International Journal of Pharmaceutics, 29 (1986) 95–102
Elsevier

IJP 00969

Research Papers

A physicochemical study of the morphology of progesterone-loaded poly (D,L-lactide) microspheres

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(Received August 20th, 1985)
(Modified version received October 24th, 1985)
(Accepted October 29th, 1985)

Key words: progesterone – poly(D,L-lactide) – microencapsulation – solvent evaporation process – solid dispersion – glass transition temperature – progesterone – poly(D,L-lactide) miscibility – differential thermal analysis

Summary

The solvent evaporation process has been used to form progesterone-loaded microspheres from poly(D,L-lactide). Methylene chloride was the casting solvent. The microspheres evaluated had an initial progesterone payload of 23 wt. percent. Though the fabricated microspheres were dried under vacuum, the chlorine and the thermogravimetric analyses indicate that 2.8–3.5% CH_2Cl_2 is present inside the microspheres. The differential thermal and X-ray analyses show the absence of detectable crystalline progesterone domains in the poly(D,L-lactide) matrix. The annealing experiments carried out above glass transition temperature (T_g), provide direct evidence that progesterone and poly(D,L-lactide) have little mutual miscibility. The drug is not dissolved in the polymer but forms a metastable molecular dispersion. In addition, thermal analyses establish that the T_g event of annealed and unannealed microsphere samples changes on storage at 22°C. Upon storage, this event tends to develop an endothermic peak. This is characteristic of amorphous polymers stored in the vicinity of T_g.

Introduction

The preparation of small, drug-loaded polymer particles has received much attention in recent years. Several processes for forming such particles exist. The particles may be called microcapsules, microspheres, nanoparticles, or pseudolattices. Although some effort has been made to characterize the morphology and structure of these particles, much characterization work remains to be done. This is particularly true for situations where the drug is solubilized at some point in the fabrication process. Solubilization often occurs when microspheres are formed by the solvent evaporation process. In this process, drug of low water solubility is added to a polymer–solvent mixture and the solvent is volatilized to give solid, drug-loaded polymer particles. If the drug is normally a crystalline solid that is insoluble in the volatile casting solvent, it could retain in the final polymer microparticles the same form it had when added to the polymer–solvent solution. If the drug dissolves in the casting solvent, it could crystallize in the polymer matrix as the solvent evaporates to form a

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dispersion of crystalline drug in the polymer matrix. Alternately, it could remain as a molecular dispersion of drug in the polymer matrix. Any state between these two extremes is possible. If a drug remains molecularly dispersed in a polymer, the polymer may act as an inert carrier for the drug or it may interact strongly with the drug.

Since the state in which drug molecules exist inside a polymer particle can significantly affect drug release rate and storage stability, this paper presents results of a characterization study carried out with drug-loaded poly(D,L-lactide) microspheres prepared by solvent evaporation. The use of this type of process to form poly(D,L-lactide) microspheres was first described by Beck et al. (1979).

Materials and Methods

Materials

Two poly(D,L-lactide) (PLA) samples were used. Sample A had a GPC M.W. of 60,000 (Pressure Chemicals, Pittsburgh, PA) and was prepared by Dr. N. Mason, Washington University, St. Louis, MO. Sample B was obtained from the Southern Research Institute, Birmingham, AL, and had an inherent viscosity of 1.14 dl/g (30°C; 0.5 g PLA/dl chloroform). Methylene chloride (CH_2Cl_2) (J.T. Baker Chemicals, Phillipsburg, NJ), progesterone (Sigma Chemicals, St. Louis, MO), and partially hydrolyzed (88%) polyvinyl alcohol (Vinol 205, Air Products and Chemicals, Allentown, PA) were used as received.

