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Effects of storage on the physicochemical properties and release characteristics of progesterone-loaded poly(*l*-lactide) microspheres

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Summary

Changes in the physicochemical properties of poly(*l*-lactide) microspheres that occurred during storage were studied by X-ray powder diffraction, differential scanning calorimetry and scanning electron microscopy, in order to elucidate the factors that affect the stability of drug release characteristics. Progesterone-loaded microspheres with amorphous polymer matrices were stored at 50 and 30°C under desiccated and moist atmospheres. The surface morphology did not change significantly during storage under any of the conditions studied. Storing the microspheres at a temperature above the glass transition temperature (T_g) of the polymer under moist conditions caused polymer matrix crystallization. The drug release rate of stored microspheres was faster than that of microspheres before storage, which indicates that matrix crystallization increased the drug release rate. In contrast, the polymer matrices of microspheres stored at a temperature below the T_g remained amorphous after 7 months storage under both moist and desiccated conditions. The T_g of microspheres stored at a high relative humidity decreased considerably during storage, which may be attributable to the plasticizing effect of water absorption by microspheres as well as to the decreased polymer molecular weight resulting from decomposition of polymer molecules. The drug release of microspheres stored under moist conditions was faster than that of those stored dry and appeared to correlate with the polymer matrix T_g. The hydrolysis of polymer molecules may decrease the rigidity of the polymer matrices, as indicated by the decrease in the T_g. This may result in rapid drug release.

Introduction

Biodegradable polyesters, such as poly(*l*-lactide) and poly(*d*,*l*-lactide/glycolide), have been used as controlled-release drug delivery systems, the drug release characteristics of which are affected by many factors (Benita et al., 1984; Benoit et al., Spenlehauer et al., 1986; Bodmeier and McGinity, 1987; Sato et al., 1988; Jalil and Nixon, 1989, 1990a; Redmon et al., 1989; Kishida et al., 1990; Izumikawa et al., 1991). We reported that the degree of crystallinity of progesterone-loaded poly(*l*-lactide) microspheres correlated closely with the release rates. Microspheres with amorphous polymer matrices exhibited much slower drug release than those with crystalline polymer matrices (Izumikawa et al., 1991).

Although it is important to elucidate the fac-

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tors that affect the stability of drug release characteristics of these systems, only a few workers have described the effects of storage on the physicochemical properties of polyester microspheres. The crystallinity of progesterone dispersed in poly(*d*,*l*-lactide/glycolide) matrices changed during annealing at 60 and 80°C (Rosilio et al., 1991). Storage of poly(*d*,*l*-lactide) microcapsules at 37°C for 6 months caused microcapsular shrinkage and reduced the release rate (Jalil and Nixon, 1990b). Crystallization of poly(*l*-lactide/glycolide) matrices during storage in aqueous media at 37°C for 40 weeks has been reported (Vert et al., 1991).

In this study, we investigated the stability of the drug release characteristics of poly(*l*-lactide) microspheres during storage and focused on the changes in their physicochemical properties, in particular the polymer matrix crystallinity and surface morphology. The effects of change in crystallinity and the glass transition temperature of the polymer matrices on their release characteristics are discussed.

Materials and Methods

Materials

Poly(*l*-lactide) and progesterone were obtained from Kokusan Chemical Co. (Tokyo) and Sigma (St. Louis, Mo) respectively. The weight average molecular weight of poly(*l*-lactide) was determined to be 13 000 by gel-permeation chromatography using polystyrene as molecular weight standard. The polydispersity of the polymer was about 2.6. All the other chemicals used were of reagent grade.

