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Long-term delivery of all-*trans*-retinoic acid using biodegradable PLLA/PEG-PLLA blended microspheres

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Abstract

All-*trans*-retinoic acid (atRA) has been proved to be effective against several malignancies in human clinical trials. However, in many patients who were treated with atRA, the cancer relapsed after a brief remission. One reason for such relapse is that atRA is metabolized by specific P450s that are induced in the liver during prolonged atRA treatments. In order to overcome such a drawback of atRA, we prepared biodegradable microspheres to provide continuous release of atRA for a long period of time. These biodegradable microspheres were prepared by poly(L-lactide) (PLLA) and polyethylene glycol (PEG)-PLLA diblock copolymers (PLE) in various blending ratios to control the release rate of atRA. As the PLE content in microsphere was increased, the density of the hydrophilic PEG block of PLE on microsphere surfaces increased and the microspheres were dispersed well in PBS without any surfactants. Various release patterns of atRA were obtained according to PLE and atRA contents in the microspheres. Especially, the pseudo-zero-order release profiles were observed for 5 weeks when the contents of PLE and atRA in the microspheres were above 4 wt.%.

Keywords: All-*trans*-retinoic acid; Biodegradable microspheres; Polyethylene glycol-poly(L-lactide) diblock copolymer; Poly(L-lactide)/polyethylene glycol-poly(L-lactide) blending

1. Introduction

All-*trans*-retinoic acid (atRA), an active metabolite of retinol, plays essential roles in the regulation of differentiation and proliferation of epithelial tissues (Gillis and Goa, 1995). It has been proved that atRA is effective in the treatments of epithelial and hematologic malignancies such as breast cancer (Toma et al., 1997), lung cancer (Kalmekerian et al., 1994), head and neck cancer (Giannini et al., 1997), ovarian adenocar-

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cinoma (Krupitza et al., 1995), and acute promyelocytic leukemia (APL) (Huang et al., 1988). In particular, Hong et al. reported that RA is effective in preventing the second primary cancer of head and neck squamous cell carcinoma (HNSCC) (Hong et al., 1990).

However, in spite of its pronounced effects, the clinical applications of atRA are limited due to the retinoid acute resistance (Frankel et al., 1992; Muindi et al., 1992a). All-*trans*-retinoic acid induces complete remission in a high proportion of the patients with APL; however, the cancer relapsed in many patients after a brief remission in spite of continued atRA treatments. Patients who relapsed from remission that was initially induced by atRA became clinically "resistant" to further atRA treatments. A recent study found that a continuous exposure to atRA in rhesus monkeys resulted in the induction of atRA metabolism and increased levels of cellular retinoic acid (RA) binding protein (Adamson, 1994). Muindi et al. reported that the recurrence of cancer might be due to the diminution of the atRA concentration in plasma (Muindi et al., 1992a,b). Pharmacokinetic studies have demonstrated that the area under the curve (AUC) of atRA decreases over time when it is orally administered on a chronic daily basis (Muindi et al., 1992b). Achkar et al.

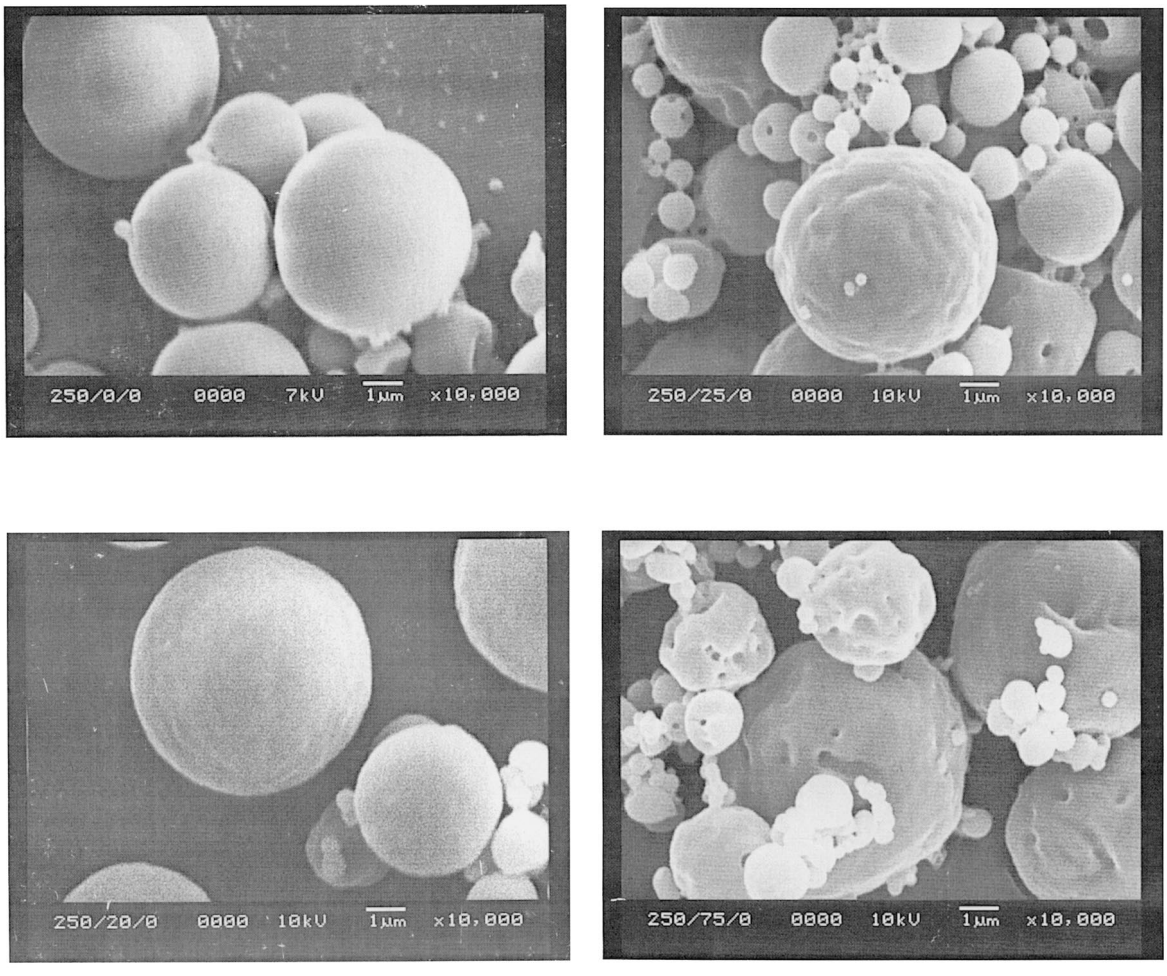

Fig. 1. Scanning electron micrographs showing morphological changes of microspheres by different contents of PLE: (a) 0 wt.%, (b) 8 wt.%, (c) 10 wt.%, and (d) 30 wt.%.

