Characterization of Drug-Loaded Poly(*d*,*l*-lactide) Microspheres

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Abstract □ Lomustine and progesterone have been incorporated in biodegradable poly(*d*,*l*-lactide) microspheres by evaporating dichloromethane from stirred dichloromethane-in-water emulsions. Spherical microspheres with lomustine or progesterone payloads ≤23% were obtained. Higher lomustine payloads gave irregularly shaped particles. Microspheres with ≤68% progesterone were obtained, but free drug crystals formed on the surface of such microspheres. Increased agitation rates decreased mean microsphere size. Addition of drug to the dichloromethane phase increased average particle size relative to that obtained with drug-free microspheres prepared under the same experimental conditions. Complete evaporation of the dichloromethane, while the medium was continuously stirred, promoted formation of free drug crystals in the aqueous phase. Increased emulsifier concentrations did not significantly enhance drug incorporation efficiency within the microspheres. Shelf-life stability of lomustine and progesterone was reduced by incorporation in the microspheres, presumably due to their molecular dispersion in the poly(*d*,*l*-lactide).

Keyphrases □ Poly(*d*,*l*-lactide) microspheres—loaded with lomustine, progesterone, effect of emulsifier concentrations, agitation rates on formation, stability □ Microspheres—poly(*d*,*l*-lactide), progesterone, effect of emulsifier concentration, stability

Parenteral controlled-release systems which are capable of being targeted to tissue sites and to deliver their active ingredient over a long period of time are of considerable interest (1). These potential sustained-release drug-targeting dosage forms require the use of biodegradable polymers as drug carriers. Candidate biodegradable materials include gelatin (2), albumin (3, 4), poly(lactide-glycolide) copolymers (5), and polylactide (6–9). Progesterone has been incorporated in poly(*d*,*l*-lactide) microspheres in order to design an injectable long-acting delivery system. Such a dosage form administered *in vivo* yielded promising pharmacological action (10). Since little has been reported about the physical properties of such microspheres, a study of their properties as a function of the preparative conditions was carried out. Poly(*d*,*l*-lactide) microspheres containing either progesterone or lomustine were prepared. The ultimate objective is to use the latter to produce tumor embolism by intra-arterial infusion. This therapeutic method has been shown by Kato *et al.* (11), using mitomycin C microcapsules, to be an effective means of combining vascular mechanical obstruction and slow release of active mitomycin C within tumors.

EXPERIMENTAL SECTION

Materials—Poly(*d*,*l*-lactide) was supplied by D. N. Mason[1] and had a mean mol. wt. of 61,000. Two polymeric emulsifiers were used: 88% hydrolyzed polyvinyl alcohol[2] and methylcellulose (10 and 400 cps grades)[3]. Progesterone[4] and lomustine[5] [*N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea] were used as supplied. Dichloromethane[6], Analar grade, was employed without further purification.

Methods—Microspheres were formed at atmospheric pressure by a process similar to that described by Beck *et al.* (10). The procedure involved placing, in a 400-mL glass beaker, 250 mL of water that contained polyvinyl alcohol or methylcellulose. A solution that contained 20 mL of CH_2Cl_2, 927 mg of poly(*d*,*l*-lactide), and a known weight of drug (280–2,000 mg), was poured rapidly into the aqueous phase as it was stirred at a constant rate by a stirrer fitted with a digital revolution counter[7]. The resulting emulsion was agitated at 22° C for a known period of time during which the CH_2Cl_2 evaporated. In a few cases, the system was agitated continuously until CH_2Cl_2 evaporation was complete. This is the continuous evaporation process. For most runs, the interrupted evaporation process was used; agitation was stopped before CH_2Cl_2 evaporation was complete, the partially dried microspheres were allowed to settle, and the aqueous phase that contained the polymeric dispersing agent was replaced with deionized water by three washing and decantation steps. Once the microspheres were resuspended in emulsifier-free water, they were agitated 7–17 h so that CH_2Cl_2 evaporation could proceed to completion. Removal of the polymeric emulsifier from the aqueous phase before completion of CH_2Cl_2 evaporation minimizes formation of free drug crystals in the aqueous phase or on the surface of the microspheres (12).

