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Effects of the characteristics of chitosan on controlling drug release of chitosan coated PLLA microspheres

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Chitosan has been shown to be a biomaterial with good biocompatibility, and is highly biodegradable. This study investigated the effect of post-coating PLLA microspheres with different chitosans on the initial burst and controlling the drug release of the microspheres. Without chitosan, 19.2% of encapsulated lidocaine would release from PLLA microspheres within the first hour (R_1), and the time of 50% release (T_{50}) was 25 h. After the microspheres were coated with chitosan of viscosity (η) 384 ± 10 cp, R_1 and T_{50} could be reduced and prolonged to 14.6% and 90 h, respectively, for all tested molecular weights (Mw) of chitosan. In the case of the same Mw of chitosan being applied, the efficacy of reducing the initial burst of drug release was higher for a lower degree of deacetylation (D.D.). With chitosan in acetic acid solution, coating the microspheres with high Mw and high viscosity could most effectively reduce the initial burst and control drug release of PLLA microspheres. For example, the microspheres coated with chitosan solution of Mw 800 kDa and η of 1479 cp, R_1 and T_{50} could be reduced and prolonged to 7.4% and 245 h, respectively. The study indicated that manipulating the viscosity of the chitosan solution was the most important factor in contributing to controlling the drug release of chitosan post-coated PLLA microspheres.

Keywords: Chitosan, PLLA, microsphere, controlled drug release.

Introduction

PLLA (poly-L-lactide) and its derivatives have been widely applied as carriers for drug delivery (Geolewski *et al.* 1993, Gupta *et al.* 1997, Leach and Mathiowitz 1998). Since they are biodegradable and shown not to cause adverse tissue reactions, there is no needed to retrieve the carrier when the drug is depleted.

The release of drug from PLLA microspheres is affected by many factors such as morphological surface, particle size and the kind of drug being encapsulated. In an earlier study, different solvent evaporation processes during fabrication of the microspheres would affect the above-mentioned characteristics (Chung *et al.*

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2000). In addition, drug loaded PLLA and PLGA microspheres showed a high initial burst during the early stage of drug releases (Kreitz *et al.* 1997, Huang *et al.* 1999, Chung *et al.* 2000, Yang *et al.* 2000). To reduce the burst, PLLA microspheres coated with different concentrations of gelatin have been proposed, and shown to effectively reduce the phenomenon (Huang *et al.* 1999), but this did not control the release of drug.

Chitosan [poly(1,4-β-D-glycopyrano-samine)] is one of the polysaccharides which show antimicrobial (Muzzarelli *et al.* 1990) and biocompatible behaviour (Hirano and Nagano 1989, Lehr *et al.* 1992). Most chitosan is obtained from chitin, extracted from shrimp or crab shells, and further deacetylated by heating and hydrolysis of chitin (Yamamoto 1984). Since it is highly biocompatible and easily biodegradable, chitosan has been used as a material for medical applications such as surgical sutures, artificial skin and an immunosuppressant (Chandy and Sharma 1990, Okamoto *et al.* 1993, Chang *et al.* 1996). Recently, the chitosan gel bead has received attention as a matrix for drug delivery systems (Shiraishi *et al.* 1993, Sezer and Akbuga 1995, Jameela *et al.* 1998) because it possesses different reactive groups, and is degraded to non-toxic products by enzymes such as lysozyme. Two techniques are usually applied to fabricate chitosan microspheres (Yao *et al.* 1995). One involves the phase separation of chitosan from an aqueous solution by a counterion (simple coacervation) (Chandy and Sharma 1992) or oppositely charged macromolecule (complex coacervation) (Polk *et al.* 1994). The other technique involves the emulsification of an aqueous solution of chitosan in a non-solvent, followed by cross-linking of the polymer with glutaraldehyde to solidify the dispersed chitosan droplet (Akbuga and Durnaz 1994, Jameela and Jayakrishnan 1995).

In a non-eroding biodegradable microsphere, drug is preferentially released from the surface, while embedded drug needs to diffuse through different thicknesses of polymer matrix. Multi-walled microspheres might achieve the objective of drug release control (Pekarek *et al.* 1994). To control the release of microspheres with a high initial burst, choosing a suitable biodegradable polymer and manipulating the outer structure to form a double-walled sphere is worth studying. Here, chitosan was chosen to post-coat PLLA microspheres, with lidocaine as a model drug, to control drug release, and also to enhance the biocompatibility of PLLA microspheres. The effect of different molecular weight, D.D. and viscosity of chitosan solution on the structure and drug release of double-walled microspheres were investigated.