Microspheres with amorphous polymer matrices were prepared by the reduced pressure-solvent evaporation method (RSE), as described in our previous paper (Izumikawa et al., 1991). 1 g poly(*l*-lactide) and 111 mg progesterone were first dissolved in 10 ml dichloromethane, and then added to 250 ml aqueous gelatin 1%(w/v) solution containing 3 M NaCl, which was stirred at 400 rpm. Dichloromethane was removed by stirring under reduced pressure (about 200 mmHg) at 25°C for 3 h. The microspheres in the 45–90 μm sieve fraction were collected, washed with cold water and freeze-dried. It has been reported that progesterone in the RSE microspheres are molecularly dispersed in the matrices at this drug loading (Izumikawa et al., 1991). In order to remove any residual dichloromethane, these microspheres were heated at 50°C for 2 days, after which the residual dichloromethane content was determined to be less than 50 ppm by gas chromatography (packing, silicon DCQF-1 20% Chromosorb P 60/80; column, glass 3 mm × 2 m; temperature, 60°C; carrier, nitrogen 40 ml/min). The release characteristics of the microspheres and surface morphology of their polymer matrices were not affected by this treatment.

Microspheres with crystalline polymer matrices were also prepared by the atmosphere-solvent evaporation method (ASE), which is similar to the RSE method except that solvent evaporation was carried out under atmospheric pressure for 16 h, as described in our previous paper (Izumikawa et al., 1991).

Stability test

Samples of RSE microspheres were placed in 10 ml glass vessels, which were put into 50 ml screw capped bottles, in which the relative humidity (RH) was adjusted with silica gel (for 0% RH) or a saturated solution of LiCl (11% RH), NaBr (50% RH) or NaCl (75% RH). The bottles were capped tightly and stored in a thermostated water bath maintained at 30 or 50°C.

Characterization of microspheres

The surface morphology of the microspheres was examined by scanning electron microscopy (JEOL JSM-840A, Tokyo), as described previously (Izumikawa et al., 1991). Samples were prepared by sputter-coating with Au/Pd in an ion coater (Eiko IB-3, Tokyo) at 40 mA for about 2 min, at a pressure of < 0.2 Torr. Micrographs were obtained at an intensity of 15 kV.

The crystallinity of the polymer matrices was examined by differential scanning calorimetry (DSC) and X-ray powder diffraction, as described previously (Izumikawa et al., 1991). The DSC curves of the microspheres were obtained using a Shimadzu DS-40 system (Kyoto) and samples in sealed aluminum pans were heated at 2°C/min. The glass transition temperature (T_g) of the polymer matrices was measured as the midpoint of the transition curve. The X-ray powder diffraction diagrams were measured using a Rigaku Denki RAD-2C system (Tokyo) with Ni-filtered Cu-Kα radiation (30 kV, 10 mA) at a scanning speed of 2°/min.

The progesterone content of the microspheres were assayed by high-performance liquid chromatography (HPLC) as described previously (Izumikawa et al., 1991). Microsphere samples (10 mg) were dissolved in 2 ml acetonitrile, which contained betamethasone valerate as an internal standard (0.5 mg/ml), the poly(*l*-lactide) in the solution was precipitated by adding 8 ml methanol, followed by centrifugation at 3000 rpm for 5 min, the resultant supernatant was diluted with an aqueous solution of 70% acetonitrile and injected into a Hitatch model 655A high-performance liquid chromatograph (Tokyo), equipped with a TSK gel ODS-80TM column (4.6 mm × 150 mm, TOSOH, Tokyo), which was maintained at 35°C. The mobile phase solvent was an aqueous solution of 70% acetonitrile, which was delivered at a flow rate of 1 ml/min, and the column eluate

