Influence of Protein Content on the Physicochemistry of Poly(ε-caprolactone) Microparticles

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ABSTRACT: The aim of this work was to determine the influence of protein content on the physicochemical properties of protein-loaded poly(ε-caprolactone) (PCL). To achieve this goal, bovine serum albumin (BSA), an example of protein was encapsulated within PCL matrix by emulsion solvent evaporation method. The polymer matrix's rheological properties, hydrophobicity, molecular weight (M_W), and thermal behavior were determined. Particle characteristics such as BSA loading, surface area, mean size, morphology, and *in vitro* release profile were also assessed. After encapsulation process, the polymer crystallinity and crystallization point were markedly increased suggesting that a nucleation phenomenon occurred. The increase in PCL M_W (from 46. 7 to 179.4 kDa) led to an increase of both particle size and encapsulation efficiency that was consistent with rheological data. The increase of protein content from 1.6 to 11.5% (w/w) influenced considerably particle's specific surface area and decreased the rate of protein release. Together, these results suggest that beside the nature of the carrier polymer, protein content may have implication on their controlled release, the coating of particle by protein, and on the carrier polymer chemistry and degradation. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 1042–1050, 2006

Key words: GPC/SEC; microencapsulation; polyesters; proteins; thermal analysis

INTRODUCTION

To overcome the formulation and the administration problems consecutive to the development of new biopharmaceuticals, one of the frequent alternative methods investigated is the microencapsulation technology.[1–14] However, most of these investigations on protein and peptide encapsulation were conducted with polylactide (PLA), polyglycolide, or their copolymers (PLGA) as polymer matrix.[15–25] As a result, there has been a wide range of knowledge about the physicochemical parameters governing the entrapment and the release profile of proteins from PLA and PLGA microparticles. For instance, earlier reports claimed that the degradation of PLGA microparticle caused a significant pH decrease from pH 7.4 to below pH 3.0 after aqueous incubation, resulting in great hydrolytic damage of the protein released or entrapped into microparticles.[5,8] To our knowledge, there are relatively fewer investigations especially on the physicochemistry of protein-loaded poly(ε-caprolactone) (PCL) microparticles.[26] PCL is a biodegradable polyester that exhibits certain desirable characteristics. It is a semicrystalline aliphatic polymer with relatively lower melting point, higher permeability to low molecular weight drugs, and lack of toxicity. It also has considerably lower cost when compared with other biodegradable polyesters such as PLA and PLGA.[27,28] It has been shown that dispersion of a wide variety of bioactive agents into PCL matrix may be a valuable alternative for their controlled delivery.[4,10,12,13,29–33] However, the physicochemical principles underlying the molecular pharmaceutics of protein within PCL matrix remained to be elucidated. As contribution to this long-term goal, it is important to enhance our understanding of the physicochemical properties of such macromolecules (protein and carrier polymer) mixture for rationale drug formulation and delivery. We have previously determined the physicochemical properties of protein-loaded poly-(ε-caprolactone) (PCL) microparticles exhibiting an oily core.[10] The present work is a contribution to the physicochemical characterization of PCL microparticles without an oily core. Specifically, the rheological, water/polymer interfacial, mesoscale, and thermal analysis of such systems were investigated.

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MATERIALS AND METHODS

Materials

Poly(ε-caprolactone) (PCL) of different molecular weight (M_W); 46.7, 69.6, 84.8, and 179.4 kDa (referred

TABLE I
Native Carrier Polymer/Water Interfacial Data

Type of polymers	Contact angle (θ°)	Cosθ	Spreading coefficient ($S_{W/P}$, in mN/m)	Work of adhesion (W_a, in mN/m)
PCL_a	78.6	0.1976	−57.76	86.22
PCL_b	75.8	0.2453	−54.33	89.65
PCL_c	74.2	0.2722	−52.40	91.59
PCL_d	75.2	0.2554	−53.60	90.38
R203	67.7	0.3794	−44.67	99.31
R207	68.4	0.3681	−45.50	98.49
RG756	69.2	0.3551	−46.42	97.55

Contact angle (θ) was average 10 measurements. $S_{w/p}$, spreading coefficient of water over polymer; W_a, work of adhesion of water to polymer. Additional informations on physicochemical properties of these polymers were shown in Table II.