Materials and methods

Materials

Lidocaine (Mw 234.34) and PVA (degree of polymerization of 2000) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Poly-L-lactide (Mw 85 000–160 000) was purchased from Sigma Chemical Company (St. Louis, USA). Dichloromethane (ultra pure) was purchased from Alps Chemical Company (Hsin-Chu, Taiwan). Wet diosmosis membrane (MwCO 1000) was obtained from Spectrum Medical Industries Inc., CA, USA. All other chemicals were LC grade and used as received.

Preparation of PLLA microspheres

The emulsion-solvent evaporation method was applied to fabricate PLLA microspheres. The process was similar to that in previous reports (Huang *et al.* 1999, Chung *et al.* 2000); 0.5 g PLLA was dissolved in dichloromethane and 0.05 g lidocaine (as a model drug) was added. Then, 0.05% PVA solution was poured into the above-mentioned PLLA solution and emulsified by an ultrasonicater (GE50T, Cole-palmer Co., Illinois, USA) at 4 °C. After emulsification, solvent was fully evaporated with a mechanical stirrer at room temperature. The drug-loaded microspheres were recovered by centrifugation, and then rinsed with distilled water. After drying at room temperature, the microspheres were further dried for 6 h at 40 °C.

Post-coating PLLA microspheres with chitosan

Chitin was prepared by the method of Chen *et al.* (1994) from shrimp waste. Chitosan was prepared by the method of Yamamoto and Amaike (1997). After 3% chitosan of different Mw or D.D. was dissolved in 1% acetic acid, 2 mg of drug loaded PLLA microspheres were poured into the chitosan solution and stirred until completely mixed. A syringe with a needle (27G) was used to inject into 1N sodium hydroxide solution that contained 26% alcohol to form spherical gels. After 30 min, the spherical gels were removed and rinsed with distilled water until neutral conditions, and dried for 24 h at room temperature. The hypothetical structure of drug loaded microspheres with the double-walled model is shown in figure 1. In this process, the drug loss from the chitosan microspheres were minor, so it is assumed that the amount of drug encapsulated into chitosan post-coated PLLA microspheres is the same as that of uncoated ones.

Viscosity measurement of chitosan solution

Viscosity of chitosan solutions was measured by a HAAKE RS-100 cone/plate viscometer (HAAKE MESS-Technik, Karlsruhe, Germany), with a cone angle of 1° at 25 °C. The detailed description of the viscometer and its applications has been reported (Chung *et al.* 1998). Chitosan solution (1 ml) was sheared at a fixed shear stress of 7.2 Pa, and the viscosity of steady flow of the chitosan was calculated. A fixed viscosity of chitosan solution for different Mw and D.D. chitosan was adjusted by varying the concentration of chitosan dissolved in acetic acid.

Scanning electron microscopy (SEM) analysis

SEM was performed using a Hitachi (Model S-4700, Tokyo, Japan) microscope. Microspheres were sprinkled on to double-sided tape, sputter-coated with gold and examined in the microscope at 10 kV.

In vitro *release*

Two milligrams of PLLA microspheres (chitosan coated or non-coated) was placed in a diosmosis membrane (MwCO 1000 and 8 cm length) with 0.2 ml PBS (0.025 M. pH 7.5). The diosmosis membrane was put in a beaker filled with 50 ml PBS, and shaken in a water bath at 65 rpm and 25 °C. PBS solution (1.5 ml) was pipetted out from the beaker at predetermined time perods and refilled. The

