# Effects of *in vitro* degradation on properties of poly(DL-lactide-*co*-glycolide) pertinent to its biological performance

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Poly(DL-lactide-*co*-glycolide) (PLGA) is a widely used biodegradable polymer. In this study, effects of *in vitro* degradation on its dynamic mechanical properties, thermal behavior, molecular weight, fluid uptake and dimension stability were investigated. The *in vitro* experiment was conducted at 37 °C in phosphate buffer solution with pH = 7.4 in a dynamic incubator. The dynamic mechanical experiments were performed on rectangular specimens under three point bending. For an aging period of 6 weeks, the dynamic mechanical properties were found to decrease significantly with the increased time. The absorbed fluid had a significant effect on polymer storage modulus. The molecular weight decreased with aging time, and was found to be different at the center and the surface, being higher at the center in the beginning and lower thereafter. The polydispersity did not change significantly with degradation. The fluid uptake and specimen geometric dimension increased proportionally with aging time. The glass transition and thermal decomposition temperatures decreased with the increased degradation time.


## 1. Introduction

Poly(lactide), poly(glycolide) and their copolymers represent a major class of biodegradable materials with wide medical applications in areas, such as wound closure, controlled release systems, orthopedics and tissue engineering [1–6]. As a result, many studies have been conducted on their biological, physical and chemical properties. These include biocompatibility [7, 8], mechanical properties [9–12], morphology [13, 14], thermal characterization [15–17], and degradation mechanisms *in vitro* and *in vivo* [18–21]. However, little attention was directed towards studying the dynamic mechanical behaviors and degradation effects of these polymers, in spite of their importance in most of the cited applications. More specifically most polymer implants encounter cyclic loading in the biological environment. Meanwhile, responses to dynamic forces cannot be easily predicted from experimental data of more traditional static testing although both properties may be similarly affected by the physicochemical impacts of degradation. Another less explored area is the study on effects of degradation on thermal properties, e.g. glass transition and thermal stability, of the polymer. This justified our pursuit of the present study, which was to examine the effects of *in vitro* degradation on physical properties of poly(DL-lactide-*co*-glycolide), with an emphasis on dynamic mechanical properties and thermal behaviors. The dependence of molecular weight, dimension and fluid uptake on degradation was also determined.

## 2. Materials and methods

### 2.1. Materials and preparation of test specimens

Poly(DL-lactide-*co*-glycolide) (PLGA) (Lot #5083-269) was purchased from Medisorb Technologies International, Cincinnati, Ohio, USA. This is an amorphous polymer with high inherent viscosity. The raw polymer was received in the form of small granules. Analysis of the polymer showed an inherent viscosity of 0.73 dl/g and a lactide to glycolide ratio of 75 to 25. Using DSC analysis at a heating rate of 10 °C/min under nitrogen purge, a glass transition temperature of 52 °C was determined for the raw polymer.

For the purpose of this study, the raw polymer was compression-molded into sheets between two Teflon plates at 85 °C under a compressive force of 4 MPa using a Carver laboratory press. The temperature was maintained for 5 min and then the plates were cooled to room temperature using running water. Mold release agents were not used during compression-molding. After compression-molding, the weight average molecular weight ($M_w$) of the polymer, as determined by gel permeation chromatography, changed from 83 300 to 77 100, i.e. a reduction of about 7.5%. The polydispersity

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($M_w/M_n$) increased from 1.02 to 1.58. This was probably caused by thermal degradation. However, the thermal properties of the polymer were not significantly affected by heat-processing. The compression-molded polymer sheets appeared transparent with smooth surfaces. For the *in vitro* study, the edges of the polymer sheets were smoothed using sand paper to yield rectangular bars with a dimension of 18 × 4.5 × 1.3 mm (length × width × thickness). Such prepared specimens were used for degradation study without further treatment. Before use, the polymer samples were placed in glass jars, sealed and kept in the freezer.