to hereafter as PCL_a, PCL_b, PCL_c, and PCL_d) were obtained from Solvay Interox, U.K. (for PCL_a, PCL_b, and PCL_d) and Union Carbide, N.V., Benelux (for PCL_c only available from this supplier at that time). Poly(D, L-lactide)s or (PLA, Resomer® R203 and R207) and 75/25 poly(D, L-lactide-*co*-glycolide) or Resomer® RG756 were obtained from Boerhinger Ingelheim KG (Germany). The physicochemical characteristics of all these polymers are summarized in Table I and II. These native carrier polymers were used without further purification. The following chemicals were also used as received from the suppliers: bovine serum albumin fraction V (BSA, 67 kDa) and Folin-Ciocalteu reagent (Merck, Germany), polyvinyl alcohol (PVA, 87–89% hydrolyzed, M_w 13–23 kDa, Aldrich Chemical Co.); methylene chloride, chloroform, acetone (reagent grade, UCB, Belgium); sodium dodecyl sulfate (SDS, 95% base, Sigma Chemical). The other chemicals were reagent grade and were used as obtained from their respective suppliers.

Characterization of native carrier polymers

Rheological studies

The intrinsic viscosity [η_i] of all polymers was determined at (25 ± 0.2)°C in THF, using a Desreux-Bischoff type viscometer as previously reported.[30] Briefly, samples from different suppliers were initially about 200 mg per 10 mL, then diluted at 200 mg/15 mL, and 200 mg/20 mL at the end. The specific viscosity of the polymer was determined at each concentration. The intrinsic viscosity was calculated by extrapolating the specific viscosities to infinite dilution. The plastic viscosity was determined as follows. The rheogram of different types of 5% (w/v) PCL dissolved in methylene chloride (O phase) was evaluated using a rheometer Mettler RM 180 (Rheomat, Switzerland) at 25°C (system of measure 50, program 1, volume of the system: 38 mL). Care was taken to minimize solvent evaporation during the experiments. The plastic viscosities (ν) of these polymer solutions were finally expressed in mPa s using the eq. (1) and the rheometer manufacturer's protocol:

$$v = (F - f)/G \quad (1)$$

where F (in dyn/cm^2), f (dyn/cm^2), and G (sec^{-1}) were the shear stress, the yield value, and the shear rate, respectively.[34]

Contact angle measurements

The water contact angles with the film of the carrier polymer (θ) were measured at ~25°C, using the sessile

TABLE II
Physico-chemical Properties of Polyesters Tested

	Rheology	SEC/GPC results			DSC results			
Polymer	[η_i] (dL g^{-1})	M_w (kDa)	M_n (kDa)	M_w/M_n	T_{fus} (°C)	ΔH_{fus} (J g^{-1})	ΔS_{fus} (J g^{-1}K^{-1})	% Crystallinity
PCL_a	0.434	46.7	33.3	1.4	65	98.61	0.29	70.70
PCL_b	0.521	69.6	49.7	1.4	63.3	97.74	0.29	70.20
PCL_c	0.654	84.8	53.0	1.6	62.7	74.89	0.22	53.70
PCL_d	0.908	179.4	112.2	1.6	61.2	83.97	0.27	60.20
R203	0.173	21.5	11.3	1.9	—	—	—	—
R207	0.823	199.8	124.8	1.6	—	—	—	—
RG756	0.445	78.2	48.8	1.6	—	—	—	—

[η_i] , intrinsic viscosity; M_w, weight-average molecular weight; M_n, number average molecular weight; M_w/M_n, polydispersity index; T_{fus}, melting temperature; H_{fus}, enthalpy of fusion; S_{fus}, entropy of fusion; –, amorphous material.

drop technique at room temperature (~25°C) and an image analysis system as previously described.[35,36] The principle of the measurement was derived from Young equation.[34] Briefly, different polymer surfaces were prepared by dip-coating a glass slide with a 10% (w/v) polymer solution in methylene chloride and curing. The water droplet volume was always kept in the range of 1–3 μL to prevent gravitational distortion of the spherical profile. The water was taken from the purification system just a few minutes before the measurements. Each determination was performed by averaging the results of at least 10 drops. The standard deviation on the measurements was about 3°. The spreading coefficient of water over the carrier polymer ($S_{W/P}$) and its work of adhesion to the carrier polymer (W_a) were computed as defined by Martin et al.,[34] using eqs. (2) and (3), respectively:

$$S_{W/P} = \gamma_w(\mathrm{Cos}\ \theta - 1) \quad (2)$$

$$W_a = \gamma_w(1 + \mathrm{Cos}\ \theta) \quad (3)$$

where γ_w = 71.99 mN/m was the surface tension of water at 25°C[37] and Cosθ, the cosine of the contact angle (θ).