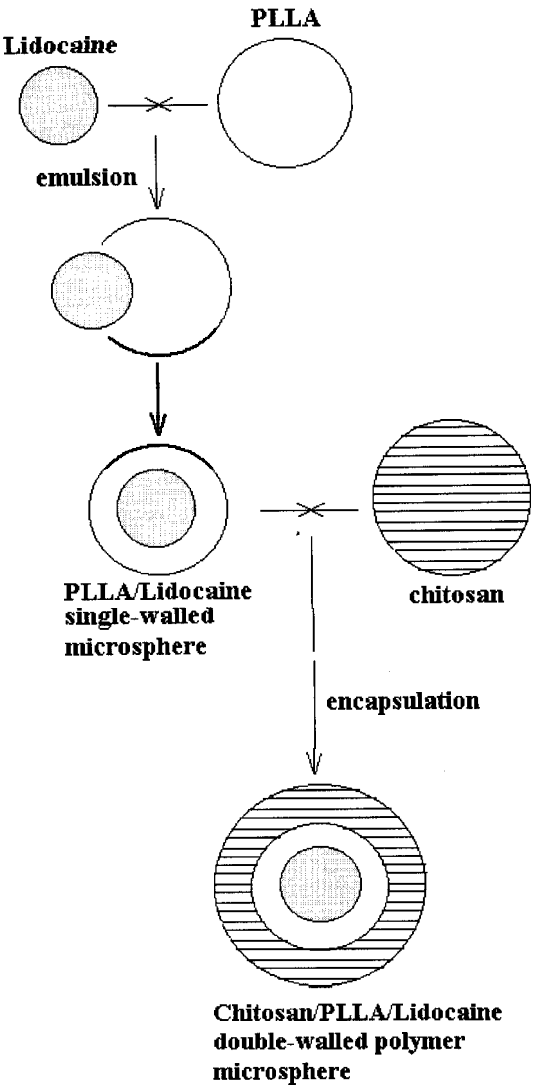

Figure 1. The hypothetical structure of drug microspheres.

lidocaine concentration was determined using a UV/VIS spectro-photometer (Jasco V-530, Kobe, Japan) at 215 nm, and also verified with capillary electrophoresis, as in earlier reports (Huang *et al.* 1999). The data presented are mean ± SD.

Results and discussion

Characteristics of chitosan solution

The different characteristics of chitosan solution are shown in table 1. The viscosity of chitosan solutions increase with decreasing D.D. and increasing Mw of chitosan. Chen *et al.* (1996) and Chen and Tsain (1997) have shown that the higher

Table 1. Effects of different Mw, viscosity, concentration, and degree of deacetylation of chitosan on drug release of chitosan coated PLLA microspheres.

Codes	Mw ($\times 10^4$ dalton)	D.D. (%)	Concentration (%)	Viscosity (cp)	R_1 (%)	T_{50} (h)
PLLA	8.5–16	—	—	—	19.2	25
C20	20	92.4	3	86	18.2	48
C50	50	83.5	3	464	13.1	105
C80(I)	80	88.6	3	252	15.7	90
C80(II)	80	88.6	5	1054	9.8	200
C80(III)	80	60.0	3	1479	7.4	245

D.D. chitosan have larger pore sizes, which may be due to the higher chain flexibility than that of the lower D.D. one. However, it is suggested that larger pore sizes of high D.D. chitosan may be due to more amino groups instead of acetyl groups, and, consequently, cause a smaller intermolecular density than that of lower D.D. The viscosity of chitosan solution increased with increasing intermolecular density at a fixed volume of chitosan. Moreover, the viscosity of chitosan solution increased with increasing the molecular weight of chitosan and concentration of chitosan.

Morphology of chitosan/PLLA double-walled microspheres

Chitosan microspheres were prepared by phase separation of chitosan from NaOH aqueous solution. From SEM photographs, it was found that the surface of drug free microspheres were fairly smooth and spherical (figure 2(*a*)). However, the surface of the drug-loaded microspheres appeared to be rough (figure 2(*b*)), which might be due to the mixing and hardening process when PLLA microspheres were blended with chitosan to form a uniform drug distribution. Figure 2(*c*) shows the inner surface of cross section of chitosan layer. In another view (figure 2(*d*)), the wrinkle type of crevice was clearly seen (i.e. position of arrow). When aqueous (e.g. PBS) diffuses into the interior of microsphere by capillary or infiltration, the drug might be dissolved and drawn out on the same path.

Release behaviour of drug from chitosan matrix formed at constant viscosity

In studying controlled drug release by using chitosan as a matrix, some investigators considered the effects of different D.D. or Mw of chitosan (Sezer and Akbuga 1995, Chen *et al.* 1996, Chen and Tsaih 1997). Whether the viscosity of chitosan solution would also affect drug release from the chitosan matrix has not been investigated. Chitosan solutions of different Mw but with the same viscosity (i.e. 384 ± 10 cp) were used to coat PLLA microspheres. The results showed that the release of drug was not dependent on Mw or D.D. of chitosan (figure 3). Without chitosan coating 19.2% of encapsulated lidocaine would release from PLLA microspheres within the first hours (R_1), and T_{50} is $\sim$25 h, similar to earlier studies (Chung *et al.* 2000). Chitosan post-coated PLLA microspheres did reduce initial burst of drug release and T_{50} values (table 1).