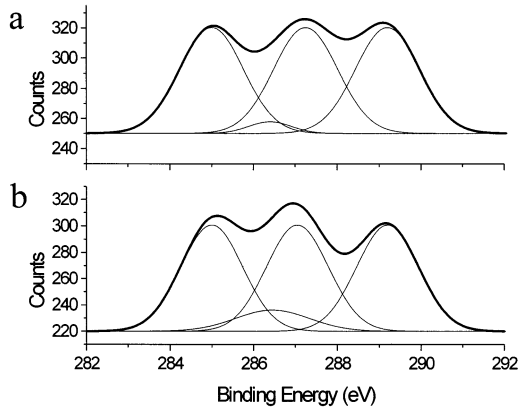

Fig. 2. C_{1s} XPS spectra of (a) the microspheres containing 4 wt.% PLE, and (b) the microspheres containing 8 wt.% PLE.

reported that the rapid decrease of the half-life of atRA in a continuous oral administration was due to catabolism by cytochrome P450s that were induced by atRA (Achkar et al., 1994).

In order to overcome the retinoid resistance, several methods such as the following have been proposed: an intermittent dosing schedule (Adamson et al., 1993), co-administration of P450 inhibitor (Wouters et al., 1992; Achkar et al., 1994), and formulations such as microemulsions (Takino et al., 1994), liposomes (Masini et al., 1993), and nanoparticles (Ezpeleta et al., 1996).

In this study, biodegradable microspheres were proposed for maintaining the atRA concentration in plasma for a long period. When RA-loaded microspheres are administered subcutaneously, metabolism in the hepatic microsomes can be bypassed, thereby reducing the rapid induction of the specific P450's. In addition, the effective plasma level of atRA can be maintained for a long time by pseudo-zero-order release of atRA from microspheres, thereby overcoming acute resistance of this drug. The long-term delivery of atRA would greatly improve the chemoprevention effect of atRA in the treatment of second primary cancer. In this study, polyethylene glycol (PEG)-poly(L-lactide) (PLLA) diblock copolymers (PLE) were physically blended into the PLLA microspheres in various ratios to control the release pattern of atRA.

2. Materials and methods

2.1. Materials

Mono-methoxy PEG (MPEG, M_n 5000), L-lactide, and poly(vinylalcohol) (PVA, 98% hydrolyzed, average M_w 13 000–23 000) were obtained from Aldrich Chemical Co. (Milwaukee, WI). L-lactide was purified by recrystallization twice from ethyl acetate, and MPEG was dried at 60°C for 8 h under vacuum. Stannous octoate and atRA were obtained from Sigma Chemical Co. (St. Louis, MO). PLLA (Res L206, M_w 110 000) was purchased from Boehringer Ingelheim Co. (Ingelheim, Germany).

2.2. Synthesis of polyethylene glycol-poly(L-lactide) diblock copolymer

PLE was synthesized by a solution polymerization as described by Stevels et al. (Stevels et al., 1995). In brief, L-lactide (6.507 g) and MPEG (2 g) were dissolved in toluene (45 ml) at 70°C under nitrogen atmosphere. Stannous octoate (65 mg) was added to the mixture as an initiator. After refluxing for 24 h at 110°C, the produced PLE was cooled and the solvent was removed under reduced pressure. PLE was dried overnight at 40°C under vacuum. The dried PLE was purified by the precipitation from an acetone/diethyl ether (1:4 v/v) mixture and then from the methanol/hexane (4:1 v/v) mixture. The purified PLE was finally dried under vacuum.

The number average molecular weight of PLE was determined by calculating the ratio of peak areas between the PLLA block and the PEG block of PLE in the ^{1}H-NMR spectrum (JEOL JNM-LA 300 WB, 300 FT-NMR, Tokyo, Japan) since the molecular weight of the PEG block of PLE was already known. The molecular weight distribution of PLE was determined by gel permeation chromatography (GPC, Waters Co., Milford, MA). GPC measurements were carried out with three columns (Styragel HR1, HR3, and HR4, Waters Co., Milford, MA) in series, and tetrahydrofuran was used as an eluent (1 ml/min). The columns were calibrated according to polystyrene standards. Internal and column temperatures were kept constant at 35°C.

2.3. Preparation and characterizations of the poly(L-lactide)/polyethylene glycol-poly(L-lactide) diblock copolymers microspheres containing atRA

PLLA/PLE microspheres containing atRA were prepared using the solvent evaporation technique in oil-in-water emulsion. PLLA (250 mg), PLE (0, 1, 2, 4, 8, 10, 20 and 30 wt.%), and atRA (0, 2, 4, and 8 wt.%) were dissolved in 5 ml of dichloromethane. This mixture was injected into 40 ml of an aqueous solution containing 2 w/v% of PVA while being mixed vigorously by a homogenizer (Ultra-Turrax T25, Janke and Kunkel IKA-Work, Staufen, Germany) at 24 000 rpm. After homogenization for 10 min, the resulting suspension was gently stirred for 2 h at 40°C with a magnetic stirrer to evaporate the dichloromethane. The microspheres were collected by centrifugation at 15 000 rpm for 10 min. The obtained microspheres were washed with distilled water four times and freeze-dried.

The effect of PLE on the morphology of microspheres was investigated using a scanning electron microscopy (SEM, JSM-5800 scanning microscope, JEOL, Tokyo, Japan). The PEG block of PLE on the microsphere surfaces was analyzed with X-ray photoelectron spectroscopy (XPS) (SS12803-S, S-probe, Surface Science Instrument,