Molecular weight characterization

The molecular weights (M_w, M_n) and polydispersity index (M_w/M_n) of PCL solid samples were determined by size exclusion chromatography (SEC or GPC), using a Waters 590 solvent delivery module. The polymers were dissolved in THF (2 g/L) and filtered through a 0.45 μm filter, after which 50 μL was injected. The THF solution was stabilized by BHT (2, 6-di-*tert*-butyl-4 methyl phenol) to avoid peroxide formation. Two ultrastyragel linear columns (Part n° 106081, Waters®) were used with THF as the eluting solvent at a flow rate of 1.0 mL min^{-1} at room temperature with a refractive index detector (Waters 410). The SEC procedure was calibrated using polystyrene standards of different molecular weights (Polymer Laboratories). The data were analyzed using the Waters' Millennium 2010 Chromatography Manager Software. In our operating condition, the coefficient of variation on each M_w and M_n measurement was 2–3% (for M_w) and 5–10%, respectively.

Thermal analysis of native polymer

The melting point and the enthalpy of fusion of the polymers were determined by differential scanning calorimetry (Perkin–Elmer DSC7).[30,38–40] Briefly, samples of (10 mg were put into aluminum pans. The instrument was calibrated with an indium standard, and measurements were carried out from 0 to 150°C under nitrogen at a scan rate of 10°C/min. To erase previous thermal history of samples, before each measurement, the temperature was raised up to at least 20°C above T_g than cooled back to 0°C at the same scan rate. The crystallinity of the PCL was calculated from totally crystalline PCL for which the enthalpy of fusion is 139.5 J g^{-1},[41] and the crystallinity of the PLA may be measured from the enthalpy of melting for 100% crystalline PLA, which is 203.4 J g^{-1}.[42] At the crystalline melting point, solid and liquid polymers are at equilibrium,[34] Therefore, one could estimate the entropy of fusion using eq. (4)

$$\Delta S_{\mathrm{fus}} = \Delta H_{\mathrm{fus}}/T_{\mathrm{fus}} \quad (4)$$

where ΔH_{fus} is the enthalpy of fusion and T_{fus} of the melting point after conversion of temperature in Kelvin unit.

Preparation of PCL microparticles

The protein-loaded microparticles were prepared by a modification of method described in details elsewhere.[10,32,37] Briefly, typically a solution of BSA in deionized water (the internal aqueous phase W_1, 1 mL) was emulsified with a 5% w/v polymer containing dichloromethane solution (the organic O phase, 10 mL), using a mixer Ultraturrax (IKA, T25, Van der Heyden, Belgium) at 8000 rpm during 5 min. Then, 2.5 mL of the resulting W_1/O emulsion was emulsified in the same conditions with a 5% (w/v) PVA containing aqueous solution (the external aqueous phase W_2, 50 mL) to produce a (water-in oil)-in water emulsion ($W_1/O/W_2$). This $W_1/O/W_2$ emulsion was then stirred magnetically (700 rpm) at room temperature and pressure for 18 h to allow solvent evaporation and microparticles formation. The solidified microparticles were isolated by centrifugation at 4000 rpm for 10 min (Biofuge 15R, Heraeus Instruments, Germany), washed three times with deionized water, and freeze-dried.

Characterization of PCL microparticles

Morphological analysis

The external morphology of the microparticles was analyzed by optical and scanning electron microscopy (SEM). The optical microscope (Leitz Wetzlar, Germany) was used at magnification 1000×. For SEM, the freeze-dried particles were redispersed in water, air-dried, and coated with gold–palladium under argon atmosphere. Examination was carried out under a scanning electron microscope (Hitachi *S*-570, Japan) equipped with an image analyzer.

Particle size analysis

The freeze-dried particles were redispersed in a 0.9% (w/v) sodium chloride filtered solution (filter of 0.22 μm diameter) and sized with a Coulter Multisizer II (Coulter Electronics, Ltd, Luton, U.K) equipped with a 100 μm aperture. Experiments were performed in triplicate. The particle size was expressed as volume mean diameter (Vmd) in μm.