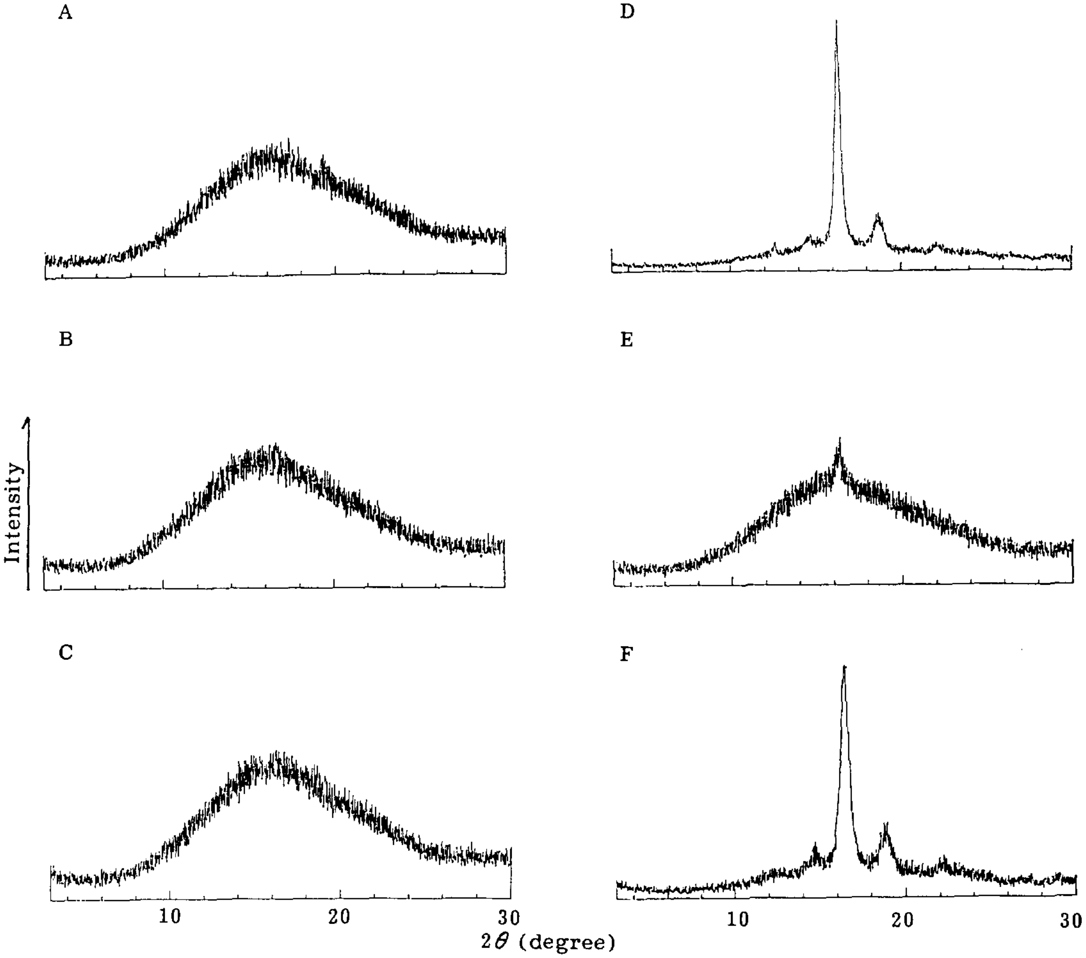

Fig. 1. X-ray powder diffraction scan of progesterone-loaded poly(*l*-lactide) microspheres (A–E) and scan of poly(*l*-lactide) powder (F): (A) before storage, (B) stored at 30°C and 75% RH for 7 months, (C) stored at 30°C and 0% RH for 7 months, (D) stored at 50°C and 11% RH for 6 months, (E) stored at 50°C and 0% RH for 6 months.

was monitored at 240 nm. The progesterone content in the microspheres was determined to be 10.0 (w/w)% and was not changed during storage under conditions studied.

Release studies were carried out according to the second method of the Japan Pharmacopoeia XII dissolution test as described in our previous paper (Izumikawa et al., 1991). Microsphere samples (50 mg) were added to 900 ml of 50 mM phosphate buffer, pH 7.4, containing 0.1% Tween 80. The solution was maintained at 37°C and stirred with a paddle at 100 rpm. Portions (4 ml) of the solution were removed through a 0.45 μm membrane filter at appropriate intervals and the volume of the removed sample was replaced. The progesterone in the solution (1 ml) was diluted with 5 ml of 3 M NaCl and extracted with chloroform (3 ml). The chloroform was removed under nitrogen, the residue was dissolved in an aqueous solution of 70% acetonitrile and injected into the HPLC system. All the release rates were determined in duplicate.