Figure 3 also shows that 94 and 87% of drug release for non-coated PLLA microspheres and chitosan post-coated ones at the end of the release study, respectively. One could estimate that the encapsulation efficiency for the process

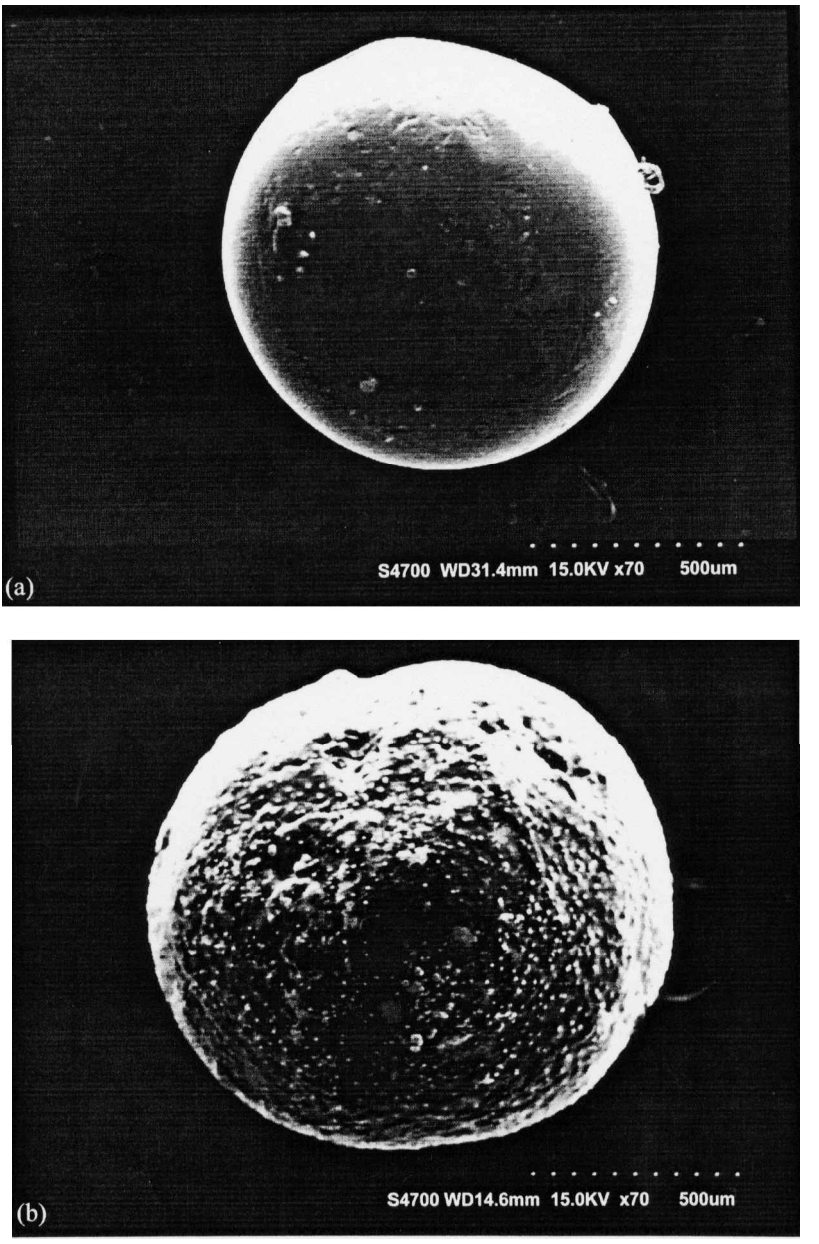

Figure 2. SEM of chitosan: (*a*) free drug loaded; (*b*) drug loaded.

of chitosan post-coated PLLA microspheres were over 92%, as the assumption in the materials section.