Methods

PLA microspheres were prepared by the solvent evaporation process described previously (Benita et al., 1984). The weight of PLA was fixed at 1.0 g. For progesterone-loaded microspheres, the weight of progesterone was fixed at 0.28 g. Samples 1–4 were formed at 200 rpm agitation; Samples 5–7 were formed at 150 rpm agitation. For all samples, the process was interrupted before CH_2Cl_2 evaporation was complete in order to remove the aqueous phase emulsifier (polyvinyl alcohol). The interruption of CH_2Cl_2 evaporation was determined experimentally by trial and error. CH_2Cl_2 evaporation was then taken to completion in emulsifier-free water. All microsphere samples were isolated by filtration followed by in vacuo drying at 22°C for 20 h.

Thermal analyses were carried out with a Rigaku simultaneous differential thermal-thermogravimetric analyzer (DTA/TGA) (Rigaku, Tokyo, Japan) and a Perkin-Elmer Model 4C Differential Scanning Calorimeter (DSC). Samples for DTA/TGA analyses were heated at 10°C/min in a nitrogen or air atmosphere; samples for DSC analyses were heated at 20°C/min in a nitrogen atmosphere. Recorded glass transition temperature (T_g) values are mid-point values. The samples were either stored continuously at 22°C after fabrication or subjected to heat treatment. Heat-treated samples were kept at 110°C in vacuo for 22 h, quench-cooled by immersion in liquid nitrogen, and subsequently stored at −20°C or 22°C. During the 110°C heat treatment step, all microsphere samples evaluated fused together to form a continuous polymer mass.

X-Ray powder diagrams of microspheres were obtained by Prof. L. Gulbranson (Washington University, St. Louis, MO) with a Norelco diffraction unit (3 h of copper Kα radiation).

The chlorine content of microsphere samples was determined by Galbraith Laboratories, Knoxville, TN.

Results

Table 1 lists the 7 samples characterized in this study. The microspheres of Samples 1–4 were 100–250 μm diameter whereas those of Samples 5–7 were 250–350 μm diameter. Free progesterone crystals were not detected in the aqueous phase used as the suspending medium for forming the progesterone-loaded microspheres. The progesterone was retained by the microspheres. Assays of numerous microsphere samples prepared by the procedure used in this study consistently gave progesterone loadings of 22.1–23 wt. percent (Benita et al., 1984; Benoit, 1983). Accordingly, the 23 wt. percent progesterone loadings reported in Table 1 are calculated from the weight of progesterone and PLA used to form the microsphere samples.

TABLE 1

EFFECT OF 22°C STORAGE ON THERMAL EVENTS OF PLA MICROSPHERES

Microsphere sample	PLA sample	Progesterone loading (wt. %)	Time kept at 22°C (days)	T_g (PLA) (°C)	Nature of T_g event	T_m (prog) (°C)	TGA wt. loss (%) *
1	A	None	3	38	Diffuse	–	4.7
1	A	None	70	57	Endo peak	–	2.9
2	B	None	2	?	Too diffuse	–	4.4
2	B	None	4	45	Diffuse	–	3.3
2	B	None	7	45	Diffuse	–	3.1
3	A	23	0	?	Too diffuse	None	3.5
3	A	23	5	35	Diffuse	None	1.8
3	A	23	70	45	Endo peak	None	2.5
4	B	23	14	?	Too diffuse	None	2.9
4	B	23	45	35	Diffuse	None	3.2
4	B	23	83	37	Diffuse	None	2.9
7	A	23	32	50	Endo peak	120	2.8
6	B	23	12	45	Diffuse	None	3.4
5	B	23	32	?	Too diffuse	None	3.6

* TGA wt. loss measured from 25 to 180°C.

