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Degradation of poly(D,L-lactic acid) microspheres: effect of molecular weight

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Abstract

Degradation behaviors of two poly(D,L-lactic acid) (PDLA) microspheres prepared from two different molecular weights (17000 and 41000) were examined. The degree of the degradation was quantitatively estimated by employing an aqueous gel permeation chromatography (GPC) which allowed the determination of the overall amount of water soluble degradation products released out into aqueous medium. At the initial stage of incubation, short chain oligomers, which were produced in an ultrasonication treatment step during the microsphere preparation were immediately released out. Critical weight average molecular weight of the water soluble oligomers ranged from 1050 to 1150. The polymer degradation behaviors of the microspheres during the 53-day incubation were greatly affected depending on the molecular weight of raw polymers as evidenced by significantly different trends of time-dependent molecular weight change. The lower molecular weight polymeric microsphere exhibited a significant degradation with reduced glass transition temperature, while the higher molecular weight polymeric microsphere did not show any detectable change in the degradation until 53 days. It was found that the water hydration in the low molecular weight PDLA microsphere immediately allowed the polymer morphology to change from a glassy to rubbery state by lowering the glass transition temperature below the incubation temperature. This led to the more susceptible physical state to the degradation. On the other hand, the high molecular weight microsphere was in the glassy state until they started to degrade.

Keywords: Poly(D,L-lactic acid); Molecular weight effect; Microsphere; Degradation; Biodegradable polyester

1. Introduction

Aliphatic polyesters such as poly(L-lactic acid) and its copolymers with glycolic acid or stereoisomer, D-lactic acid, have been extensively utilized as drug delivery carriers for various drugs because of their biodegradable and biocompatible properties [1–3]. Drug encapsulated microspheres have been used for injectable controlled drug delivery systems which do not require a retrieval procedure of device after usage. Although a number of studies have been directed toward the drug release kinetics from variously formulated microspheres [4], degradation mechanism of the polymers was not studied in detail. In general, the degradation rate of aliphatic polyesters has been known to be determined by their molecular weight and structure such as amorphous/crystalline morphology and hydrophilic/hydrophobicity [5]. More hydrophobic and crystalline polymers exhibit slow degradation rate due to the low hydration degree in the microspheres, and this is related to the water accessibility to the hydrolytically unstable ester linkages in the polymer backbone. It has been believed that the microspheres degrade homogeneously via bulk erosion [6], although

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recent studies show a heterogeneous degradation for a large device [7].

PDLA is a copolymer (50/50 molar ratio) of two stereoisomers, D- and L-lactic acid. Due to its amorphous morphology, it degrades much faster than the homopolymer of D- or L-lactic acid which has a crystalline structure. The effect of PDLA molecular weight on the degradation has been little examined in the previous studies. One study reports that PDLA cylindrical devices having molecular weight range from 1500 to 3500 show marked difference in degradation profiles [8]. Normally, decrease in polymer molecular weight lowers glass transition temperature (T_g) that determines a glassy and a rubbery state of the polymer below and above it, respectively. When the dry and glassy polymeric microspheres having T_g above the incubation temperature are placed in the aqueous media, water hydration allows the T_g to shift to the lower temperature [9]. This is due to the plasticization effect of water on the polymer. If the T_g is lowered below the incubation temperature, the initial glassy microspheres become the rubbery state with hydration which makes polymer chain segments more mobile. In contrast, if the lowered T_g is above the incubation temperature, the hydrated microspheres are still in the glassy state. Thus, it is anticipated that the molecular weight that influences the T_g shift plays an important role in the overall degradation profiles of PDLA.

In this study, two microspheres made of different molecular weights of PDLA were used to compare their degradation behaviors. Organic and aqueous phase gel permeation chromatography (GPC), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and L-lactic acid assay were employed to assess the degradation behaviors and their morphological change with respect to the difference in molecular weights.

2. Materials and methods

2.1. Materials

Two kinds of PDLA were purchased from different manufacturers: low molecular weight; Polysciences Co. (Lot No. 94059), and high molecular weight; Medisorb Co. (Lot No. S9315D066). Eighty-eight percent hydrolysed polyvinylalcohol (M_w 25000) was obtained from Polysciences Co. Other chemicals including methylene chloride and chloroform were reagent grade.

2.2. Preparation of microspheres

Microspheres were prepared by an in-water solvent evaporation method. One gram of polymer dissolved in 5 ml of methylene chloride was first emulsified in 20 ml of 1% PVA solution saturated with methylene chloride by a sonication for 30 s using a Braun Sonic 2000 (power output 20 W with a needle probe 40 T). This sonication step was used to generate fine microdroplets of O/W emulsion. The above solution was added into 400 ml of 0.1% PVA solution in a 1-liter beaker under rapid stirring condition. The stirring continued for 3 h at room temperature to evaporate the solvent. The hardened microspheres were centrifuged and washed three times with deionized water. They were then vacuum dried at least for 2 days to remove any residual solvent as much as possible, since the small amount of the residual solvent in the microsphere could alter the polymer morphology and subsequently affect the degradation behavior. The dried microspheres were kept at -20°C under dessication until use. The size distribution of microspheres was relatively broad with less than 10 μm in average diameter.

2.3. Degradation studies

Ten mg of PDLA microsphere was placed in 1 ml of PBS buffer, pH 7.4 using an eppendorf centrifuge tube, and equilibrated at 37°C in an incubator under a static condition. The hydrated microspheres were not stirred in order to simulate an in vivo situation. At various time intervals, the tube was centrifuged to separate the supernatant from microsphere pellet. The supernantant containing the water soluble degradation products was used to determine the molecular weight and the concentration of L-lactic acid. The microsphere pellet was dried under vacuum and then used for DSC, SEM and GPC studies.