On completion of CH_2Cl_2 evaporation, the microspheres were isolated by filtration, washed, and dried under reduced pressure at 22° C for 20 h. The dried microspheres were sieved and stored in a desiccator until used. The preparative parameters varied in this study include: polymeric emulsifier and emulsifier concentration, amount and type of drug incorporated in the microspheres, rate of agitation, and length of agitation. Theoretical microsphere drug contents (or payloads) specified in this paper represent the percent by weight drug carried by a microsphere if all the drug and all the poly(*d*,*l*-lactide) added to an encapsulation system were incorporated in the microspheres. Actual or measured drug contents were usually lower than the theoretical values due to drug loss to the aqueous phase during CH_2Cl_2 evaporation.

Microsphere Evaluation—*Progesterone Content*—Progesterone-loaded microspheres (30 mg) were dissolved in 2 mL of CH_2Cl_2. Ethanol (23 mL) was added to precipitate the poly(*d*,*l*-lactide). The resulting suspension was centrifuged at 20,000 rpm for 10 min. The clear supernatant was diluted with ethanol and 10 μL of the resulting solution was injected into an HPLC[8]. The chromatograph operating conditions were: C_8 reverse-phase column (10-μm spheres); methanol eluant; eluant flow rate 2 mL/min; 254 nm detector. Under these conditions, progesterone had a retention time of 97 s. The progesterone content of sample solutions was obtained from a progesterone calibration curve constructed from standard progesterone solutions.

Lomustine Content—Lomustine-loaded microspheres (30 mg) were dissolved in 2 mL of CH_2Cl_2. Ethanol (23 mL) was added to precipitate the poly(*d*,*l*-lactide). After centrifugation at 20,000 rpm for 10 min, the clear supernatant (10 μL) was subjected to HPLC analysis. The HPLC operating conditions were the same as those used for progesterone assays, except that the eluant was an acetonitrile-water mixture (75:25, v/v). The lomustine retention time was 143 s. Control experiments with cyclohexylamine and 2-chloroethanol, two known lomustine degradation products, established that neither compound gave an interfering peak within 240 s of elution. Lomustine contents of all samples were obtained from a calibration curve constructed from standard lomustine solutions.

Microscopy Studies—Optical and scanning electron microscopy were used to evaluate the drug incorporation and surface characteristics of the microspheres prepared under the various conditions used. The optical microscope[9] and the electron microscope[10] were equipped with cameras.

RESULTS AND DISCUSSION

Characterization of the Microspheres Formed—The presence of an emulsifying agent is vital to the successful formation of individual spherical microspheres by the solvent evaporation process. The emulsifiers used in this

[1] Chemical Engineering Dept., Washington University, St. Louis, Mo.
[2] Vinol 205; Air Products and Chemicals, Allentown, Pa.
[3] Methocel; Dow Chemical Co., Midland, Mich.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Roger Bellon Laboratories, Neuilly/Seine, France.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Model RZR-2000; Heidolph Elektro, Kelheim, Germany.
[8] SP 8000; Spectra Physics, Santa Clara, Calif.
[9] American Optical, Rochester, N.Y.
[10] Model HHS-2R; Hitachi, Tokyo, Japan.

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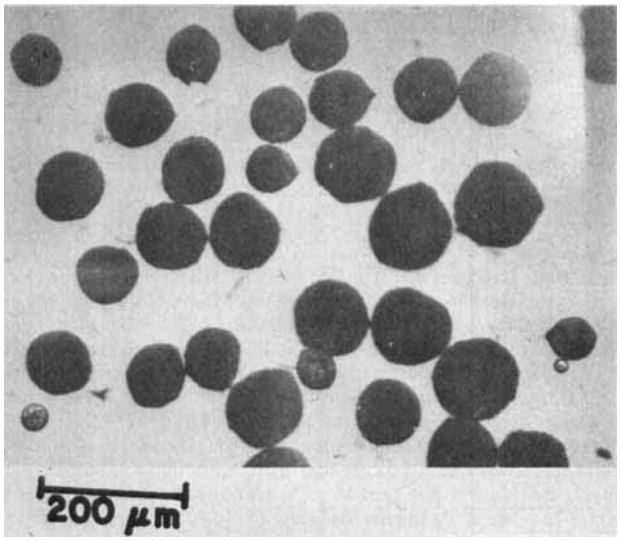