## 2.2. *In vitro* degradation

Phosphate buffer GAL-PAC (Cat. #936-4GP) was purchased from Sigma Scientific, USA. The material came in the form of fine white powder. According to the manufacturer's suggestion, the phosphate buffer solution (PBS) was prepared by dissolving the solid powder into distilled water in a powder to water ratio of 16.84 g to 1 L, which resulted in a buffer solution of 0.1 mol/L and pH = 7.4 at 25 °C.

The rectangular polymer samples of known weight were transferred into glass jars filled with PBS (two samples per jar), which were then placed into an incubator. The incubator was set at 37 °C and constantly shaken at 60 rpm. The PBS was changed every day for the first three days, every other day for the next six days, and every week for the rest of the experiment. No measurable changes in pH values were observed. The experiment was conducted for 6 weeks, beyond which the polymer samples became too weak to perform dynamic mechanical testing.

The properties of the polymer samples were determined at seven aging time intervals of 0, 1, 2, 3, 4, 5 and 6 weeks. At each time interval, samples were removed from the PBS and the fluid on their surfaces was wiped off. Half of the samples (defined as wet samples) were subjected immediately to dynamic mechanical analysis. The other half (defined as dry samples) were stored in ambient condition (good air circulation) for at least 48 hours before mechanical testing. Vacuum dry was not used as it was found that the process could damage the aged specimens.

## 2.3. Dynamic mechanical analysis

The dynamic mechanical properties of the polymer samples were evaluated by dynamic mechanical analysis (DMA) under three point bending on a Perkin Elmer DMA 7e analyzer. The samples were placed on a 15 mm three point bending platform and the load was applied through a 5 mm knife probe tip. The experimental parameters were: static load = 320 mN, dynamic load = 290 mN and frequency = 1 Hz. First, the polymer samples were pre-loaded by a static load of 10 mN, cooled to − 1 °C, held for several minutes to reach equilibrium, and then scanned from − 1 to 70 °C at heating rates of 5 °C/min under helium purge.

## 2.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to study the effects of degradation on glass transition behaviors of the polymer samples. A Perkin Elmer Pyris 1 DSC analyzer with a computer data system (Pyris Series) was used. The samples weighing 5 mg were placed in a two-part aluminum pan, compacted and scanned under nitrogen purge. No efforts were made to examine the differences in thermal properties between the center and the surface as the samples were cut through thickness direction. Each DSC experimental cycle consisted of four steps: (1) holding at − 15 °C for 1 min; (2) heating from − 15 to 200 °C at 10 °C/min; (3) cooling from 200 to − 15 °C at 10 °C/min; and (4) heating from − 15 to 200 °C at 10 °C/min. For the illustration of DSC results, the second and fourth steps were taken as the first and second scans, respectively.

## 2.5. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed to study thermal stability of the polymer samples at elevated temperatures using a Perkin Elmer TGA 7 analyzer. Polymer samples weighing 10 mg were placed in a platinum sample pan, and heated from room temperature to above 400 °C at 20 °C/min under nitrogen purge. The weight change profiles of the polymer samples with temperature and aging time were evaluated, from which effects of degradation on thermal stability were determined.

## 2.6. Gravimetric analysis

Polymer fluid uptake during degradation was evaluated by gravimetric analysis using an analytical balance with an accuracy of 0.001 mg. The percent weight change was calculated by comparing the wet and dry weights of the testing samples. The fluid uptake determined by this method should be considered as the minimum since no compensation was made for polymer mass loss due to the degradation process in PBS.

