEGs] segments were also synthesized and characterized for biomaterials purposes [17].

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Chitin [poly β (1-4)-D-glucosamine] is also a biodegradable polysaccharide widely used in biomedical fields. Many studies have reported the use of chitin and its derivatives for pharmaceutical purposes, such as the delivery of anti-cancer drugs [18,19]. Chitin is more hydrophilic than PLGA, and its biodegradable property is significantly different from that of PLGA. Accordingly, drug release from the chitin/PLGA blends may be affected by their respective hydrophilicity and degradability. Recently, the study of special protein release has attracted much attention; for example, sustained release of active growth factors (such as bFGF, EGF and TGF etc.) are required in order to achieve desirable therapy for tissue engineering research due to the shortness of in vivo half-lives of the proteins [20–23], and multiple injections are necessary for the delivery of vaccine antigen due to the low effect in attaining and maintaining high antibody titers [24–28]. In the present study, we reported the preparation and characterization of a novel biodegradable protein delivery device based on the chitin/PLGAs and chitin/PLA blends. Bovine serum albumin (BSA) is used as a model protein to examine the release behavior of prepared chitin/PLGAs and chitin/PLA microspheres. The feasibility of using the chitin/PLGAs and chitin/PLA blends for the incorporation of BSA in a controlled-release dosage form is investigated for the improvement of BSA release capability. Depending on the variation of selected non-solvents, the temperature of coagulant and LA/GA content of the PLGA polymer, the miscibility of chitin/PLGA blends can be modified, and the prepared microspheres are sustainable to release the protein for several weeks or with multiple phases of release, making them potentially useful as delivery systems for pharmaceutical proteins and vaccine antigens.

2. Experimental

2.1. Materials

Chitin was purchased from Tokyo Chemical Industry (Japan). PLGA 50/50, 65/35, 75/25, 85/15 (M_w is about 40,000, lactide/glicolide ratio is 50/50, 65/35, 75/25 and 85/15) and chitin/PLA were obtained from Polyscience (USA). Bovine serum albumin was purchased from Sigma Chemical Company, Inc. (USA). All other reagents and solvents used were of reagent grade.

2.2. Preparation of chitin/PLGAs and chitin/PLA blended solutions

Chitin solution (1.0% by weight) was prepared by suspending chitin powder (2 g) in 200 ml of dimethylacetamide(DMAc)-5 wt% lithium chloride(LiCl) solution. The chitin/DMAc-LiCl mixed suspension was

stirred by a mechanical stirrer (IKA, RW 20) and refluxed at 120°C to dissolve the chitin powder, until a brown solution was derived. The chitin/PLGA 50/50, 65/35, 75/25, 85/15 and chitin/PLA blend solutions were prepared respectively by dissolving PLGAs and PLA in the prepared chitin solution. The ratios of chitin/PLGAs and chitin/PLA blends are 1:1. The chitin/PLGAs and chitin/PLA blend solutions were then sonicated to remove the trapped air bubbles for later use.

2.3. Preparation of chitin/PLGAs and chitin/PLA blend microspheres by wet phase-inversion

2.3.1. Effect of selected non-solvent

To prepare microspheres, the chitin/PLGA 50/50 blend solution was injected dropwise through a syringe (NIPRO 2004-08, Japan) into the coagulation bath. The bath contained a large amount of various non-solvents respectively such as methanol, ethanol, *n*-propanol, *n*-butanol and water, which provides a sink of coagulation for completely replacing DMAc-LiCl solution from the droplet of chitin/PLGA 50/50 blend. The gelled microspheres were allowed to harden in the coagulation bath for 6 h. After coagulation, the microspheres were filtered, rinsed with deionized water again and dried in air overnight, then stored in desiccator for future analyses.

2.3.2. Effect of water temperature

The chitin/PLGA 50/50 blend solution was dropped through a syringe into different temperature of water bath to prepare microspheres. The temperature of water varied from 40°C to 90°C, which provided a warm coagulation bath for replacing DMAc-LiCl solution from the chitin/PLGA 50/50 blend droplet. The gelled microspheres were allowed to harden in the cool coagulation bath (25°C) for 6 h. After coagulation, the microspheres were filtered, rinsed with deionized water again and dried in air overnight, then stored in desiccator for future analyses.

2.3.3. Effect of LA/GA of PLGA

The chitin/PLGA 50/50, 65/35, 75/25, 85/15 and chitin/PLA blend solutions were dropped through a syringe into the water bath, respectively. The temperature of water was kept at 25°C, which provided a sink of coagulation for completely replacing of DMAc-LiCl solution from the chitin/PLGAs and chitin/PLA blend droplets. The gelled microspheres were allowed to harden in the coagulation bath for 6 h. After coagulation, the microspheres were filtered, rinsed with deionized water again and dried in air overnight, then stored in desiccator for future analyses.

2.4. Preparation of protein-loaded chitin/PLGA 50/50 microspheres

Bovine serum albumin was dispersed in the chitin/PLGAs and chitin/PLA blend solutions by stirring to prepare a homogeneously mixed BSA/chitin/PLGAs and chitin/PLA solution. The BSA/chitin/PLGAs and BSA/chitin/PLA solutions were respectively dropped through a syringe into the coagulation bath according to the process for preparation of raw chitin/PLGAs and chitin/PLA microspheres as described above. During the coagulation of polymeric chitin/PLGAs and chitin/PLA solutions, BSA is entrapped in the phase separating chitin/PLGAs and chitin/PLA microspheres. The microspheres were filtered, rinsed with deionized water again and dried in air overnight, then stored in desiccator for drug-release analyses.

2.5. X-ray study

The crystalline forms of the chitin microspheres after the phase separation were determined by a D-8 diffractometer (Bruker), which has an X-ray generator of 3 kW, and $\text{Cu K}\alpha$ radiation. The samples were scanned at $2^\circ/\text{min}$ under the diffraction angle 2θ in the range of $1\text{--}70^\circ$.

2.6. Scanning electronic microscopy (SEM)

The chitin/PLGAs and chitin/PLA microspheres prepared by coagulation in non-solvent (wet phase-inversion method) were attached onto a double-sided adhesive tape and fixed to an aluminum stage. The microspheres were cut by a razor, then were sputter-coated with gold in a thickness of 500×10^{-8} cm using a Hitachi coating unit (IB-2 coater). Subsequently, the morphologies of surface and cross-section of the microspheres were examined using a Hitachi S-3500N scanning electron microscopy.

2.7. Differential scanning calorimetry (DSC)

DSC (Perkin-Elmer Pyris-1) thermograms were taken using a standard aluminum pan. Nitrogen was used as a sweeping gas, and the heating rate was $5^\circ\text{C}/\text{min}$. Samples (5 mg) were loaded in a pan without further treatment. The initial and end temperatures are -50°C and 100°C , respectively. All the samples for DSC analysis were original chitin/PLGAs and chitin/PLA microspheres without loading with protein.

2.8. Hydration of chitin/PLGAs and chitin/PLA blend microspheres

The water uptake capacity of each chitin/PLGAs and chitin/PLA microspheres were determined by the

hydration of microsphere in deionized water at room temperature. The chitin/PLGAs and chitin/PLA microsphere (200 mg) were placed in deionized water for a required period of time. At preset time intervals, hydrated samples were taken and weighed after blotting the surface water with a filter paper, then weighed immediately on an electronic balance. The percentage of water content of the chitin/PLGAs and chitin/PLA microsphere was calculated as follows:

$$P_{\text{WC}}\% = [(W_e - W_o)/W_o] \times 100\%$$

P_{WC} is the percentage of water content of the chitin/PLGAs and chitin/PLA microsphere at equilibrium. W_e denote the weight of chitin/PLGAs and chitin/PLA microsphere at equilibrium of water uptake and W_o is the initial weight of chitin/PLGAs and chitin/PLA microsphere. Each water uptake experiment was repeated 3 times and the average value was taken as the percentage of water content.

