# *In Vitro* Degradation and Release Profiles of Poly-DL-Lactide-Poly(ethylene glycol) Microspheres with Entrapped Proteins

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**ABSTRACT:** Poly-DL-lactide (PLA) and poly-DL-lactide-poly(ethylene glycol) (PELA) were produced by bulk ring-opening polymerization using stannous chloride as initiator. PLA, PELA microspheres, and PELA microspheres containing the outer membrane protein (OMP) of *Leptospira interrogans* with the size of 1.5–2 $\mu$m were prepared by a solvent evaporation process. *In vitro* degradation and release tests of PLA, PELA, and OMP-loaded PELA microspheres were performed in pH 7.4 buffer solution at 37°C. Quantitatively, the degree of degradation was monitored by detecting the molecular weight reduction, by evaluating the mass loss and the apparent degradation rate constant, and by determining the intrinsic viscosity and poly(ethylene glycol) content of retrieved polymer, while the release profile was assessed by measuring the amount of protein presented in the release medium at various intervals. Qualitatively, the morphological changes of microspheres were observed with scanning electron micrography. The observed relative rates of mass loss versus molecular weight reduction are consistent with a bulk erosion process rather than surface erosion for PELA microspheres. The introduction of hydrophilic poly(ethylene glycol) domains in copolymer PELA and the presence of OMP within microspheres show critical influences on the degradation profile. The OMP-loaded PELA microspheres present triphasic release profile and a close correlation is observed between the polymer degradation and the OMP release profiles. It is suggested that the polymer degradation rate, protein diffusion coefficient, and the water-swollen structure of microspheres matrix commonly contribute to the OMP release from PELA microspheres. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 140–148, 2000

**Key words:** poly-DL-lactide-poly(ethylene glycol);degradation; degradation mechanism; protein release; release mechanism

## INTRODUCTION

Polymers find increasing application in the pharmaceutical industry as matrices for drug delivery systems. One approach to these advanced pharmaceuticals involves polymers that degrade *in vivo* after or concurrently with drug release. Biodegradable polymers often have low toxicity, are not tissue reactive, and do not require surgical removal from the host after delivery of the bioactive agent is complete. Among the various types considered as degradable carriers for drugs, hydrolytically labile polyesters have evoked consid-

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erable interest. In particular, lactide and glycolide homo- and copolymers (PLGAs) have been investigated extensively as matrices for releasing contraceptives, anesthetics, chemotherapeutics, proteins, and vaccines.[1,2] Results have been promising in many substances and several controlled release delivery systems, for example, Zoladex (Zeneca), Decapeptyl (Ipsen Biotech), and Prostap SR (Lederle) have been licensed for use in humans in Europe and the USA.[3] Nevertheless, PLGAs show some drawbacks, resulting from their hydrophobic nature. As to hydrophilic drugs, such as peptides, proteins, and antigens (usually composed of proteins and glycoproteins), the difference in physico-chemical properties with hydrophobic PLGA matrix has profound consequence on the protein encapsulation efficiency during the preparation procedure, and the protein stability during manufacture, storage, and release process. For example, the protein–polymer interactions may lead to formation of pharmacologically inactive high molecular weight aggregates, by rearrangements of the three-dimensional protein structure in a reversible or irreversible fashion.[4] In addition, during the initial protein release phase *in vivo*, the hydrophobic PLGA prevents the penetration of water into the center of the samples, thus forming an acidic micro-environment due to the accumulated acidic breakdown products, such as lactic and glycolic acid end groups.[5] This acid environment, combined with the elevated temperature and hydrophobic surfaces, may provide conditions in which proteins are unlikely to survive for a long time.

For manipulating the hydrophilicity/lipophilicity of poly-DL-lactide (PLA), the second component poly(ethylene glycol) (PEG), which has been widely used to improve the biocompatibility of the blood contacting materials, is introduced into PLA to form ABA block copolymer poly-DL-lactide-poly(ethylene glycol) (PELA).[6] The biodegradable and biocompatible nature of PELA[7] make it a suitable candidate polymer for the development of control delivery systems of water-soluble drugs, peptides, and vaccines. The hydrophilic domains of PELA copolymer acting as a protein stabilizer or surface modifier of hydrophobic PLA network, could promote the stability of proteins, increase the protein loading efficiency, and decrease the amount of emulsifier used in PELA microspheres preparation.[8] We recently reported the preparation of PELA microspheres with entrapped human serum albumin, outer membrane proteins (OMP) of *Vibrio cholera* and *Leptospira interrogans*, which showed effective immunization results by oral and subcutaneous inoculations.[8–10] *In vitro* investigation provides a convenient method to inspect the biodegradable characteristics, and quite a few communications have been presented to describe the time dependence of molecular weight changes in PLA and PLGAs, then to probe the degradation mechanisms *in vitro* and *in vivo*.[11,12]