Figure 1—*Photomicrograph of lomustine-loaded microspheres prepared by the continuous evaporation process at 250 rpm with 0.05% (w/w) methylcellulose as emulsifier. Theoretical lomustine content: 35% (w/w). Magnification: 100×.*

study, partially hydrolyzed polyvinyl alcohol and methycellulose, gave suitable microspheres, although there was some difference in their performance. When lomustine was dissolved in the CH_2Cl_2 phase, along with poly(*d,l*-lactide), both emulsifiers gave spherical microspheres as long as the initial lomustine concentration was not >280 mg/20 mL of CH_2Cl_2. Higher lomustine concentrations gave irregularly shaped particles (Fig. 1).

Dichloromethane-poly(*d,l*-lactide) solutions that contained ≤2000 mg of progesterone/20 mL of CH_2Cl_2 formed stable emulsions in water when methylcellulose was the emulsifier. Higher progesterone concentrations gave misshapen particles or unstable emulsions. For this reason, poly(*d,l*-lactide) microspheres with a >68.3% (w/w) progesterone payload were not prepared. When polyvinyl alcohol was the emulsifier, solutions containing >0.5 g of progesterone/20 mL of CH_2Cl_2 consistently formed unstable emulsions. Accordingly, poly(*d,l*-lactide) microspheres with progesterone payloads >35% (w/w) were not prepared with this emulsifier.

Although polyvinyl alcohol and methylcellulose formed suitable CH_2Cl_2-in-water emulsions for a range of initial lomustine and progesterone solutions, spontaneous crystallization of these drugs occurred either in the aqueous phase or on the surface of the microspheres when either emulsifier was left in the aqueous phase until CH_2Cl_2 evaporation was complete. With lomustine, the free crystals usually appear as well-defined rods in the aqueous phase (Fig. 1). With progesterone, the free crystals also appear in the aqueous phase, but as very fine particles.

Crystal formation is eliminated or greatly reduced for 50–600-μm microspheres that have drug payloads ≤25% (w/w) if agitation is stopped well before solvent evaporation is complete and the emulsifier is removed from the aqueous phase. Solvent evaporation is then taken to completion in emulsifier-free water. If the emulsifier is removed too soon, the microspheres agglomerate because they contain too much CH_2Cl_2. If the emulsifier is removed too late, free crystals appear. Thus, there is an optimal time interval during which emulsifier removal can occur without microsphere agglomeration or free crystal formation (12). This time interval (or window) is defined experimentally for each formulation. For microspheres prepared at an agitation rate of 150 rpm, the time interval typically varied from 200 to 240 min.

Microspheres that contain ≤25% (w/w) progesterone or lomustine are spherical and have very smooth surfaces, if free crystals do not form. Figure

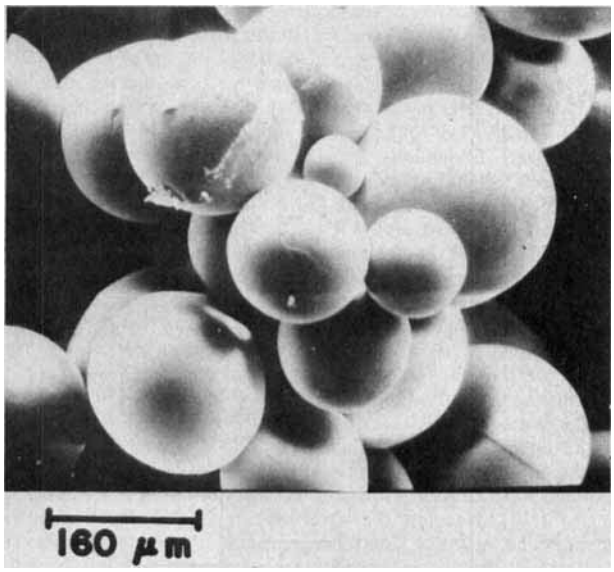

Figure 2—*Scanning electron micrograph of microspheres prepared by the interrupted evaporation process at 150 rpm with 0.27% (w/w) polyvinyl alcohol as emulsifier. Theoretical lomustine content: 23.2% (w/w). Magnification: 125×.*