Release behaviour of drug from chitosan matrix formed at varying viscosity

Figure 4 shows that the initial burst has been reduced when the molecular weight of chitosan increases from 200 kDa to 800 kDa with varying viscosity. In

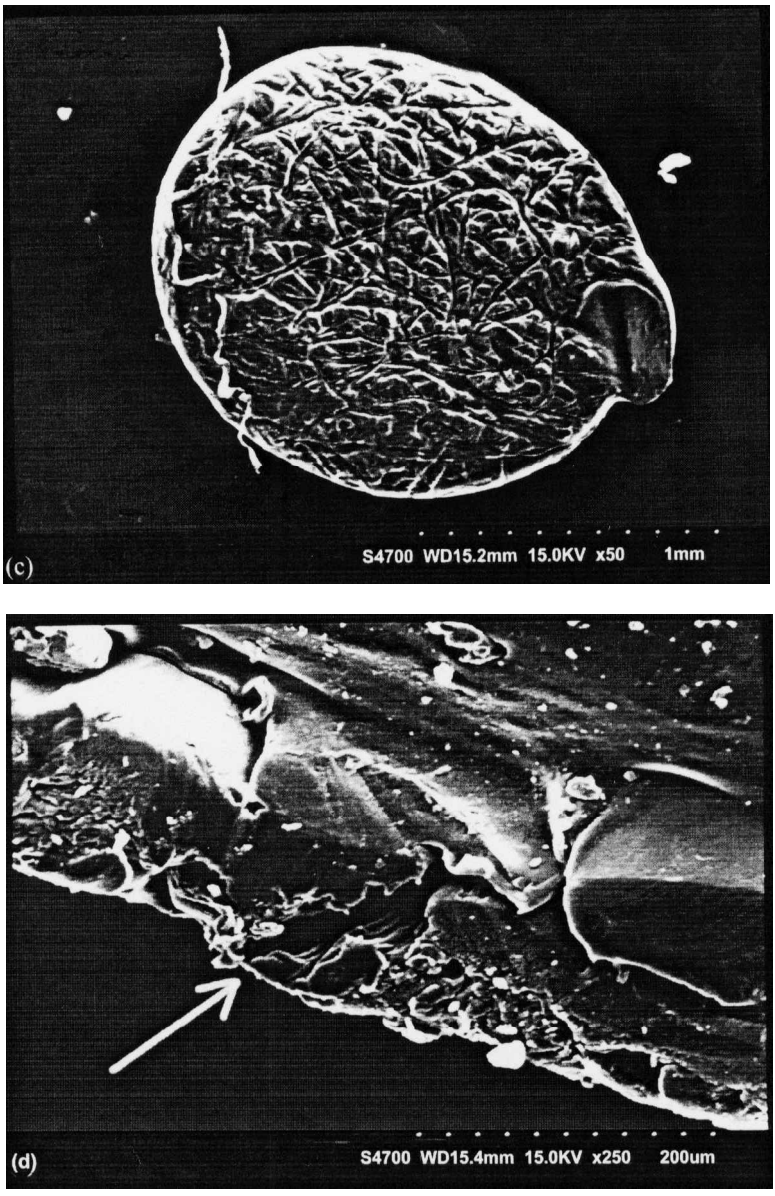

Figure 2. SEM of chitosan: (*c*) the surface of cross section; (*d*) different view of (*c*).

addition, increasing the viscosity of chitosan solution plays an important role in reducing the burst. For example, using 500 and 800 kDa Mw of chitosan to coat the PLLA microspheres, R_1 was 13.1 and 15.7% when the viscosity of chitosan solutions were 464 and 252 cp, respectively (table 1). T_{50} was also prolonged with increasing the viscosity of chitosan solution. Therefore, the release of drug of coated microspheres was more dependent on the viscosity than the Mw of chitosan. The drug release rate of chitosan coated microspheres would be closely related to the film thickness, δ, and the related equation, $\delta \propto 3Q\gamma/2\pi Rg$ (Singh and