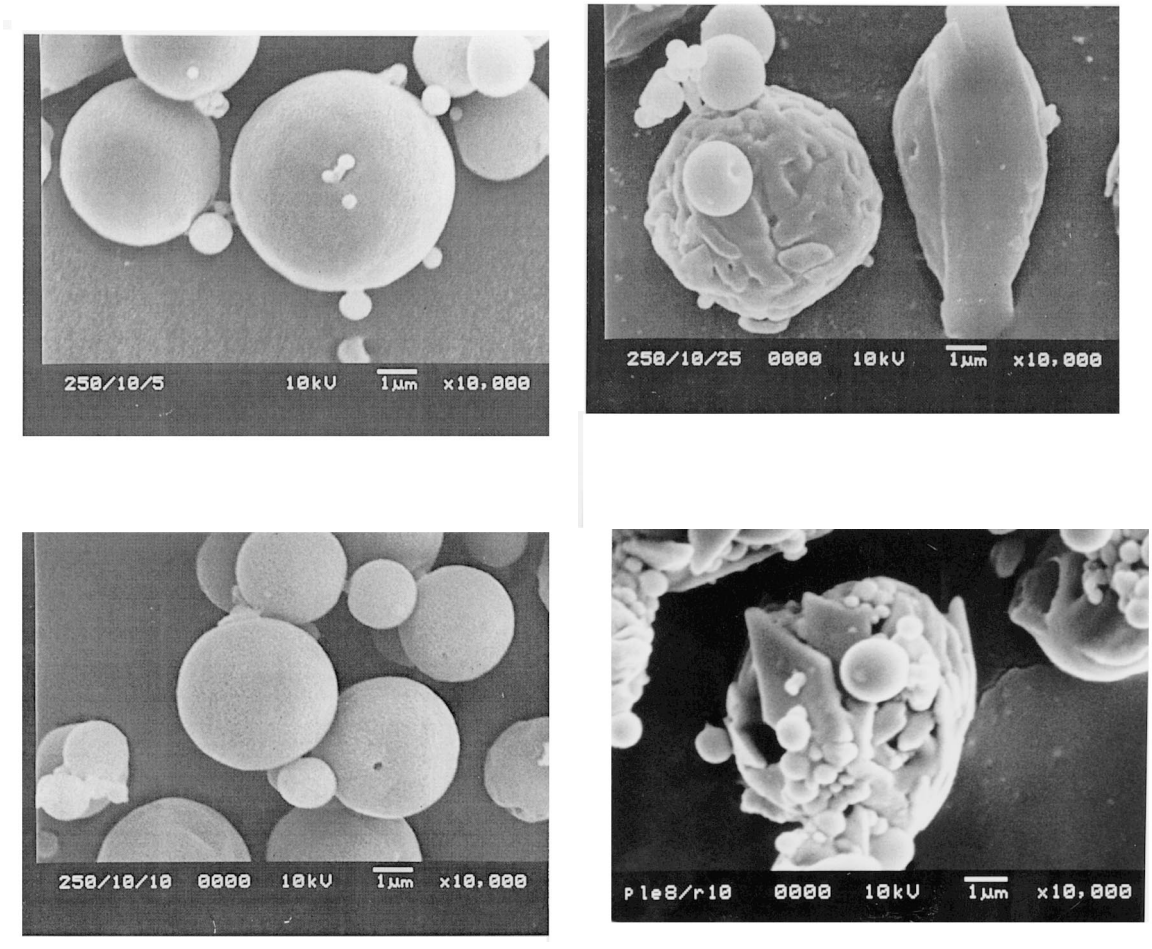

Fig. 3. Scanning electron micrographs showing morphological changes of the microspheres by drug loading: (a) 2 wt.% atRA, (b) 4 wt.% atRA, (c) 8 wt.% atRA, (d) 10 wt.% atRA.

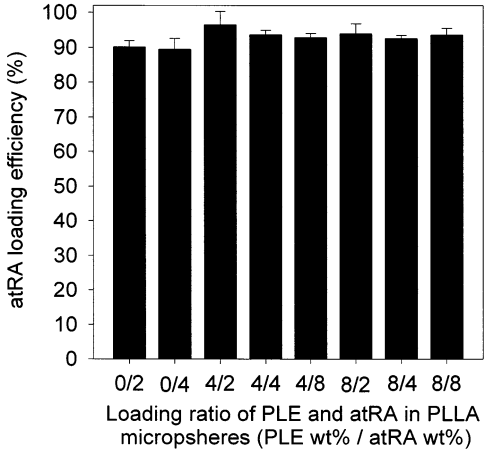

Fig. 4. Drug loading efficiencies according to the contents of PLE and atRA.

Table 1
Size and morphology of PLLA/PLE microspheres

Samples	Size (μm) ± S.D.[a]	Shape and surface morphology of microspheres
PLE0/RA2	5.45 ± 1.66	Spherical/smooth
PLE4/RA2	5.19 ± 1.67	Spherical/smooth
PLE8/RA2	5.49 ± 1.94	Spherical/smooth
PLE0/RA4	5.68 ± 1.57	Spherical/smooth
PLE8/RA8	5.58 ± 1.95	Irregular/irregular

[a] S.D. – standard deviation.

Mountain View, CA), employing an electron take-off angle of 35° normal to the surface. A thermal analysis of the microspheres was performed with a differential scanning calorimetry (DSC 2010, TA instruments Inc., New Castle, DE) to evaluate the miscibility of PLE and PLLA in microspheres. The sample was scanned from 5 to 200°C at a heating rate of 10°C/min. In order to determine the amount of atRA in the microspheres, the microspheres were first dissolved in dichloromethane, and the absorbance was measured at 365 nm. All procedures were performed in a dark room. The size of microspheres was measured by a Field Flow Fractionator (F-1000 Universal Fractionator, LLC, SLC, UT) as described by Moon et al. (Moon et al., 1999). In this analysis, the carrier liquid contained 0.05% sodium dodecyl sulfate and 0.02% sodium azide.

2.4. Drug release and degradation study

Since atRA is highly hydrophobic and has a low solubility in PBS, the amount of released atRA from microspheres was calculated from the remaining amount of atRA in the microspheres at each time. The atRA-loaded microspheres of 10 mg were enveloped in each of cellulose acetate membrane, whose molecular weight cut-off is 300 000 Da, and immersed in a shaking water bath at 37°C, which contained 40 L of PBS. The PBS medium was exchanged with fresh solution periodically in order to maintain the sink condition. The released amount of atRA from the microspheres was analyzed according to the method described by Giordano et al. (Giordano et al., 1993). On the 1st, 3rd, 6th, 9th, 14th, 21st and 35th day, the microspheres were dissolved in dichloromethane, and the amount of remaining atRA was determined by the absorbance at 365 nm. All procedures were performed in a dark room. The degradation test of the microspheres was performed under the same condition as mentioned above. Morphological changes of the microsphere surface over time were analyzed by SEM. The cross-sections were also examined to confirm the disintegration of the inner parts of microspheres. Microspheres were embedded in gelatin, sectioned at 20 μm thickness on a cryostat, and analyzed by SEM.

3. Results

The chemical structure and the molecular weight of PLE were determined by ^{1}H-NMR spectrum as mentioned in the literature (Zhu et al., 1990; Youxin and Kissel, 1993). The ^{1}H-NMR spectrum showed resonances at 1.58 ppm (CH_3 doublet) and 5.19 ppm (CH quartet), which belonged to the PLLA block. The signal at 3.65 ppm ($O-CH_2-CH_2$ singlet) is characteristic of methylene units in the PEG block. The average number of molecular weights of PLE calculated from the ^{1}H-NMR spectrum was 32 500 Da, where the molecular weights of PEG block and

PLLA block were 5000 and 27 500 Da, respectively. The molecular weight distribution (M_w/M_n) of PLE determined by GPC was 1.46. These data confirmed that PLE was successfully synthesized and had a narrow molecular weight distribution.