2.9. Degradation of chitin/PLGAs and chitin/PLA blend microspheres

The degradation study of the chitin/PLGAs and chitin/PLA microspheres was conducted in vitro by incubating the microspheres in deionized water within a test tube with known weights, respectively. The test tubes were placed in shaking water bath. The temperature was kept at a 37°C . At predetermined time intervals, the chitin/PLGAs and chitin/PLA microspheres were separated from the medium and dried in air overnight. The biodegradation rate was expressed as the weight loss of the chitin/PLGAs and chitin/PLA microspheres after incubation. Each biodegradation experiment was repeated 3 times and the average value was taken as the remaining weight of chitin/PLGAs and chitin/PLA microspheres. The variation of morphologies and chemical compositions of the chitin/PLGAs and chitin/PLA microspheres after degradation were examined by SEM (Hitachi S-3500N) study.

2.10. Protein release from chitin/PLGAs and chitin/PLA blend microspheres

BSA-loaded chitin/PLGAs and chitin/PLA microspheres (0.5 g) were incubated in pH 7.4 of PBS buffer solution (20 ml) in a test tube. The temperature of incubation medium was maintained at 37°C . Agitation was provided using a shaking water bath (at 80 St/min throughout the dissolution run. After a predetermined period, the amount of BSA in the incubation medium was analyzed spectrophotomerically at 595 nm by mixed with the reagent of Bio-rad Protein Assay (Bio-rad Chemical Co., USA). The Bio-rad Protein Assay is a dye-binding assay in which a differential color change of a dye occurs in response to various concentrations of

protein. The Coomassie Brilliant Blue G-250 dye shifts from 465 to 595 nm when binding to protein occurs. Thus, Beer's law can be applied for accurate quantitation of the dye-albumin complex solution.

3. Result and discussion

3.1. Effect of solubility parameters of non-solvents on phase separation

Fig. 1 shows the X-ray powder diffraction of chitin microspheres prepared by using various non-solvents. The diffraction peaks from X-ray analysis demonstrated that crystallinity of chitin microspheres prepared from various non-solvents are in the order of n -propanol < ethanol < methanol < water. The result could be attributed to the different crystallinity of those microspheres, which may be conditioned by the solubility difference between solvents and non-solvents. It is shown in Table 1 that the alcohols with higher carbon number (n -propanol and ethanol) have smaller $\Delta\delta_{s-n}$, whereas water has a largest $\Delta\delta_{s-n}$. This suggests that there is a faster exchanging rate between n -propanol and

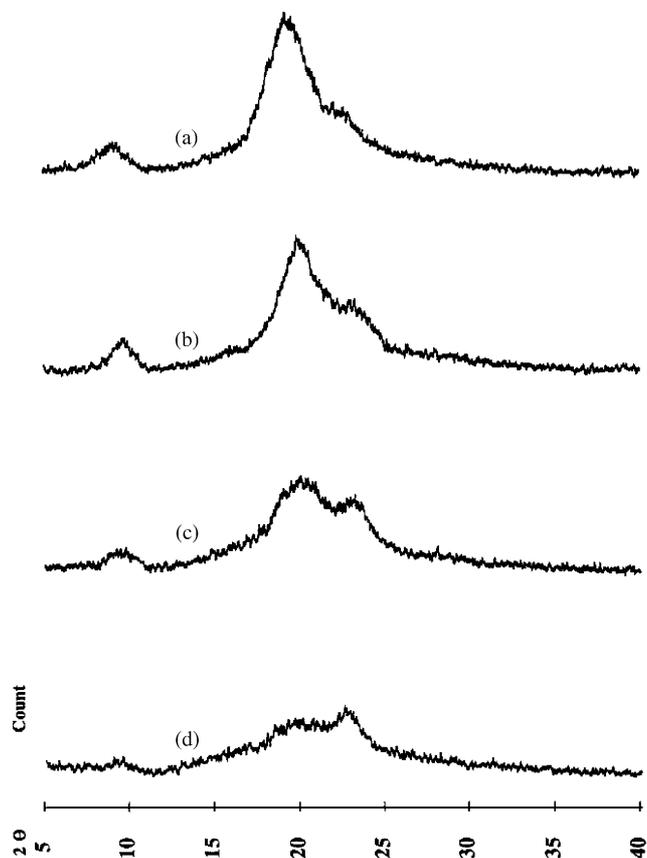


Fig. 1. X-ray powder diffraction of chitin microspheres prepared by coagulation in different non-solvents: (a) coagulation in n -propanol, (b) coagulation in ethanol, (c) coagulation in methanol, (d) coagulation in water.

Table 1

The solubility parameter of various non-solvents and the solubility parameter differences between DMAc and non-solvent or chitin (MPa)^{1/2}

Non-solvent	δ_n	$\Delta\delta_{s-n}$	$\delta_{n(H)}$	$\Delta\delta_{s-n(H)}$	$\Delta\delta_{n-c}$
Water	47.9	25.8	42.4	32.2	23.1
Methanol	28.8	6.7	21.8	11.6	4.0
Ethanol	26.0	3.9	19.4	9.2	1.2
n -propanol	24.3	2.2	17.4	7.2	0.5

δ_s , δ_n , δ_c , $\delta_{s(H)}$ or $\delta_{n(H)}$ is the solubility parameter of solvent (complete parameter), non-solvent (complete parameter), chitin (complete parameter), solvent (hydrogen bonding term) or non-solvent (hydrogen bonding term), and $\Delta\delta_{s-n}$, $\Delta\delta_{n-c}$, or $\Delta\delta_{s-n(H)}$ is solubility parameter difference between solvent and non-solvent, non-solvent and chitin, or hydrogen bonding term of solubility parameter difference between solvent and non-solvent.

δ_c value (24.8) of chitin is calculated from Hoy's data.

δ_s value and $\delta_{s(H)}$ value of DMAc are 22.1 and 10.2 respectively; the $\Delta\delta_{s-c}$ is 2.7.

DMAc but a slower exchanging rate between water and DMAc. The lower the rate of non-solvents to diffuse into the polymer solution, the slower the precipitating rate of the polymer, allowing the growth of an ordered crystalline in the chitin gel.

It is well known that the ratio of the solvent outflow (J_2) to the coagulant (non-solvent) inflow (J_1) may play an important role in determining the phase separation of polymeric structures. As reported by several studies [29], the path enters the gelation gap of the polymer–solvent–non-solvent triphase diagram when the ratio of J_2/J_1 is large; however, it intersects the liquid–liquid phase separation region when the ratio of J_2/J_1 is small. Gelation and liquid–liquid demixing result in the formation of dense and porous structures of polymeric matrix, respectively. The ratio of the solvent outflow (J_2) to the coagulant inflow (J_1) is also a major factor to influence the thickness of skin layer [29]. As demonstrated in Fig. 2, the morphologies of chitin/PLGA 50/50 beads prepared by coagulated in various non-solvents are different. This can be explained by noting that the solvent/non-solvent/polymer system has a smaller $\Delta\delta_{s-c}$ (solubility difference between solvent and chitin) and $\Delta\delta_{s-n}$ (solubility difference between solvent and non-solvent). Table 1 shows the solubility parameters of solvent, non-solvent and chitin. The $\Delta\delta_{s-n}$ and $\Delta\delta_{s-n(H)}$ between the solvent and non-solvent pairs of DMAc–methanol, DMAc–ethanol, DMAc– n -propanol, and DMAc–water are [6.7, 11.6(s-m)], [3.9, 9.2(s-m)], [2.2, 7.2(s-m)] and [25.8, 32.2(s-l)] respectively [30]. The smaller $\Delta\delta_{s-n}$ means a faster exchanging rate of solvent but the larger $\Delta\delta_{s-n(H)}$ suggests a weak hydrogen bonding between solvent and non-solvent. Table 2 shows the group contributions (Hoy's data) for the estimation of solubility parameter of chitin and PLGAs. The smaller $\Delta\delta_{s-c}$ but larger $\Delta\delta_{n-c}$ means that chitin has