Bovine serum albumin entrapment in PCL microparticles

The protein extraction method was adapted from the one used previously by Hora et al. (4). Typically, 100 mg of the freeze-dried microparticles, accurately weighted, was dispersed in 3 mL of 1*M* sodium hydroxide solution containing 5% (w/v) SDS. After alkaline degradation of the polymer under continuous stirring at room temperature and pressure overnight, the protein concentration in the supernatant was determined by the method described by Lowry et al.[43]

Thermal analysis of PCL microparticles

The thermal analysis of the microparticles was performed according to the same procedure described above for the polymer. The weight of PCL involved was adjusted by subtracting the weight of protein content from the initial weight of the freeze-dried microparticles.

Surface area analysis

Surface area analysis was carried out by using a Flow-Sorb II 2300 Surface Area Analyzer, Micromeritics Instrument (Norcross, GA).[34] The freeze-dried samples (200 mg) were previously degassed at room temperature for 15 h. The instrument was calibrated with 1 mL of liquid nitrogen corresponding normally to 2.84 m^2. The surface areas were measured under argon/nitrogen atmosphere (30/70 v/v) at a relative pressure of nitrogen $P/P_0 = 0.3$. The single point BET method was applied for the calculations.[34] The surface area values were expressed as mean of adsorption and desorption area.

In vitro protein release study

The *in vitro* release of BSA was performed on BSA-loaded PCL_c particles with different BSA loadings (1.6, 9.6 and 11.5% w/w) as follows. The freeze-dried microparticles (500 mg) were suspended in 3 mL of 0.1*M* phosphate buffer solution (PBS, pH 7.4, at 37°C) in 10 mL sealed glass vial and shaken horizontally (Shaker model Grants instruments, Cambridge, England) at 60 rpm. The PBS contained 0.01% (w/v) sodium azide as preservative agent. At predetermined time intervals, the amount of BSA released in the supernatant was assayed by the method of Lowry et al.[43]

Statistical analysis

Most experiments were performed in replicate as specified in appropriate section. The quantitative data were analyzed by ANOVA using Instat software version 3.0 for Windows as previously described.[37] Tukey-Kramer multiple comparison tests were performed as well: a probability level (P value) of < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Physicochemical characterization of native carrier polymers

Table I and II exhibit the physicochemical properties of the characterized polyesters. On the basis of the contact angle data in Table I, it was noteworthy that PCL/water interfacial data were significantly different from those of PLA and PLGA ($P < 0.05$). Within the same group of polyesters, these data were not statistically different ($P > 0.05$). Moreover, the spreading coefficients of water over these two groups of polyesters ($S_{W/P}$) were negative indicating non spontaneous spreading of water over these polymers. However, the $S_{W/P}$ values of water over PLA and PLGA were smaller in absolute values suggesting more favorable spreading when compared with those of PCLs. These data were consistent with the relatively lower work of adhesion of water of the latter polymers suggesting weaker interaction between water and PCLs compared to that with PLA and PLGA polymers.[34] These observations provide quantitative evidence of the difference in hydrophobicity between both polyester groups and may be explained as follows. The longer hydrocarbon chain in the PCL backbone made it more hydrophobic than PLA and PLGA.[44] Indeed, PCL was made of ε-caprolactone ($C_5H_{14}O_2$), which was more hydrophobic than lactic acid ($C_3H_6O_3$) and glycolic acid ($C_2H_4O_3$). In addition, these chemical formula clearly showed that PLGA and PLGA structures contain more oxygen atom, an substance that is known to be an excellent H-bond acceptor, especially when interacting with water molecules.[45] The relatively higher hydrophobicity of PCL may be particularly desirable in the design of gene and/or oral vaccine delivery system for which the particle uptake by cells is critical for success. For example, it has been shown that hydrophobic particles undergo enhanced uptake by M-cells of Peyer's patches in the gut-associated lymphoid tissue.[46]

Table II shows DSC and SEC data. The increase of intrinsic viscosity data was correlated with polymer

TABLE III
Influence of the Type of Polymer on Particles Characteristics

Type of PCL	BSA loading (% w/w)	Entrapment efficiency (%)	Particle size (μm)	Morphological analysis
PCL_a	0.45 ± 0.09	9.1 ± 1.9	6.5 ± 0.1	Discrete spheres
PCL_b	0.59 ± 0.02	11.9 ± 0.5	7.4 ± 0.4	Discrete spheres
PCL_c	1.60 ± 0.66	32.5 ± 1.0	13.9 ± 0.8	Discrete spheres
PCL_d	2.87 ± 0.11	57.9 ± 0.1	32.3 ± 0.3	Agglomerated and irregular