2.4. Gel permeation chromatography (GPC)

Molecular weights of the two PDLA raw materials, microspheres during the degradation, and their degradation products in the aqueous phase were determined

by a gel permeation chromatography using a Hewlett Packard 1050 pump with a Shodex RI-71 refractive index detector. For the determination of molecular weight for the polymer and microspheres, the following conditions were adopted: the column was Shodex K803; chloroform as a mobile phase; a flow rate of 1 ml/min. The microspheres were dissolved in chloroform, filtered, and then injected with a 20 μl sample size. Weight and number average molecular weights were calculated from the GPC curve using a series of polystyrene standards. For the determination of molecular weight of water soluble degradation products, the following conditions were used: the column was Shodex OHPak Q802; water as a mobile phase; a flow rate of 1 ml/min. The column packed with polyvinylalcohol beads which can detect up to M_w 5000 was thermally equilibrated at 50°C. The sample was filtered and then injected with a 20-μl sample size. Average molecular weights were calculated using a series of polyethyleneoxide standards.

2.5. *Differential scanning calorimetry (DSC)*

Measurements of glass transition temperature (T_g) were performed with a Perkin Elmer 7-Series differential scanning calorimetry. All the samples were placed in aluminum pan which were scanned from −35 to 200°C with a heating rate of 20°C/min. All the DSC thermograms were obtained from the first heating cycle. Nitrogen was used as a sweeping gas.

2.6. L-*Lactic acid assay*

L-Lactic acid concentration in the aqueous medium was measured by an enzymatic method using an L-lactic acid assay kit obtained from Sigma Co.

2.7. *Scanning electron microscopy (SEM)*

Surface morphologies of microspheres during the incubation period were observed by a Amary 120 scanning electron microscopy (SEM). The samples were coated with gold particles.

3. Results and discussion

The PDLA microspheres prepared by the solvent evaporation method have a relatively small size distribution, and most are less than 10 μm in diameter. GPC profiles of the two PDLA microspheres are shown in Fig. 1. It can be seen that both GPC traces have a bi-modal profile of molecular weight (M_w) distribution. They exhibited the presence of an appreciable fraction of oligomers which follows a main peak. Average molecular weights calculated from these curves are as follows: for the low M_w microsphere, M_w (weight average) 7400; M_n (number average) 900; M_w/M_n 8.2 and for the high M_w microsphere, M_w 18000; M_n 1300; M_w/M_n 13.8. The large differences between M_w and M_n which result in a large polydispersity value are due to the bi-modal distribution of GPC traces caused by the presence of a significant amount of low M_w fraction in the microspheres. To elucidate whether they were originated from raw materials used as received or they were generated by hydrolysis during the relatively short period of the microsphere preparation, the M_w distribution of raw polymer samples was determined. It was found that their GPC traces (Fig. 1) showed a narrow M_w distribution without any detectable oligomer fraction. Weight average molecuar weights of the two polymers obtained from Polysciences and Medisorb were 17000 and 41000, respectively. Thus, it is clear that during the microsphere preparation, the hydrolysis reaction occurs with the formation of oligomers. Since the processes of the solvent evaporation procedure and the following drying process permit the polymer to have relatively short exposure time with water, the polymer might degrade a little, if any. Acidic impurities in the raw polymer might play an additional role in the degradation. However, the brief sonication step to prepare the O/W emulsion may be mainly responsible for this degradation. It has been reported that the ultrasonication degraded various biodegradable polymers such as polyanhydride and poly(lactic-co-glycolic acid), which have been utilized as stimuli-responsive drug delivery systems [10,11]. The bi-modal behavior of M_w distribution was observed to varying extents in many different microspheres regardless of their M_w, composition, and crystallinity. In particular, substantial amount of oligomers was produced in the samples of amorphous polymers as compared to semi-crystalline polymers. These short chain fragments could not be removed during the three times of the washing step in the preparation. The reason for the sonication treatment in our preparation method is to prepare small size monolithic microspheres with less than 10 μm in diameter