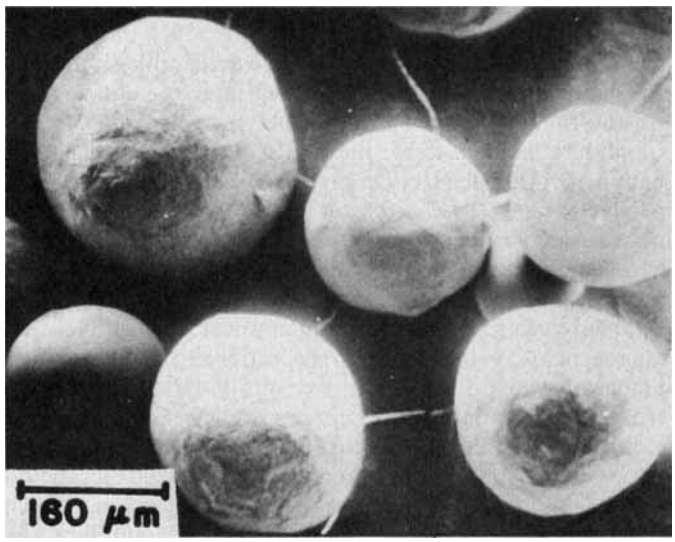

Figure 3—*Scanning electron micrograph of microspheres prepared by the continuous evaporation process at 150 rpm with 0.27% (w/w) polyvinyl alcohol as emulsifier. Theoretical lomustine content: 23.2% (w/w). Magnification: 125×.*

Table I—Drug Content of Poly(*d,l*-lactide) Microspheres Formed by the Solvent Evaporation Process [a]

Drug	Evaporation Process	Agitation Rate, rpm	Formation of Free Drug Crystals	Measured Drug Content, %[b]	Drug loss, %[c]
Lomustine	Continuous	150	+	20.2	12.9
		150	+	17.8	23.3
	Interrupted	150	−	22.0	5.2
		150	−	20.4	12.1
Progesterone	Continuous	150	+	21.4	7.6
		250	+	20.5	11.7
	Interrupted	300	−	22.3	3.9
		300	−	24.0	—

[a] All microspheres had a theoretical drug content of 23.2% (w/w) and were made with 0.27% polyvinyl alcohol as the emulsifier. [b] Mean of three determinations. [c] [(Theoretical drug content − measured drug content)/(theoretical drug content)] × 100.

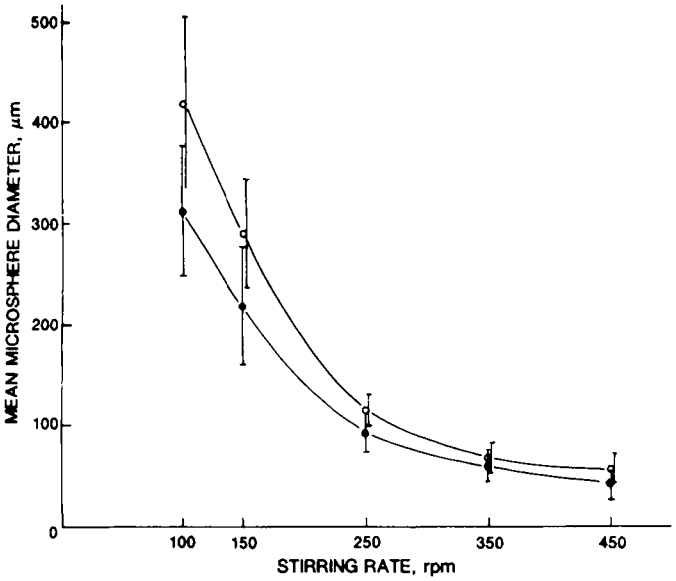

Figure 4—*Effect of stirring rate on mean size of drug-free and progesterone-loaded (23.2%, w/w) microspheres prepared with 0.27% (w/w) polyvinyl alcohol as emulsifier. Key: (●) empty microspheres; (○) progesterone-loaded microspheres.*