Values are expressed as average ± standard deviation ($n = 3$). Polymer properties are in Table I and II.

molecular weight and may be explained by the Mark-Houwink equation. The latter eq. (5) usually describes the correlation between intrinsic viscosity $[\eta_i]$ (reduced viscosity extrapolated to infinite dilution) and M_W (M).[30,34]

$$[\eta_i] = KM^a \quad (5)$$

Where parameters K and a are constants for a given polymer solvent combination at a given temperature. The knowledge of the viscosity of the polymer is also important in drug formulation because the viscosity is proportional to the relaxation time (an indicator of the molecular mobility), based on the Maxell equation.[40] The polydispersity index (M_w/M_n) of each PCL polymer tested in this study was within the range (1.4–1.9). The PCL melting points were found to be consistent with literature values.[29] However, there was no correlation between PCL M_W and DSC data probably because of to the difference in the polymer suppliers and therefore in their manufacturing process. It was not possible to obtain the required PCL M_W for this study from one supplier. Nevertheless, it was noteworthy that PCL_c of M_W 84.4 kDa gave the lowest enthalpy and entropy of fusion and crystallinity. Additional study may be necessary to elucidate these observations. Because of the amorphous nature of PLA and PLGA used, there was no data regarding their melting point, enthalpy of fusion, and percent crystallinity.

Physicochemical characterization of protein-loaded microparticles

Influence of polymer properties on encapsulation process

Table III and Figure 1 showed the influence of PCL M_W and the plastic viscosity of the polymer containing organic phase on particle characteristics. The particle size increased linearly between 6.5and 32.3 μm with the viscosity of the organic phase [Fig. 1(a)]. But there was a semilog correlation between the viscosity and percent encapsulation efficiency in the range 9.1–57.9% [Fig. 1(b)]. The linear relationship observed with particle size was also consistent with previous studies using similar method of preparation using PLGA as polymer.[3,14] These results may be explained by the reduced emulsification efficiency (rate of agitation) consecutive to higher medium viscosity thereby leading to larger droplets (protomicroparticles). In fact, even if controlled largely by the rate of agitation, particle size is also dependent on the viscosity, interfacial tension, and density of microparticle materials.[47] However all other parameters remaining empirically the same, it was postulated that increasing the M_W of the PCL led to an increase in the viscosity of the

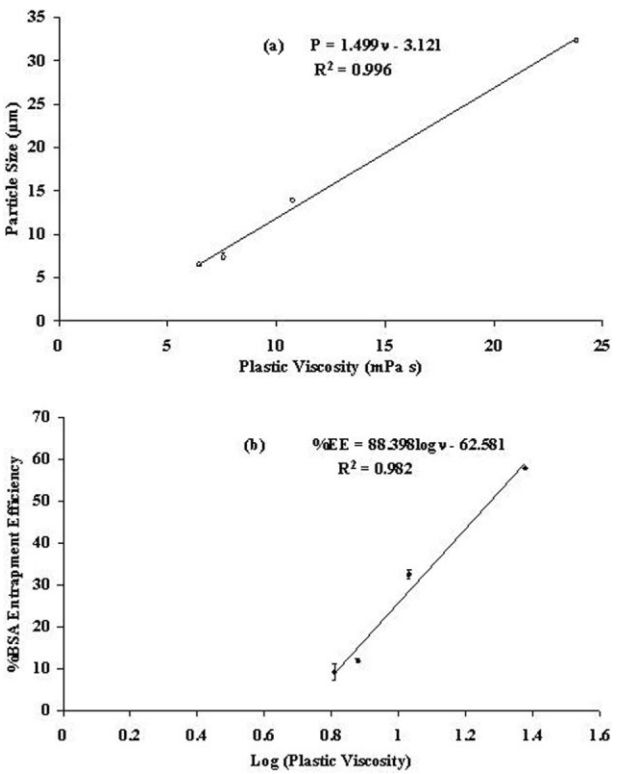


Figure 1 Correlation between the plastic viscosity (v) of the polymer in organic solution versus the obtained particle size (P) and percent entrapment efficiency (%EE). All the batches were prepared strictly in the same condition, beside the unique variable under consideration. The figures referred to (a) linear correlation between plastic viscosity and P; (b) semi log-correlation between plastic viscosity and %EE.