Therefore, this report describes a study on the *in vitro* degradation of PELA in the form of microspheres that is presently used for antigen delivery system. PELA microspheres with a diameter of 1.90 $\mu$m, containing OMP of *Leptospira interrogans* were prepared by solvent extraction method. For comparison, the degradation of PLA microspheres was also included in the present work. These microspheres under investigation were incubated for 14 weeks in pH 7.4 buffer at 37°C. On removal of microspheres from the degradation medium, the following parameters were determined as a function of degradation: the mass loss and the surface morphology of recovered microspheres, the molecular weight and polydispersity, the intrinsic viscosity and the PEG content of the retrieved polymers. The OMP release profile from PELA microspheres was also investigated.

## EXPERIMENTAL

### Materials

PELA copolymer with 10% of PEG content and PLA homopolymer were prepared by bulk ring-opening polymerization of lactide/PEG or lactide using stannous chloride as initiator.[6] The block polymer structure of PELA was determined by $^{13}$C-NMR as described previously. *Leptospira interrogans* antigen, outer membrane protein (OMP) (molecular weight, 39 kDa) was granted from West China University of Medical Sciences, Chengdu. All other chemicals and solvents were of reagent grade or better.

### Preparation and Characterization of Microspheres

PLA and PELA microspheres were prepared by oil-in-water (o/w) organic solvent evaporation, whereas OMP-loaded microspheres were produced by water-in-oil-in-water (w/o/w) based on solvent extraction process, as reported previously.[8] After the complete removal of organic solvent, the microspheres were collected by centrifugation (Tomy Seiko Co., Japan). The resultant microspheres were rinsed with distilled water and cen-

trifuged three more times, then lyophilized overnight and stored at 4°C. The microsphere size was determined with laser diffraction particle size analyzer (Shimadzu SALD-2009, Japan), or by measuring the diameters of at least 500 particles shown in scanning electron micrography (SEM) (Amray, USA). The amount of OMP entrapment was measured by placing 100 mg of microspheres in 1.5 mL of dichloromethane and extracting the OMP three times with 1.5 mL of double distilled water. The OMP content of the extracted solution was determined using Bradford's method,[13] compared with a standard curve of data obtained by assaying known concentrations of OMP solutions.

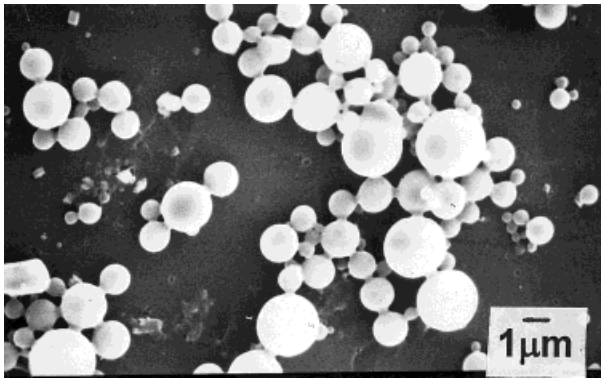


**Figure 1** The scanning electron micrograph of OMP-loaded PELA microspheres.

### *In Vitro* Degradation of Microspheres

Preweighed microspheres were placed in individual test tubes containing 10.0 mL of pH 7.4, 0.1*M* phosphate buffered saline (PBS). The tubes were kept in a thermostated shaking water bath (Jiangsu Taichang Medical Apparatus Co., China) that was maintained at 37°C and 60 cycles/min. The degradation medium was removed weekly from the vessel containing microspheres by centrifugation and replaced with the same volume of fresh PBS. At predetermined time intervals, triplicate samples for each kind of microsphere were recovered, rinsed with distilled water to remove any residual buffer salts, and dried to constant weight in a vacuum desiccator.