## 2.7. Gel permeation chromatography

Molecular weight and polydispersity of the PLGA samples were determined using gel permeation chromatography (GPC) analysis. The experiments were carried out on a Perkin Elmer Advanced LC Sample Processor (ISS 200) equipped with a PE Series 200 LC Pump, PE Series LC refractive index detector, and a PE Nelson 900 Series Interface. The polymer samples were dissolved in high performance liquid chromatography (HPLC)-grade tetrahydrofuran (THF) at a concentration of 5 mg/mL. The polymer solution was filtered with a 1 cc syringe equipped with a 0.45 μm PTFE filter to remove any insoluble components, and eluted through a mixed bed column (PE PLgel, 5 μ, mixed bed) at a flow rate of 1 μL/min. Turbochrom 4 software on a Digital Celebris 466 computer was used to perform data analysis. The molecular weight was determined relative to polystyrene standards (Polysciences, Inc., USA).

### 2.8. Dimension change

As a result of degradation and fluid uptake, the dimension of the polymer samples changes with aging time. This kind of dimension change was evaluated by measuring polymer sample size before and after degradation using a caliper with an accuracy of 0.01 mm. Then, the percent volume changes were calculated by reference to the sample original dimensions. It was found that the relatively large variation in dimension change could result only in the late stages of experiments.

## 3. Results and discussion

### 3.1. Physical appearance

During the course of degradation study, the polymer samples remained transparent for the first three days, then gradually became white and opaque. This was happening as the polymer was slowly swollen by fluid, and the crack and crazing were formed. However, if heated to above 100 °C and cooled to room temperature, the polymer samples became transparent again. This may indicate that crystallization did not occur despite the increase in molecular chain mobility due to the reduction in molecular weight.

### 3.2. Dynamic mechanical properties

To fully evaluate the effects of *in vitro* degradation on dynamic mechanical properties of PLGA, the degraded polymer samples were tested in both wet and dry states. For the wet state, the samples were tested immediately following their removal from the PBS. The samples were said to be dry when the weight difference was less than 0.5% for a 24-hour period during drying in ambient conditions. Normally, the drying process took more than 48 hours. From dynamic mechanical analysis, the storage modulus $(E)$ and the mechanical loss factor (damping) $(\tan\delta = \frac{E''}{E'})$ were determined, where $E''$ is loss modulus. The results are presented in Figs. 1–4. Figs. 1 and 2 illustrate the change of $E$ with temperature and degradation time for the wet and dry samples, respectively. Generally speaking, for both wet and dry samples, $E$ decreased rapidly with degradation time. By 4 weeks, $E$ was reduced to half of the initial value at room temperature, but a reduction of more than 90% was observed at 37 °C, which is particularly important to biomedical applications. The higher the temperature, the lower the $E$. This is due to the softening of the polymer with increasing temperature. The sharp drop in $E$ was observed when the temperature was near the glass transition temperature $(T_g)$ of the polymer samples. The temperatures at which this sharp drop in $E$ happened got lower with increasing aging time, indicating the decrease of $T_g$. But this kind of sudden drop was not observed after 4 weeks by looking at Figs. 1 and 2, which shows that the $E$ at 5 and 6 weeks decreased at almost constant rates. Comparing Figs. 1 and 2 clearly indicates that dry samples yield higher $E$ than wet samples for aging time up to 5 weeks. This result strongly suggests that the fluid would not only degrade the polymer, but also function as a plasticizer. Even after one week, the $E$ of the wet sample showed a more than 70% reduction at 37 °C compared to a less than 10% reduction for dry samples. This result indicates that because of the dual function of the fluid, the mechanical strength of the amorphous PLGA quickly decreases in aqueous conditions such that long-term implantation for load-bearing applications may not be appropriate. It can also be inferred from the above results that although dry samples showed a higher $E$ than wet ones, the data from the latter will be closer to real conditions.