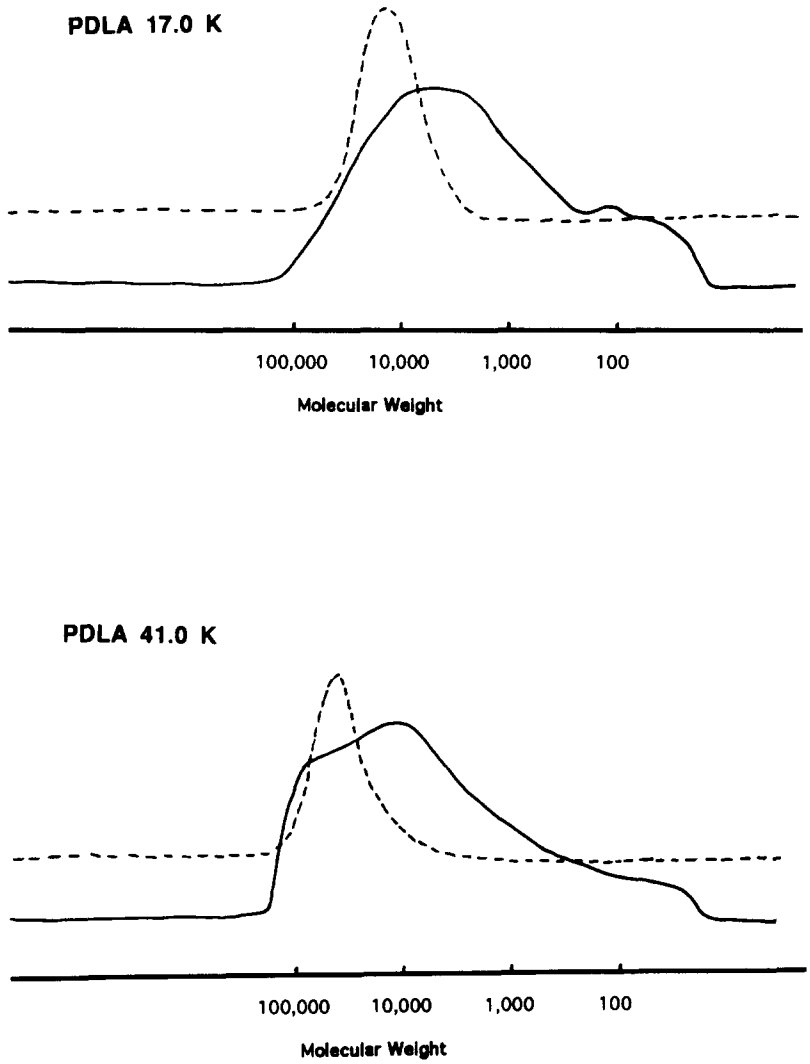

Fig. 1. GPC elution profiles of two PDLA microspheres and raw polymers as received from manufacturers. Solid line, microspheres; dotted line, raw polymers.

which can be easily phagocytized by macrophage cells [12]. In the recent experiment to confirm the sonication-induced degradation, it was found that the sonication not only accelerated the degradation of PLGA dissolved in organic solvent, but also broke apart the microspheres suspended in the aqueous medium. These results will be reported in a separate paper in the near future. In many cases of microsphere and microcapsule formulations [13,14], ultrasonication has been used to generate fine emulsion microdroplets. One should carefully take into account its effect on the physical properties of the microspheres.

The degradation behaviors of the two microspheres incubated in PBS buffer at 37°C were analysed. By using an aqueous phase GPC, M_w of water soluble oligomers released into the bulk medium could be determined. Fig. 2 shows the progressive change of the GPC profiles over the incubation time. It can be seen that upon the incubation, water soluble oligomers having weight average M_w 1050 – 1150, rapidly diffuse out; the critical M_w of lactic acid oligomers which can

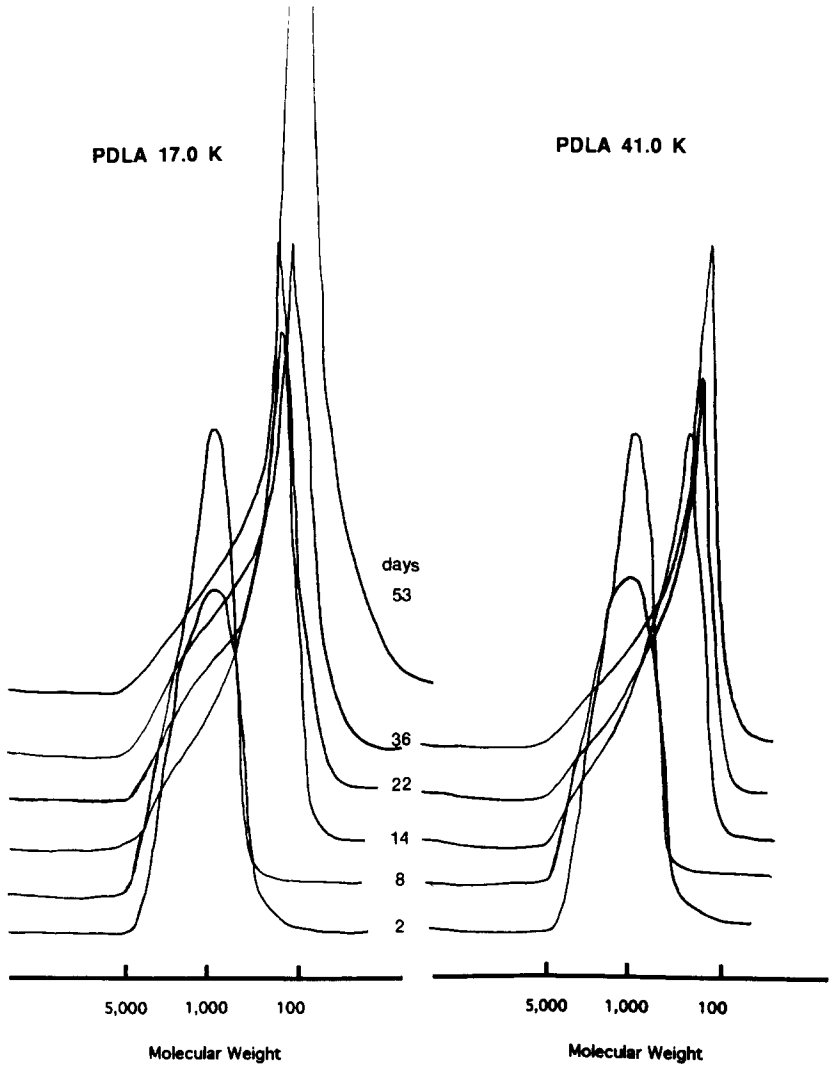

Fig. 2. Aqueous GPC elution profiles of water soluble degradation products released from the two PDLA microspheres at different time intervals.