Table 1 also describes the nature of the DTA PLA glass transition temperature (T_g) event for each sample after a known storage time at 22°C. The nature of this event varied considerably. In several cases, the DTA T_g event was too diffuse for a meaningful T_g value to be assigned. In other cases, it was diffuse, but the inflection in the DTA scan was sufficient for one to assign a meaningful T_g. In some cases, the DTA T_g event was well-defined and had an endothermic peak associated with it. Fig. 1 contains three DTA scans that illustrate each of these types of T_g events. Curve A

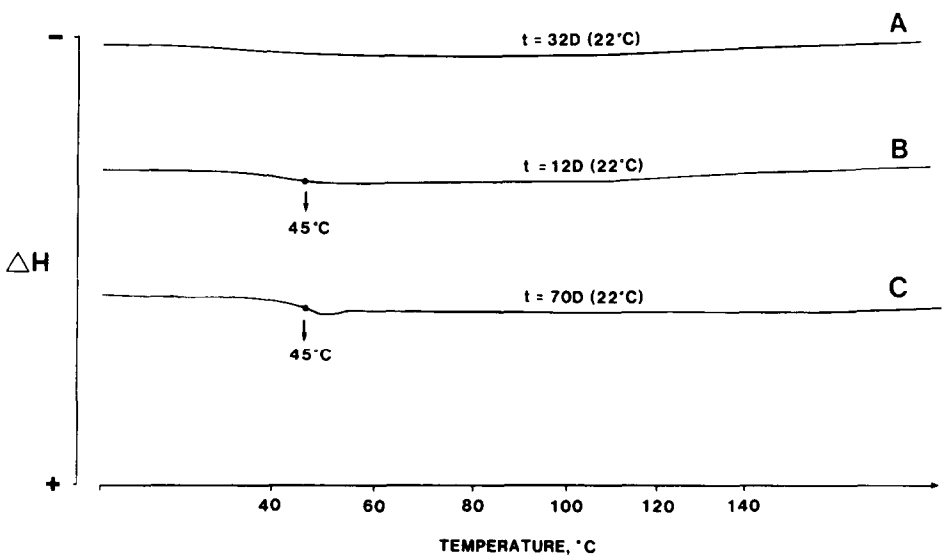

Fig. 1. DTA scans that illustrate the types of PLA T_g events observed in this study: A, T_g event too broad and diffuse to assign a meaningful T_g value; B, T_g event that is diffuse; C, well-defined T_g event with an endothermic peak. All samples contained 23 wt. percent progesterone.

shows a T_g event too broad and diffuse to give a definitive T_g event. Such a T_g event is classified as too diffuse in Table 1. Curve B is a diffuse PLA T_g event to which a reasonable T_g value can be assigned. T_g events like this are classified in Table 1 as diffuse. Curve C illustrates a well-defined T_g event which has an endothermic peak associated with it. This type of T_g event is classified in Table 1 as endo.

Only Sample 1 in Table 1 had a T_g event that fell at 57°C, the T_g value normally reported for D,L-PLA (Benita et al., 1984). All of the other samples characterized had a PLA T_g event located at a significantly lower temperature. For Samples 4–6, the PLA T_g event was too diffuse or diffuse at all storage times evaluated. Fig. 2 illustrates the change in T_g that Sample 4 experiences during 83 days storage at 22°C. Fig. 3 illustrates the T_g aging behavior of Sample 2 for 7 days. Samples 3 and 7 gave T_g events with an endo peak after 22°C storage for 70 and 32 days, respectively. Sample 7 had a well-defined endothermic event at 120°C that is attributed to progesterone fusion. None of the other samples characterized had a progesterone fusion event sufficiently well-defined to be detected by DTA. The absence of crystalline progesterone in Sample 4 was confirmed by X-ray analysis.

The last column of Table 1 gives the TGA weight loss that each sample experienced when heated at 10°C/min from 25°C to 180°C. These losses ranged from 1.8 to 4.7%. If one assumes that

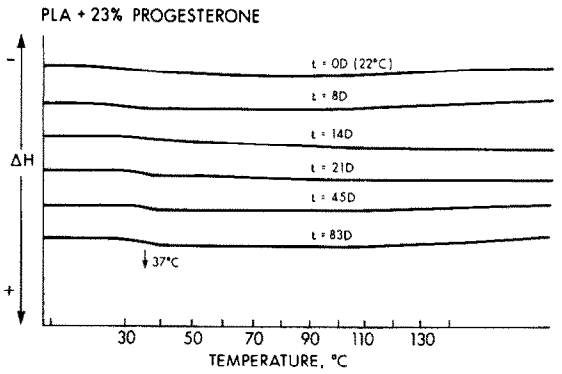

Fig. 2. Aging of PLA T_g for PLA microspheres loaded with 23 wt. percent progesterone and stored at 22°C.