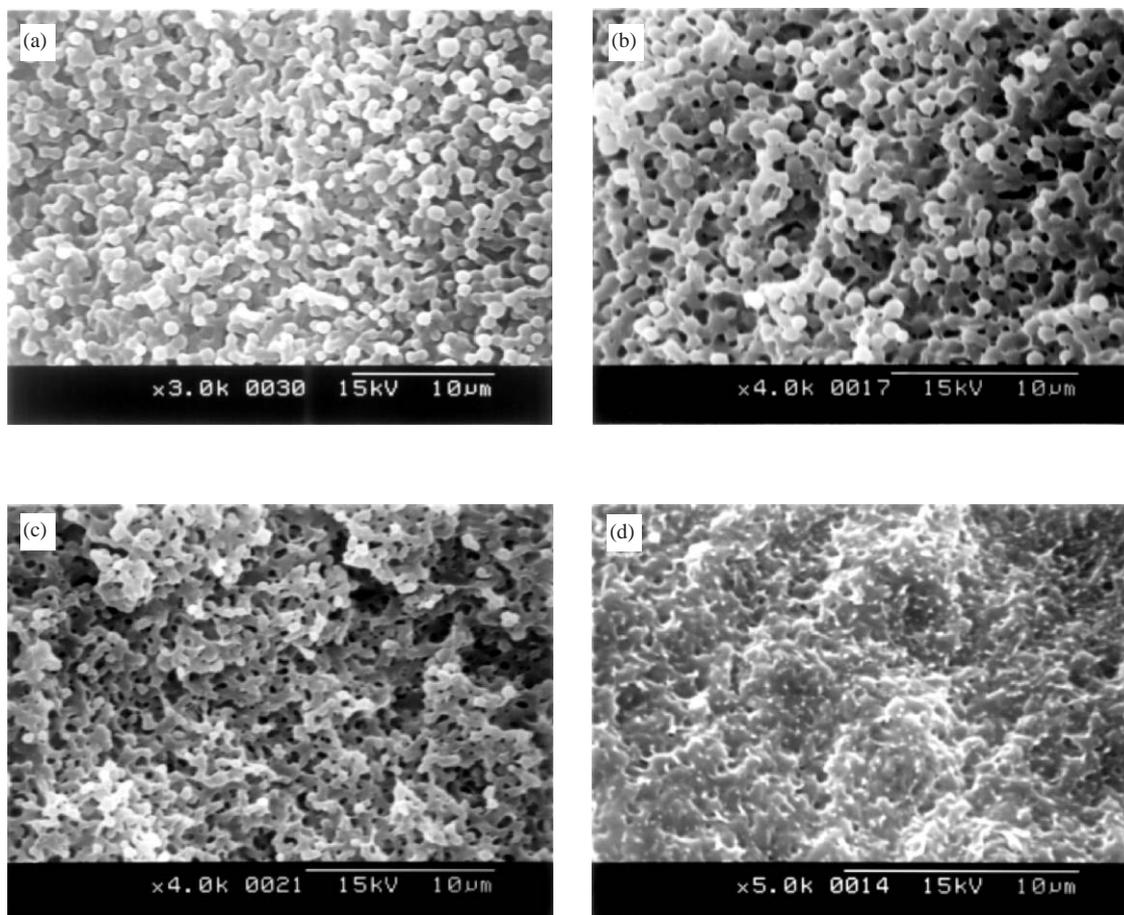


Fig. 2. SEM micrographs (cross-section) of phase separated chitin/PLGA 50/50 microspheres prepared by coagulation in different non-solvents: (a) coagulation in *n*-propanol, (b) coagulation in ethanol, (c) coagulation in methanol, (d) coagulation in water.

Table 2
Group constants of Hoy for the estimation of solubility parameter of chitin and PLGAs

Group	Molar attraction constants (MPa) ^{1/2}	
	Small	Hoy
–CH ₃	437	303
–CH ₂ –	274	271
>CH–	58	177
–O–	143	235
–OH	—	462
–(C=O)–NH–	—	906
Ring –4	—	159
–5	215–235	43
–6	194–215	–48
–(C=O)–O–	—	672

Solubility parameter of polymers are calculated by: $\delta_p = \rho \Sigma G/M$; G is Hoy's group molar attraction constants, ρ is polymer density and M is molecular weight of polymer unit.

δ_p value of chitin calculated from Hoy's data is 24.8.

a stronger interaction with DMAc but not with non-solvents. These results indicate that the solvent/non-solvent/chitin system has a stronger tendency towards

gelation, therefore results in a dense matrix. As shown in Fig. 2, one can find significant skin layer on the microspheres prepared by coagulated in alcohols (methanol, ethanol and *n*-propanol); however, the skin-layer is not observed from the microspheres prepared by coagulating in water. The faster solvent–non-solvent exchange due to the smaller $\Delta\delta_{s-n}$ between the solvent and non-solvent pairs of DMAc-alcohols are probably responsible for the formation of skin-layers.

From the cross-section of chitin/PLGA 50/50 microspheres (Fig. 2), it reveals that the microspheres are composed of numerous PLGA particulates (dispersion phase) dispersed in the chitin matrix (continuous phase). The thermodynamic compatibility between PLGA 50/50 and chitin is expected to depend on the miscibility of two types of polymers. Table 3 shows both the $\Delta\delta_{p-c}$ (solubility difference between PLGAs and chitin) and $\Delta\delta_{p-w}$ (solubility difference between PLGAs and water) estimated according to the parameters of group molar attraction constants (Hoy's data) in Table 2. Since the difference in the solubility parameter values of PLGA 50/50 and chitin is obvious, and the existence of hydrogen bonding between PLGA 50/50 and chitin is

Table 3

The solubility parameter of various PLGAs and the solubility parameter differences between PLGAs and chitin (water) (MPa)^{1/2}

Polymer type	δ value of polymer unit		δ value of polymer (MPa) ^{1/2}	$\Delta\delta_{p-c}$	$\Delta\delta_{p-w}$
	LA (16.0)	GA (19.1)			
PLA	100%	0%	16.6	8.2	31.3
PLGA (85/15)	85%	15%	17.3	7.5	30.6
PLGA (75/25)	75%	25%	18.4	6.4	29.5
PLGA (65/35)	65%	35%	19.7	5.1	28.2
PLGA (50/50)	50%	50%	21.4	3.4	26.5

Solubility parameters of chitin, PLA and PLGAs are calculated by: $\delta_p = \rho \Sigma G/M$; G is Hoy's group molar attraction constants, ρ is polymer density and M is molecular weight of polymer unit.

$\Delta\delta_{p-c}$, or $\Delta\delta_{p-w}$ is solubility parameter difference between PLGA and chitin, and PLGA and water.

not significant, high degree of phase separation can be observed from the chitin/PLGA 50/50 blends. The phase-separation of chitin/PLGA 50/50 microspheres prepared by coagulating in alcohols (methanol, ethanol and *n*-propanol) is more significant than the microsphere prepared by coagulating in water. The larger $\Delta\delta_{p-w}$ between the PLGA 50/50 and water is responsible for the decreased miscibility of the chitin/PLGA 50/50 blend.

3.2. Effect of temperature on phase separation

It is reported that increase in overall crystallinity of polylactides caused by the relaxation and recrystallinity could be achieved by thermal treatment [31]. However, only chain relaxation occurred when the PLGA samples were heated up to 60°C. This is due to the reason that PLGA 50/50 is a non-crystal polymer. As reported in our previous studies, the DSC studies of chitin/PLGA 50/50 microspheres exhibit an endothermo peak around 50°C which is characterized as the glass transition temperature (T_g) of PLGA 50/50 [32]. As shown in Fig. 3, it was noted that an increase of glass transition temperature of PLGA from 46°C to 53°C (in blend polymer) results from the increase of water temperature from 40°C to 90°C. Heat treatment promotes the intermixing of chitin and PLGA phase, T_g was supposed to increase because of the reduced segmental motion of random coil chains in amorphous regions.