Table II—Effect of Stirring Rate on Lomustine Microsphere Content and Size [a]

Stirring Rate, rpm	Measured Drug Content, %[b]	Drug Loss, %[c]	Mean Particle Size, μm ± *SD*
150	20.2	12.9	289.9 ± 88.3
250	19.8	14.7	102.5 ± 25.1
350	19.7	15.1	71.0 ± 16.6
450	19.9	14.2	58.2 ± 15.6

[a] All microspheres had a theoretical content of 23.2% (w/w) lomustine and were made by the continuous evaporation process with 0.27% polyvinyl alcohol as the emulsifier. [b] Mean of three determinations. [c] [(Theoretical drug content − measured drug content)/(theoretical drug content)] × 100.

2 illustrates the type of smooth surface obtained. There is no evidence of macroscopic pores. Formation of free progesterone crystals seems to have no effect on the surface structure of poly(*d,l*-lactide) microspheres when the theoretical progesterone payload is ≤31% (w/w). This is not the case for lomustine-loaded microspheres. Figure 3 is a scanning electron micrograph of such microspheres that had a theoretical lomustine payload of 23% (w/w) and were made under conditions that allowed free lomustine crystals to form. The surface is rippled and rough. In most cases, free lomustine crystals are not visible on the surface of the microspheres.

As long as drug payloads are kept below ~25% (w/w) for lomustine and 32% (w/w) for progesterone, most free drug crystals that form either float in the water phase or are so loosely attached to the microsphere surface that they wash off during the isolation step. The loss of free drug crystals reduces the amount of lomustine or progesterone incorporated in the microspheres. Measured lomustine and progesterone losses in such cases range from 7.6 to 23.2% (Table I). Drug losses are reduced when microspheres are prepared by the interrupted evaporation process and free drug crystals do not form. However, efficiency of drug incorporation always is relatively high whether or not free crystals form. Large amounts of progesterone and lomustine are not partitioned from the CH_2Cl_2 phase into the aqueous phase during the multihour fabrication process. Since lomustine is sensitive to water, it is significant that extensive degradation did not occur during fabrication of the microspheres.

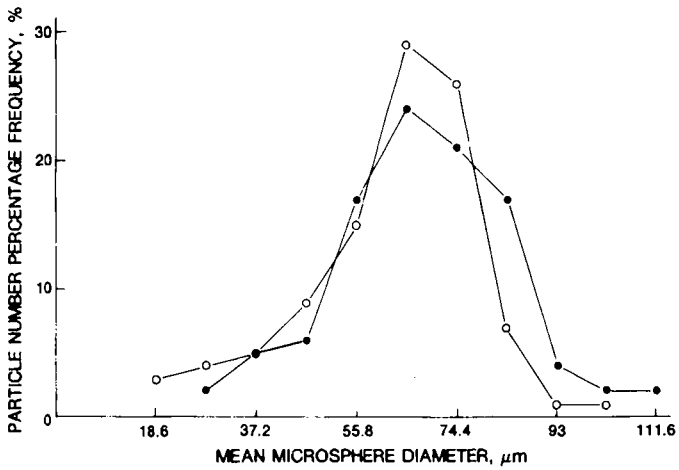

Figure 5—*Size distribution of drug-free and progesterone-loaded (23.2%, w/w) microspheres prepared at 350 rpm by the continuous evaporation process with 0.27% (w/w) polyvinyl alcohol as emulsifier. Key: (○) empty microspheres; (●) progesterone-loaded microspheres.*

Effect of Stirring Rate—Increasing the stirring rate decreases the mean diameter of the microspheres and reduces the width of the size distribution (Fig. 4, Table II). Incorporation of progesterone (23%, w/w) within the microspheres gives larger microspheres than drug-free microspheres prepared under identical conditions. However, the mean size and size distribution of drug-free and progesterone-loaded microspheres converge as the agitation rate is increased to 350 rpm (Fig. 5). The larger size of progesterone-loaded microspheres formed at lower agitation rates may reflect an increase in viscosity of the poly(*d,l*-lactide)–CH_2Cl_2 solution caused by the progesterone. Stirring rate had no detectable influence on drug content of the microspheres.