TABLE IV
Influence of BSA Loading on Particle Characteristics

BSA loading (% w/w)	DSC Results			Surface area (g m^{-2})	Particle size(μm)	% Burst release at day 0
	T_{fus}	T_c	% Cr			
0 (Pure PCL)	62.7	20.5	53.7	ND	ND	ND
0 (Empty MP)	60.1	30.7	66.9	2.94	9.2 ± 0.2	ND
1.6 ± 0.6	60.2	31.2	55.1	1.27	13.9 ± 0.8	24.2 ± 0.9
9.6 ± 0.3	59.7	30.7	60.1	2.03	13.3 ± 0.4	25.8 ± 0.6
13.5 ± 0.6	59.8	30.6	57.5	2.93	12.4 ± 0.4	19.7 ± 0.4

Size and release values are expressed as average ± standard deviation (n = 3). Particles were prepared from PCL_c (84.8 kDa). *T*fus, melting point; *Tc*, crystallization point; % Cr, per cent crystallinity; MP, microparticles; ND, not determined.

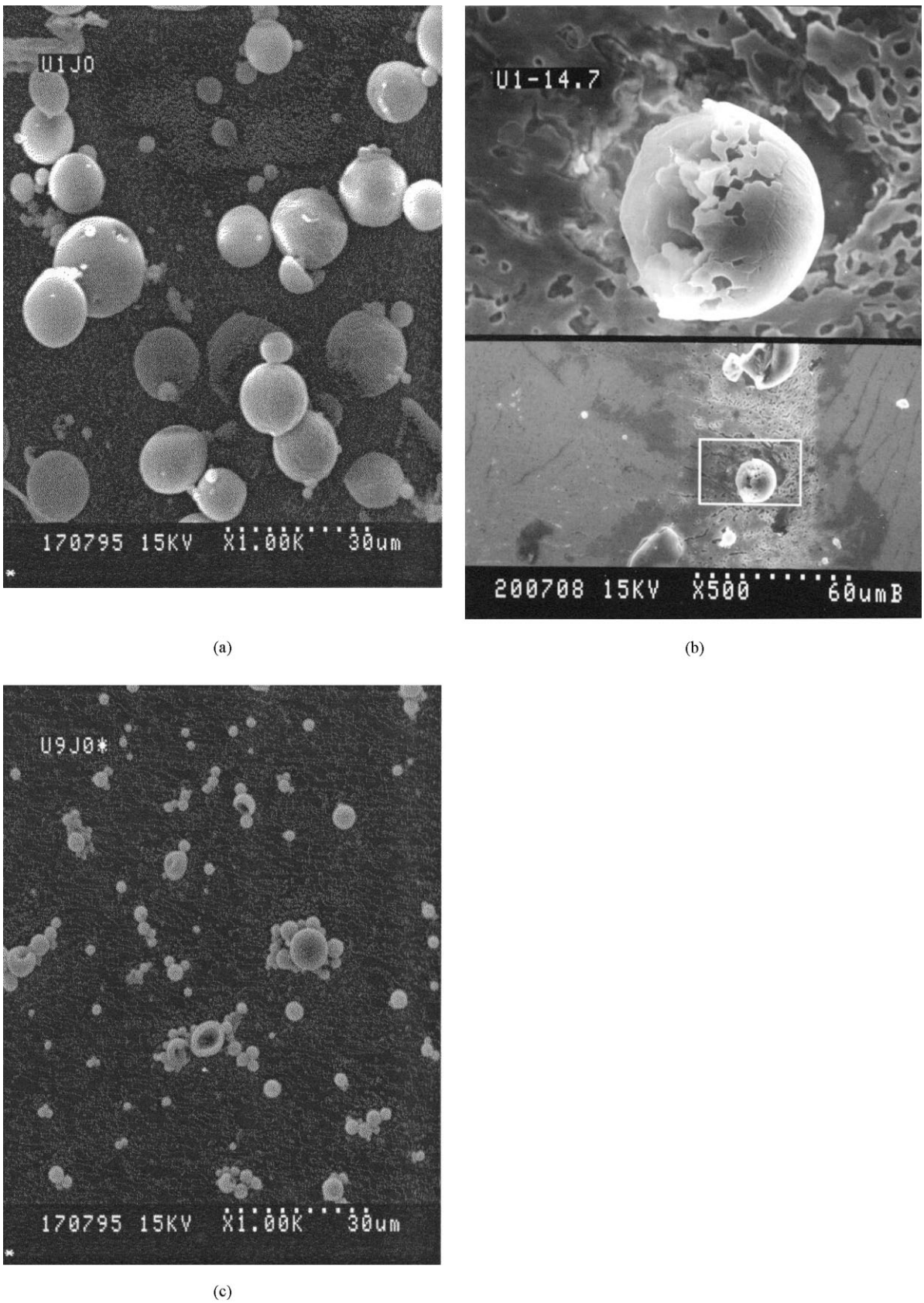
organic phase, which reduced protein diffusion into the external aqueous phase before microparticles hardening.[6,48] All the particles were smooth, spherical, and discrete except those obtained with PCL_d with highest M_W (Table III). For the same initial BSA loading, at low M_W, the particles were smaller, spherically shaped, and discrete but at high M_W, particles obtained were larger, irregularly shaped and more agglomerated. These results support those obtained with PLA by Jalil and Nixon.[49] Future study should investigate whether there is any relationship between polymer M_W and DSC behavior of the polymer.