Figs. 3 and 4 illustrate the effects of degradation on tan δ and its changes with temperature. Fig. 3 is the result for the wet samples and Fig. 4 the result for the dry samples. Both figures indicate the dependence of tan δ on

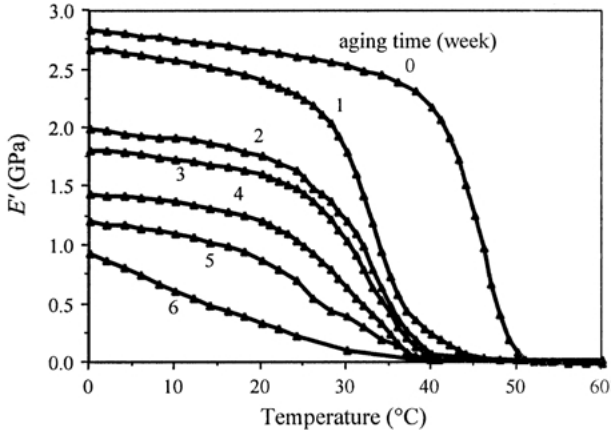


*Figure 1* Effects of degradation on storage modulus $(E)$; wet samples.

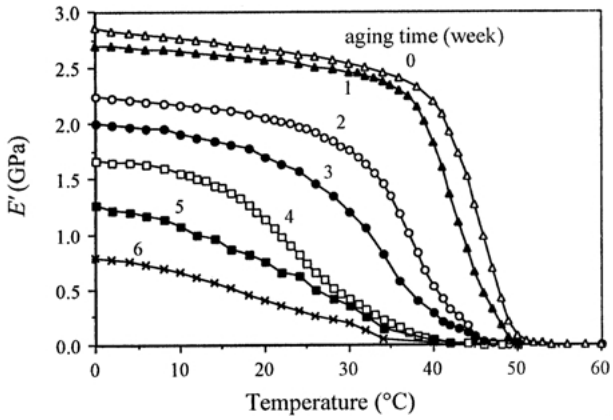


*Figure 2* Effects of degradation on storage modulus $(E)$; dry samples.

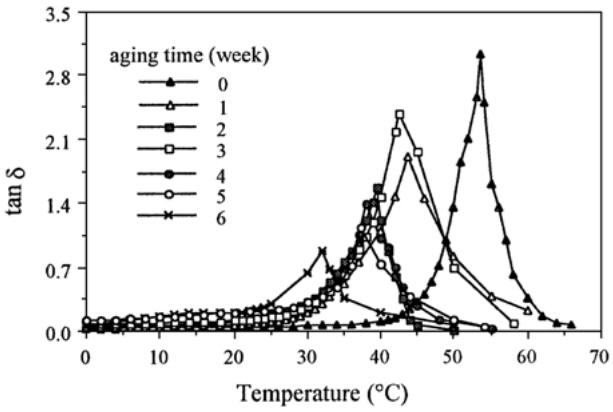


*Figure 3* Effects of degradation on loss angle (tan δ); wet samples.

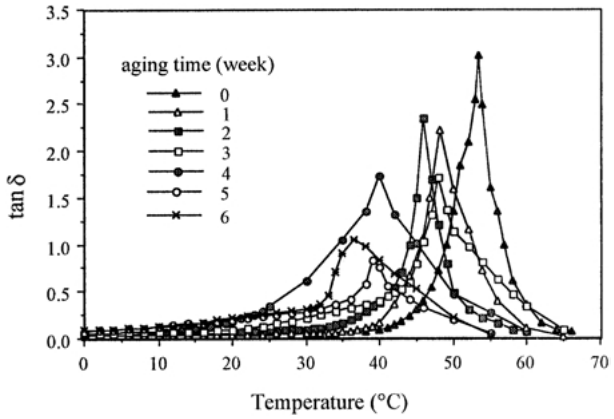


*Figure 4* Effects of degradation on loss angle (tan δ); dry samples.

