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Physical Aging of Progesterone-Loaded Poly(D,L,-lactide-co-glycolide) Microspheres

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Purpose. To study the interactions between a polymeric matrix and a drug during storage at a temperature lower than the glass transition temperature of the polymers.
Methods. Poly(lactide-co-glycolide) microspheres loaded with different progesterone ratios were stored at 4, 20 and 40°C. DSC-scans were recorded at regular intervals, depending on the storage temperature.
Results. The physical aging of the polymeric matrix, as monitored by the amplitude of the endotherm associated with the glass transition, is slowed down by crystalline progesterone. The development of the progesterone polymorphic depends on the interface/volume ratio of the crystals.
Conclusions. For polymeric drug delivery systems, the determination of all studies parameters must take into account an effect of dispersed drugs which are more sensitive as the storage temperature is lower than the glass transition temperature of the matrix.

KEY WORDS: poly(lactide-co-glycolide); differential scanning calorimetry of physical aging; progesterone; polymorphism.

INTRODUCTION

The physical aging of polymers corresponds to the relaxation toward the equilibrium of samples in a non equilibrium glassy state (1). Although no physical interpretation of this phenomenon is presently fully satisfying, it is clearly associated with the time dependent variation of the free volume, and with the modification of the properties that depend upon it, like diffusional properties (2–5). Consequently, physical aging during storage may modify the performance of polymeric drug delivery systems.

The influence of drug loading upon this phenomenon is difficult to foresee as it may act in different ways. When drug molecules occupy a fraction of the volume they slow down the matrix physical aging (3). The same effect may be associated with the reduction of chain mobility if drug crystals interact with polymer chains (6,7). Conversely, a molecularly dispersed drug may act like plasticizer and increase the free volume (8), resulting in a faster aging.

We report in this paper the results obtained with two different poly(d,l,-lactide-co-glycolide) microspheres loaded with α-progesterone. The first copolymer contained 60 wt% lactide and 40 wt% glycolide (PLG 60/40), and the second one, used for a first insight into the influence of the composition of copolymer matrix, contained 85 wt% lactide and 15 wt% glycolide (PLG 85/15). Progesterone crystals exist within two main polymorphic forms, α and β (9). The evolution of these forms was studied along with aging of the polymeric matrix, as a previous work (10) had evidenced a variation of their relative amounts during storage.

MATERIALS AND METHODS

Loaded and unloaded samples were first prepared as microspheres, by a solvent (methylene chloride) evaporation process. The highest progesterone loading was equal to 50% w/w for 60/40 PLG, and to 65% w/w for 85/15 PLG. For the sake of text simplicity, samples loaded with X% w/w of progesterone will be referred to as X% samples. The 85/15 and the 60/40 PLG were prepared by Phusis (St. Ismier, France) by ring-opening polymerization of 85/15 or 60/40 w/w mixtures of D,L-lactide and glycolide (11). Materials, preparation of microspheres, determination of their progesterone content, chlorine analysis, and X-ray powder diagrams have been previously described (10). The microsphere size range was determined by optical microscopy measurements performed on a 50 microspheres samples for each batch. All measured diameters were contained between 25 and 50 μm.

Heat Treatment

The metallic sample pans used for Differential Scanning Calorimetry (DSC) were aluminium sample pans from Perkin-Elmer (ref. 0219-0041). They were washed with boiling methanol, using a Soxhlet extractor, for one hour. Samples used for the study of the T_g endotherm (typical weight: 3–4 mg) were weighted (Mettler ME 30, readability 1 μg) in those sample pans and stored at 80°C for 24 hrs. The coherent polymer masses obtained after this treatment were then quench cooled by putting the capsules on a metallic surface maintained at −20°C, and stored in a dessicator in the presence of P_2O_5, at 4, 20 or 40°C. The 80°C treatment was sufficient to over rule the previous thermal story of the samples, as it was demonstrated by the identity of DSC measurements performed on so treated microspheres either after their preparation or after a one year of storage at room temperature. Samples used for the investigation of progesterone physical state changes in the microspheres after storage were kept for 11 months at 4°C after fabrication, without thermal treatment at 80°C.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were performed using a Model 990 scanning calorimeter (Du Pont, Wilmington, DE, USA). Samples were introduced in the measurement cell at a preset temperature equal to 4°C for those stored at this temperature, and equal to 20°C for those stored at 20 or 40°C. They were heated at 10°C/min in a nitrogen atmosphere. Reported glass transition temperatures (T_g values), energy associated with the glass-transition (ΔH values, J/g PLG), and difference between the specific heats, at constant pressure, of the equilibrium and the glassy state of the polymer (C_e - C_g, J/g PLG) were measured as shown in Fig. 1.