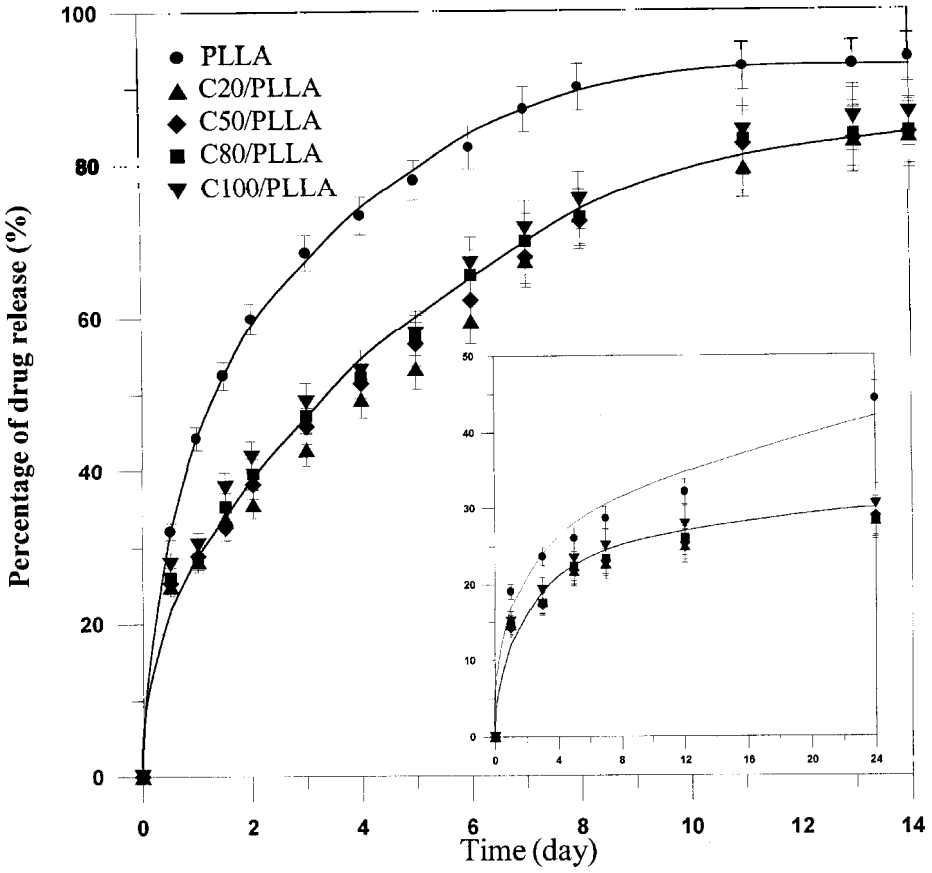

Figure 3. Effects of different Mw, but with the same viscosity on drug release of chitosan coated PLLA microspheres. Key: Mw of chitosan (●) non-coated; (▲) 200 kDa; (♦) 500 kDa; (■) 800 kDa; (▼) 1000 kDa. The chitosan solution viscosity is 384 ± 10 cp at 25 °C. The drug releases of the microspheres in an early stage are shown in the right hand corner (i.e. 24 h).

Stockar 1996). The thick film formed by the higher viscosity of chitosan would slow down release rate of drug molecules from microspheres by increasing diffusion.

Figure 5 shows the morphology changes of different Mw chitosan coated microspheres after 14 days of drug release tests. Degradation of the chitosan layers was observed within 14 days for low viscosity chitosan (e.g. 86 and 252 cp) coated PLLA microspheres (Figure 5(*a*) and (*b*)). This might contribute to the higher release rates of the drug. The morphology for PLLA microspheres coated with higher viscosity chitosan solution (e.g. 464 cp) was still intact over 14 days (figure 5(*c*)). The result shown on figure 5(*c*) was similar to the report by Jammeela *et al.* (1995) who reported that, after 7 days incubation in lysozyme solution, chitosan microspheres appeared to have little degradation. Pangburn *et al.* (1982) also reported that cross-linked chitosan microspheres with glutaraldehyde did not degrade to a significant extent *in vivo* in 3 months.