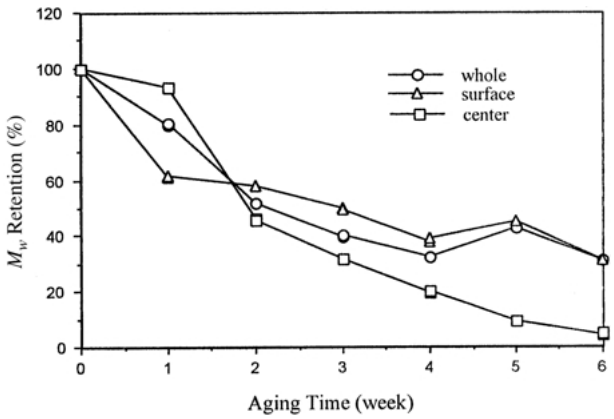


*Figure 6* Effects of degradation on $M_w$.

degradation time and temperature. The loss peaks became small and shifted towards lower temperatures when the degradation time was increased. This also indicates the decrease in the damping properties of the polymer. Since the loss peaks are indications of glass transition, the results elucidate that the $T_g$ of the polymer would decrease with increase in aging time. Such reduction suggests that the use temperature of the polymer should get lower for load-bearing applications, indicating that the $T_g$ of either raw materials or nonage samples may not be a good representation for the actual design of medical devices, or this kind of change must be taken into consideration.

## 3.3. Molecular weight and polydispersity

The changes in molecular weight of the polymer due to degradation were evaluated using GPC analysis. The GPC analysis was done on three regions of the samples, i.e. the samples taken at the surface, at the center and the samples containing both surface and center portions (whole). The typical GPC chromatograms are illustrated in Fig. 5. This figure shows the results for a sample aged for 4 weeks. It clearly indicates the differences in molecular weight at different locations, being higher at the surface. The changes in $M_w$ are illustrated in Fig. 6. As shown in this figure, two GPC runs were performed for each aging time. Generally speaking, the $M_w$ decreased with the increasing degradation time. At 6 weeks, more than 50% reduction in $M_w$ was observed. At short degradation time (2 weeks), $M_w$ was lower at the surface than at the center. After that period, however, $M_w$ started to show a higher value at the surface than at the center, which agrees with the data reported in literature [22]. The molecular weight decreases led ultimately to the decrease in mechanical strength and $T_g$.

Fig. 7 illustrates the changes in $M_w/M_n$ with degradation time. It is clear from this figure that the change of this parameter with time is limited (about 10% variation), suggesting a degradation mechanism of random ester bond breakage for poly(DL-lactide-*co*-glycolide) during degradation under the conditions of this study.

## 3.4. Fluid uptake and dimension change

The fluid uptake of the polymer samples during degradation is illustrated in Fig. 8. As shown in this figure, the fluid uptake increased proportionally with aging time. The results indicate that the maximum fluid uptake at 6 weeks was about 30%. Such an amount of fluid uptake will not only plasticize the polymer, but also swell and craze it. The swelling of the polymer samples aged in PBS was illustrated by its volume change. The results are also displayed in Fig. 8. Like fluid uptake, the percent volume change increased with increase in aging time and the increase was faster than the fluid uptake, indicating the formation of cracks. At 6 weeks, a volume increase of about 60% was observed.

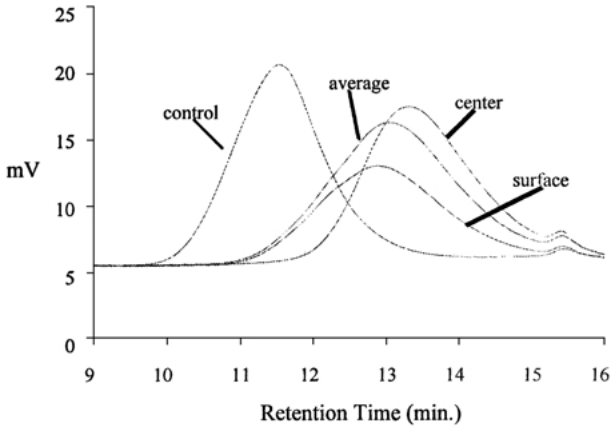


*Figure 5* GPC chromatograms for the control and the samples aged for 4 weeks.