The effect of PLE on the morphology of the microspheres, when microspheres were prepared by PLLA and PLE, is shown in Fig. 1. The microspheres displayed smooth surfaces when PLE was contained below 8 wt.%, whereas, the microsphere surfaces became irregular when the PLE content was above 10 wt.%. To evaluate the miscibility of PLE and PLLA in the microspheres, the first heating curve of the microspheres in DSC was analyzed (data not shown). Glass transition temperature (T_g) of PLLA was lowered from 64.3 to 57°C by blending 8 wt.% of PLE, and cold crystallization temperature (T_{cc}) of the polymer was also lowered from 93.2 to 88.5°C. In addition, the degrees of crystallinity of microspheres increased with the increase of PLE content by 12.9% by blending 8 wt.% of PLE as described in our previous study (Choi et al., in press).

PLLA microspheres, which do not contain PLE, did not disperse in PBS, but could be dispersed only in the presence of surfactant. In con-

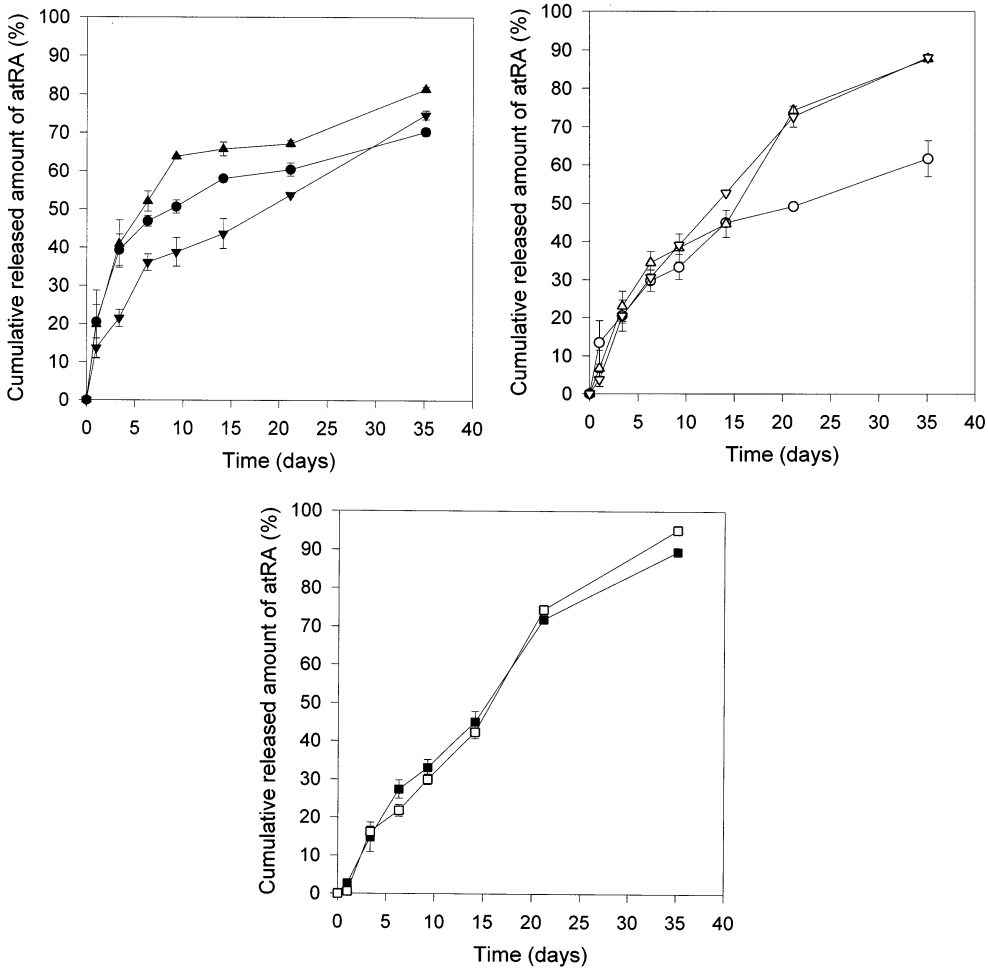

Fig. 5. The release profiles of atRA according to the contents of PLE and atRA: (a) 2 wt.% atRA, (●) PLE0/RA2, (▲) PLE4/RA2, (▼) PLE8/RA2; (b) 4 wt.% atRA, (○) PLE0/RA4, (△) PLE4/RA4, (▽) PLE8/RA4; and (c) 8 wt.% atRA, (■) PLE4/RA8, (□) PLE8/RA8.