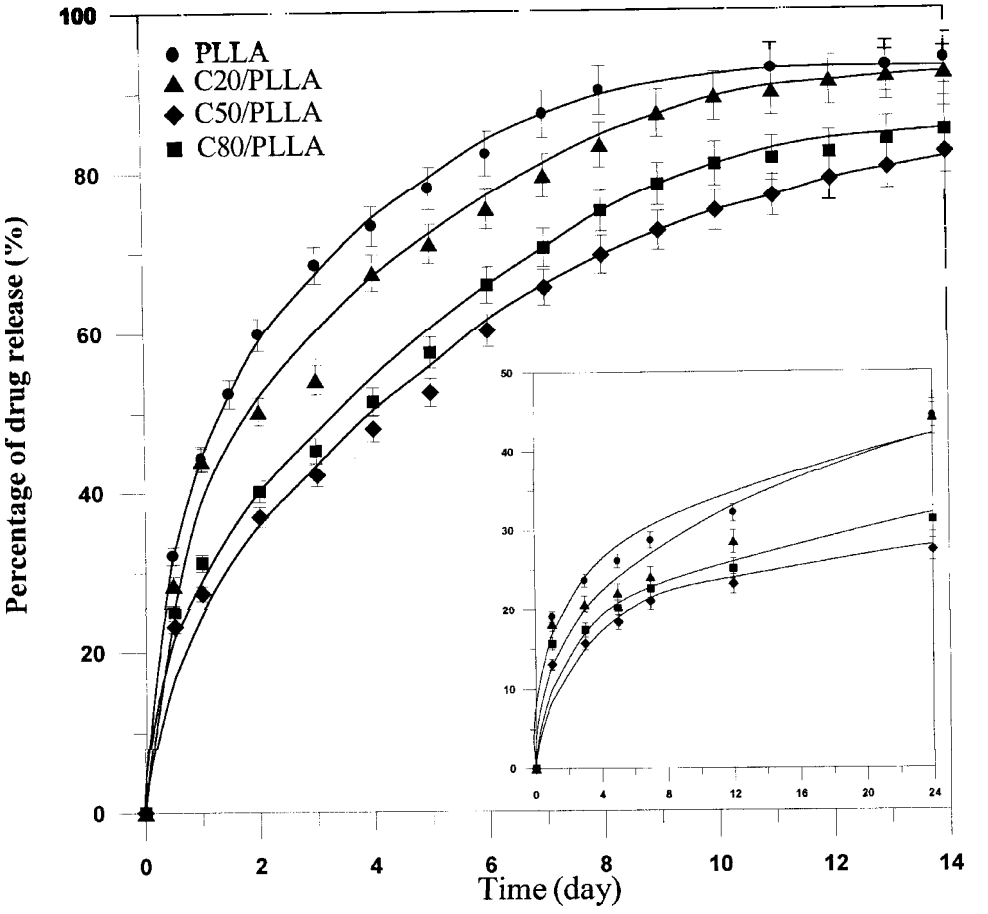

Figure 4. Effects of different Mw and viscosity of chitosan on drug release of chitosan coated PLLA microspheres. Key: chitosan (●) non-coated; (▲) Mw 200 kDa, viscosity 82 cp; (♦) Mw 500 kDa, viscosity 464 cp; (■) Mw 800 kDa, viscosity 252 cp.

To confirm that controlling the viscosity of chitosan solution would play an important role in controlling the drug release behaviour of post-coated microspheres, the same Mw chitosan (800 kDa), but with varying viscosity, was used to post-coat PLLA microspheres. The results show that the initial burst and drug release rate were highly reduced as the viscosity of chitosan solution increased (figure 6). For example, R_1 was reduced to 9.8 and 7.4%, and T_{50} was prolonged to 200 and 245 h for the slutions with viscosities of 1054 and 1479 cp, respectively. It was also found that the release of drug from chitosan coated microspheres was nearly linearly proportional to the release time. Mi *et al.* (1997) have proposed that high viscosity of chitosan may increase the adhesion force between chitosan and drug and result in reducing drug release from chitosan microspheres. The same explanation has been applied in Singh and Ray (1999). They showed that the release rate of glucose through a chitosan membrane decreased with increasing film thickness of chitosan.