Fig. 4 shows the SEM micrographs of the cross-section view of all chitin/PLGA 50/50 microspheres prepared by coagulating in different temperatures of water. As described above, chitin and PLGA 50/50 were immiscible according to the estimation of their solubility parameters. Being immersed in water, PLGA 50/50 precipitates from the chitin/PLGA 50/50 blend droplet much quicker than chitin because that water used as a coagulant for PLGA 50/50 is a even poorer solvent (non-solvent) than when it is used for chitin.

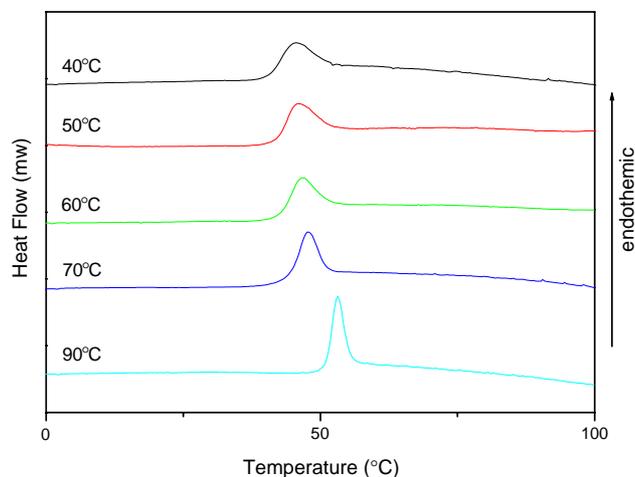


Fig. 3. DSC thermogram of chitin/PLGA 50/50 blend microsphere prepared by coagulation in 40°C, 50°C, 60°C, 70°C and 90°C of water.

Owing to these reasons, a novel microsphere containing numerous PLGA 50/50 particulates dispersed in chitin matrix can be obtained by the coagulation of chitin/PLGA 50/50 blend (homogeneously dissolved in DMAc/LiCl solution) in water. The domain size of PLGA phase significantly decreases from several micrometers to hundreds of nanometers with the increase of water temperature from 40°C to 90°C, suggesting the decreased phase-separation of these chitin/PLGA blends. The miscibility of phase-separated chitin-PLGA increases as a result of the migration of mobile PLGA polymeric chain into amorphous regions of chitin via elevation of water temperature.

3.3. Effect of LA/GA ratio on phase separation

As described previously, the miscibility of chitin and PLGAs is dependent on the related solubility parameter of both polymers. The solubility parameter of PLGAs and chitin/PLA estimated according to the parameters of Hoy's group molar attraction constants is shown in Table 3. Due to its additional methyl group, lactic acid (L) is more hydrophobic than glycolic acid (G). Hence, as more glycolic acid units are incorporated into the polymer chain, solubility parameter of PLGA decreases, leading to the decrease of solubility difference between chitin and PLGA ($\Delta\delta_{c-p}$). Fig. 5 shows the DSC analysis of various chitin/PLGAs blends prepared by coagulating in water. It can be observed that the glass transition temperature (T_g) of original PLGA increases from 48°C to 53°C with the increase of LA/GA ratio from 50/50 to 85/15. Chitin/PLA has a higher T_g (54°C) than all PLGA polymers. The larger steric hindrance of methyl group on lactide is responsible for the increased T_g of higher LA/GA ratio of PLGA. An obvious shift of T_g of PLGAs in the chitin/PLGAs blends was also noted

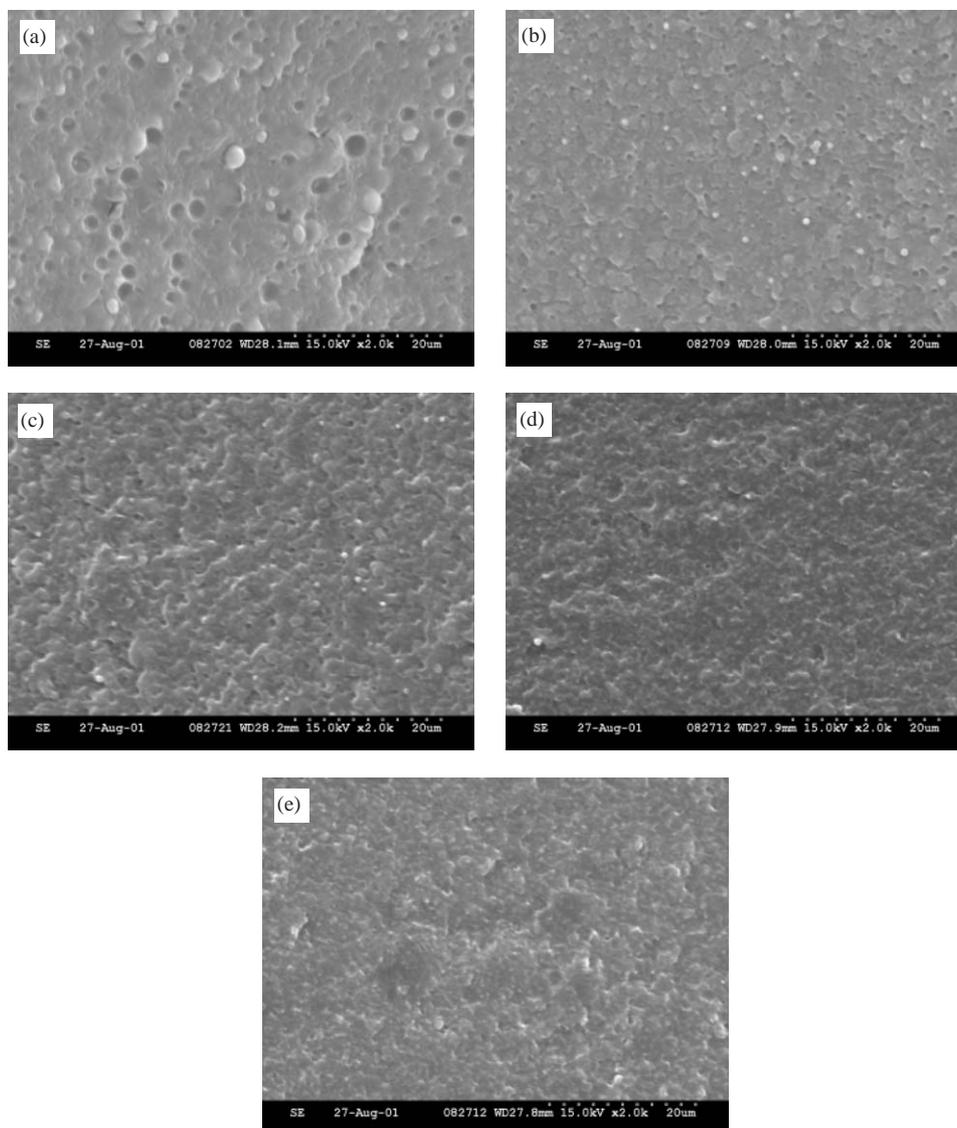


Fig. 4. SEM micrographs (cross-section) of chitin/PLGA 50/50 microspheres prepared by coagulation in different temperature of water: (a) coagulation in 40°C of water, (b) coagulation in 50°C of water, (c) coagulation in 60°C of water, (d) coagulation in 70°C of water, (e) coagulation in 90°C of water.

from 48–53°C (original PLGAs) to 58–65°C (PLGA in blend polymers) due to the intermixing of PLGA with chitin phase. The observed double T_g value of chitin/PLA blend can be attributed to the presence of two different polymer domains during the blending: a crystal and amorphous blend region [8]. Double T_g values are only found in the chitin/PLA blend probably due to the high crystallinity of PLA.

Fig. 6 shows the SEM micrographs of the cross-section view of the chitin/chitin/PLA and various chitin/PLGAs microspheres with different LA/GA ratio (LA/GA ratio are 50/50, 65/35, 75/25 and 85/15) prepared by coagulating in water. In five types of chitin/PLGA microspheres, the domain size of PLGA phase significantly increases with the increase of LA/GA ratio of

PLGA. As described previously, chitin is more immiscible with chitin/PLA than its PLGAs counterparts according to the estimation of their solubility parameters. After the phase separation, aggregation into larger domain is possible for the increase of LA/GA ratio of PLGAs (especially for chitin/PLA) in the chitin/PLGAs blend, due to the great driving force for solidification.