be solubilized in water can be determined by using this method. It should be mentioned here that the M_w calculated above is a relative value on the basis of poly(ethyleneoxide) standards. Thus, the number of repeating units in the water soluble oligomers can be approximately estimated: 14–15 lactic acid units. The GPC profiles up to 8 day incubation period show a unimodal M_w distribution suggesting that the released oligomers have a relatively narrow range of M_w distribution. As the oligomers in the bulk medium are subject to the hydrolysis, it can be seen that the chromatogram gradually becomes a bi-modal M_w distribution with a growing low M_w fraction. The high M_w oligomer fraction decreased at the same time. This indicates that the oligomers in the aqueous medium further degraded towards monomeric D- or L-lactic acid unit, while there was insignificant amount of freshly released high M_w oligomers from the microspheres during the later stage of the incubation. From the above GPC traces, the total amount of water solubilized degradation products released can be determined by measuring the peak area at different incubation periods, assuming that refractive indices for PDLA oligomers are the same regardless of their M_w. This method allows to estimate the degrada-

tion progress of the PDLA microspheres. Fig. 3 shows the plot of GPC peak area vs. incubation time. It can be seen that the peak area for the low M_w PDLA microspheres increases continuously, while there is no significant change in the peak area of the high M_w sample. Since the amount of degradation products in the aqueous medium per unit time can be regarded as a polymer degradation rate, it can be concluded that the low M_w microsphere (PDLA 17.0 K) degrades faster than the high M_w one (PDLA 41.0 K). The PDLA 41.0 K microsphere exhibits virtually no apparent degradation during the study period. In addition, it is of interest to note that the amounts of initially released oligomers are similar between the two, indicating that the amount of oligomer formation during the sonication procedure was little affected by the M_w of PDLA. However, significant variations can be observed between amorphous and semi-crystalline polymers as noted above. From the GPC traces, average molecular weights of the degradation products were calculated and then plotted as a function of incubation time as shown in Fig. 4. It can be seen that initially released oligomers from the two microspheres further degrade to smaller fragments in a similar fashion. The degradation rate of water soluble oligomers in aqueous medium can be considered as an intrinsic degradation rate of PDLA in the solution state, compared to the apparent PDLA degradation kinetics in the solid state (i.e., microsphere) which include various degradation steps such as water hydration, hydrolysis, and diffusion. Since the high M_w microsphere does not show any further significant release of degradation products except for the burst release of

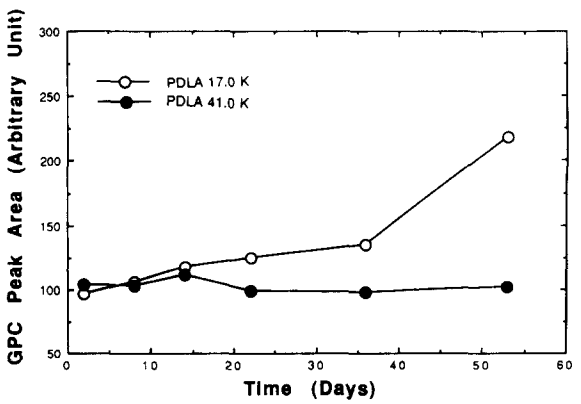

Fig. 3. Aqueous GPC peak area as a function of incubation time.

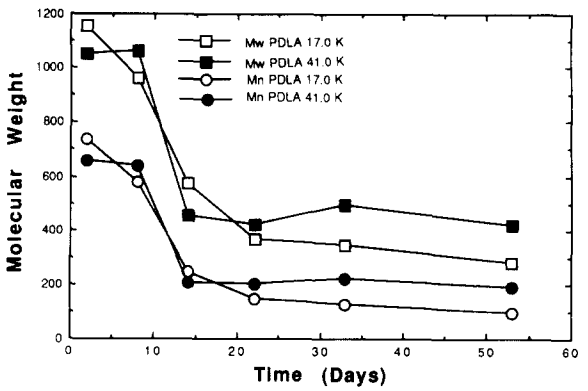

Fig. 4. Change in the average molecular weight of water soluble degradation products as a function of time.

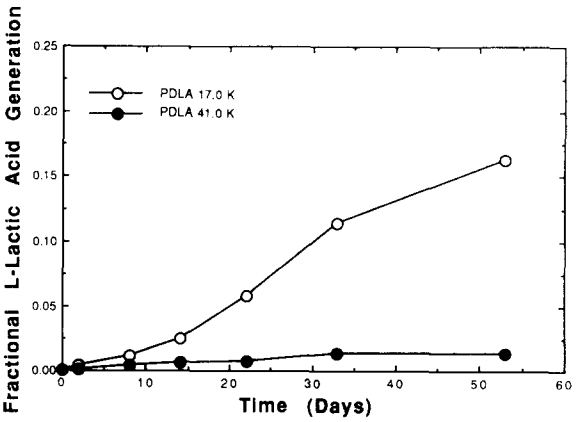

Fig. 5. L-Lactic acid concentration in the releasing medium as a function of time.

oligomers at day 2 as seen in Fig. 3, the aqueous degradation kinetics for the PDLA oligomers can be approximately estimated as follows. The initially released 14–15 monomeric units are broken down into hexamer or heptamer at day 14. These values are calculated based on the weight average M_w. This indicates that in the solution state, approximately one ester linkage in 14–15 monomeric units in the oligomer backbone is hydrolysed during the initial 2-week period. Thereafter, no apparent progress of degradation reactions occurs.