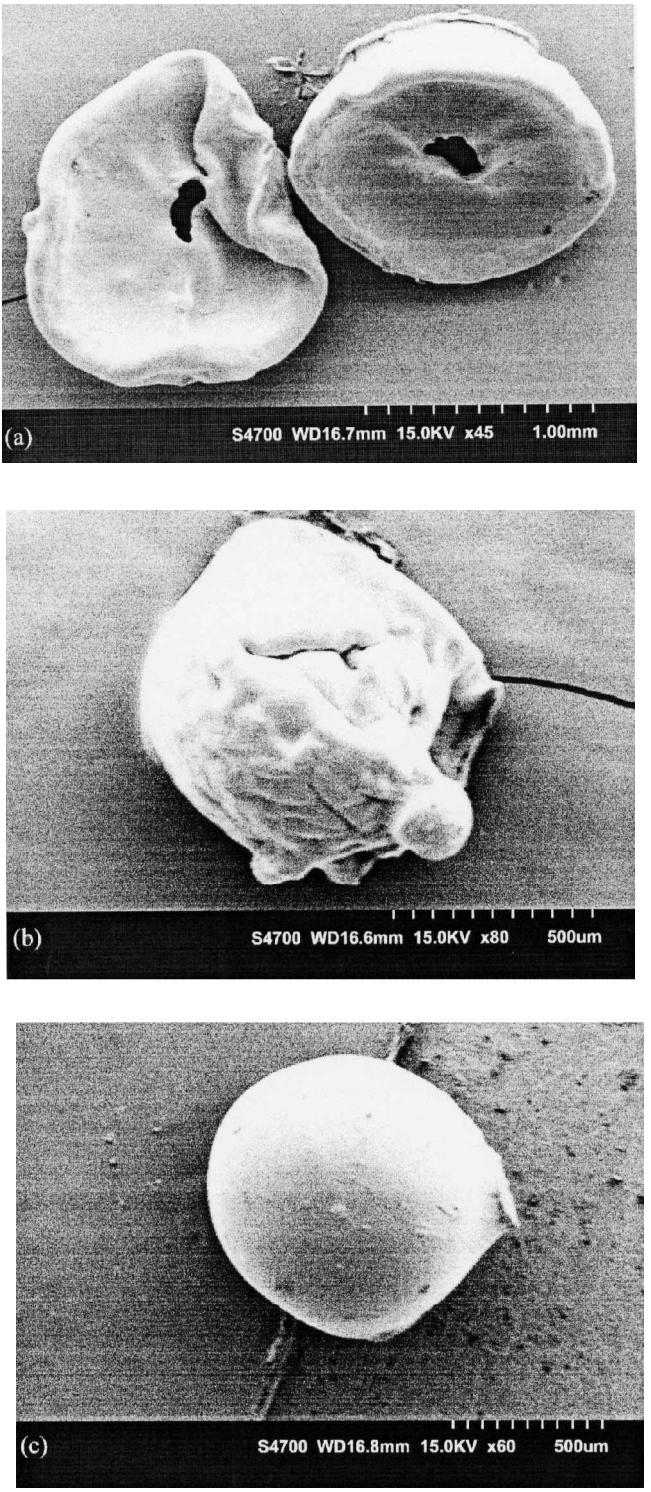

Figure 5. SEM of chitosan microspheres degradation after 14 days: (*a*) Mw 200 kDa, viscosity 82 cp; (*b*) Mw 800 kDa, viscosity 252 cp; (*c*) Mw 500 kDa, viscosity 464 cp.

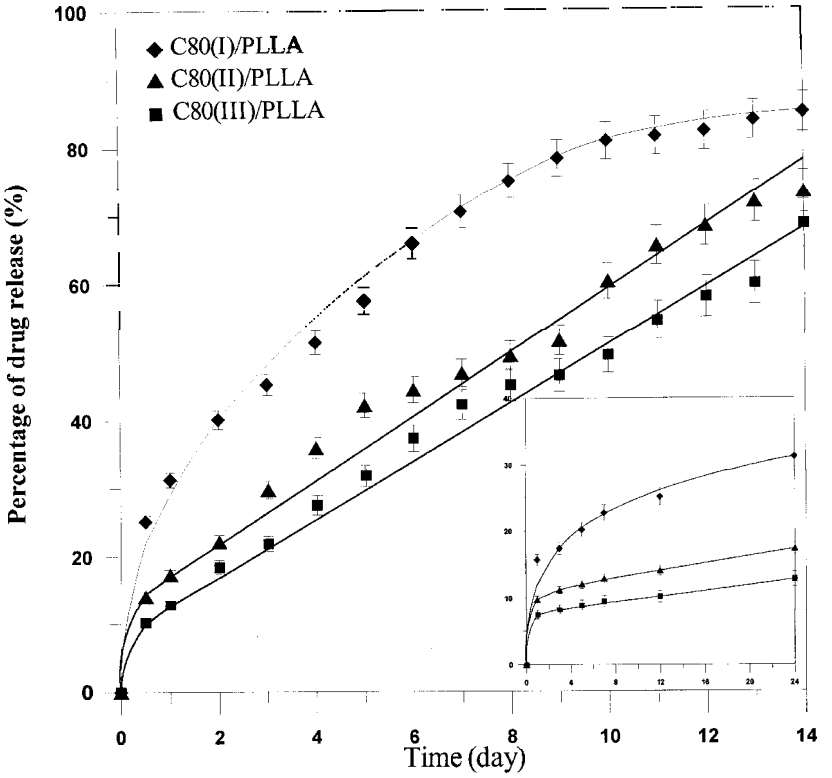

Figure 6. Effects of different viscosity, but with the same Mw of chitosan, on drug release of chitosan coated PLLA microspheres. Key: viscosity of chitosan (♦) 252 cp; (▲) 1054 cp; (■) 1479 cp. The chitosan Mw is 800 kDa.

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