3.4. Hydration and degradation studies of chitin/PLGAs microspheres

Under equilibrium conditions, the hydration capability of a polymer matrix is mainly governed by its hydrophilicity or crystallinity. Water molecules could

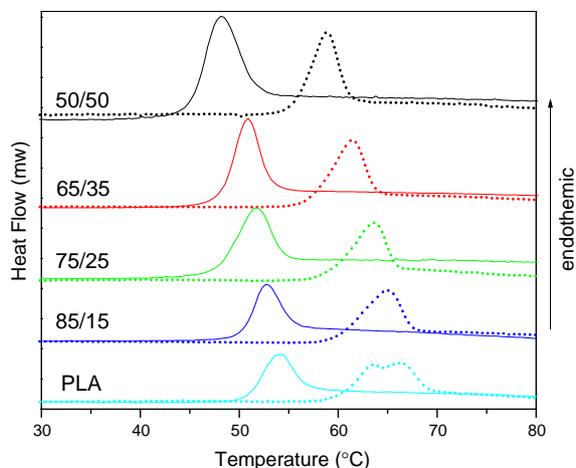


Fig. 5. DSC thermogram of chitin/PLGA 50/50 microsphere prepared from different LA/GA ratio of PLGAs: line (—) is the T_g of original PLA and PLGAs; dot line (...) is the T_g of PLA and PLGAs in chitin/PLA and chitin/PLGAs blends.

only be adsorbed to amorphous but not crystal region of a polymer matrix. It is encouraging to find that the hydration degrees of chitin/PLGA 50/50 microspheres prepared in different temperatures of water are increased with the increase of water temperature (Fig. 7(a)). The increased water temperature is responsible for the increased miscibility of hydrophobic PLGA 50/50 into amorphous region of hydrophilic chitin. PLA is more hydrophobic than PLGAs, and it is a semicrystalline polymer. Blending of chitin/PLA or PLGA with chitin is expected to produce different degrees of matrix hydration depending on the choice of chitin/PLA or various LA/GA ratios of PLGAs. Fig. 7(b) shows the hydration degrees of microspheres prepared from chitin/PLA blend and different LA/GA ratio of chitin/PLGA blends. All of the chitin/PLGA blends showed high water uptake capability relative to chitin/PLA blends, which is attributed to the relatively

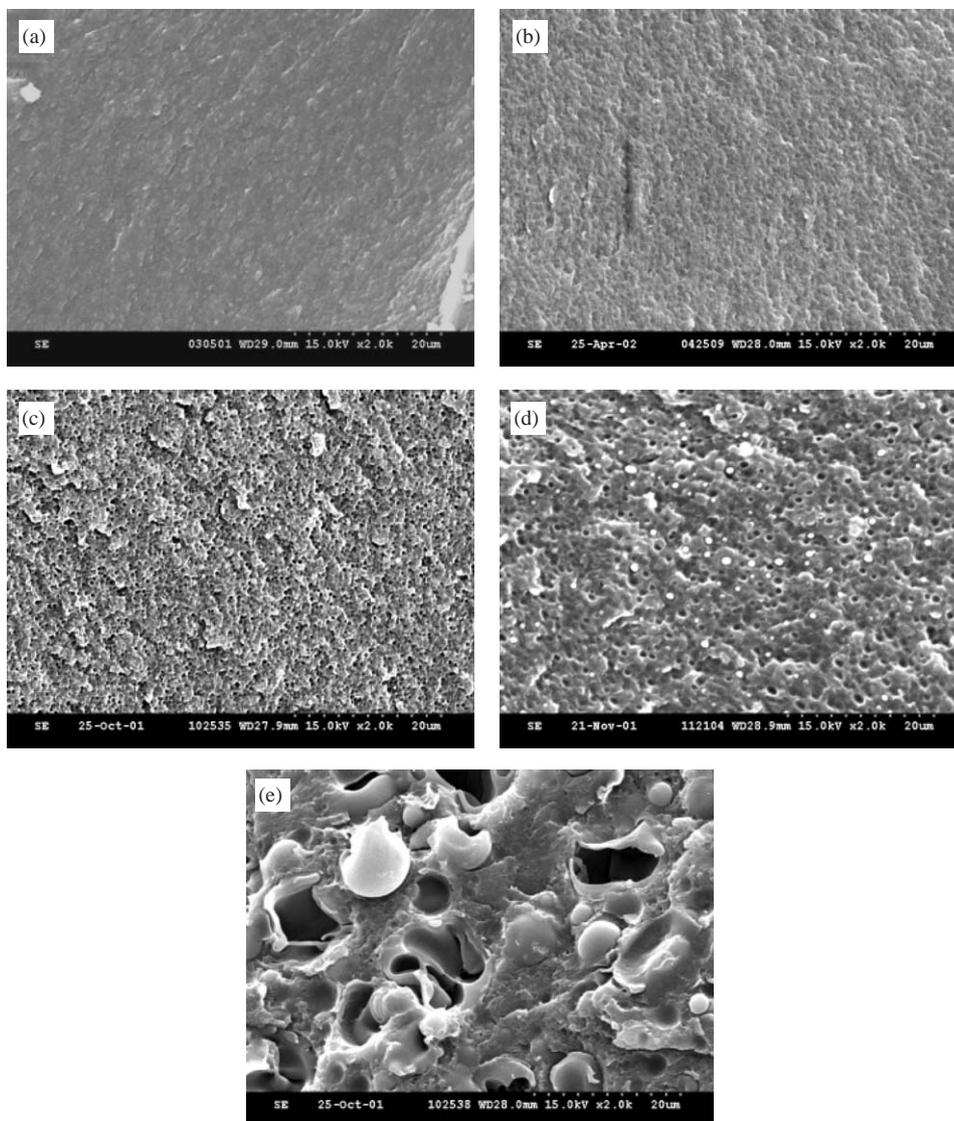


Fig. 6. SEM micrographs (cross-section) of chitin/PLGA 50/50 microsphere prepared from different LA/GA ratio of PLGAs; LA/GA ratio: (a) 50/50, (b) 65/35, (c) 75/25, (d) 85/15, (e) 100/0 (PLA).

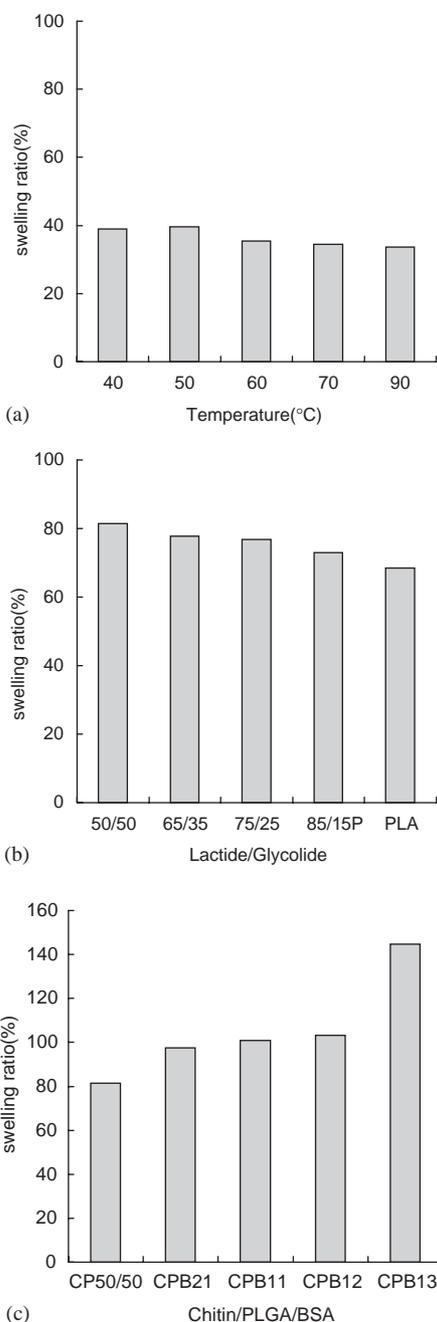


Fig. 7. Hydration degrees of chitin/PLGA 50/50 microspheres: (a) prepared by coagulation in different temperature of water (40°C, 50°C, 60°C, 70°C and 90°C), (b) prepared from different LA/GA ratio of PLGAs (50/50, 65/35, 75/25, 85/15, 100/0), (c) prepared with different chitin/PLGA to BSA ratios: CPB21(2:1), CPB11(1:1), CPB12(1:2), CPB13(1:3).