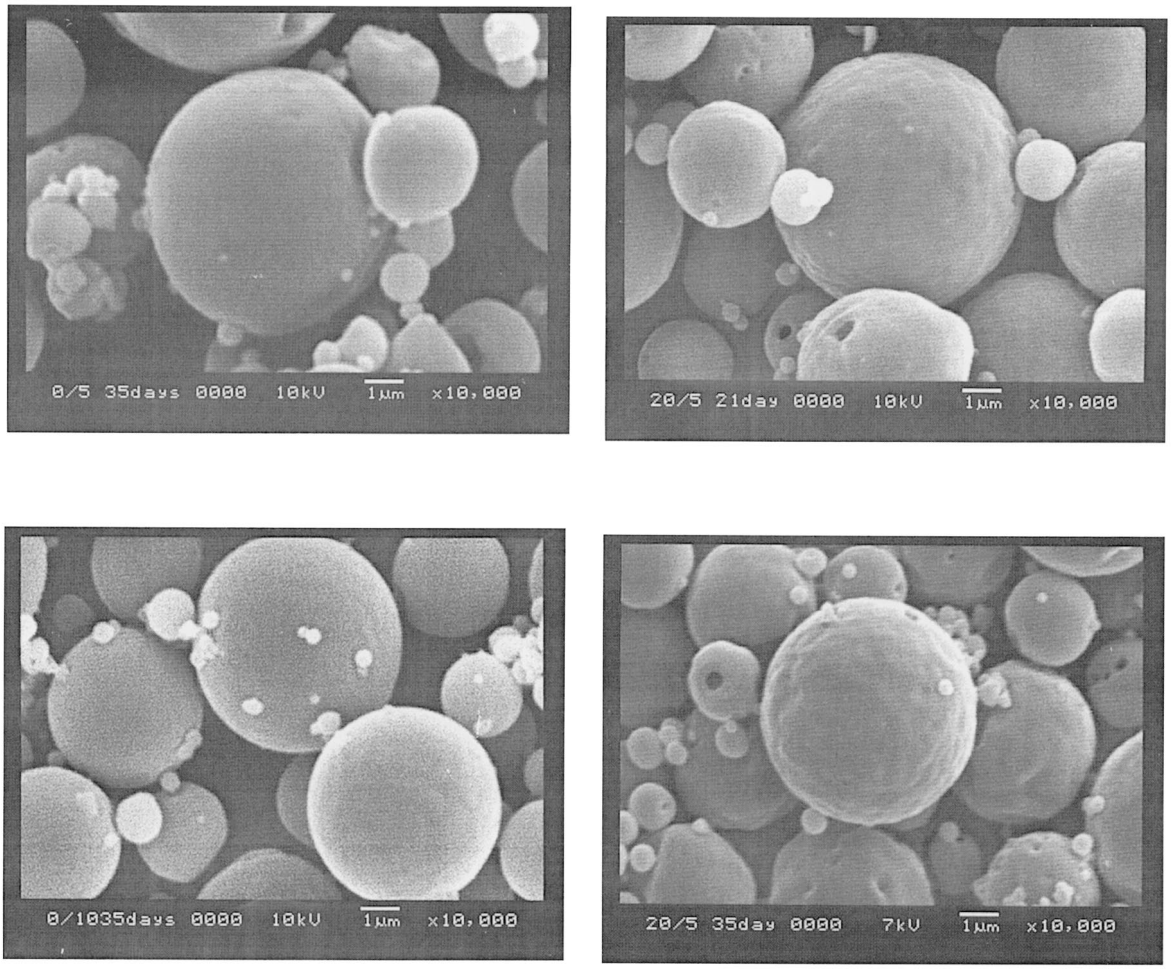

Fig. 6. Morphological changes of microspheres by degradation: (a) PLE0/RA2 (at 5 weeks), (b) PLE0/RA4 (at 5 weeks), (c) PLE8/RA2 (at 3 weeks), (d) PLE8/RA2 (at 5 weeks), (e) PLE4/RA8 (at 3 weeks), (f) PLE4/RA8 (at 5 weeks), (g) PLE8/RA8 (at 3 weeks), (h) PLE8/RA8 (at 5 weeks), (i) PLE8/RA4 (at 3 weeks), and (j) PLE8/RA4 (at 5 weeks).

trast, PLLA/PLE microspheres dispersed well in PBS without the addition of any surfactants. It was also found that as the PLE content was increased, the dispersion of the microspheres in PBS improved. The XPS analysis confirmed that this good dispersity is due to the PEG block of PLE on the microsphere surfaces, as shown in Fig. 2. Each peak in the XPS data was assigned by comparing the data reported by Shakesheff et al. (Shakesheff et al., 1996). The characteristic peak of the PEG block was observed at 286.4 eV of the C_{1s} signal. Peaks at 285, 287.05, and 289.2 eV were assigned to the binding energy of –CH–, –CO–, and $-CO_2-$ in PLLA, respectively. The ratio of areas between the peaks C–C–O (PEG block of PLE) and CH (PLLA block of PLE and matrix PLLA) was calculated from the XPS data. Their values were 8% for PLE4 microspheres and 23% for PLE8 microspheres. These values were higher than those of the theoretical values, which were 2% for PLE4 microspheres and 3.8% for PLE8 microspheres. The XPS method analyzes the composition within a thickness of 100 Å from the microsphere surface. It can be therefore said from the XPS data that the content of PLE is higher near the microsphere surface (in the range

of 100 Å from the surface) than in the region beyond 100 Å from the microsphere surface.

The morphology of microspheres, which were prepared by PLE and PLLA, was also affected by the amount of loaded atRA. When the loading amount of atRA was below 4 wt.%, the shape of the microspheres was spherical, and their surfaces were smooth (Fig. 3 (a) and (b)). At the 8 wt.% atRA loading condition, leaf-shaped microspheres were partially obtained (Fig. 3 (c)). At above 10 wt.% of atRA in the microspheres, atRA was not completely encapsulated inside the microspheres, and observation by SEM revealed small crystallines of atRA on some microsphere surface (Fig. 3 (d)). When the loading amount of atRA was below 8 wt.%, the loading efficiency of atRA was above 90%, and the efficiency was not affected by the contents of PLE and atRA (Fig. 4). The average size of the microspheres was around 5 μm, and the size of microsphere did not change by altering the content of PLE and atRA, as shown in Table 1.

The release pattern of atRA from the microspheres was dependent upon the contents of PLE and atRA (Fig. 5). The PLE0/RA2 microspheres, which contained 2 wt.% of atRA without PLE, showed a pseudo-first-order release profile of atRA; that is, a burst effect at the early stage was