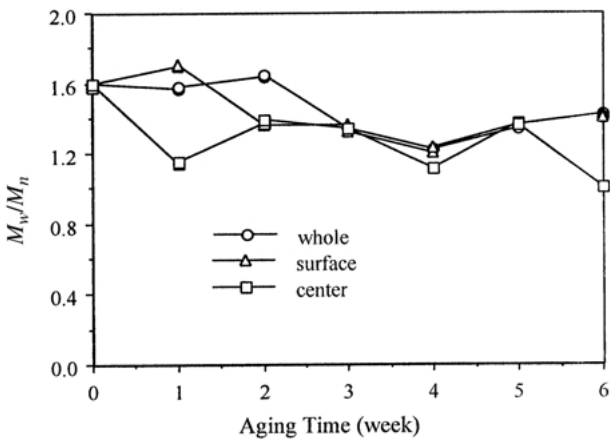


*Figure 7* Effects of degradation on $M_w/M_n$.

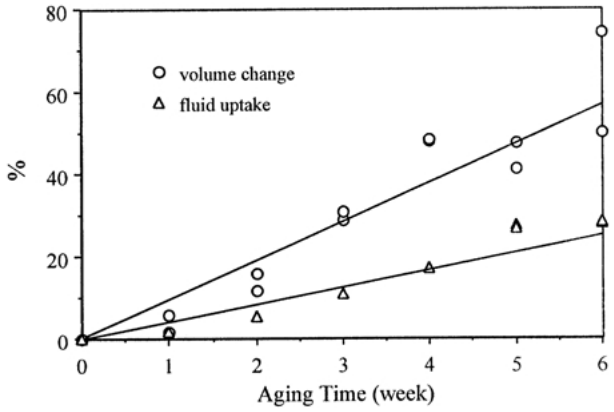


*Figure 8* Fluid uptake and dimension change as a function of aging time.

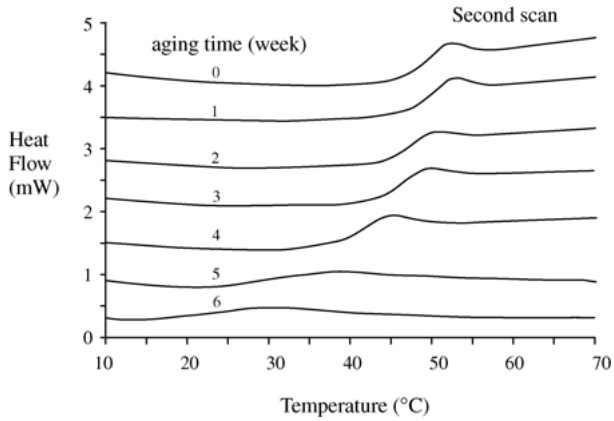


*Figure 10* Effects of degradation on DSC thermograms: second scan.

## 3.5. Thermal properties

The thermal properties of the polymer were discernibly affected by the *in vitro* degradation process. For amorphous PLGA, two factors may contribute to this process, namely molecular weight and fluid uptake. In this study, the thermal analysis of all polymer samples was conducted on practically dry specimens, so changes in molecular weight would be the major contribution. The effects of fluid uptake on thermal properties will be discussed elsewhere. The effects of degradation on shapes of the DSC thermograms are illustrated in Figs. 9 and 10. Fig. 9 depicts the DSC thermograms of the first scan and Fig. 10 corresponds to the second scan. Comparing Figs. 9 and 10 reflects the significant differences in DSC thermograms for the first and second scans. These differences are due to the effects of physical aging and fluid uptake. Although the samples were dried, it was difficult to remove all the fluid in the polymer samples using the present method. However, the physical aging effect and the residual fluid were eliminated during the first DSC heating cycle. Both figures show that as aging time increased the glass transitions became less discernible and $T_g$ appeared at a lower temperature. These results are consistent with the DMA data, although the latter yielded a higher $T_g$. The dependence of $T_g$ on degradation time is displayed in Fig. 12. It is clear from this figure that for aging time up to four weeks the $T_g$ showed a limited drop (several degrees); however, after 4 weeks, $T_g$ decreased rapidly and by 6 weeks a $T_g$ of about 28 °C was observed, which represents a more than 20 °C drop as compared to the initial value. Fig. 11 illustrates the TGA thermograms. Similarly, the heat resistance of the polymer decreased with increasing aging time. The effects of degradation on onset thermal degradation temperature $(T_d)$ are illustrated in Fig. 12. As for $T_g$, $T_d$ values dropped slowly at the beginning, but the rate increased after 2 weeks. At 6 weeks, a drop of about 20 °C in $T_d$ was observed. These results indicate that the stability of the polymer at elevated temperatures decreases with increasing aging time, which is due mainly to the molecular weight reduction.