The content of terminal carboxyl groups in the poly(*l*-lactide) was determined by an acid-base titration method (Metrohm E682, Switzerland). Microsphere samples (20 mg) were dissolved in 10 ml dichloromethane, 10 ml benzyl alcohol was then added to the solution, which was titrated against a solution of 2.5 mM potassium hydroxide in benzyl alcohol. All the determinations were carried out in duplicate or triplicate.

Results and Discussion

Effect of storage on crystallinity and rigidity of polymer matrices of the microspheres

The RSE microspheres obtained by the reduced pressure-solvent evaporation method had amorphous polymer matrices and smooth surface morphology, as reported in our previous paper (Izumikawa et al., 1991). The surface morphology did not change significantly during storage under any of the experimental conditions (data not shown). The glass transition temperature (T_g) of these microspheres before storage was about 42°C.

Fig. 1 shows the X-ray diffraction scans of the microspheres with amorphous polymer matrices stored under various conditions. Those stored at 30°C and both 0 and 75% RH for 7 months exhibited no diffraction peaks, which indicates that the poly(*l*-lactide) remained in the amorphous state throughout. In contrast, the microspheres stored at 50°C and 11% RH for 6 months exhibited diffraction peaks at $2\theta = 17$, 19 and 22°. A small diffraction peak at $2\theta = 17°$ was also observed with the microspheres stored at 50°C and 0% RH for 6 months. These peaks coincided with those obtained with poly(*l*-lactide) powder and were, therefore, ascribed to crystalline poly(*l*-lactide). Crystallization of the polymer matrices of microspheres stored at 50°C and 11% RH occurred more rapidly than in those stored at 0% RH, which suggests that moisture accelerates the crystallization process.

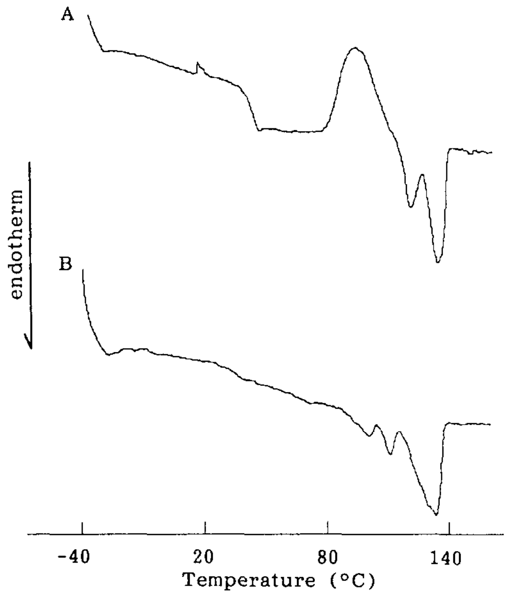

Fig. 2. DSC curves of poly(*l*-lactide) microspheres: (A) before storage, (B) stored at 50°C and 11% RH for 6 months.

Thermal analysis of the stored microspheres supported the X-ray diffraction data. Before storage, the microspheres subjected to DSC exhibited an exothermic peak at about 94°C, as shown in Fig. 2A, which was ascribed to matrix transformation from the amorphous to the crystalline state during DSC (Izumikawa et al., 1991). A similar exothermic peak was observed with microspheres

TABLE I

Effect of storage on glass transition temperature, crystallization peak temperature and heat of crystallization of microspheres, and content of terminal carboxyl groups in the polymer