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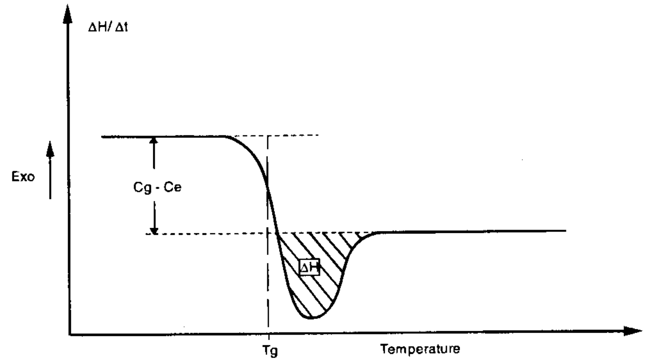

Fig. 1. Determination of the thermal parameters related to the glass-transition event. T_g : glass-transition temperature; C_g and C_e: constant pressure heat capacity below and above the glass-transition temperature.

Each reported result is the average of at least three measurements. In order to reduce the error on aging time at 20 and 40°C storage temperatures, multiple sets of experiments were organized for all samples. For each set, only one measurement was done for each aging time, allowing an error inferior to 10 minutes on this parameter. Such a procedure was not used for storage at 4°C, as the relative error generated by consecutive DSC measurements was generally negligable, due to the difference between storage times and the time required for these measurements. For each loading ratio, different batches of microspheres, all prepared in the same experimental conditions, have been used throughout this study and the overall reproducibility for the reported results was equal to: ± 0.5°C for T_g, and ± 10 % for ΔH and for C_e- C_g.

The crystalline forms of progesterone were differentiated knowing their melting temperatures (respectively 131°C and 120°C for the α- and β- form) and heats of fusion (respectively 21.3 kJ/mole and 17.5 kJ/mole) (10). The reproducibility of the amounts of the two polymorphic forms was equal to ±5 %.

RESULTS

The influence of progesterone loading upon the variation of ΔH with storage time is illustrated in Fig. 2. The results for 60/40 PLG stored at 4,20 and 40°C are reported respectively in Figs. 2A, 2B and 2C while Fig. 2D is related to 85/15 PLG stored at 40 °C. ΔH values were found to increase with aging for all studied samples, however, the time dependence courses varied with the storage temperature.

All 60/40 PLG samples stored at 4°C exhibited an increase in ΔH values with time (Fig. 2A). Except for the 10% sample, a "jump" was observed between 10 and 11 days of storage, followed by apparent plateau values. When the storage was continued for 11 months, ΔH values continued to increase up to the results reported in Table I.

For samples stored at 20°C, no apparent plateau was attained during the first 72 hours of aging (Fig. 2B), however, the values of ΔH measured after 11 months were equal to those reported for 72 hours. When the 60/40 PLG samples were stored at 40°C, plateau values were reached in less than 5 hours and were much lower than for samples stored at 4 or 20°C (Fig. 2C). Eleven months of aging at that temperature did not further modify the ΔH values (Table I).