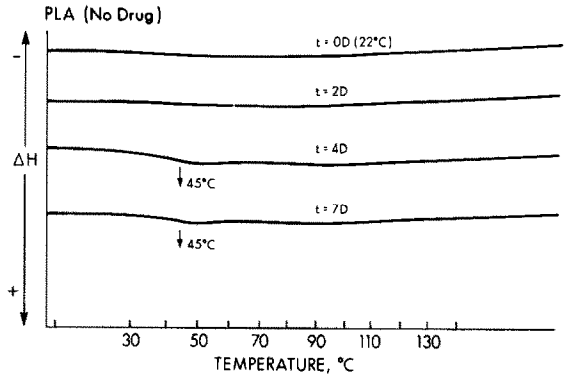

Fig. 3. Aging of PLA T_g for progesterone-free PLA microspheres stored at 22°C.

they are due solely to volatilization of entrapped CH_2Cl_2 and all entrapped CH_2Cl_2 is released from the samples during the TGA analysis, the samples contained 1.8–4.7 wt. percent CH_2Cl_2. In order to test this assumption, chlorine and TGA analyses were run on two PLA samples not listed in Table 1. One drug-free sample had a CH_2Cl_2 content of 3.06% based upon the chlorine analysis and a 3.0% TGA weight loss. The second contained 28.6% progesterone and had a CH_2Cl_2 content of 2.19% based upon the chlorine analysis. Its TGA weight loss was 1.12%. Although the TGA and chlorine data do not agree precisely in both cases, the values obtained are sufficiently similar to say that the TGA weight loss data of Table 1 provide a reasonable estimate of the CH_2Cl_2 content of the samples listed there. In most cases, 2.8–3.5% CH_2Cl_2 is present.

Table 2 summarizes the nature of the PLA T_g event and progesterone melting event for three microsphere samples annealed in vacuum at 110°C for 22 h. During this heat treatment, the microspheres fused together to form a continuous polymer melt. After 22 h at 110°C, the melt was quench-cooled and subsequently stored at 22 or −20°C for a known period before being characterized.

All heat-treated samples stored at 22°C developed a measurable T_g event within a few days, but differences in the nature of the event exist. The T_g event of Sample 1, a drug-free sample, remained diffuse after 7 days. The two samples that contained 23% progesterone developed an endo T_g

TABLE 2

EFFECT OF STORAGE HISTORY ON THERMAL EVENTS OF HEAT-TREATED PLA MICROSPHERES *

Microsphere sample	PLA sample	Progesterone loading (wt. %)	Storage History		T_g (PLA) (°C)	Nature of T_g event	T_m (prog), (°C)	TGA wt. loss (%) **
			Time (days)	Temp. (°C)				
1	A	None	0	22	56	Diffuse	–	0
1	A	None	4	22	55	Diffuse	–	0
1	A	None	7	22	52	Diffuse	–	0
1	A	None	7	−20	?	Too diffuse	–	0
1	A	None	21	−20	?	Too diffuse	–	0
1	A	None	90	−20	?	Too diffuse	–	0
7	B	23	0	22	?	Too diffuse	122	1.7
7	B	23	4	22	53	Endo peak	123	0
7	B	23	12	22	55	Endo peak	121	0.3
7	B	23	17	22	52	Endo peak	120	0
4	B	23	3	22	53	Endo peak	130	0
4	B	23	14	22	54	Endo peak	130	0
4	B	23	0	−20	~ 49	Diffuse	129	0
4	B	23	21	−20	~ 50	Diffuse	128	0
4	B	23	70	−20	~ 49	Diffuse	128	0

* All samples were annealed at 110°C in vacuo for 22 h before quench-cooling and subsequent storage at 22 or −20°C.
** TGA wt. loss from 25 to 180°C.

event within 4 days. Fig. 4 illustrates the T_g aging behavior of Sample 4 over a 14-day period at 22°C.