hydrophobic property and high crystallinity of chitin/PLA. Additionally, it is found that the water content increases with the decrease of the LA/GA ratio of PLGAs. Fig. 7(c) shows the hydration degrees of chitin/PLGA 50/50 microspheres prepared from different blend ratios of chitin/PLGA to BSA content (2/1, 1/1, 1/2 and 1/3). BSA is hydrophilic and probably signi-

Table 4

The effects of chitin/PLGAs to BSA ratios and temperatures of water on BSA content in chitin/PLGAs microspheres

Chitin/PLGAs to BSA ratios	BSA contents BSA (mg)/microsphere (g)	Temperature of water (°C)	BSA contents BSA (mg)/microsphere (g)
2:1 (CPB21)	37	40	98
1:1 (CPB11)	98	50	Not determined
1:2 (CPB12)	259	60	102
1:3 (CPB13)	378	70	63
		90	31

The temperatures of water in the batches of different chitin/PLGAs to BSA ratios are kept at 40°C.

The chitin/PLGAs to BSA ratio in the batches of different temperatures are kept at 1:1.

ficant contributor to the overall hydrophilicity of the microsphere. All of the chitin/PLGA 50/50 microspheres containing BSA showed higher water uptake capabilities comparing to original chitin/PLGA 50/50 microsphere. However, the increase of hydration degrees of the chitin/PLGA 50/50/BSA microspheres is limited, expect for the blend ratio of chitin/PLGA to BSA content is 1:3. BSA was mostly partitioned into water during coagulation due to its hydrophilic property. It leads to have a low degree of BSA loading in the chitin/PLGA 50/50 microspheres (Table 4), and reduces the effect of incorporated protein on the hydration of prepared chitin/PLGA 50/50 microspheres.

Fig. 8(a) shows the degradation of chitin/PLGA 50/50 microspheres prepared by coagulating in different temperature of water. Despite the different miscibility of the prepared chitin/PLGA 50/50 microspheres, the microspheres demonstrated a similar rate of weight loss. Water accessibility to the ester backbone bond and the hydrolytic breakage of these bonds will determine the rate of PLGA degradation. PLGA 50/50 is relatively hydrophilic, and has a very low degree of crystallinity in its structure. When PLGA 50/50 is blended with a hydrophilic polymer in good miscibility, it is expected to bring about a rapid increase in the PLGA 50/50 hydrolysis rate due to the soakage of PLGA 50/50 with water in the same phase. However, in our study, it is found that the blend microspheres with lower chitin/PLGA 50/50 ratio (chitin:PLGA ratio is 1:3), the adsorbed water in chitin phase will not contribute significantly to hydrolytic breakage of the PLGA 50/50 polymeric chain. Fig. 8(b) shows the degradation of microspheres prepared from different LA/GA ratio of PLGAs in chitin/PLGA blends. The chitin/PLGAs microspheres showing a reduced rate of weight loss with the increase of LA/GA ratio of PLGA, suggests that the degradation of chitin/PLGA microspheres is reduced after being blended with higher LA/GA ratio of

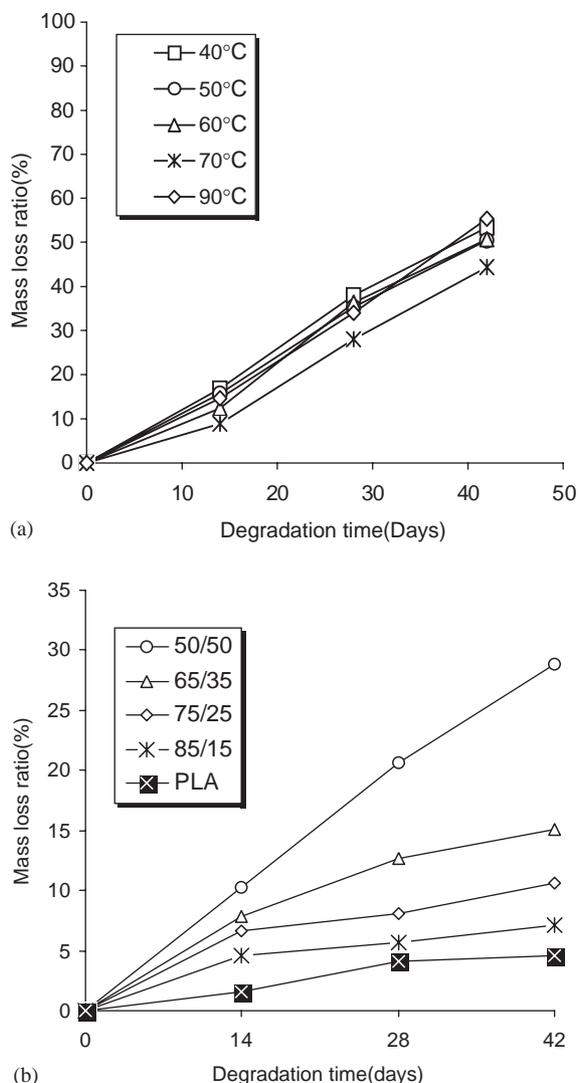


Fig. 8. Degradation (weight loss) of chitin/PLGA 50/50 microspheres: (a) prepared by coagulated in different temperature of water, (b) prepared from different LA/GA ratio of PLGAs.

PLGAs. These results seem to be consistent with the expectation based on the result described above (the increase of LA/GA ratio of PLGAs increase the phase separation of chitin/PLGA blends).

Figs. 9 and 10 respectively show the SEM micrographs of various chitin/PLGAs microspheres after different periods of degradation. Under 3 to 5 weeks of degradation, there are no obvious pores on the surface of chitin/PLGA 50/50 microspheres with lower chitin/PLGA 50/50 ratio (chitin:PLGA ratio is 1:3) which are prepared by coagulation in different temperatures of water (Figs. 9(a)–(b)). It reveals that heat treatment has less effect on the degradation of these microspheres. However, it is of interest to find that surface morphology of the chitin/PLGA 50/50 microspheres demonstrates significantly different degradation

behaviors depending on the chitin/PLGA blend ratio (Figs. 9(c)–(f)). For the examination of various chitin/PLGA 50/50 blend microspheres, one can find that the morphology of chitin/PLGA 50/50 microsphere with lower chitin/PLGA ratio (chitin:PLGA ratio is 1:3) remains intact; however, its counterpart with higher chitin/PLGA ratio (chitin:PLGA ratio is 3:1) becomes porous after 16 weeks of degradation. With the increase of chitin/PLGA ratio, the polymer chains of PLGA 50/50 can be excessively intermixed with the hydrophilic amorphous region of the chitin phase by heat treatment, leads to increasing the hydrolytic degradation of the PLGA 50/50. Fig. 10 shows that the surface morphology of chitin/PLGAs microspheres with lower LA/GA ratio of PLGAs becomes more porous than that of microspheres with higher LA/GA ratio under one to five weeks of degradation. The erosion of PLGAs with higher LA/GA ratio from the blend matrices is slower than that with higher LA/GA ratio counterparts. The hydrophobicity and crystallinity of PLGAs increases with increasing the LA/GA ratio of PLGAs, leading to the decreased erosion rate of the chitin/PLGA microspheres.