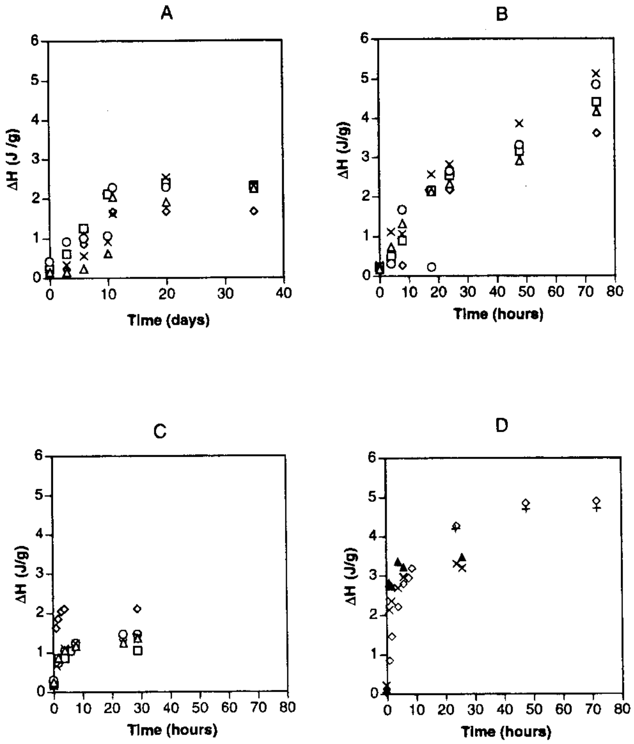

Fig. 2. Dependence of the endotherms associated with the glass-transition on aging time. Progesterone loading : ◇ 0%, □10%, + 16.5%, ○ 23%, × 35%, △ 50%, ▲ 65%. 2A : 60/40 PLG stored at 4 °C. 2B : 60/40 PLG stored at 20°C. 2C: 60/40 PLG stored at 40°C. 2D: 85/15 PLG stored at 40°C.

The 85/15 PLG samples stored at 40°C (Fig. 2D) exhibited much higher plateau values than the 60/40 PLG samples, after longer aging times, varying from 4 hours for 65% samples up to 48 hours for 0 and 16.5% samples.

As for ΔH changes, the influence of progesterone loading upon the variation of T_g with aging time for 60/40 PLG samples stored at 4, 20 and 40°C, and for 85/15 PLG stored at 40°C is illustrated in Fig. 3.

The main feature of Fig. 3A is the pronounced diminution of T_g measured after 3 and 6 days of storage, for 0, 10 or 23% samples, followed by its increase to a plateau value after 20 days (0 and 10%) or 10 days (23%). For 35 and 50% samples, the change in T_g was reduced to a decrease of a few degrees, down to a plateau value reached on the third day.

This decrease of a few degrees, followed by a plateau, was also observed for all 60/40 PLG loaded samples stored at

Table I. Values of ΔH for 60/40 PLG After 11 Months of Aging

Progesterone loading (%)	ΔH (J/g)		
	4°C	20°C	40°C
0	5.3	3.6	2.1
10	5.9	4.4	1.0
23	6.5	4.8	1.5
35	5.0	5.1	1.4
50	2.0	4.1	1.3

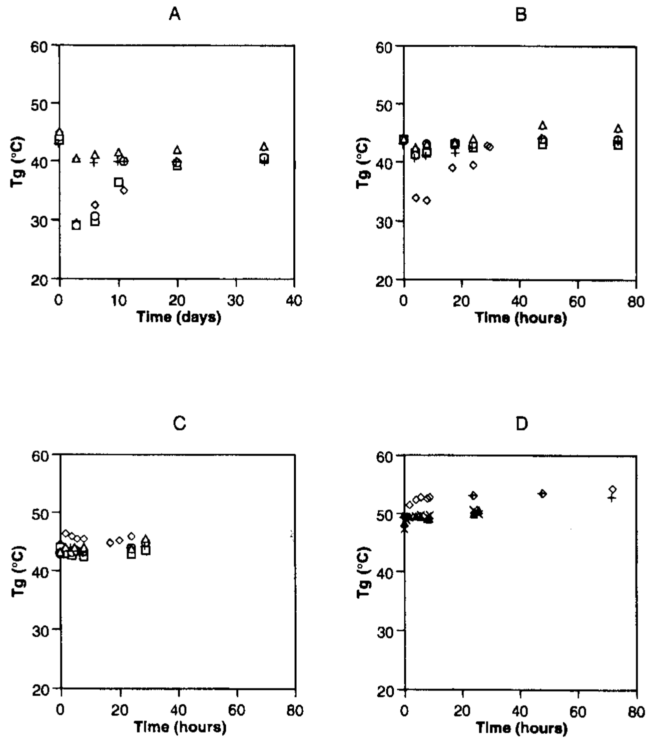

Fig. 3. Dependence of the glass-transition temperature on aging time. Symbols and legends as for Figure 2.