The T_g event of heat-treated samples stored at −20°C did not change over the storage times examined. The progesterone-loaded sample had a diffuse T_g event at 49°C whereas the T_g event of the drug-free sample was too diffuse to define. All heat-treated samples that contained 23% progesterone have a progesterone melting event. For Sample 4, this event falls at 128–130°C. It was not present before heat treatment. For Sample 7, this event fell at 120°C before and after the 110°C heat treatment. The annealing process had no effect upon it.

The last column of Table 2 shows that most TGA scans of heat-treated samples recorded zero weight loss. Two out of 4 TGA scans of Sample 7 had a finite weight loss. In this case, some entrapped CH_2Cl_2 could still be present, although the amount is greatly reduced from what was present before heat treatment.

DSC scans for heat-treated Sample 7 were obtained after 0, 3 and 7 days storage at 22°C. The progesterone melting event measured after 0 days at 22°C is broad and skewed toward lower temper-

TABLE 3

ESTIMATED FRACTION OF PROGESTERONE IN ANNEALED PLA SAMPLES THAT IS CRYSTALLINE

Sample	Storage at 22°C (days)	Melting range (°C)	Fraction of prog. crystallized (%)
Progesterone	–	127–138	100
Progesterone (23%)/PLA (77%) *	0	110–143	80
Progesterone (23%)/PLA (77%) *	3	128–140	55
Progesterone (23%)/PLA (77%) *	7	128–140	54

* Annealed at 110°C for 22 h in vacuo before quench-cooling and subsequent storage at 22°C.

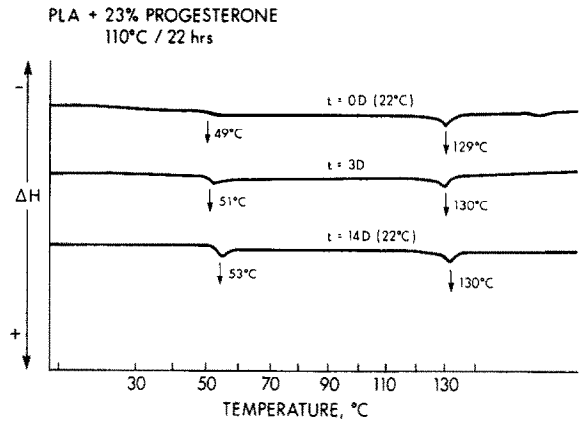

Fig. 4. Aging of PLA T_g for sample stored at 22°C following 22 h storage at 110°C. Progesterone loading: 23 wt. percent.

atures. This event sharpens measurably upon 3 and 7 days storage at 22°C. DSC provided heat of fusion values for each progesterone fusion event from which the fraction of progesterone that crystallized during the 110°C annealing process was estimated. Table 3 contains these estimates. It was assumed that the progesterone crystal domains formed in the PLA matrix have the same heat of fusion as free progesterone crystals. This is a reasonable assumption for samples stored 3 and 7 days at 22°C since their progesterone melting curves are similar to that found for free progesterone crystals. It is a questionable assumption for the sample stored 0 days at 22°C because its progesterone melting curve is significantly broader than that of free progesterone crystals. This may account for why the sample stored zero days at 22°C was calculated to have a higher percent crystallinity than the samples stored 3 and 7 days.