3.5. Protein release

Fig. 11(a) shows the in vitro release of BSA from chitin/PLGA 50/50 microspheres which are prepared by coagulating in different temperatures of water. It was found that BSA released from the chitin/PLGA 50/50 microspheres demonstrates a triphasic pattern. The pattern of BSA release was similar for all the blends, with a rapid rate of initial release and incomplete release pattern. It has been reported that proteins encapsulated within PLGA often show an incomplete release pattern due to the protein aggregation and non-specific adsorption [33]. The initial drug release (the first phase) was followed by a slower, continuous release for up to 250 days. The initial quicker release is governed by the rate of water transport in the hydrophilic chitin phase. Due to the fact that chitin phase is fast hydrated, BSA is eluted from chitin/PLGA 50/50 microspheres quickly in this period of release. The dissolution of BSA on the microsphere surface is also responsible for the initial burst release. The continuing period (the second phase) of protein release is mostly dependent on the degradation of hydrophobic PLGA phase. Due to the well miscibility of chitin/PLGA 50/50 prepared by coagulation in higher temperature of water, the adsorbed water in the polymer bulk will contribute more significantly to the degradation of PLGA 50/50. As a result, the increased temperature of water for the decreased phase separation of chitin/PLGA 50/50 blends clearly enhanced the release of BSA. A sudden increasing release rate of BSA is observed at 250 h post-operation, suggests that this period (the third phase) of bulk hydrolysis

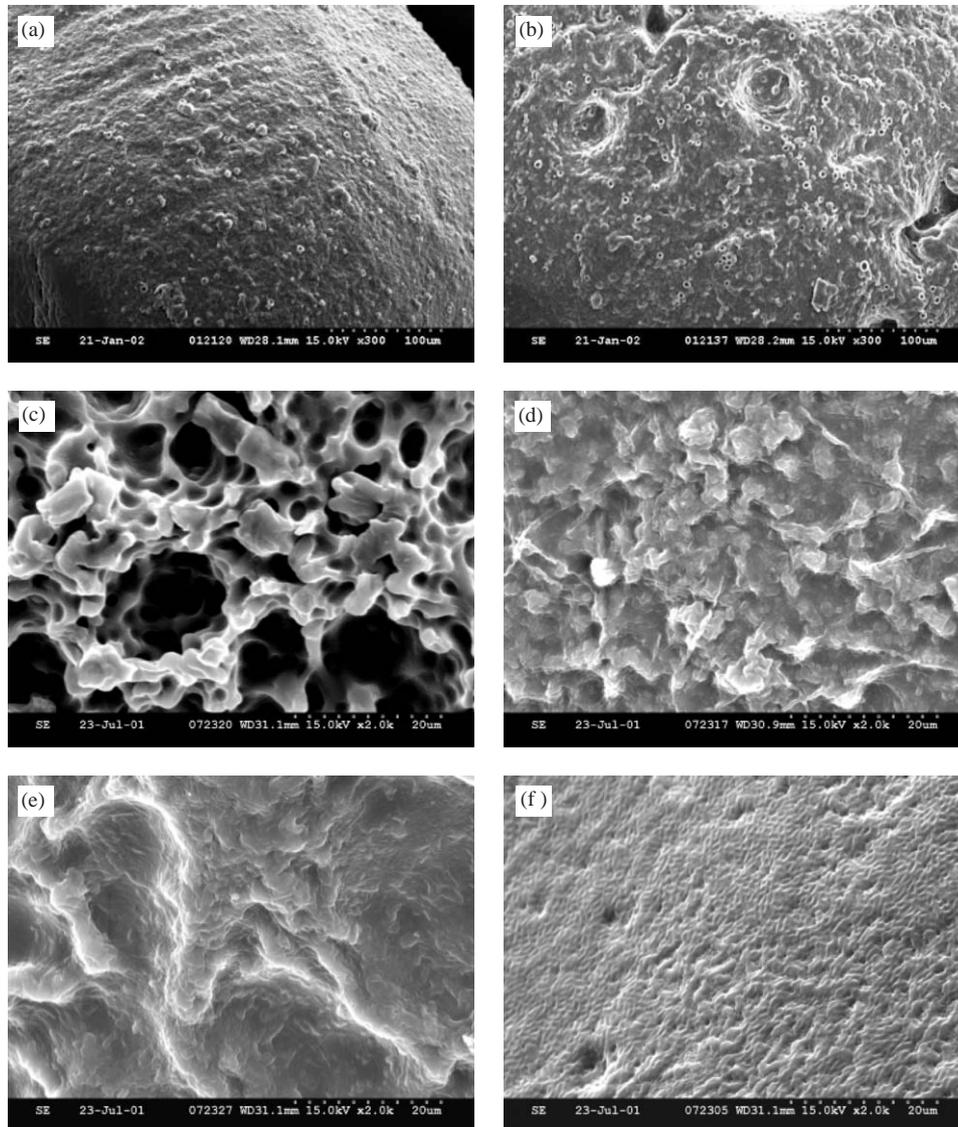


Fig. 9. SEM micrographs of degraded chitin/PLGA 50/50 microsphere (chitin: PLGA ratio is 1:3) prepared by coagulation in different temperature of water: (a) 40°C, (b) 90°C and different chitin/PLGA 50/50 ratios of microspheres prepared by coagulation in 90°C of water, (c) chitin/PLGA 50/50 ratio is 3/1, (d) chitin/PLGA 50/50 ratio is 2/1, (e) chitin/PLGA 50/50 ratio is 1/1, (f) chitin/PLGA 50/50 ratio is 1/2.

makes PLGA 50/50 particulates less durable for the protection of BSA from dissolution. It is believed that the decrease (caused by degradation) of PLGA 50/50 molecular weight to a critical limit will lead to a great alteration of the physical properties of the polymer, and enhance the diffusion of the protein from the microspheres.

Fig. 11(b) shows the BSA release profiles of the chitin/PLGAs microspheres prepared with different LA/GA ratios of PLGAs. The rate of BSA release from the chitin/PLGA 50/50 blend microsphere was faster than that of chitin/PLGA 85/15, 75/25 and 65/35. This result indicates that the rate of BSA release increases with the increase of GA content in PLGAs. It is due to the difference in degradation rate of the PLGA polymer: the

higher the GA content, the faster the degradation rate. Chitin/chitin/PLA microsphere has a slower BSA release rate as compared to its chitin/PLGAs counterparts. Since PLGAs are amorphous, it is thought that the extent of water hydration and the rate of degradation in the matrix are greater than those of the semi-crystalline chitin/PLA. Within 350 h of release period, the suddenly increased BSA release (the third phase of release; the second burst) could only be found from the chitin/PLGA 50/50 microspheres. It is reported that it takes 1–3.5 months for PLGA 65/35, PLGA 85/15 and PLA, respectively, to incur the second burst of release [34,35]. PLGA 50/50 take even more shorter time to induce the second burst. Thus the hydrolysis-labile ester linkages in PLGA 50/50 are more accessible to water

