Comparison of the Enthalpic Relaxation of Poly(Lactide–*co*-Glycolide) 50:50 Nanospheres and Raw Polymer

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ABSTRACT: The phenomenon of enthalpic relaxation was evaluated for poly(lactide-*co*-glycolide) (PLGA, 50:50), in terms of storage of nanospheres for use as a controlled drug delivery system. Samples were stored for different times and temperatures below the glass transition temperature (T_g). Relaxation occurred at a significant rate up to 15 degrees below the T_g of 39.2°C. The effect of polymer morphology was considered by comparing the relaxation kinetics of the raw polymer with that of nanospheres formed using a novel technique. The nanospheres were shown to have a larger change in heat capacity at the glass transition and a longer average relaxation time than that of the raw polymer, and the relationship between these two parameters was discussed. For both the raw polymer and the nanospheres, relaxation was found to occur at a significant rate at room temperature. The storage of this system at subambient temperatures was therefore deemed important for maintaining the physicochemical properties of the system. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 1868–1872, 2002

Key words: relaxation; poly(lactide-*co*-glycolide) (PLGA); kinetics; nanospheres; differential scanning calorimetry (DSC); glass transition

INTRODUCTION

When a polymer is quenched below the glass transition temperature (T_g), the polymer chains are not in their equilibrium conformation and in time gradually relax towards equilibrium. This process is thermodynamically driven and is known as enthalpic relaxation. The enthalpic relaxation of polymers has been well documented[1–6] and results in a change in the mechanical properties with the polymer becoming more brittle and having a reduced elongation to break,[7] a process known as physical aging. The extent of physical aging can be measured by studying the kinetics of this process by holding the polymer below the glass transition at temperature T_a for time t_a. The magnitude of the relaxation can also be quantified by calibration with a known standard. Enthalpic relaxation is nonlinear in time and can be described as the relaxation of a wide range of molecular processes that have a distribution of relaxation times.

When a sample is held at a constant temperature T_a the change in enthalpic relaxation increases with time up to a limit ΔH_∞ at which point the glass is in equilibrium with the liquid at the aging temperature. The extent of physical aging is defined as $(\Delta H(t_a)/\Delta H_\infty)$ in the Cowie–Ferguson equation:[8]

$$(\Delta H(t_a)/\Delta H_\infty) = (1 - \phi(t_a)) \quad (1)$$

and is related to time by the KWW (Kohlrausch, Williams, and Watts)[9,10] equation

$$\phi(t_a) = \exp(-t_a/\tau)^\beta \quad (2)$$

for which τ is the average relaxation time and β is an inverse measure of the breadth of the relaxation spectrum ($0 \leq \beta \leq 1$). A value of 0 implies an infinite number of relaxation processes and a value of 1 implies a single relaxation process.

This model can be used to determine ΔH_∞, β, and τ. By evaluating these parameters for different materials it is possible to determine the effect of chemical structure on detailed relaxation processes. Enthalpic relaxation has been extensively studied in the past using differential scanning calorimetry (DSC).[6,8] The change in physical properties has been quantified and the effect on many engineering applications considered. The effect of enthalpic relaxation on drug delivery devices has also been noted in the literature. A review by Guo[11] reported the importance of studying the physical aging of polymers and stability of dosage forms and concluded that the extent of physical aging was affected by the water content of the dosage forms and is highly influenced by the difference in storage

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temperature. Other additives in the dosage forms would also have a pronounced effect on the polymer physical aging and the stability of the dosage forms.

Polymeric micro- and nanospheres have been investigated as possible delivery systems for many drugs. The advantages of encapsulating the drug in a polymer matrix include its protection from the acidic environment of the stomach, the need for fewer administrations and an increase in patient compliance, and a controlled method of drug delivery that facilitates sustained plasma levels that are free of the wild fluctuations typical of bolus administrations. For many types of drug delivery, the ability to store the system and, therefore, the encapsulated drug, in a stable form for extended periods of time is also an advantage. For instance, many drugs encapsulated within a polymeric system are able to be retained in their most stable form as solid particulates within the polymeric matrix. Because drug delivery systems must necessarily be stored in a manner that will prolong the activity of the particular therapeutic agent, considerations must also be made when contemplating an appropriate storage environment, including humidity and temperature. If the sample becomes exposed to water, in addition to the possible degradation considerations, the T_g may be lowered, thereby altering the kinetics of relaxation.[12] An additional consideration is the storage of the drug delivery devices by the end user. Because this storage is commonly at room temperature, the changing physical properties of the polymer at this temperature with time must be considered. If enthalpic relaxation results in loss of adhesive properties, there man also be an in accumulation of moisture, thereby significantly affecting the stability of drugs to degradation.[13] Enthalpic relaxation may also affect the dissolution properties, which in turn would compromise oral bioavailability.[14] For example, water permeabilities of cellulose acetate and ethyl cellulose and the dissolution rate of HPMCP decreased with physical aging time after being quenched from above the T_g to below the T_g.