Influence of protein content on particles characteristics

Table IV showed the influence of BSA content on particles characteristics such as thermal behavior obtained DSC, surface area, and particle size. All parameters remaining the same, we also observed that increasing the initial BSA loading (data not shown) lead to lower encapsulation efficiency because of larger protein concentration gradient between the emulsion droplet phase and continuous/external aqueous phase (W_2) as previously reported with other biomaterials.[31] After the protein entrapment, the following three observations were made as follows. (i) Compared to that of native PCL, the melting point (T_{fus}) of PCL particles was slightly reduced, according to the van't Hoff's equation, which expresses that the melting point of a pure solute is higher than that of a solution. Because there is a linear relationship between T_{fus} and glass-transition temperature (T_g),[50] it was reasonably speculated that the T_g was also slightly reduced probably because of plasticizer effect of residual moisture. This speculation was also supported by the commonly accepted fact that the T_g value of water (−137°C)[39] was lower than that of pure PCL (−60°C).[29] Therefore, the $T_{g(mix)}$ of binary mixture (PCL/water) may have decreased based on Gordon Taylor equation. Such analysis became more complex if one considered the T_g of other components involved in the formulation. Future in-depth analysis of T_g effect will be important because the William-Landel-Ferry equations describe molecular mobility near and above the T_g.[40] In addition to the enthalpy relaxation, such studies may contribute to rationalize the protein formulation and delivery process from polymeric carriers such as PCL. However, this was not the scope of the present work. (ii) Compared to that of native PCL, the crystallization point of PCL particles increased markedly suggesting that a phenomenon of nucleation had occurred. These observations were also consistent with our previous results obtained with superoxide dismutase-loaded PCL particle.[38] (iii) Compared to empty MP, the presence of protein in the matrix reduced the percent crystallinity without a considerable influence on T_c. Additional investigations will be needed to elaborate on these observations. Figures 2(a), 2(c) showed a typical SEM of 1.6 and 9.6% w/w protein loading, respectively. The 13.5% w/w protein loaded microparticles were morphologically finer than the 9.6% loading protein-loaded particles (data not shown) as evidenced by their relatively higher specific surface area. These SEM and the specific surface area data (Table IV) together suggest that the increase of BSA or protein content considerably increased particle specific surface area but surprisingly decreased the rate of protein release as shown in the next section. However, the coulter counter size data was not discriminating between these low and high protein content samples. This may be explained by the fact, despite the use of dispersion methods, clusters of strongly agglomerated small particles may be detected as coarse particles by the coulter principle that is based on electrical sensing zone method. In contrast, the BET method based on nitrogen molecule adsorption provided a clearer insight into particle specific surface area that was consistent with SEM data.

Influence of protein content on *in vitro* release profile

The Figure 3 showed the cumulative release profile of BSA from PCL particles presenting different loadings (1.6, 9.6, and 11.5%) for over 180 days. For all these formulations, the protein release profile was consistently biphasic: a burst release followed by a continu-



(a) (b)

(c)

Figure 2 Scanning electron micrograph (SEM) of microparticles from PCL_c produced by a (water-in oil)-in water emulsion solvent evaporation. BSA or protein loading was either 1.6 or 9.6% (w/w)-loaded microparticles. The figures referred to (a) = 1.6% BSA loaded particles at day 0, (b) = 1.6% protein-loaded particles after 180 days in the release medium, and (c) = 9.6% protein-loaded particle at day 0. The magnification was ×1000 (for 2a, c) and ×500 (for the upper part of 2b).