Discussion

The absence of detectable crystalline progesterone residues in various PLA microspheres formed by the interrupted solvent evaporation process demonstrates that this technique can successfully prevent crystallization of progesterone during PLA microsphere fabrication. Nevertheless, free progesterone crystals form in the aqueous phase or on the surface of the microspheres if the water-soluble emulsifier is not removed from the aqueous phase before CH_2Cl_2 evaporation is complete. Crystallization clearly occurred in one of the samples listed in Table 1, even though an effort was made to avoid this. When the progesterone content is increased, crystallization occurs inside the microspheres, on the surface of the microspheres, or in the aqueous dispersion phase. At sufficiently high progesterone loadings, the latter two events will occur whether or not the emulsifier is removed. The tendency for the progesterone to spontaneously form free drug crystals in a variety of samples suggests that it has low miscibility with PLA. A sparsely water-soluble drug that is highly miscible with PLA would be expected to remain in the PLA rich phase and not spontaneously crystallize outside this phase.

With drugs that are freely soluble in CH_2Cl_2, crystallization inside the PLA microspheres is repressed at low drug loadings because of the nature of the solvent evaporation process. The first step of this process is to form a dilute drug–PLA–CH_2Cl_2 solution of relatively low viscosity. This solution is dispersed in an aqueous phase and the CH_2Cl_2 is allowed to evaporate. As evaporation progresses, the CH_2Cl_2 phase becomes an increasingly concentrated drug–PLA–CH_2Cl_2 solution which also becomes increasingly viscous. Since PLA has a T_g of 57–59°C, the viscosity of the PLA-containing phase will increase to infinity at 22°C as the CH_2Cl_2 concentration approaches zero. If the drug–PLA–CH_2Cl_2 phase is sufficiently viscous when the saturation solubility of drug in CH_2Cl_2 is reached, and if the rate at which the remaining CH_2Cl_2 evaporates is sufficiently high, drug crystallization in the viscous polymer matrix is effectively prevented. In this manner, drug that has little mutual miscibility with PLA can be molecularly dispersed in PLA. The dispersion is a metastable state formed because the drug does not have time to crystallize in the polymer phase before the PLA sets up as an organic glass. If the drug and PLA are mutually miscible, a true molecular solution of drug in PLA is formed, like a solution of a plasticizer in a polymer. Cases of partial drug–PLA miscibility fall between these extremes.

Complete repression of drug crystallization in a

PLA is favoured when the drug has high solubility in the casting solvent. An effort was made to determine the saturation solubility of progesterone in CH_2Cl_2 that also contained PLA. This was done by adding increasing amounts of progesterone and PLA to a fixed weight of CH_2Cl_2. A solution that contained 39.8 wt. percent progesterone and 16.3 wt. percent PLA (56.2 wt. percent total solids) remained homogeneous. Further addition of progesterone and PLA was not made because the solution was too viscous. Nevertheless, it is clear that progesterone has high solubility in CH_2Cl_2. The presence of PLA in the CH_2Cl_2 appeared to enhance progesterone solubility. Addition of PLA to a concentrated solution of progesterone in CH_2Cl_2 caused this solution to change from a turbid system to an optically clear system. Although PLA and progesterone appear to have little mutual miscibility in a solvent-free system, clarification of the concentrated progesterone–CH_2Cl_2 solution by PLA indicates that some type of interaction exists, at least in the presence of CH_2Cl_2.

The above comments emphasize that the absence of crystalline drug in a drug-loaded PLA microsphere formed by solvent evaporation does not necessarily mean that the drug is miscible with PLA. Other criteria must be used to differentiate between systems in which a drug is dissolved in PLA from those cases where it forms a metastable molecular dispersion. This can be done by examining how a drug affects the T_g of PLA. If it forms a molecular solution with PLA, the PLA T_g event will be broadened and shifted to a lower temperature. If there is little mutual miscibility the drug will have minimal effect on the T_g of PLA provided the drug loading is low. When the drug becomes the predominant component, this may no longer be the case.