In polymers such as poly(lactide-*co*-glycolide) (PLGA), where the glass transition is just above room temperature, the effect of enthalpic relaxation is an additional consideration when determining storage conditions. If relaxation is occurring over the time period of storage, the physical properties of the polymer will change and may affect the release characteristics, possibly causing either inactivation of the encapsulated molecule or bolus dumping of the drug. It is therefore essential to not only characterize the relaxation parameters of the raw polymer but also of the nanospheres that will be used as the delivery system. It is also essential to characterize the polymer and nanospheres fully in the absence of any drug.

EXPERIMENTAL

Materials

PLGA was supplied by Boehringer Ingelheim (MW 12,000). Blank nanospheres were fabricated from PLGA by a phase inversion technique.[15] Briefly, a 2% polymer solution in dichloromethane was quickly introduced into a nonsolvent bath of petroleum ether at a solvent:nonsolvent ratio of 1:50. The resulting nanospheres were then collected with a high-pressure filtering system. Additional aliquots of raw polymer were pulverized into a fine powder and also tested.

Method for determining the extent of relaxation

All measurements were made on a Perkin-Elmer DSC-7 with an intracooler attachment, and the data were collected on a PC. The instrument was calibrated for enthalpy and temperature using the melt of ultra pure indium (28.42 J/g). A standard glass of the sample was formed by heating above the T_g to remove thermal history and then quenching in the DSC at a standard cooling rate of 100°C/min. The T_g of PLGA was taken to be the midpoint of the step change in the heat capacity and determined to be 39.2 ± 0.1°C. Samples of polymer were held at 5, 8, 10, and 15°C below the T_g for 15, 30, 60, 120, 240, 480, 960, 1440, 2880, and 4320 min. The extent of relaxation was determined by heating through the T_g at 10°C/min, quenching to form the standard glass at 100°C/min, and then reheating through the T_g at 10°C/min. The quenched polymer trace was subtracted from the aged polymer and the change in enthalpy on relaxation thus determined.

RESULTS AND DISCUSSION

Various samples were prepared so that the relaxation kinetics could be compared and the effect of fabrication on the relaxation parameters noted. All samples were treated identically in the DSC. A standard glass was produced by quenching amorphous PLGA through the glass transition at 100°C min^{-1}. This glass had a T_g of 39.2 (±0.1°C) after correction for thermal lag. Because physical aging can occur up to 50°C below the T_g, it is apparent that enthalpic relaxation will also occur at room temperature. This result has implications for predicting how the material properties of PLGA will change on storage and should be taken into account when considering its potential applications. The change in heat capacity at the glass transition was also measured and found to be larger for the nanospheres than the raw polymer (0.562 compared with 0.501 J/g, respectively). This result has previously been attributed to the fabrication technique as a result of the rapid introduction of the polymer solution into nonsolvent during nanosphere formation.[16]

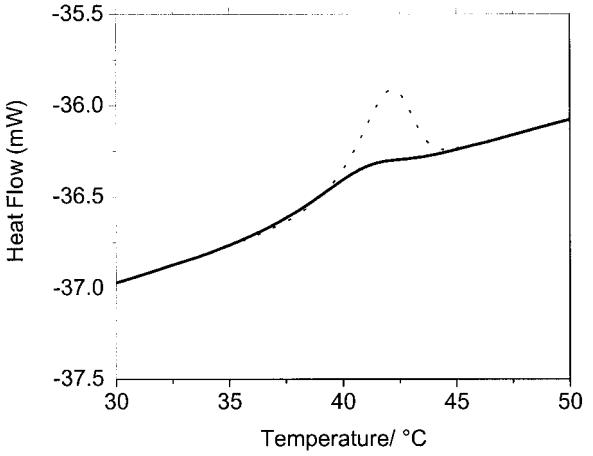

Figure 1 Enthalpic relaxation observed as a peak superimposed on the glass transition of PLGA.