20°C (Fig. 3B). The unloaded samples exhibited a marked diminution of T_g during the first part of aging, with a minimum between 3 and 8 hours, and reached the same plateau value than the progesterone loaded samples after 30 hours. As in Fig. 3A, the T_g values measured after the longest periods of storage for 50% samples were about 2°C higher than for the other samples.

Contrarily to the 60/40 PLG samples stored at 4 and 20°C, microspheres stored at 40°C (Fig. 3C) displayed almost no change in T_g with time, and exhibited the highest T_g in the absence of progesterone. When 85/15 PLG samples were stored at the same temperature (Fig. 3D), 0 and 16.5% samples also showed a higher T_g than the 35 and 65 % samples. A noticeable difference with 60/40 PLG samples is that T_g plateau values were higher than the initial values.

The change in the physical state and the crystalline forms of progesterone after 11 months storage at 4°C is summarized in Table II. At 10 and 16.5% loading, progesterone remained molecularly dispersed and no endotherm was observed between 120 and 130°C. At a higher drug loading, either well defined fusion peaks, corresponding to α and/or β form, or a broad peak centered at 124°C, corresponding to an inseparable mixture of the two crystalline polymorphs were observed. When the relative amounts of α and β forms could be determined, it clearly appeared that the 85/15 PLG matrix favoured the formation of the β form, while in the 60/40 PLG matrix, the α form remained the dominant or the only crystalline form.

DISCUSSION

The chemical stability of the copolymers investigated during aging may be inferred from a recent work (12). The measurement by size exclusion chromatography of the molecular mass of 50/50 and 75/25 PLG nanoparticles showed that storage at 4°C for one year, or at 20 or 37°C for a few days, did not produce any marked alteration of the polymer chains. Thus, the observed changes in T_g or ΔH values may be related only to the physical aging process illustrated in Fig. 4.

When a polymer is cooled from a temperature above T_g, its structural relaxation in the fluid state is fast enough to allow the enthalpy to vary along the equilibrium curve, corresponding to a constant pressure heat capacity C_e. Below T_g, this is no longer true. The structure of the glassy state appears to be frozen, with a slower variation of the corresponding enthalpy, which in Fig. 4 was considered as constant during the time necessary to reach the annealing temperature T_a, and a constant pressure heat capacity C_g. During annealing, the enthalpy decreases slowly towards the extrapolated equilibrium curve. If the sample is reheated above T_g, its enthalpy will first remain equal to the value reached at the end of storage, and then will increase suddenly in the glass transition region, where the polymer representative point moves upward in the enthalpy/temperature diagram and joins the equilibrium curve, resulting in the endothermal variation of enthalpy (13,14).

For a given storage temperature (i.e. T_2), ΔH will be greater for the longer aging time (i.e. $\Delta H(T_2,t_2) > \Delta H(T_2,t_1)$), with a maximal value reached when the sample enthalpy representative point lies on the extrapolated equilibrium curve (enthalpy equal to $H_e(T_a)$). As the diminution of the enthalpy occurs more slowly when the storage temperature decreases, it is easy to understand that for a given aging time (i.e. t_1) ΔH will be greater for the samples stored at the higher temperature (i.e. $\Delta H(T_1,t_1) > \Delta H(T_2,t_1)$). This effect may be overbalanced by a sufficient difference in storage times, as illustrated in Fig. 4, by the comparison of $\Delta H(T_2,t_2)$ with $\Delta H(T_1,t_1)$. The variations of ΔH for 60/40 PLG stored at different temperatures agree well with these predictions. For a given storage temperature, ΔH values increase with aging, and if we compare the results obtained at 4, 20 and 40°C after 4 hours of storage (Fig. 2), we have the relation $\Delta H(40°C) > \Delta H(20°C) > \Delta H(4°C)$. For the longest storage times, this order is reversed (Table I), except for the samples stored at 4°C with a progesterone loading equal to 50%. When comparing these results with those obtained for 85/15 PLG stored at 40°C, one must keep in mind that the glass-transition temperature for this polymer is in the range 50–53°C (Fig. 3D), while it is in the range 40–45°C (Fig. 3A–3C) for 60/40 PLG. The change of ΔH for 85/15 PLG stored ~10°C below T_g should thus be compared either to that of 60/40 PLG stored ~20° below T_g (i. e. at 20°C), or to that corresponding to a storage at a few degrees below T_g (i. e. at 40°C). A comparison of Fig. 2B, 2C and 2D shows clearly that the time dependence and the amplitude of ΔH variations for 85/15 PLG stored at 40°C are closer to those for 60/40 PLG when stored at 20°C than when stored at 40°C.