### Estimation of Degradation

The degree of degradation was estimated from the decrease of intrinsic viscosity and molecular weight, from the mass loss, from the change of the amount of PEG content, and from the morphological changes of microspheres. Each point of the plots describing the degradation data represents the mean of the triplicate samples.

Mass loss was determined gravimetrically by comparing the dry weight remaining at a specific time with the initial weight. SEM (Amary) was used to observe the surface morphological changes. Samples of fresh microspheres and microspheres from the degradation experiments were dissolved in tetrahydrofuran (THF) and filtered to eliminate unsolved proteins. The molecular weight and polydispersity of the retrieved polymer were determined by gel permeation chromatography in THF at 30°C with an Ultrastyragel linear column (Waters, USA) and a refractive index detector (R401,Waters), and calibrated with polystyrene standards. The intrinsic viscosity was measured with an Ubbelohde viscometer on 0.4 g/dL solution of PELA in THF at 30°C. The PEG contents of PELA at different degradation periods were evaluated from the integral height of hydrogen shown in $^1$H-NMR (Varian FT-80A, USA) as described previously.[9]

### *In Vitro* OMP Release Test

The rate of OMP release from PELA microspheres was determined by incubation OMP-loaded PELA microspheres into a test tube containing 10.0 mL 0.1*M*, pH 7.4 PBS. The release tests were performed three times independently. These tubes were allowed to store in the same water bath as mentioned in degradation test. At appropriate intervals, 1.50 mL of release medium was collected by centrifugation and 1.50 mL of fresh PBS was added back to the test tube. The amount of OMP was measured by the Bradford's protein assay as described above.

## RESULTS

### Characterization of Microspheres

The SEM spectrum of PELA microspheres containing OMP is shown in Figure 1, and the results of the characterization studies conducted on the three microspheres are provided in Table I. PLA and PELA with similar weight-average molecular weight ($M_w$), produced by optimization of the process parameters, were chosen as the matrix polymers for microsphere preparation. The average diameter of each sample of microspheres was 1.5–2 $\mu$m, which is suitable for parenteral vaccination because of their efficient prevention of capillary clogging. OMP-loaded PELA microspheres

**Table I Characteristics of Microspheres Under Investigation**

| Property | PLA Microspheres | PELA Microspheres | OMP-Loaded PELA Microspheres |
|---|---|---|---|
| $M_w$ (kDa)[a] | 58.1 | 58.9 | 58.9 |
| $M_n$ (kDa)[a] | 1.90 | 2.18 | 2.18 |
| $M_w/M_n$[a] | 3.05 | 2.70 | 2.70 |
| D ($\mu$m)[b] | 1.95 | 1.82 | 1.90 |
| SD[b] | 0.289 | 0.251 | 0.263 |
| D ($\mu$m)[c] | 1.86 | 1.57 | 1.59 |
| [$\eta$] (dL/g)[d] | 0.389 | 0.393 | 0.393 |
| PEG content (%)[e] | — | 11.50 | 11.50 |
| OMP Entrapment (%)[f] | — | — | 5.79 |

[a] Molecular weight and polydispersity determined by GPC.
[b] The average diameter (D) and its standard deviation (SD) measured by laser diffraction particle size analyzer.
[c] Particle size determined by SEM spectrum.
[d] Intrinsic viscosity determined with an Ubbelohde viscometer.
[e] Estimated from the $^1$H-NMR spectrum.
[f] OMP extracted from microspheres and evaluated by Bradford's protein assay.

with the size of 1–2 $\mu$m could reach the immunization-related tissues, such as the intestinal payer's patches, liver, and spleen following oral administration,[14] which is known as a more convenient and safer vaccination way. As seen from Table I, the OMP entrapment of 5.79% with the loading efficiency of 85% was achieved for PELA microspheres, whereas OMP entrapment of below 1.0% was obtained for PLA microspheres prepared by the similar process.[8] This effect may be caused by the increased phase compatibilities between the protein and PELA block polymer containing hydrophilic domains. The values of particle size showed evident differences between the two measuring methods, laser diffraction, and SEM spectrum. Microspheres were suspended in distilled water by supersonication to evaluate the size in laser diffraction method, whereas microspheres in a vacuum-dried state were required for SEM analysis. It showed particle sizes of PLA, PELA, and OMP-loaded PELA microspheres increase about 4.8%, 15.9%, and 19.5% after hydration, respectively. These observations suggest that the water-swollen effect of PELA microspheres resulted from the hydrophilic PEG domains.