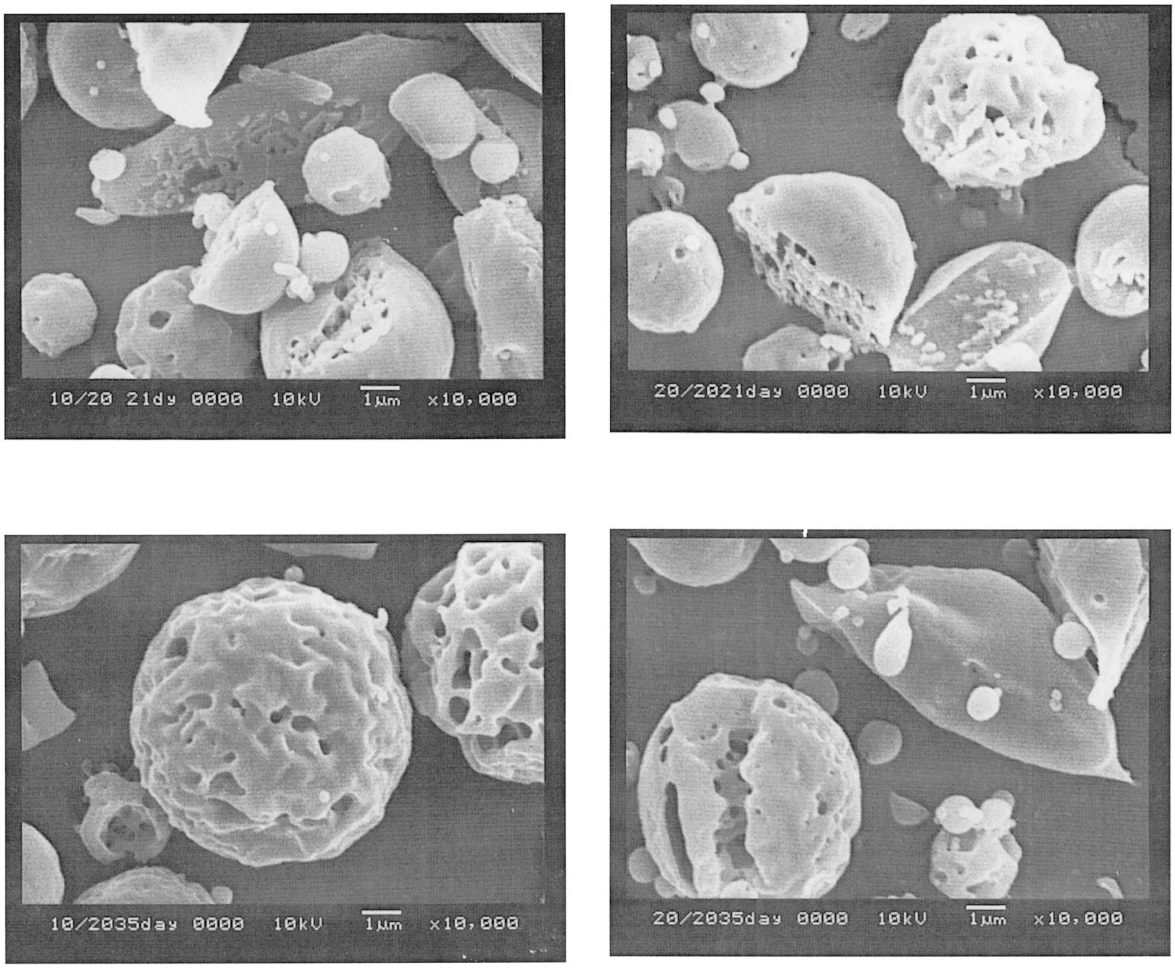

Fig. 6. (*Continued*)

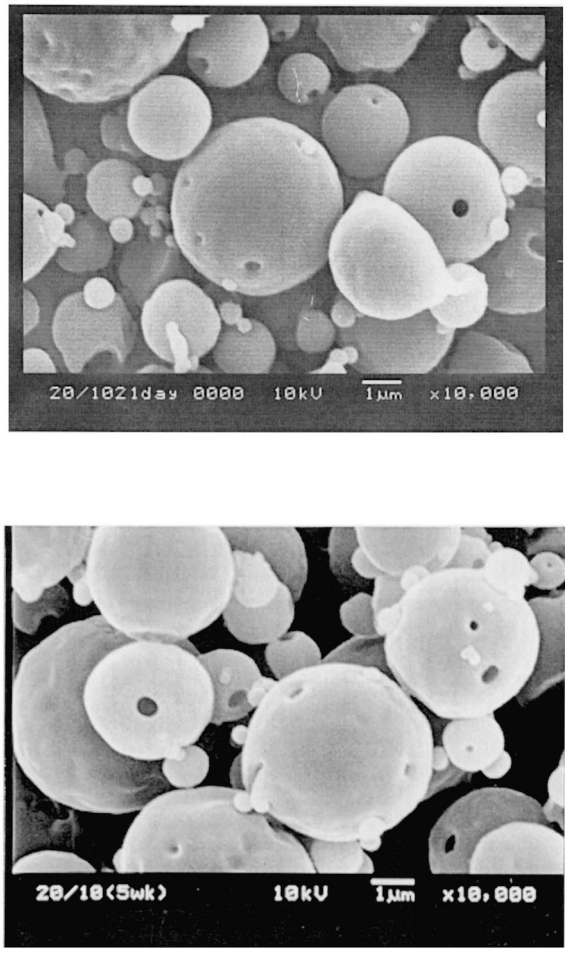

Fig. 6. (*Continued*)

followed by a slow release of atRA. In contrast, the PLE8/RA8 microspheres, containing 8 wt.% atRA and 8 wt.% PLE, presented a nearly constant release rate of atRA (i.e. pseudo-zero-order release profile) for 5 weeks. The cumulative released amount of atRA for 5 weeks was about 95%. As the contents of PLE and atRA were increased, the release pattern of atRA shifted from a first-order to a pseudo-zero-order.

The effects of atRA and PLE on microsphere degradation were investigated by SEM (Fig. 6). PLE0/RA2 and PLE0/RA4 microspheres were slightly degraded in 5 weeks. In contrast, the microspheres of PLE4/RA8 and PLE8/RA8 showed substantial degradation in 3 weeks, and severe disintegration after 5 weeks. Such morphological changes of microspheres over time indicate that the difference in the atRA release patterns according to the contents of PLE and atRA were the results of a difference in microsphere degradation.

However, PLE8/RA4 microspheres showed a pseudo-zero-order release pattern of atRA although the surfaces of PLE8/RA4 microspheres were only slightly degraded for 5 weeks. This phenomenon could be explained by observing the cross-sectional view of microspheres, as shown in Fig. 7. There were initially several pores inside the PLE0/RA4 and the PLE8/RA4 microspheres that appeared during the microsphere preparation. In

the case of the PLE0/RA4 microspheres, these vacuoles remained almost intact even after 5 weeks. However, it was observed that the inside the PLE8/RA4 microsphere was severely disintegrated after 3 weeks. This result indicates that the degradation of these microspheres proceeds faster in the inside than at the surfaces.

The GPC analysis of these microspheres indicated that the decrease in the molecular weight of PLE8/RA4 microspheres was faster than that of PLE0/RA4 microspheres (Fig. 8). Especially, the initial rate of degradation was faster for PLE8/RA4 than for PLE0/RA4, and the degradation rate decreased when the degradation time was greater than 3 weeks.

4. Discussion

When the PLE content in the PLLA/PLE microspheres was below 8 wt.%, the degree of crystallinity increased as the PLE content in the microspheres was increased. The PEG block of PLE has a large free volume and offers space for