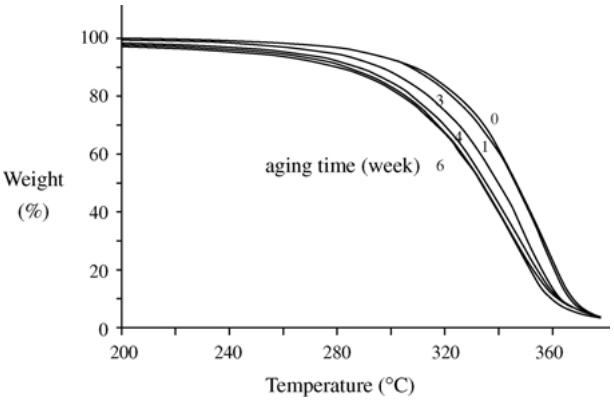


*Figure 11* Effects of degradation on TGA thermograms.

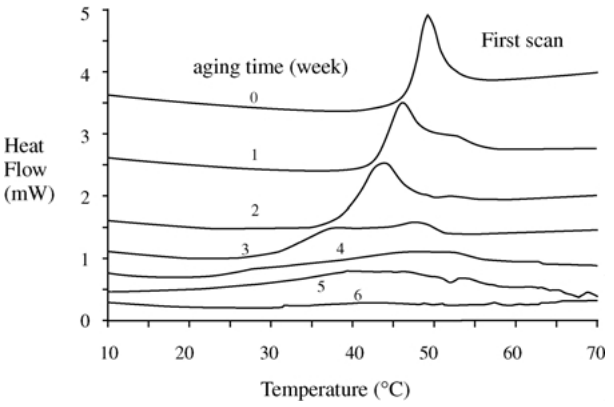


*Figure 9* Effects of degradation on DSC thermograms: first scan.

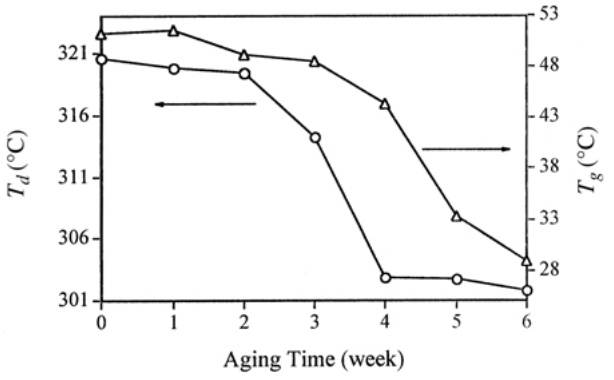


*Figure 12* Changes of $T_g$ and $T_d$ with aging time; $T_g$ is from the second scan.

## 4. Conclusions

Effects of *in vitro* degradation on dynamic mechanical properties, thermal behavior, molecular weight, fluid uptake and dimension stability of poly(DL-lactide-*co*-glycolide) were demonstrated. The *in vitro* degradation in PBS significantly decreased the dynamic mechanical properties ($E$ and $\tan\delta$) and thermal parameters ($T_g$ and $T_d$) and molecular weight of the polymer. The fluid uptake and size of the polymer increased with increased aging time.


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