Condition	Time (months)	T_g (°C)	T_c (°C)	H_c (mJ/g)	[COOH] (mmol/g)
50°C 11%RH	0	41.8	93.4	19	0.21
	1	40.8	93.8	19	
	2	42.4	92.8	20	
	3	39.1	86.2	16	
	4	41.3	86.2	13	
	6	N.D.	N.D.	N.D.	0.31
50°C 0%RH	0	41.8	93.4	19	
	1	42.7	96.4	20	
	2	42.0	94.1	21	
	3	41.6	94.1	19	
	4	42.7	93.9	20	
	5	45.1	96.4	18	
	6	45.6	96.2	20	
30°C 75%RH	0	41.8	93.4	19	
	1	39.6	90.8	20	
	3	37.8	88.7	21	
	5	37.2	89.1	19	0.30
	7	34.8	89.0	18	0.35
30°C 50%RH	0	41.8	93.4	19	
	1	41.8	93.4	19	
	3	42.3	92.4	20	
	5	44.6	93.3	20	0.24
	7	43.3	93.1	21	0.23
30°C 0%RH	0	41.8	93.4	19	
	1	45.6	96.3	17	
	3	46.1	95.1	21	
	5	48.8	95.2	19	
	7	50.6	98.9	17	

N.D., not detected.

stored at 30°C, whereas the exothermic peak of microspheres stored at 50°C and 11% RH became smaller as the storage time increased and disappeared after 6 months, as shown in Fig. 2B.

The effects of storage on the T_g, crystallization peak temperature (T_c) and heat of crystallization (H_c) estimated from the DSC exothermic peak areas are summarized in Table 1. No significant change of the H_c was observed with the microspheres stored at 30°C, in which the matrices did not crystallize during storage for up to 7 months. The H_c of the microspheres stored at 50°C and 11% RH decreased during storage, which indicates that the polymer matrices crystallized during storage. The T_g of the microspheres stored at 50°C did not change appreciably. The T_g of the microspheres stored at 30°C and both 0 and 50% RH increased, whereas that of the microspheres stored at 30°C and 75% RH decreased. The T_c values of the stored microspheres varied similarly to the T_g values except that they decreased during storage at 50°C and 11% RH. The T_g and T_c increases observed with the microspheres stored at 30°C and both 0 and 50% RH may be ascribed to an annealing effect (Jalil and Nixon, 1990b), which renders the polymer matrices more rigid. The T_g and T_c decreases observed with microspheres stored at 30°C and 75% RH may be attributed to the plasticizing effect of water absorption by the microspheres.

The content of terminal carboxyl groups increased in the microspheres during storage at 50°C and 11% RH and at 30°C and 75% RH, which indicates that the polymer molecules decomposed and that the polymer molecular weight decreased during storage. The T_g of poly(*d,l*-lactide) has been demonstrated to depend on its molecular weight (Omelczulk and McGinity, 1992). The T_g and T_c decreases of the microspheres stored at 30°C and 75% RH may also be attributable to the polymer decomposition of molecules.

Crystallization of the polymer matrices did not occur in the microspheres stored at 30°C, as this temperature was lower than the T_g. The T_g and T_c decreases of the microspheres during storage at 30°C and 75% RH, however, suggest that the physicochemical properties of the polymer matrices underwent changes which may have resulted in matrix crystallization if the microspheres had been stored for longer.

Effect of storage on drug release from the microspheres

Fig. 3 shows typical release profiles for the microspheres stored under various conditions. Prior to storage, the microspheres exhibited a slow initial release phase, which was followed by faster release. The microspheres stored under humid conditions at 50 and 30°C released progesterone faster and the duration of the initial slow release phase was shorter than prior to storage. Fig. 3 also shows the release profile of the ASE microspheres with crystalline polymer matrices, which were prepared by the atmosphere-solvent evaporation method. The ASE microspheres released progesterone very rapidly. The release rate from the RSE microspheres in which the matrices crystallized during storage at 50°C and 11% RH was slower than that of the ASE microspheres.