The two polymeric microspheres have a discrepancy in the concentration of one end product of degradation, L-lactic acid in the aqueous medium. As shown in Fig. 5, oligomers from the low M_w microspheres are degraded to the monomer, L-lactic acid, higher amount

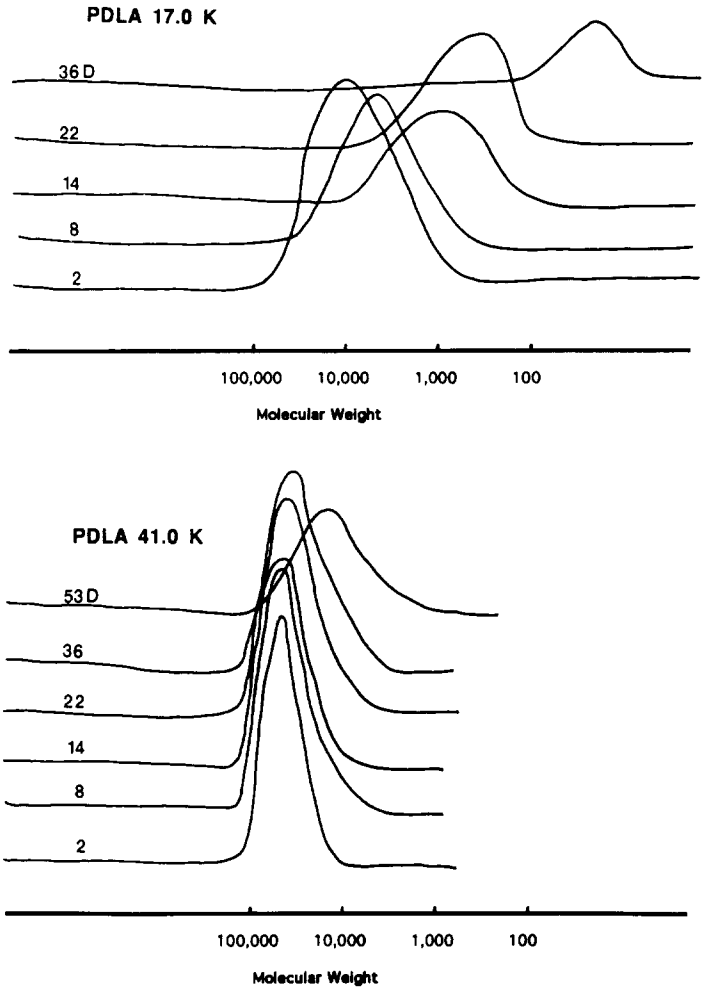

Fig. 6. GPC elution profiles of the two PDLA microspheres as a function of time.

than those from the high M_w microsphere which does not degrade into any significant amount of L-lactic acid. After 53 day incubation in medium, the former ends up with the presence of 16.3% of one stereoisomer (L-lactic acid), out of the total number of L-lactic acid repeating unit of the polymeric microsphere, while the latter has only 1.3% L-lactic acid value. No time-dependent change of L-lactic acid concentration for the PDLA 41.0 K microsphere implies that the majority of end degradation products during the study period is composed of tetramer or pentamer of D- and/or L-lactic acid as judged from the Fig. 4. These data suggest that the 14–15 mers that are initially released from the two microspheres have different structural conformations in the aqueous solution. Thus, it is likely that the aqueous hydrolysis reaction of these oligomers produces the difference in L-lactic acid concentration. In this point of view, the L-lactic acid concentration in the releasing medium can not be used as an index for the polymer degradation. Inherently slow degradation kinetics of the oligomers released from PDLA 41.0 K microspheres may be related to the formation of more compact crystalline structures of D- or L-enriched short chain oligomers in the aqueous medium. This postulation is based on the fact that crystalline structure of poly(L-lactic acid) has either 1/3 or 10/3 helical structure

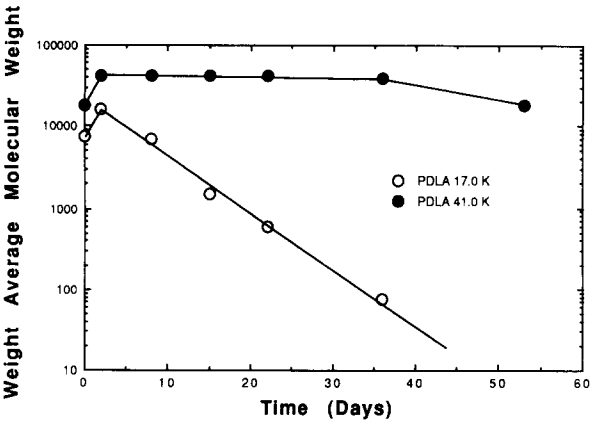

Fig. 7. Plot of weight average molecular weight vs. incubation time.

[15] as well as the fact that the degradation products exhibit crystalline melting behaviors in DSC studies as discussed later. In a computer-generated poly(D- or L-lactic acid) helical structure, ester groups in the polymer backbone are buried inside of the helix, while hydrophobic methyl groups are oriented outside, suggesting that the unstable ester linkages are shielded from the bulk aqueous medium and have a limited water accessibility.

GPC elution profiles of the two microspheres at various incubation periods are shown in Fig. 6. The 17.0 K microsphere exhibits gradual decrease of M_w with the appearance of a broad M_w distribution, while the 41.0 K microsphere shows no such change in M_w along with its distribution until 53 days. These data agree well with the results obtained from the aqueous GPC elution profiles. Fig. 7 shows the plot of M_w vs. incubation time. It can be seen that the M_w of the 17.0 K microsphere decreases linearly with time. It is also noticeable that after day 2, the M_w increases slightly for both microspheres which is due to the rapid release of the low M_w fragments as mentioned above. It should be noted that all the GPC peaks, although they become broader at the later stage of incubation, do not show any discernible bi-modal M_w distribution which may directly indicate the heterogeneous degradation mechanism [7, 16]. The absence of the bi-modal GPC peaks, however, doesn't necessarily indicate the homogeneous bulk degradation, either. More detailed discussion