### Evaluation of Microspheres Degradation *In Vitro*

Figure 2 shows the number-average molecular weight ($M_n$) decrease versus the degradation time. For PELA microspheres independent of the OMP entrapment, the $M_n$ decreases changed rapidly initially but slowed at longer incubation intervals. During the predetermined time, the $M_n$ of PELA microspheres showed 67.0% loss, whereas 74.3% of $M_n$ loss was observed for OMP-loaded PELA microspheres. In contrast, less amount of $M_n$ reduction (38.4%) but with constant decrease rate was detected over 14 weeks' degradation of PLA microspheres. A slow increase in polydispersity ($M_w/M_n$) was observed after the degradation of PLA and PELA microspheres (shown in Fig. 3).Over 14 weeks of degradation, there was approximately 10% increase in $M_w/M_n$ of PLA microspheres, whereas 30% increase was detected for PELA and OMP-loaded PELA microspheres.

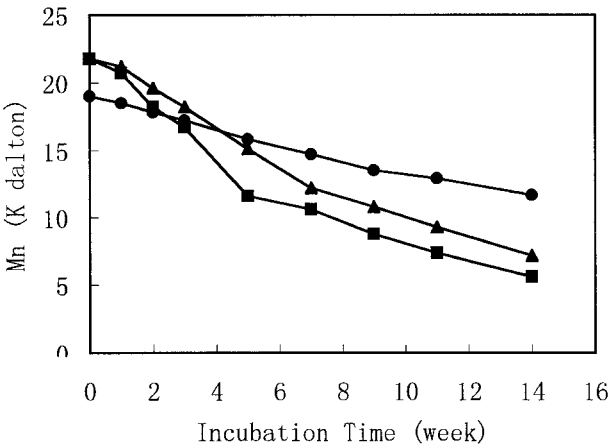


**Figure 2** *In vitro* molecular weight breakdown for polymers retrieved from PLA (●), PELA (▲), and OMP-loaded PELA (■) microspheres incubated in PBS at 37°C.

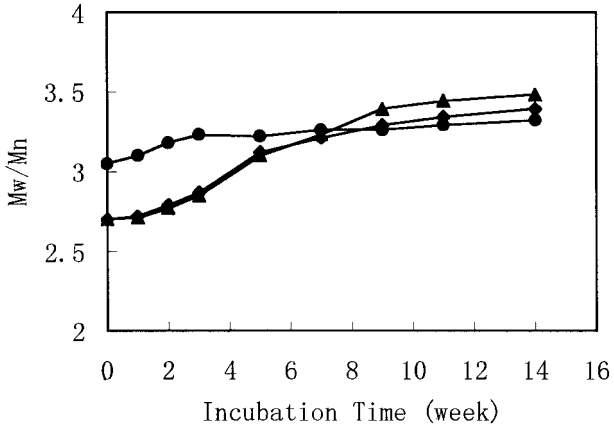


**Figure 3** Molecular weight polydispersity ($M_w/M_n$) as a function of time for polymers retrieved from PLA (●), PELA(♦), and OMP-loaded PELA (▲) microspheres degraded in PBS at 37°C.

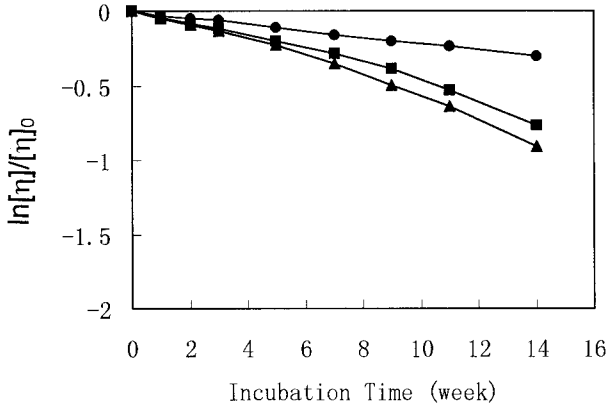


**Figure 5** Semilogarithmic relationship between the intrinsic viscosity [$\eta$] of polymers retrieved from PLA (●), PELA (■), and OMP-loaded PELA (▲) microspheres and degradation time in PBS at 37°C.