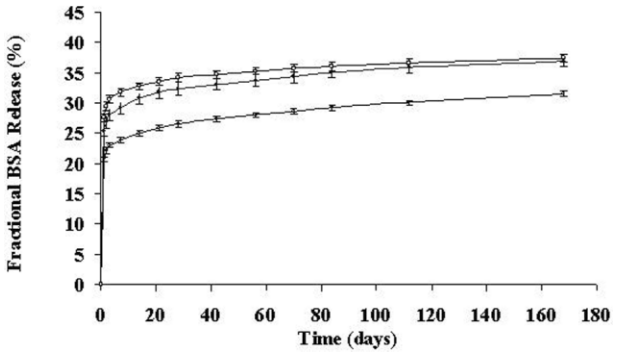


Figure 3 Analysis of the *in vitro* release profile of BSA from PCL microparticles presenting different BSA loadings: 1.6% (^), 9.6% (□), 11.5% w/w (○). The release medium was 0.1*M* PBS pH 7.4 containing 0.01% sodium azide. Error bars refer to standard deviation for $n = 3$.

ous release for 180 days. The burst release (see data in Table IV) was attributed to the protein that was poorly encapsulated within the polymer matrix. The continuous release phase may be explained by protein diffusion through matrix micropores and microchannels, according to the percolation theory as previously underlined.[10] Unlike protein-loaded PCL particles exhibiting an oily core that we have previously formulated for pulsatile release,[10] protein release profile was rather biphasic and occurred mainly by diffusion and consistent to the work of Huatan et al.[51] It was noteworthy that protein release rate from the 11.48% BSA loading formulation was lower than the 1.6 and 9.6% BSA-loaded formulation despite similar percent crystallinity and its relatively higher surface area. Because BSA contained hydrophobic (e.g., tryptophan, phenylalanine) and charged (e.g., arginine, lysine) aminoacids in its backbone[52] and PCL was highly hydrophobic, it was postulated that the increase of BSA content in the polymer matrix led to increased hydrophobic, hydrogen bonding, and electrostatic interaction between the BSA and PCL leading to the decrease in protein release rate observed with higher protein loading in the PCL microparticles. Pashley et al. demonstrated that attractive hydrophobic forces at interfaces were 10–100 folds stronger than expected by Van der Waals theory over a distance up to 10 nm.[53] The exact implication of such forces between BSA and PCL within PCL microparticles remained to be elucidated at fundamental level. The Figure 2(a,b) provided visual evidence of the morphological change in protein containing PCL particle over time. After 180-days incubation in the release medium, the PCL particles were only slightly eroded on the surface [Fig. 2(b)]. Similar results were observed with all protein formulation tested in Table IV. PCL particles appeared to be relatively stable: little surface morphological change occurred after 180-days release study probably because of the semicrystalline nature of the PCL. For all formulations tested, regardless of the protein content, the PCL biodegradation rate may have been reduced over time by the decrease in accessible ester bonds in the crystalline region. Normally, the degree of crystallinity of the PCL is known to increase over time during the degradation.[29] Moreover, study of Pitt et al. showed that significant PCL weight loss (4.6%) was not observed until 48 weeks (>180 days).[54] Drug release was mainly diffusion controlled over 180 days without major change in PCL particle morphology. Because diffusion is limited to the amorphous phase of semicrystalline polymers and the crystalline phase can additionally restrict chain motion in the amorphous phase, the value of the diffusion coefficient is dependent on the degree of crystallinity of the polymer.[50] The overall slow release of BSA from PCL may be fundamentally attributed to both the stronger physicochemical interaction forces and the increased crystallinity of PCL following polymer degradation. As initiated by Hao et al.,[33] future investigations with protein -loaded PCL should evaluate the influence of the PCL network density on the protein release rate in addition to other formulation variables.

CONCLUSIONS

This work dealt with some physicochemical parameters such as polymer/water interfacial data, rheological, thermal, and molecular weight that influenced efficient protein entrapment and release from PCL microparticles produced by double emulsion solvent evaporation. It was shown essentially that the increase in PCL molecular weight (from 46. 7 to 179.4 kDa) led to an increase of both particle size and encapsulation efficiency that was consistent with rheological data. The increase of protein content from 1.6 to 11.5% (w/w) considerably increased particle specific surface area and surprisingly decreased the rate of protein release presumably following enhanced physicochemical interaction between the protein and the carrier polymer. These results may have implications not only for controlled drug delivery but also for other applications such as protein coating of particles, polymer chemistry, and degradation.

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