The description of the modification of the glass-transition temperature with aging may be deduced from the evolution of the endotherm, as it has been shown that, for a given heating rate, the difference between the value for a fully annealed sample at a temperature T_a, $T_g(T_a)$, and the value measured after aging during a time t_a, $T_g(T_a,t_a)$, is proportional to the difference between $H(T_a, t_a)$ and $H_e(T_a)$ (13). This means that the glass-transition temperature, for a given aging temperature, will increase as the endotherm will. If this behaviour is effectively observed for 85/15 PLG (Fig. 3D), for 60/40 PLG (Fig. 3A–3C), the only fit with this

Table II. Polymorphic Transformation of Progesterone Crystals During Aging at 4°C

Progesterone loading (%)	Crystalline Form of Progesterone			
	85/15 PLG		60/40 PLG	
	0	11 months	0	11 months
10	no progesterone fusion peak			
16.5	no progesterone fusion peak			
23	broad mixture	β	$\alpha/(\alpha+\beta) = 0.83$	$\alpha/(\alpha+\beta) = 0.69$
35	$\beta/(\alpha+\beta) = 0.75$	$\beta/(\alpha+\beta) = 0.68$	α	broad mixture
50	$\beta/(\alpha+\beta) = 0.63$	$\beta/(\alpha+\beta) = 0.67$	α	α
65	$\beta/(\alpha+\beta) = 0.69$	$\beta/(\alpha+\beta) = 0.58$	n.d.	n.d.

description is a more pronounced evolution of $T_g(T_a,t_a)$ when the annealing temperature is lower. Noteworthy is the fact that T_g values measured when heating at time zero, just after quench cooling, are in the range of the highest measured T_g values, and one must wait a few hours (aging at 20°C), or a few days (aging at 4°C), to observe the minimum values of T_g when such values exist.

The values of the enthalpy change associated with the glass-transition, measured during aging, may be compared with its maximum value, ΔH_{Max}, which is obtained when the point representative of a sample stored at T_a reaches the extrapolated equilibrium curve. When reheating, the readjustement of enthalpy towards its equilibrium value starts at $T_g(T_a)$, and the corresponding endotherm may then be calculated as it is equal to $(C_e-C_g)(T_g(T_a)-T_a)$. The ratio between the measured value of ΔH and ΔH_{Max} is generally considered as an indicator of the evolution of the aging phenomena (7,15). C_e-C_g values, determined separately for each progesterone loading of 60/40 PLG, were all in the range 0.42–0.58 J/g/K, in a good agreement with the 0.54 J/g/K reported for polyethylene terephtalate (14) or poly (L-lactide acid) (16). $T_g(T_a)$ values were the plateau values, also determined for each progesterone loading. The results reported in Table III illustrate the general evolution of the ratio ΔH / ΔH_{Max} with the progesterone loading. Two facts appear clear. The first one is the lack of influence of molecularly dispersed progesterone on the polymer relaxation, as the evolution of ΔH / ΔH_{Max} is the same for the pure and for the 10% loaded polymer. The second one is the hindering effect of progesterone crystals on the polymer chains mobility, resulting, for given aging parameters, in a diminution of the enthalpies ratio with the increase of the progesterone loading. A similar effect was reported for polyethylene terephtalate (PET) crystals in partially crystalline PET (7).