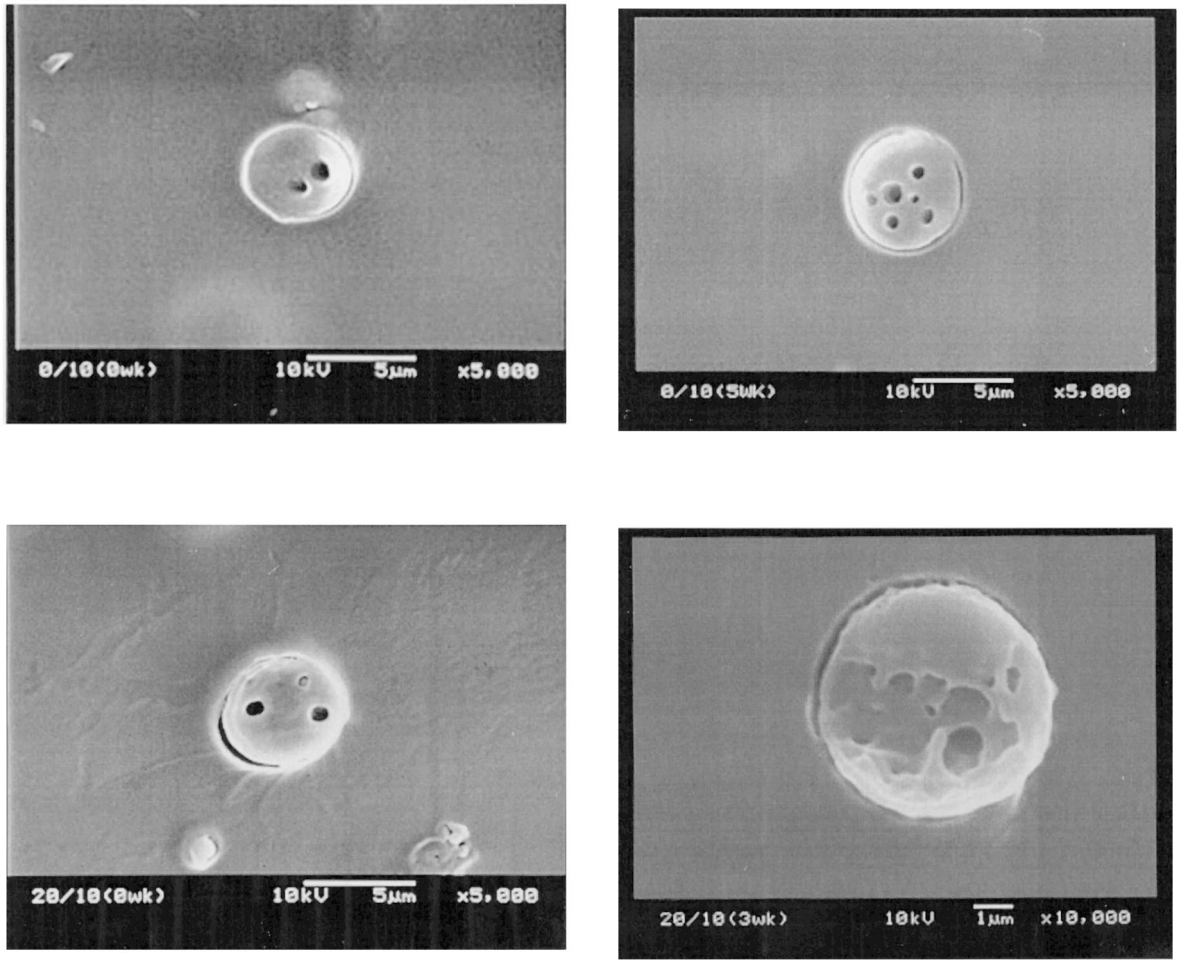

Fig. 7. Cross-sections of microspheres (SEM); PLE0/RA4 microspheres at (a) 0 week, (b) 5 weeks, PLE8/RA4 microspheres at (c) 0 week, (d) 3 weeks. Magnification of (a)–(c), 5000 ×; (d) 10 000 ×.

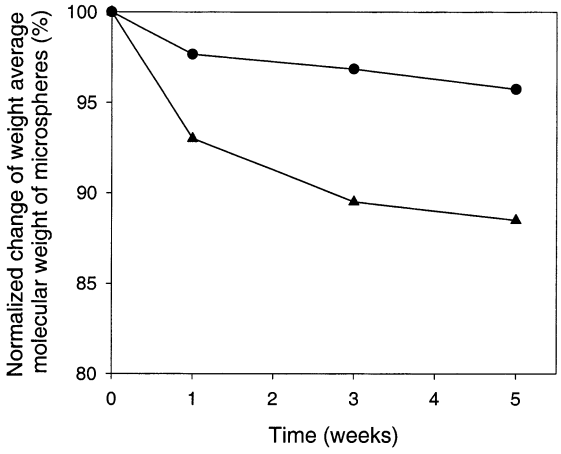

Fig. 8. Normalized change of weight average molecular weight of microspheres: (●) PLE0/RA4 microspheres, (▲) PLE8/RA4 microspheres.

the recrystallization of PLLA polymers. These phenomena indicate that PLE molecules blended well with PLLA in the microspheres (Yue et al., 1996; Yang et al., 1997). However, when the loading amount of PLE in the microspheres was above 10 wt.%, the morphology of microspheres became irregular. Loading of atRA in the PLLA/PLE microspheres was also limited to 8 wt.% because of the appearance of small atRA crystallines on the surface of some microspheres. The small crystals on the microsphere surfaces seem to appear due to the limitation of the drug solubility in PLLA polymer. Hydrophobic atRA could be soluble to some degree, but will be saturated with the increase of atRA loading. And the shape of microspheres will be changed. On the other hand, high encapsulation efficiency of atRA could be due to the low solubility in water (Szuts and Harosi, 1991) and miscibility with hydrophobic PLLA molecules. Because the solubility of RA in the PBS media is very low, most of the drug was encapsulated inside microspheres with a slight loss into the aqueous media during microsphere preparation. As a result, the encapsulation efficiency of microspheres was above 90% for all samples, with atRA being distributed in the interstices of hydrophobic PLLA molecules (i.e., PLLA of matrix and PLLA block of PLE) rather than in hydrophilic PEG blocks of PLE.

One advantage in using the PLLA/PLE microspheres was its ability to disperse well in PBS. Surfactant is necessary to disperse the PLLA microspheres in PBS since PLLA microspheres were aggregated in PBS because of the hydrophobic property of PLLA. However, the PLLA/PLE microspheres dispersed well in PBS when used without any surfactants, because the hydrophilic PEG blocks of PLEs were located at the microsphere surfaces, thereby creating a hydrophilic property to the surfaces.

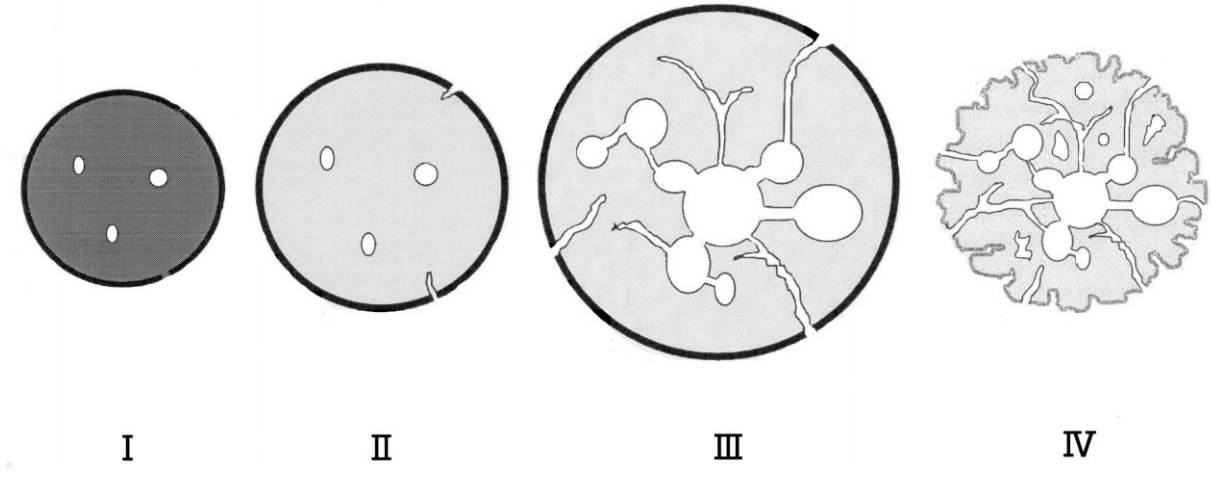

Fig. 9. Schematic diagram of degradation mechanism proposed for PLE8/RA4 microspheres.