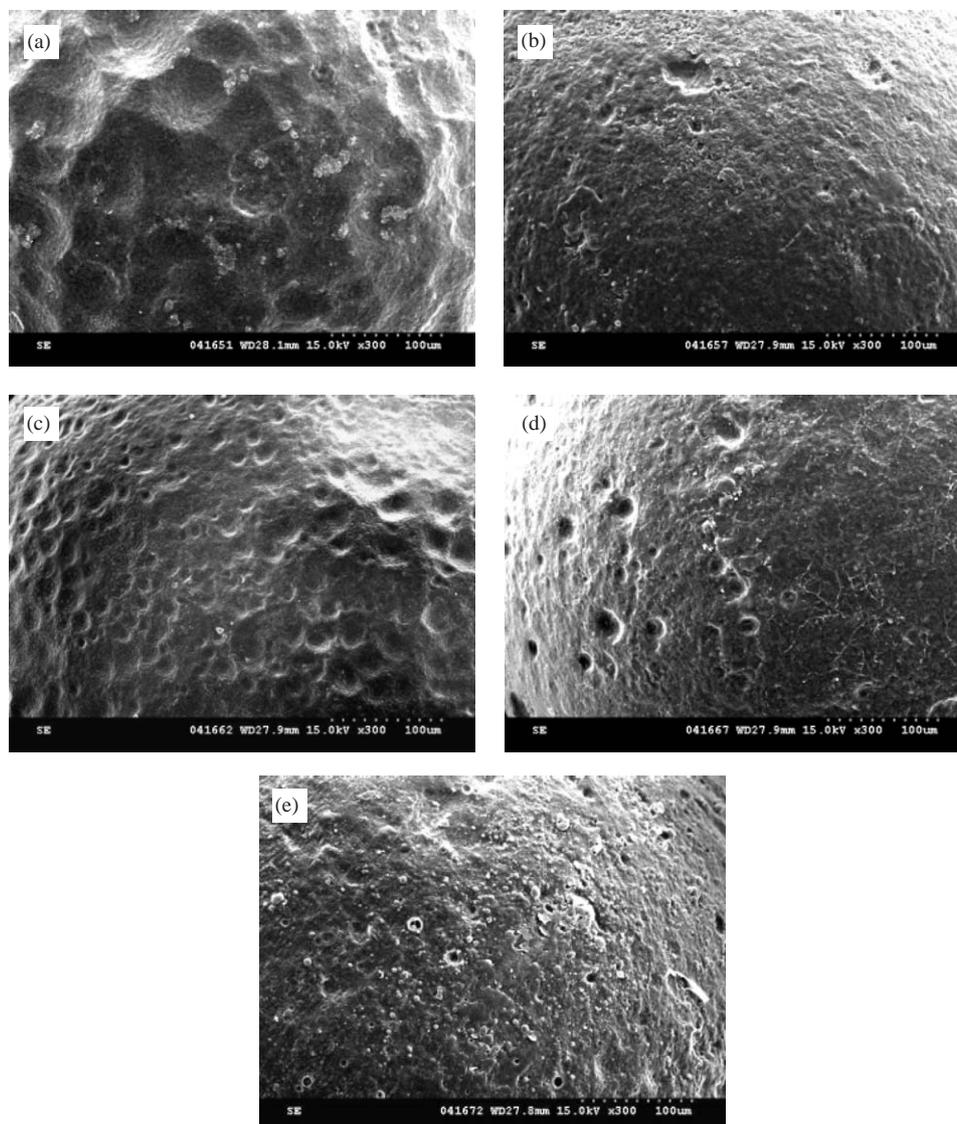


Fig 10. SEM micrographs of degraded chitin/PLGA 50/50 microspheres prepared from different LA/GA ratio of PLGAs; LA/GA ratio: (a) 50/50, (b) 65/35, (c) 75/25, (d) 85/15, (e) 100/0 (PLA).

than those in the PLGA 85/15, 75/25, 65/35 and chitin/PLA, leading to faster degradation in the former. Fig. 7(c) shows the BSA release profiles of the chitin/PLGA 50/50 microspheres prepared from different blend ratios of chitin/PLGA to BSA content (2/1, 1/1, 1/2 and 1/3). It is interesting to find that the release rate significantly increases with the increase of BSA contents in the blends. The hydrophilicity of chitin/PLGA 50/50 microspheres increases with the increase of incorporated BSA, which is responsible for the increased BSA release rate. These results suggest that the overall rate of BSA release may be dependent on the hydration of hydrophilic chitin phase and the erosion of hydrophobic PLGA phase.

In the present study, the goal of designing controlled-release systems for long-term delivery of protein was

achieved by selecting various chitin/PLGA blends with suitable hydration and degradation properties. Additionally, a triphasic pattern of release with second burst was also found in our study. It is known that sustained release of the peptide growth factor (bFGF, EGF, TGF et al.) from microspheres would prolong its half-life in a blood stream, which potentially help to enhance therapeutic effectiveness of the growth factor for local tissue regeneration. Recently, there is also a global emphasis on the development of improved vaccine strategies based on controlled antigen delivery. It is as effective to deliver antigen in a single shot as multiple shots (with the booster shots) via a designed vaccine delivery system with multiple phase of burst release (the first and second burst). The results of our study revealed that the chitin/PLGA 50/50 microspheres might prove

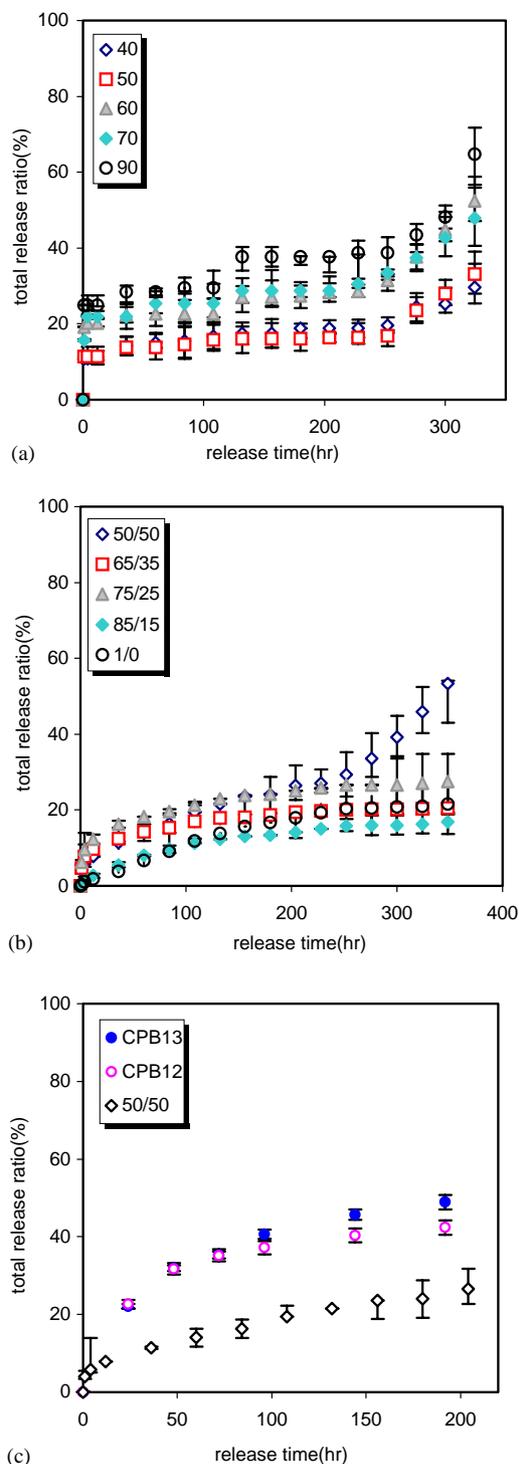


Fig. 11. Protein releasing from chitin/PLGA 50/50 microspheres (a) prepared by coagulation in different temperature of water (40°C, 50°C, 60°C, 70°C and 90°C), (b) prepared from different LA/GA ratio of PLGAs (50/50, 65/35, 75/25, 85/15, 100/0), (c) prepared with different chitin/PLGA to BSA ratios: CPB21(2:1), CPB11(1:1), CPB12(1:2), CPB13(1:3).

to be a useful and potential controlled delivery system for pharmaceutical proteins (such as growth factors) and vaccine antigens.

4. Conclusion

In this study, we prepared novel microspheres based on chitin/PLGAs and chitin/PLA blends and were developed for protein delivery system. The hydration and degradation of the blend microspheres can be adjusted by parameters such as selected non-solvents, temperature of water and ratio of polylactide to polyglycolide, leading to the variation of miscibility of chitin and PLGA phase in the blends. Formulations of the blends, which are based on the precise balance between the hydration rate of chitin phase and degradation of chitin/PLA and PLGA phase, can lead to a controllable release of bovine serum albumin (BSA). In conclusion, such a chitin/PLGAs and chitin/PLA microsphere are novel and interesting, and may be used as special protein delivery systems.

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