The polymorphic transformation of progesterone crystals during storage at 4°C (Table II) is somewhat different from

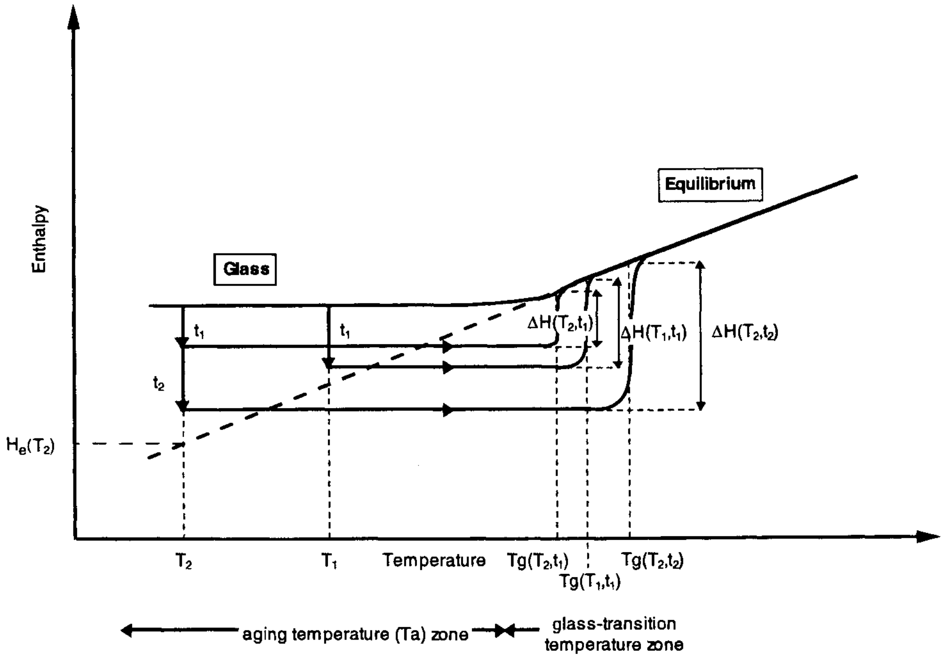

Fig. 4. Schematic enthalpy versus temperature diagram (for further explanation see text).

Table III. Influence of Progesterone Loading on the Evolution of 60/40 PLG Relaxation

	$\Delta H/\Delta H_{Max}$ (%)		
	4°C	40°C	
Aging conditions	11 months	2 hours	29 hours
Progesterone loading (%)			
0	32	65	74
10	35	61	76
23	37	39	76
35	24	40	64
50	13	41	67

what may be inferred from thermodynamic data. If the α form is thermodynamically stable at room temperature (17), the β form is metastable in the same condition and turns to the α form when standing at higher temperatures (18). One could thus expect that either no change in the crystalline form would occur at 4°C, or that it would be a transformation of the β form into the α form. This transformation is observed only for the 50 and 65% samples, while the amount of the β form increases or stays roughly constant for the 23 and 35% samples. If one reasonably assumes that at the highest loading ratios the progesterone crystals are not only more numerous but also larger in size than at the low loading ones, the variation of the crystals volume to crystals/polymer interface ratio could be involved in these different evolutions, as it has been shown (10, 19) that formation of the β-form progesterone crystals was favoured by contact with the PLG matrix.

From the results of our experiments it is possible to emphasize that two points must be taken into account by researchers in charge of further studies relative to the evolution of polymeric drug delivery systems during storage at temperatures lower than the polymeric matrix glass-transition temperature.

The first one combines our results with those obtained for two other systems, glass powder/polystyrene (6) and PET crystals/PET (7): physical aging is slowed by the presence of drugs in a crystalline form.

The second one is the striking influence of the difference between the glass-transition temperature and the storage temperature. If the absolute temperature may be considered as a critical factor for accelerated aging experiments (20), this parameter is clearly unsuitable to predict entirely the different aspects of the evolution of polymeric matrices with aging. The presence of a dispersed drug able to act as a filler will reinforce this statement, since its effect on physical aging will be more sensitive as the storage temperature will be lower.

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