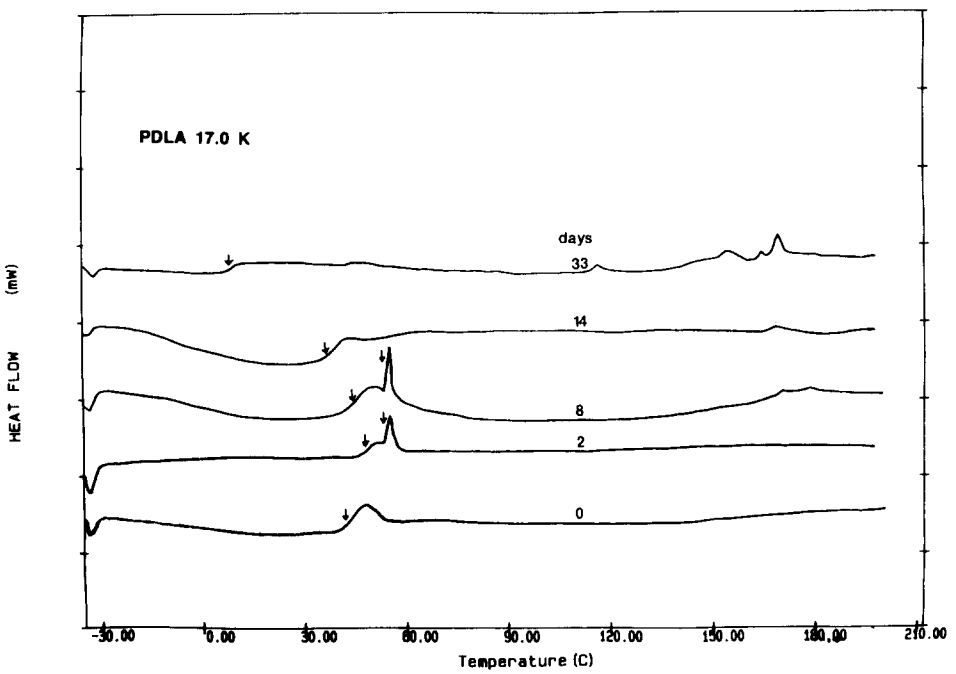

Fig. 8. DSC thermogram of 17.0 K microspheres as a function of incubation time. Dried microspheres were used for scanning. Arrow indicates the glass transition temperature.

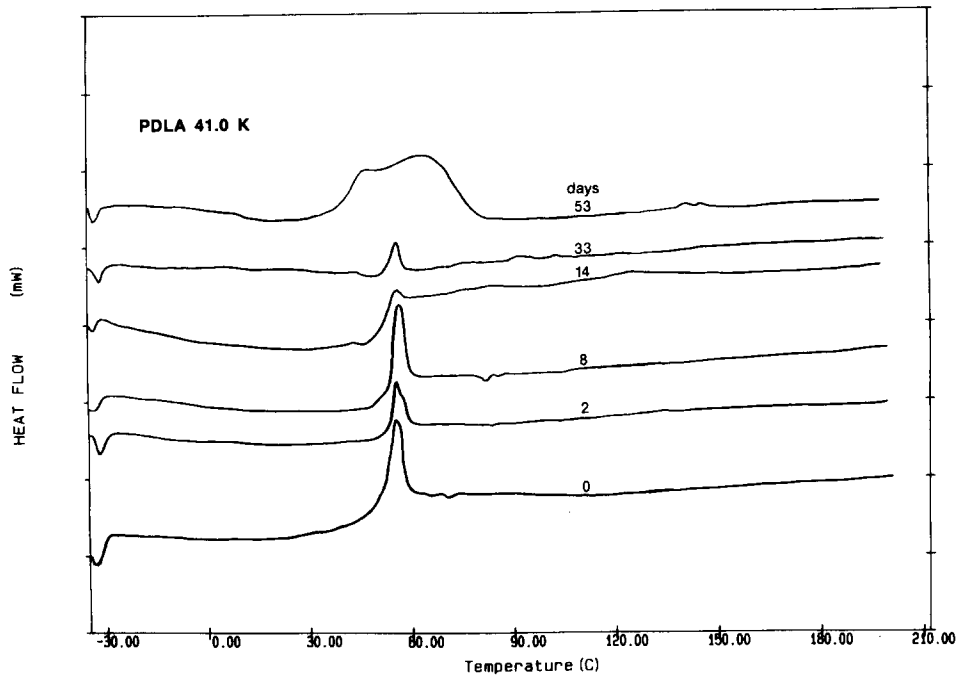

Fig. 9. DSC thermogram of 41.0 K microspheres as a function of incubation time. Dried microspheres were used for scanning.

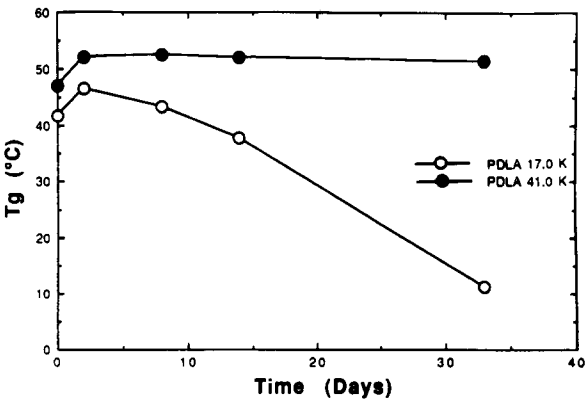

Fig. 10. Change in glass transition temperatures for two PDLA microspheres as a function of time. In the case of the transient doublet T_g's appearance, only the first T_g is plotted.

on the degradation mechanism will be presented in the subsequent paper (T.G. Park, Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition, submitted for publication).