The mass losses of all the samples during the degradation period are listed in Figure 4. No mass losses could de detected for PLA and PELA microspheres for 9 and 7 weeks, respectively. OMP-loaded PELA microspheres showed a slight mass loss during the initial 5 weeks, and exhibited a significant weight loss in the following period. PLA, PELA, and OMP-loaded PELA microspheres maintained 93.2%, 68.2%, and 52.8%, respectively, of their initial mass after exposure to PBS at 37°C for 14 weeks.

The intrinsic viscosity of all samples of microspheres decreased continuously after being exposed to PBS. Figure 5 shows the decrease in $\ln([\eta]/[\eta]_0)$ with incubation times. The intrinsic viscosity of PLA samples showed a slight reduction during the experiment period, whereas that of PELA and OMP-loaded PELA samples decreased faster. Figure 6 displays representative PEG contents of the recovered polymers from PELA and OMP-loaded PELA microspheres versus incubation time. In each case, a lag phase (characterized by constant PEG content) appeared before a slight decrease in PEG content. The extent of the lag phase was approximately 7 weeks for PELA microspheres, whereas that of OMP-loaded microspheres was 5 weeks.

SEM spectrum of OMP-loaded PELA microspheres was taken to observe the morphological changes during various degradation stages (Fig. 7). Before incubated into degradation medium, microspheres showed an overall intact outer surface with tiny pores noticeable only at close examination [Fig. 7(A)]. After 7 weeks degradation,

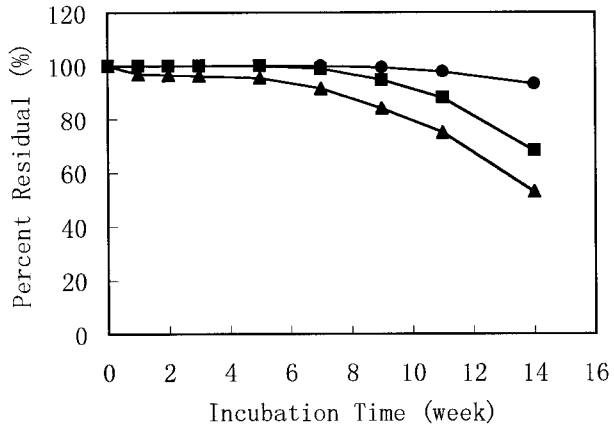


**Figure 4** The change of residual weight of PLA (●), PELA (■), and OMP-loaded PELA (▲) microspheres incubated in PBS at 37°C.

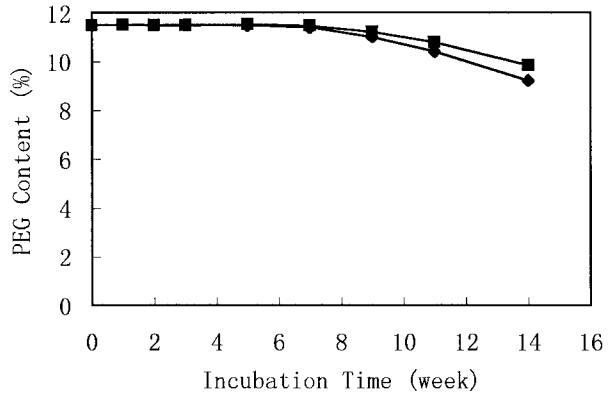


**Figure 6** PEG content changes of polymers retrieved from PELA (■) and OMP-loaded PELA (♦) microspheres during degradation in PBS at 37°C.