The standard glass was held at the aging temperature, T_a, for various periods of time, t_a, and the extent of enthalpic relaxation that had occurred was measured by heating the sample through the $\mathbf{T}_g$ at a heating rate of 10°C min^{-1} (see Figure 1). Enthalpic relaxation was observed as an endotherm superimposed on the glass transition that arises as a result of de-aging the sample on heating through the T_g. This endotherm represents the change in enthalpy of the glass stored in the sample when the polymer chains relax from higher energy conformations to lower energy ones. On subsequent reheating, the sample overshoots the glass transition, a change in enthalpy is observed before equilibrium is achieved, and the liquid rejoins the liquid line at a higher temperature. The enthalpic relaxation endotherm, so determined, increased in magnitude with increased aging time accompanied by an apparent shift in the glass transition to higher temperatures (see Figure 2, for a sample aged at 29.2°C, 10°C $< T_g$).

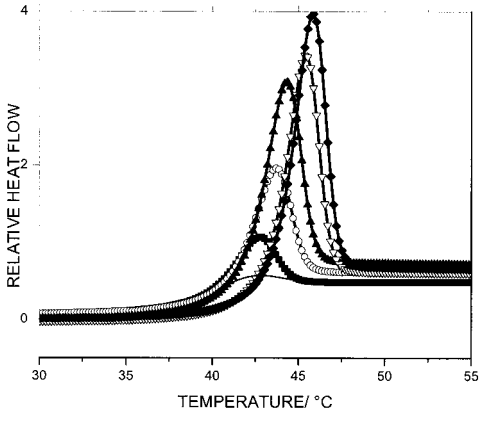

Figure 2 Development of endotherm with increased aging time for t = 0 (solid line), 30 (solid square), 120 (open circle), 240 (solid up triangle), 960 (open down triangle), and 2880 (solid diamond) min.

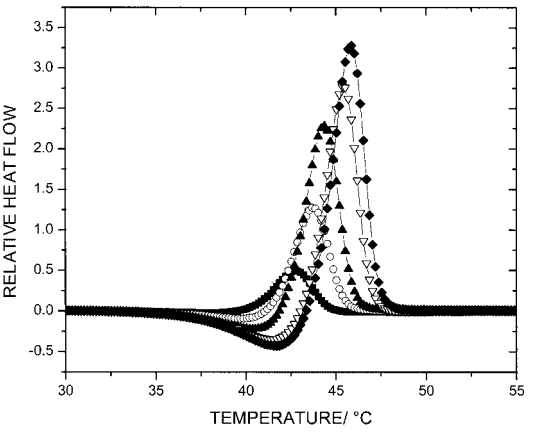

Figure 3 Development of enthalpic relaxation with time for t = 30 (solid square), 120 (open circle), 240 (solid up triangle), 960 (open down triangle), and 2880 (solid diamond) min for ΔT = 10.

The change in enthalpy was calculated by quenching the sample at the same cooling rate as used to form the standard glass (100°C min^{-1}), and then reheating the quenched sample immediately under the same experimental conditions as the original heat (10°C min^{-1}) so that a baseline of un-aged materials was obtained. By subtracting the quenched enthalpy response from that of the aged specimen, the change (i.e., ΔH_a), was determined (see Figure 3). This change is an absolute measure of the extent of enthalpic relaxation and so ΔH_a was plotted against aging time (Figure 4).

This magnitude of enthalpic relaxation was related to time using the Cowie–Ferguson and KWW relationships using a log–log plot to determine the values of β and τ, as shown in Figure 5. Analysis of this plot showed that $\log(-\ln(1 - (\Delta H_t/\Delta H_\infty)))$ was linear with $\log t_a$, with a slope of β and an intercept of $-\beta \log(\tau)$. Therefore, τ and β were compared for the raw polymer and the nanospheres (see Table I).

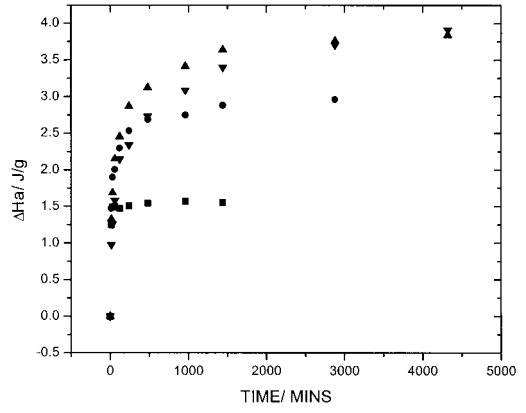

Figure 4 Degree of enthalpic relaxation with time for ΔT = 5 (square), 8 (circle), 10 (up triangle), and 15 (down triangle).