It is now clear that the two microspheres prepared from the two polymers having the relatively small M_w difference of about 24000 exhibit quite opposite degradation profiles. To elucidate the possible reason for this discrepancy, a DSC study was undertaken to see any change in the T_g in PDLA which dictates the polymer morphological state, such as glassy and rubbery, under the incubation temperature, 37°C. Figs. 8 and 9 show the DSC thermograms of dried microspheres as a function of incubation time. Obviously, T_g of 17.0 K microsphere (41.7°C) is lower than that of 41.0 K microsphere (47.0°C). It can be seen that the PDLA 17.0 K exhibits gradual decrease in T_g, while the PDLA 41.0 K does not show any change in T_g until 53 day incubation. Since the decrease in T_g is releated to the M_w decrease in the polymer backbone [17], it can be concluded that the PDLA 17.0 K indeed degraded during the study period, while the PDLA 41.0 K did not. It is of particular interest to note that the PDLA 17.0 K exhibits apparently two glass transition temperatures at day 2 and 8 (Fig. 8). For the PDLA 41.0 K, broad, but apparent two glass transitions of 41.8 and 55.2°C also appear at day 53 (Fig. 9). The two transitions may be caused by the formation of two different polymer domains within the microsphere during the degradation. These DSC results support the recently proposed heterogeneous degradation mechanism for the PDLA

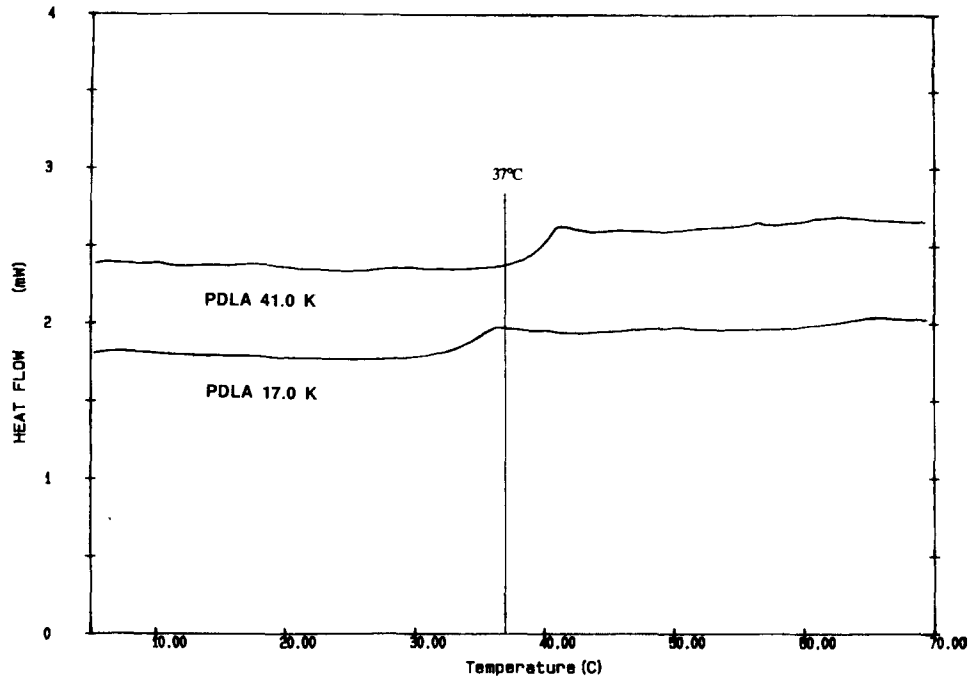

Fig. 11. DSC thermograms of two hydrated PDLA microspheres after 2 days of incubation.

film: the faster degradation in the region of inside the matrix than the shell side [7,16]. It is possible that the two glass transitions observed here are indicative of the fast and slow degradation regions in the microsphere. In other similar degradation studies of various polymers (T.G. Park, Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition, submitted for publication), transient two glass transitions that appeared below 60°C were observed. Fig. 10 demonstrates the plot of the T_g against incubation time. Here, the second transient T_g is not plotted. It can be seen that after day 2, both T_gs increase a little bit compared to those of unincubated microspheres. This suggests that the release of oligomers upon incubation allows the T_g to increase because average M_w of the polymer in the microspheres actually increases with a narrow polydispersity. The removal of water soluble small fragments enhances the integrity of molecular structure in the polymer matrix which plays a role in determining the T_g. The decreasing trends in T_g are well matched with the decreases in M_w as shown in Fig. 7. In Fig. 8, it is also observed at day 33 that between 150 and 180°C, multiple small crystalline melting peaks appear, which indicates the crystallization of D- or L-lactic acid enriched oligomers. This oligomer crystallization behavior upon the degradation of amorphous PDLA polymers is not surprising. It is possible that L- or D-lactic enriched oligomers are produced by preferential cleavage of the linkages of the two stereoisomers. This result implies that the sequential order of D- and L-lactic acid in the polymer backbone is not in a random order, but is in a segregated state. Vert also observed similar crystallization behavior in the degradation of poly(D,L-lactic-co-glycolic acid) film [7]. From the DSC results, it is more evident that the two PDLA microspheres undergo distintively different morphological states during the degradation period studied.

In order to elucidate the plausible reason for this M_w-dependent discrepancy, it is important to consider the polymer morphology in the presence of water, since water hydration in the microspheres changes the T_g and other properties. It has been reported that water acts as a plasticizer in polymers, thereby decreasing the T_g