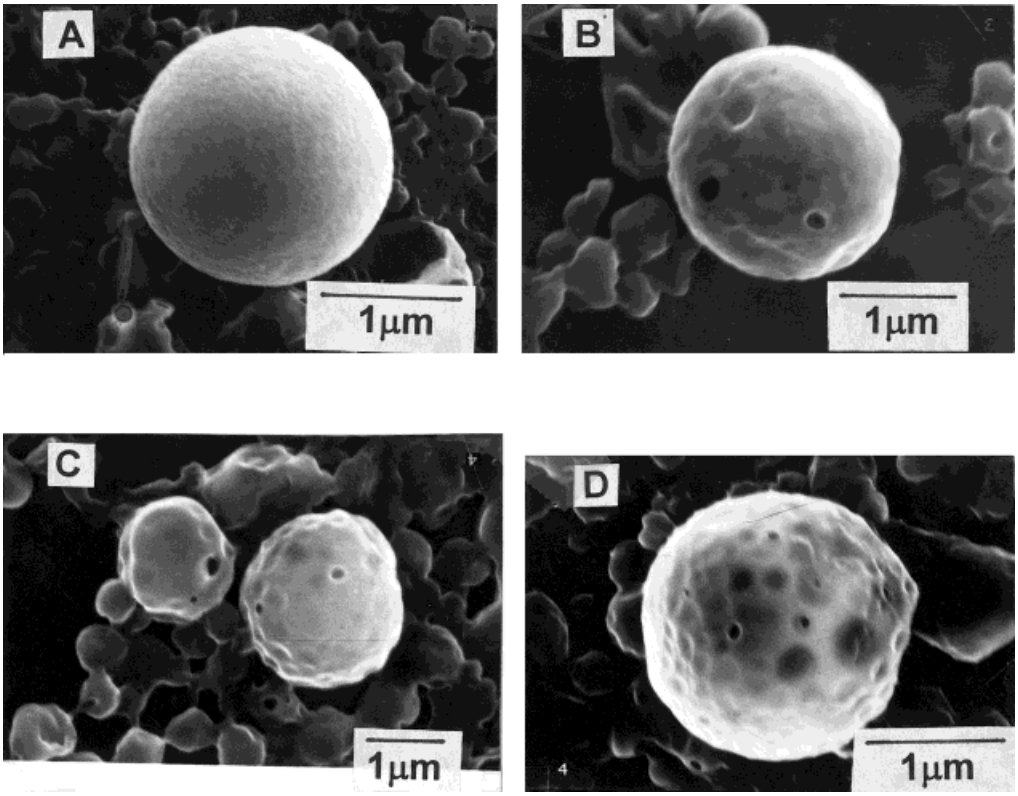


**Figure 7** The surface morphology, determined by SEM of OMP-loaded PELA microspheres (A) before degradation, (B) after 7 weeks' degradation, (C) after 14 weeks' degradation, and (D) PLA microspheres after 14 weeks' degradation.

the size of pores increased apparently, although microspheres were approximately spherical [Fig. 7(B)], whereas after 14 weeks of incubation most of the microspheres appeared highly eroded and porous [Fig. 7(C)]; meanwhile, small fragments were observed. For comparison, PLA microspheres showed apparent fissures on their surface only after 14 weeks degradation [Fig. 7(D)] and little fragments were detected.

### *In Vitro* Release Profile of OMP-Loaded PELA Microspheres

Figure 8 shows the percent release of OMP from PELA microspheres against incubation time. The release pattern was characterized as an at least triphasic process. In the initial phase, 18% of burst release during the first day was observed, and then a continuous release phase followed and proceeded for 5 weeks, whereas in the later incubation time, a quicker release phase was presented to release 30% of OMP within 1 week. The release of OMP was complete after 10 weeks of incubation.

## DISCUSSION

The possibility of controlling antigen release through the degradation time of polymeric microspheres represents an attractive feature for antigen delivery. To rationalize the design of antigen delivery microspheres, the present work aimed at

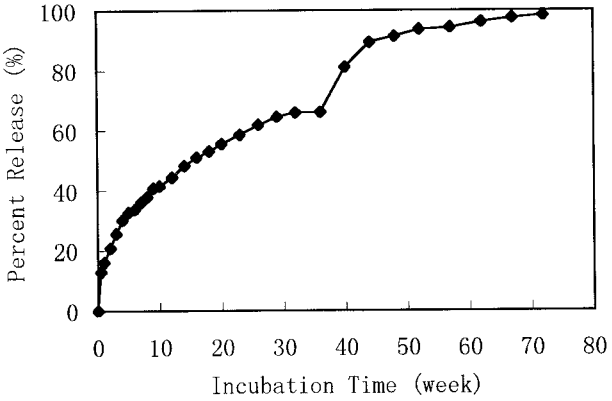


**Figure 8** Percent release of OMP from PELA microspheres incubated in PBS at 37°C.

characterizing the degradation profile of PELA microspheres, a novel antigen delivery system. Furthermore, this study aimed at a better understanding of the correlation of polymer degradation and antigen release profiles. It has been noted in PLA degradation investigation that the degradation mechanisms depend on processing conditions and sample dimensions,[12] thus data from native polymer powder or other forms of implants are not necessarily representative for microspheres degradation. Therefore, an *in vitro* degradation study was conducted with antigen-loaded PELA microspheres in the present work.