Fig. 4 shows the relationship between the T_g values of the stored microspheres and the drug release rates, which were approximated by the percentage of drug released over a 72 h period. The microspheres with lower T_g values tended to release progesterone faster than those with higher T_g values. The polymer molecule hydrolysis may decrease the rigidity of matrices, as indicated by the decrease in the T_g, which may result in rapid drug release. The decrease of the rigidity may be due to the change in physicochemical property of the matrices such as plasticity and porosity.

The release rate of microspheres stored at 50°C and 11% RH for 6 months was estimated from the release rate vs T_g profile using the T_g value of the microspheres stored at 50°C and 11% RH for 4 months, as the T_g could not be determined after crystallization had occurred. The observed drug release for 72 h (60%) was larger than the estimated drug release (40%), which suggests that polymer matrix crystallization may be another cause of rapid drug release. Matrix crystallization may affect drug distribution in the matrices and polosity of the matrices.

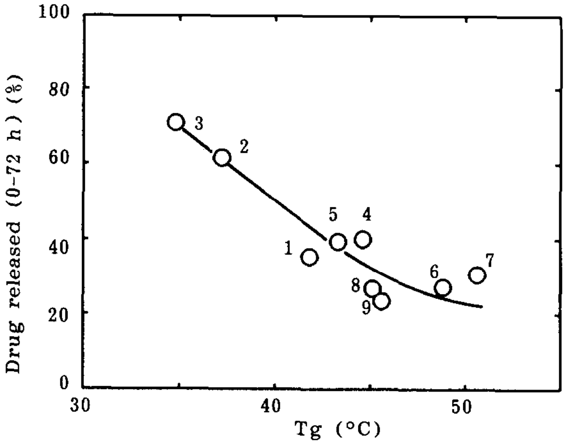

Fig. 4. Relationship between drug release (0–72 h) and T_g of poly(*l*-lactide) microspheres: (1) before storage, (2) stored at 30°C and 75% RH for 5 months, (3) stored at 30°C and 75% RH for 7 months, (4) stored at 30°C and 50% RH for 5 months, (5) stored at 30°C and 50% RH for 7 months, (6) stored at 30°C and 0% RH for 5 months, (7) stored at 30°C and 0% RH for 7 months, (8) stored at 50°C and 0% RH for 5 months, (9) stored at 50°C and 0% RH for 6 months.

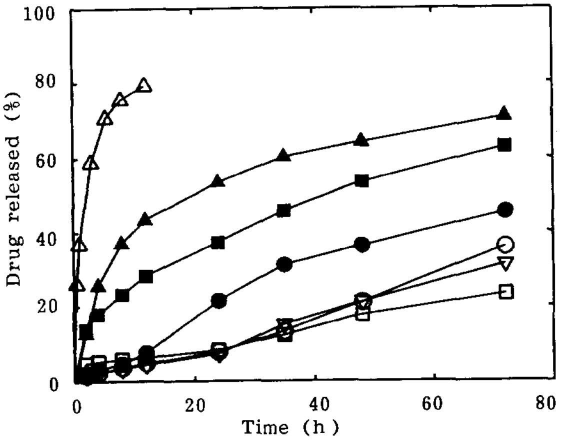

Fig. 3. Typical release profiles of progesterone from poly(*l*-lactide) microspheres: (○) before storage, (▲) stored at 30°C and 75% RH for 7 months, (●) stored at 30°C and 50% RH for 7 months, (▽) stored at 30°C and 0% RH for 7 months, (■) stored at 50°C and 11% RH for 6 months, (□) stored at 50°C and 0% RH for 6 months, (△) microspheres prepared by the atmosphere solvent evaporation method.

The decreased release rate observed during the later stages of the release study of microspheres stored at 50 and 30°C under desiccated conditions may be ascribed to the annealing effect of the polymer matrices (Jalil and Nixon, 1990b).

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