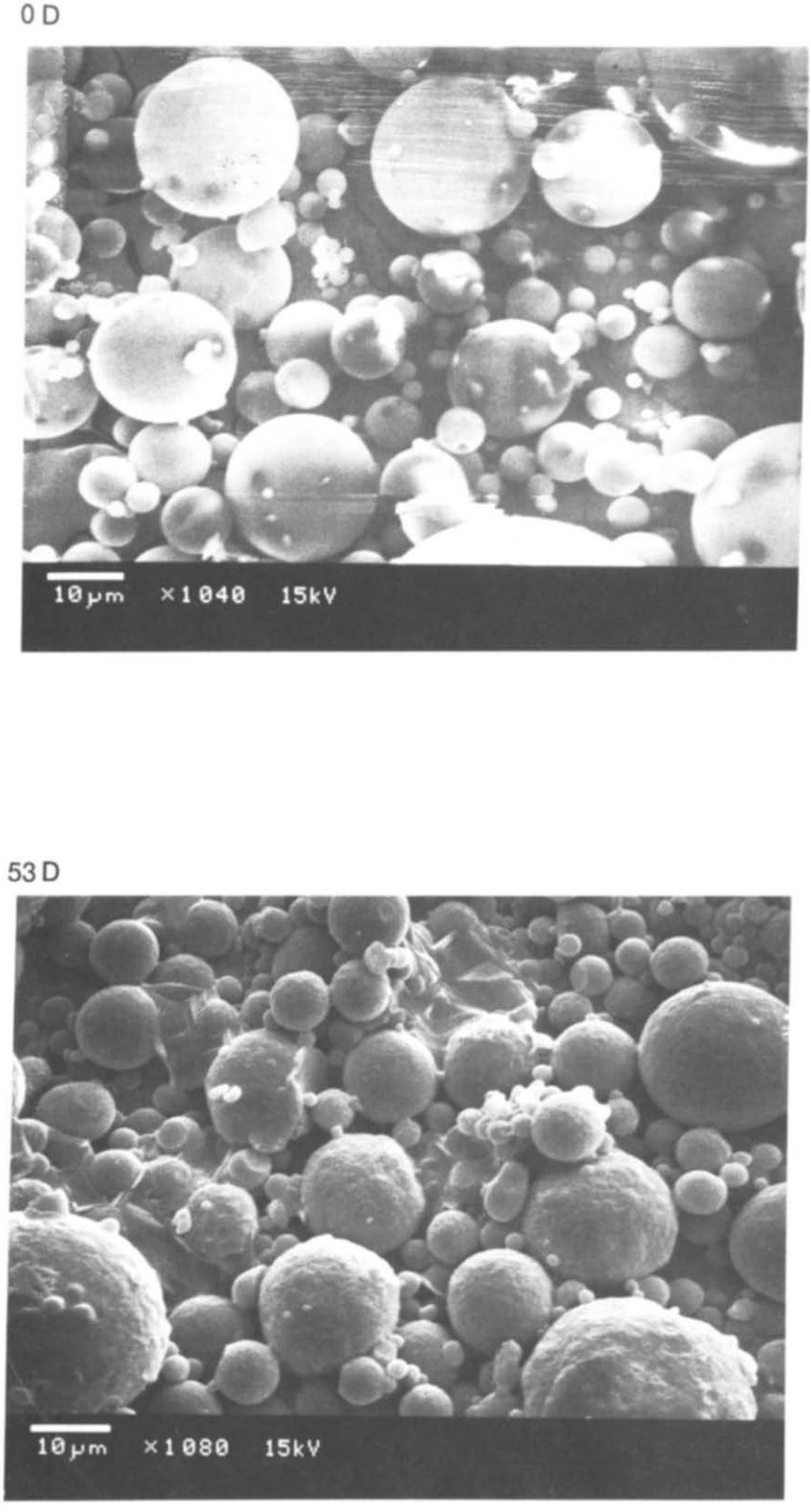

Fig. 12. SEM pictures of 41.0 K microspheres before (top) and and after 53 days of incubation in PBS buffer (bottom).

[18]. Thus, hydrated microspheres after 2 day incubation were undertaken to the DSC study. Fig. 11 shows that the T_gs of the two hydrated microspheres were lowered. The T_gs of the PDLA 17.0 K and 41.0 K microspheres are shifted down from 41.7 and 47.0°C to 34.2 and 39.4°C, respectively. Therefore, at 37°C, the PDLA 17.0 K microsphere was in the rubbery state, while the 41.0 K microsphere was in the glassy state. Different physical states in aqueous medium are clearly responsible for the different degradation behaviors observed above. In the glassy state, the mobility of polymer chain segments is restricted so that the high M_w PDLA microsphere would have a slow diffusion rate of water (slow hydration rate) with a concomittant slow diffusion-out rate of water soluble oligomers out of the microsphere. Thus, one of the most critical factors in determining the degradability of a particular microsphere may be the M_w dependent T_g under hydrated condition. This important fact has not received much attention for designing drug delivery systems.

The SEM picture of PDLA 41.0 K microspheres is shown in Fig. 12. It can be seen that there is not much change in overall size distribution of microspheres after 53-day incubation except for the formation of rough and nonporous surface, which is consistent with the fact that they did not degrade significantly. Although the PDLA 17.0 K microspheres had a similar size distribution and possessed a smooth surface like the PDLA 41.0 K before the incubation, they were irregularly distorted and lost their original spherical shape after the same incubation time. SEM picture of these microspheres could not be obtained.

In summary, it has been demonstrated that the relatively small difference in M_w of biodegradable polymer, PDLA, plays a critical role in the degradation profiles. The T_g in the hydrated state is responsible for this behavior. A slight change of the T_g near the incubation temperature dictates the polymer physical state which affects the degradation. In considering the utilization of these polymers for drug delivery device, one should thoroughly examine the M_w effect on the T_g under the hydration condition in order to deliver active ingredients for a desired period. Furthermore, the present sudy discloses several new findings which have not been examined in detail previously such as the oligomer formation during the formulation process, the critical M_w of water soluble oligomers, transient double glass transitions, and the crystallization behavior during the degradation. Detailed degradation mechanism of various PLGA microspheres will be discussed in the following paper. It appears that the degradation takes place via a more complicated procedure than one has speculated so far.

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