### *In Vitro* Degradation Profile

It is noted that even though no mass loss was observed in PLA, PELA, and OMP-loaded PELA microspheres over the initial several weeks (Fig. 4), significant changes in polymer intrinsic viscosity and molecular weight were detected (Figs. 2 and 5). Figures 2 and 4, displaying the curves of percent mass loss and $M_n$ reduction, show that the mass loss is slower than the reduction in $M_n$, suggesting that the mass loss continues until a fraction of oligomers that is soluble in degradation medium is generated from the polymers. The onset of mass loss lags behind molecular weight loss, thus PELA copolymer hydrolysis should proceed through the bulk of polymer structure, which is identical with the bulk degradation mechanism of PLA.[15] Another mechanism, surface layer hydrolysis and erosion, requires that erosion precedes molecular weight reduction of retrieved polymer.

The instant decrease in molecular weight suggests that polymer chain scission begins as soon as the sample is exposed to PBS, and the relatively lower rate of molecular weight reduction in the first week presumably reflects the intervals required for water to completely permeate into microspheres matrix. As seen from Figure 2, for PELA microspheres independent of OMP entrapment, the plots of $M_n$ reduction versus degradation shows apparent biphasic patterns. One high $M_n$ drop rate region is observed prior to the slowdown decrease region, and the breaks in $M_n$ reduction rates occur approximately at the onset of mass loss. As noted above, coincident with erosion onset, low molecular weight polymer fractions dissolve into buffer solution and become unavailable for gel permeation chromatography analysis of molecular weight on retrieved samples. It is also noted from Figure 2 that PELA microspheres become soluble (and the mass loss ensues) when the average molecular weight of the measured insoluble portion of polymer reduces to approximately 13.5 kDa.

The decrease in $M_n$ of samples retrieved from PELA microspheres was much faster than that of PLA, whereas OMP-loaded PELA microspheres were slightly faster than PELA microspheres in $M_n$ reduction (Fig. 2). The introduction of hydrophilic PEG domains into PLA promotes the water uptake and the swelling of microspheres matrix (Table I), which both enhance the hydrolysis rate of polymers. The presence of OMP in PELA microspheres leads to faster degradation; this may be attributed to the increasing hydrophilicity of microspheres matrix owing to OMP entrapped within microspheres, and the decreasing hindrance owing to porous network formed after OMP dissolved in PBS or diffused out from microspheres matrix.

Compared with PLA microspheres, the higher rate of augmentation in polydispersity ($M_w/M_n$) of polymers retrieved from PELA and OMP-loaded PELA microspheres was detected in the degradation experiment (Fig. 3). This may be related to the ether bonds of PEG fractions, which exhibit no hydrolytic activity under the present degradation conditions. Thus, a polymer chain would degrade into fragments containing PEG fraction or not, resulting in the higher molecular weight dispersion degree. Analysis of copolymer composition of PELA samples reveals that the PEG contents are kept constant initially, and then shift toward lower region upon mass loss during the degradation period (Fig. 6). This can be because degraded fragments with PEG fractions have higher hydrophilicity, which would enhance the hydrolytic reactions. In addition, the formed oligomers containing PEG domains dissolve in buffer preliminarily.

From the above discussion, the degradation of PLA and PELA can be described by penetration of water into microspheres matrix, followed by the bulk hydrolysis of ester bonds, and results in decrease in molecular weight and loss of microsphere weight. The *in vitro* degradation profile of copolymer PELA is strikingly different than that of PLA microspheres. The degradation of polyesters has been shown to take place by random hydrolysis of ester bonds, and the degradation of PLA and its copolymer can be described as follows[16]:

$$[\eta] = [\eta]_0 \exp(-\alpha k t)$$

where $\alpha$ is the exponent in the Mark-Howink formation $[\eta] = KM^{\alpha}$.