A second way to make the distinction is to determine what happens to drug-loaded PLA microspheres stored for prolonged periods at temperatures well above the T_g of PLA, but below the melting temperature of the drug. In this situation, drug trapped as a molecular dispersion in the PLA glass will have its diffusivity greatly increased due to the large increase in PLA chain mobility that occurs above T_g. Under such conditions, molecularly dispersed drug molecules immiscible with PLA can diffuse together to nucleate and grow finite progesterone crystal domains. If the drug is miscible with PLA, it will remain molecularly dispersed during and after heat treatment.

Thermal history, variations in the microsphere preparation process, and residual CH_2Cl_2 content are factors that undoubtedly affected the T_g behavior of the PLA microsphere samples listed in Table 1 and contributed to the range of T_g values reported. Accordingly, the value of these data for defining the degree of mutual miscibility of progesterone with PLA in the unannealed microspheres of this study is limited. In contrast, the T_g data obtained after progesterone-loaded (23%) PLA microspheres were treated at 110°C provide direct evidence that progesterone and PLA have little mutual miscibility. A significant fraction of the progesterone initially trapped as a molecular dispersion in the PLA glass crystallizes during the heat treatment process. Once the progesterone crystallizes, the PLA develops a well-defined T_g event that approaches the T_g found for PLA free of progesterone. The presence of progesterone in a heat-treated system does not cause a major change in the T_g event of PLA. This leads to the conclusion that PLA and progesterone have little mutual miscibility. The slightly lower T_g event observed with heat-treated, progesterone-loaded PLA (53°C vs 57°C for drug-free PLA) may be evidence that a small degree of miscibility exists. It may also reflect a lower PLA MW caused by chain scission during the 110°C annealing process.

The variable behavior of the PLA T_g event reported in Tables 1 and 2 is interesting. Entrapped CH_2Cl_2 is assumed to be a factor at least partially responsible for the variations in T_g shown, although to what degree remains to be defined. Even with this complication, one general trend is visible. The T_g event of annealed and unannealed samples changes on storage at 22°C. DTA T_g events measured immediately after the microspheres are formed or annealed can be too diffuse to assign a meaningful T_g event. As the storage time at 22°C increases, the T_g event becomes more visible and a reasonable T_g value can be assigned. In a number of cases, this event becomes well-defined and develops an endothermic peak. The de-

velopment with time of an endothermic peak at the T_g event is a phenomenon characteristic of amorphous polymers stored in the vicinity of T_g or cooled slowly through T_g from higher temperature. Two explanations for this peak have been suggested. One view is that it is due to formation of order in the polymer glass; another view is that it reflects relaxation of stresses and strains arising from non-equilibrium cooling. Petrie (1972) gives a good overview of the subject from the later viewpoint.

Regardless of the origin of this peak, it is relevant to note that progesterone-loaded (23%) samples free of crystalline progesterone retain a diffuse T_g event without an endothermic peak after 4–12 weeks of storage at 22°C. In contrast heat-treated samples with well-defined progesterone crystal domains have a T_g event with an endothermic peak after a few days storage at 22°C. Although this difference in behavior may be related to entrapped CH_2Cl_2 present in the unannealed samples, another factor to consider is the degree of molecular dispersion of progesterone in the PLA. A significant fraction of the progesterone in heat-treated samples is located in well-defined crystal domains. It is not uniformly dispersed throughout the PLA matrix. Some regions are relatively depleted of progesterone and will behave more like drug-free PLA. When the progesterone molecules are uniformly dispersed throughout the PLA, they offer the greatest resistance to the relaxation or ordering phenomenon responsible for development of a well-defined T_g event. This, in turn, could contribute to the observed difference in rate of aging of the PLA T_g event and perhaps even affect the absolute value of T_g obtained.

The effect that degree of drug dispersion and amount of drug loaded into PLA have on the T_g event of PLA warrants further investigation with drug-loaded microspheres shown to be free of residual solvent. The conditions needed to achieve this without simultaneously affecting microsphere morphology remain to be defined.

Acknowledgement

The authors gratefully thank Mr. D. Allie of the Perkin-Elmer Corporation, Cincinnati, for running the DSC scans.

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