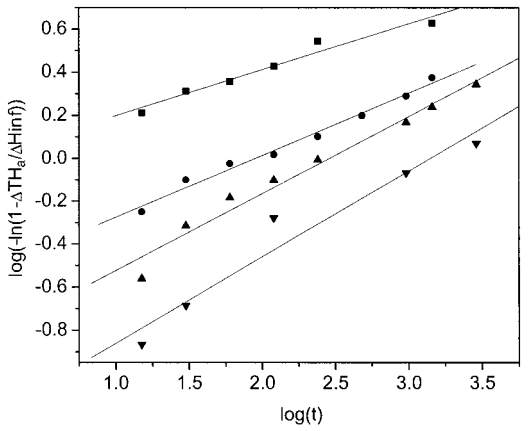

Figure 5 Data fitted to the Cowie–Ferguson equation where ΔT = 5 (square), 8 (circle), 10 (up triangle), and 15 (down triangle).

The average relaxation time was observed to increase with ΔT as did ΔH_∞. It was therefore reasonable to predict that it should take longer to reach the equilibrium enthalpic relaxation because the magnitude of the enthalpy change was larger. The average relaxation time for the nanospheres was much longer than that of the raw polymer. This difference may be related to the larger change in heat capacity at the glass transition, indicating a higher degree of disorder (greater chain entanglement) and therefore the longer time needed for relaxation to occur. So although the average relaxation time was longer (Table I) for the nanospheres, the actual change in enthalpy on relaxation was larger (Table II). The value of β was similar to that determined for other polymers including PHB (β = 0.38),[17] and PEEK (β = 0.41),[18] although the significance of this result is uncertain. The values of β for the raw polymer and nanospheres were also similar to one another, indicating that although the fabrication technique affected the relaxation time, it didn't seem to affect the relaxation distribution parameter.

The actual change in enthalpy on relaxation was then compared with the theoretical value derived from

$$\Delta H_\infty = \Delta C_P \Delta T \quad (3)$$

The value of ΔH_∞ should be expected to increase linearly with the increase in temperature difference providing the polymer was held at T_a for sufficiently long enough so that the measured change in enthalpic relaxation on aging, ΔH_a, tended towards the change in enthalpy when equilibrium has been reached, ΔH_∞. When the value of $\Delta C_P \Delta T$ was compared with the experimentally determined value (see Table II), a clear indication was seen that the value of ΔH_∞ was being progressively underestimated or that the linear extrapolation of the specific heat difference with temperature, as described in eq. 3, was not valid. Any deviation from linearity could imply that the sample had been left at T_a for an insufficient period of time to reach equilibrium and resulted in an underestimated value of ΔH_∞, or that the relationship only applied at low ΔT. During isothermal aging, the glass transition moved progressively towards equilibrium. At high temperatures, close to the T_g, this process required a short aging period. However, as the aging temperature decreased, equilibrium periods became excessively long (see Table II) and eventually became impracticably long for equilibrium to be obtained. Samples held for longer time points could be examined by using an oven rather than holding in the DSC, although this would reduce the accuracy of the temperature. This factor may also be considered for future studies and larger values of ΔT. Future studies should include a comparison of the blank nanospheres to those loaded with drug so the implications on release characteristics may be considered.

TABLE II
Theoretical and Actual Values for ΔH_∞ based on $\Delta C_P \Delta T$

	Δ			
System	5	8	10	15
Raw PLGA (t)	2.505	4.008	5.010	7.515
Raw PLGA (a)	1.57	3.00	3.95	4.30
PLGA PIN (t)	2.810	4.496	5.620	8.430
PLGA PIN (a)	1.753	3.400	4.500	5.500

CONCLUSIONS

By studying the kinetics of enthalpic relaxation for control nanospheres of PLGA, it was shown that significant relaxation occurs below the T_g and that this relaxation occurs at a different rate than that of the raw polymer. The average relaxation time, τ, for the nanospheres is approximately three times longer than

TABLE I
Values of β and τ as Determined by the Cowie–Ferguson Equation

Temp, °C	ΔT	Raw PLGA (β)	PLGA PIN (β)	Raw PLGA (τ)	PLGA PIN (τ)
34.2	5	0.18	0.21	0.3	1.2
31.2	8	0.33	0.29	30.3	89.4
29.2	10	0.38	0.36	137.2	287.7
24.2	15	0.38	0.40	454.5	1399.0

that of the raw polymer for most temperatures evaluated and increased as the temperature decreased, indicating that relaxation was occurring at a significant rate up to 15°C below the T_g. This result implies that the microspheres relax at a much slower rate and the physical properties will change more slowly at higher temperatures compared with the raw polymer. Therefore, on the timescale of material storage (e.g., 3 months), the raw polymer must be stored at −20°C (i.e., in the freezer), but the microspheres could be stored at 4°C (i.e., in the refrigerator). A more detailed study in which temperatures are lowered below the glass transition needs to be conducted to confirm these results. Therefore, this phenomenon should be considered when determining storage conditions for a polymeric-based drug delivery system.

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