$$\ln([\eta]/[\eta]_0) = -\alpha kt$$

thus, a semilogarithmic plot of $[\eta]$ versus time (Fig. 5) is linear for PLA during the whole incubation time, but for PELA only during the initial stage of degradation. The apparent rate constant ($\alpha$k) can be obtained from the slop of the plot of $\ln([\eta]/[\eta]_0)$ against time. The evaluated values of $\alpha$k are 2.18, 4.12, and 4.60 ($\times 10^{-2}$ week$^{-1}$) for PLA, PELA, and OMP-loaded PELA microspheres, respectively, which indicates that the apparent degradation rate of PELA is approximately two times higher for PELA than that of PLA samples. In the later incubation time, a significant increase in the apparent degradation rate was detected for PELA microspheres independent of OMP entrapment, and the break in such increase also appeared close to the onset of mass loss. Thus, the different degradation profiles of the investigated microspheres are associated with the apparent degradation rate constant. The earlier onset of mass loss and higher rate of molecular weight reduction of OMP-loaded PELA microspheres were owing to their higher apparent degradation rate.

### *In Vitro* Protein Release Profile

It is known that systems based on PLA and its copolymers, in an aqueous environment, undergo hydration followed by bulk erosion. During erosion, the porosity of the matrix increases and the release of protein by diffusion happens. Assuming this behavior, not only the degradation rate of the polymer, but also the initial inner structure and the dispersion pattern of protein within microspheres matrix should be considered as critical factors controlling the release process. In fact, microspheres prepared with PLA, which degrade slowly, display an initial burst release followed bya lag phase characterized as no protein release, suggesting that a large amount of protein is located close to microspheres' surface and released by diffusion before significant degradation of polymer occurs. To overcome the discontinuous and biphasic release profiles of proteins from PLA matrix, introduction of hydrophilic domains, promoting the water uptake and swelling of device is an attractive strategy. By inserting PEG into relatively hydrophobic PLA blocks, modulation of diffusion of proteins from this carrier system is anticipated.

In the present investigation, the OMP-loaded PELA microspheres revealed triphasic release profile, that is, initial burst release during the first day, gradual release of OMP over 30 days, and lately, quicker release phases (Fig. 8). It shows an 18% burst release, which is lower by far than the 80% burst release of OVA from PLGA 50:50 microspheres.[17] This may be because of the preferential localization of protein molecules within the deeper sections of microspheres matrix due to the existence of hydrophilic PEG domains of PELA. In the second phase of release, proteins gradually release from microspheres matrix for 5 weeks, showing some similarities to the diffusion of macromolecules through a hydrogel-like structure after immersion in water.[18] It is indicated that the gradual release of OMP is owing to the swollen inner structure formed by contacting with the aqueous release medium, and protein diffusion through the swollen phase. In addition, a good correlation is observed between the polymer degradation and the OMP release profile for microspheres prepared with PELA. Forty percent of OMP continuously release from microspheres in 5 weeks (Fig. 8), whereas the copolymer PELA loses 50% of its initial molecular weight with constant degradation rate during this period (Fig. 2), but the reduction in molecular weight has not reached a point at which the degradation products are sufficiently small to be soluble (Fig. 4). The similar profiles of percent OMP release and percent molecular weight reduction, thus, are probably due to protein diffusion through a combination of the swollen phase caused by decreased molecular weight, and porous network formed by hydrolysis of polymer (Fig. 7). In the third phase of release, a close correspondence between the onset of quicker release and the breakdown of microspheres matrix was detected. As seen from Figure 8, the quicker release of OMP is observed to be slightly preceded by the break in molecular weight reduction rate (Fig. 2), which is also the onset of mass loss (Fig. 4). When the reduction of the molecular weight becomes significant and the mass loss has begun, a critical increase in the porosity of the matrix (Fig. 7) is achieved and the higher release rate proceeds. Additionally, it is suggested that, besides the porous structure, OMP could diffuse through low molecular weight polymer with poor film-forming properties.

## CONCLUSION

The *in vitro* degradation profile of PELA microspheres is quite different from PLA microspheres.

The introduction of hydrophilic PEG domains in copolymer and the presence of OMP within microspheres show critical influences on the apparent degradation rate constant, the reduction rate of molecular weight, the molecular weight polydispersity, and the onset of mass loss. Compared with PLA, PELA microspheres offer an advantage because of their swelling in the aqueous medium, generating a more stabilizing environment for proteins and minimizing the initial burst release. A good correlation is observed between the polymer degradation and the protein release profiles, which indicates that the polymer degradation rate, protein diffusion coefficient and the water-swelling structure of microspheres matrix commonly contribute to the OMP release from PELA microspheres.

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