Another advantage of PLLA/PLE microspheres was that the release rate and the release pattern of atRA could be controlled by the contents of PLE and atRA. When the microspheres contained small amounts of PLE and atRA such as below 4 wt.%, the microspheres were slowly degraded and atRA was released by simple diffusion, thus showing a pseudo-first-order release profile of atRA. On the other hand, the microspheres containing relatively large amounts of PLE and atRA, such as 8 wt.%, appeared to be rapidly degraded, and showed a pseudo-zero-order release profile of atRA. In this case, the release of atRA was controlled by simple diffusion of atRA and the degradation of microspheres.

For the PLLA microspheres in this study, it is considered that the microsphere degradation was proceeded by acid-catalyzed hydrolysis of an ester bond. As shown in Figs. 6–8, hydrolysis of PLLA was observed to be affected markedly by the contents of atRA and PLE in the microspheres. Zhang et al. observed that, unlike strong acids, catalytic effect by weak acids was observed only at high concentrations (Zhang et al., 1994). It can be suggested from their report that acid-catalyzed hydrolysis can occur in the presence of carboxylic acid (i.e., the end groups of atRA and degraded PLLA) only at high concentrations in microspheres. This also explains well why the degradation of microspheres occurred rapidly only when the loading content of atRA in microspheres was greater than or equal to 4 wt.% (Fig. 6 (d), (i), and (g)). In addition, water environment around the carboxylic acids would be important for hydrolysis, because hydrolysis of polyesters is normally catalyzed by protons rather than associated carboxylic acids (Mark et al., 1986). Since hydrophobic PLLA used for microsphere preparation in this study has high molecular weight and hydroxyl end groups, the initial water absorption into microspheres would be slow. In contrast, PLE has a highly hydrophilic PEG block in its backbone, and therefore, blending of PLE in PLLA microspheres could increase water absorption into the microspheres. Especially, rapid water absorption in the initial period is to be expected when PLE is blended with microspheres (Li et al., 1998), and acid catalysis in the initial period might be possible if atRA of greater than 4 wt.% is used. This is validated from the finding that the initial rate of degradation for PLE8/RA4 microspheres was more rapid than that for PLE0/RA4 microspheres (Fig. 8). This suggestion is also supported by the results shown in Fig. 6 (b) and (i) and Fig. 7.

A relatively small decrease in the molecular weight in spite of the rapid disintegration of PLE8/RA4 microspheres means that hydrolysis of ester bonds was proceeded by chain-end scission mechanism. Shin observed that preferential mechanism of PLA hydrolysis under acidic condition is a chain-end scission and not a random-scission of backbone ester bonds (Shin, 1995). In other words, scission at the chain-end was faster than that taking place at the internal ester bonds. In the case of the hydrolysis of ester bonds by chain-end scission mechanism, lactic acids and/or low molecular weight oligomers, which are soluble in water, are produced, and weight loss of microspheres occurs by the release of these molecules into the test medium. That is, weight loss can occur with a small change in the molecular weight. For PLA, the critical molecular weight which can be solubilized in water was found to be around 1100 Da (Park, 1994).

Changes in microsphere degradation according to the contents of PLE and atRA also affect the degradation patterns of microspheres such as surface erosion and core degradation. It has been believed that PLLA and PLGA degrade via bulk erosion mechanism (Kenley et al., 1987). However, Li et al. reported on a heterogeneous degradation mechanism for large size devices (Li et al., 1990). In this case, the degradation is much faster in the center than at the surface. Park also reported other examples of heterogeneous degradation, and observed that PLGA microspheres smaller than 10 μm were degraded faster at the core than at the outer surface (Park, 1995). It was thought that heterogeneous degradation was due to the accumulation of carboxylic acid groups produced during the degradation of PLGA microspheres. That is, it is hard for inner oligomers with carboxylic end groups to diffuse out, so they remain entrapped. On the other hand, oligomers generated at the surface are released out quickly.

For PLE8/RA4 microspheres, core degradation was also more significant than surface erosion, although microsphere degradation was not so severe as PLE8/RA8 microspheres. As mentioned above, degradation of microspheres occurred fast in the initial period of degradation when the microspheres contained over 4% of atRA and PLE. Lactic acids and/or low molecular weight oligomers, produced on microsphere surface, could dissolve into test medium, eventually creating pores on the surface as seen in the Fig. 6 (i) and (j). In contrast, oligomers having carboxylic end groups in the core of microspheres might be accumulated, and the accumulated carboxylic groups would stimulate hydrolysis in the core region in addition to the hydrolysis by atRA. Moreover, it can be further postulated that the accumulation of the carboxylic end groups inside microspheres increased the water content inside the microspheres, causing both the increased osmotic pressure and the swelling. Brunner et al. also reported that the osmotic pressure inside the microspheres was increased as degradation proceeded (Brunner et al., 1999). Therefore, it can be proposed that increased osmotic pressure and swelling of microspheres stimulated the accumulated oligomers to be diffused out through surface pores, resulting in the formation of inner spaces inside the microspheres (Fig. 7 (d)). A schematic diagram of degradation mechanism proposed for PLE8/RA4 microspheres is shown in Fig. 9. Release of atRA from PLE8/RA4 microspheres could be also enhanced by the increased water content and by the formation of inner space.

Estey et al. reported that the AUC of atRA concentration versus time was maintained for 15 days by parenteral administration of liposome-encapsulated atRA to patients (Estey et al., 1996). Presumed in such mechanism is the ability of the liposomal formulation to bypass metabolism in the hepatic microsomes. PLLA/PLE microspheres containing atRA were able to constantly release atRA for 5 weeks. If these microspheres were subcutaneously administered, metabolism of atRA in the hepatic microsomes could be bypassed also, reducing the rapid induction of the specific P450's. In addition, the effective plasma level of atRA could be maintained for a long period, thereby effectively preventing the acute resistance of atRA. Furthermore, since the release pattern of atRA in the microspheres can be controlled from a first order to a pseudo-zero-order by adjusting the content of PLE and atRA, the pseudo-zero-order release profile of atRA may also be obtained in the in vivo condition.

5. Conclusion

In this study, biodegradable microspheres prepared by blending PLE and PLLA were proposed for a long-term delivery of atRA. In the condition of 8 wt.% PLE and above 4 wt.% atRA, atRA was released with a pseudo-zero-order. In addition, PLE blended PLLA microspheres showed two advantages; one was good dispersity in PBS without any surfactants, and the other was a controlled degradation by the contents of atRA and PLE.

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