Effect of Initial Emulsifying Agent Concentration—Table III contains comments about initial polyvinyl alcohol concentration effects on microsphere properties. For a constant rate of agitation, microsphere size increased as the polyvinyl alcohol concentration increased due to an increase in viscosity of the aqueous phase. The high viscosity of the 5% (w/w) polyvinyl alcohol so-

Table III—Effect of Initial Polyvinyl Alcohol Concentration on Progesterone Microsphere Yield [a]

Polyvinyl Alcohol Concentration, %	Microsphere Recovery Yield, %[b]	Comments on Microspheres Formed
0.27	77	Individual spherical microspheres; no free crystals outside
2.7	81.6	Individual spherical microspheres; no free crystals outside
5.0	95.1	Individual spherical microspheres; no free crystals outside, but aggregation

[a] All microspheres had a theoretical payload of 23.2% (w/w) progesterone and were made by the interrupted evaporation process with 0.27% polyvinyl alcohol as the emulsifier. [b] 100 × [(Weight solid isolated microspheres recovered)/(weight of progesterone and poly(*d,l*-lactide) added to system)].

Table IV—Effect of Progesterone Payload on Microsphere Properties [a]

Theoretical Progesterone Content, %	Emulsifier	Microsphere Yield, %	Comments on Microspheres Formed
23.2	0.27% PVA[b]	81.6	Individual spherical microspheres; no free crystals outside
31.7	0.27% PVA	82.0	Individual spherical microspheres; no free crystals outside
35.0	0.27% PVA	84.2	Individuals with drug crystals embedded on the surface and outside
51.9	0.27% MC[c]	95.8	Individuals with drug crystals embedded on the surface and outside
68.3	0.27% MC	99.4	Misshapen microspheres; aggregation

[a] All microspheres prepared at 300 rpm by the interrupted evaporation process. [b] Polyvinyl alcohol. [c] Methylcellulose.

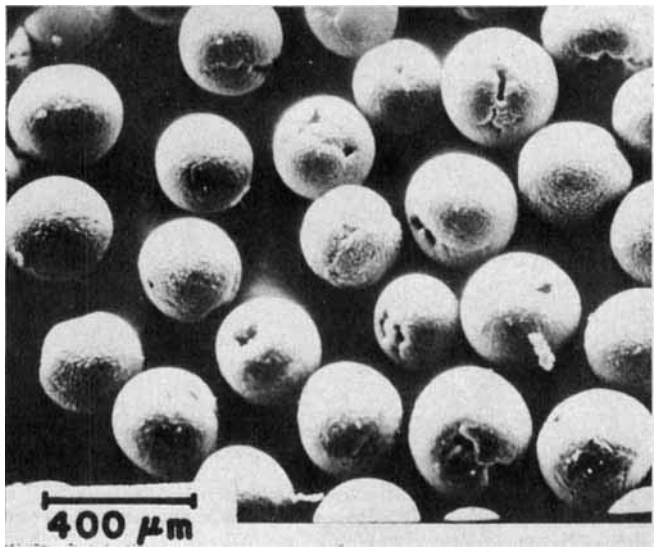

Figure 6—*Scanning electron micrograph of microspheres prepared by the continuous evaporation process at 250 rpm with 0.28% (w/w) methylcellulose (400 cps) as emulsifier. Theoretical progesterone content: 68.3% (w/w). Magnification: 50×.*

Table V—Amount of Progesterone Remaining in the Microspheres After Storage [a]

Polyvinyl Alcohol Concentration, %	Initial Mean Progesterone Content, %[b]	Mean Progesterone After Storage[b]	Progesterone Missing, %
0.27	31.7	21.5	47.4
0.27	31.7	24.8	27.8
2.5	23.6	19.0	24.2
5.0	23.2	17.8	30.3

[a] In a desiccator at 25° C for a period of 16 months. [b] Mean of three different experimental determinations.

lution hampered the washing process by prolonging microsphere sedimentation time. This led to increased microsphere aggregation.

Yields of isolated microspheres were related to final particle diameter. Microsphere batches composed of large particles were almost completely recovered. Yields of smaller particles (<100 μm) were reduced, since many small microspheres were lost during successive decantation washings.

Effect of Initial Drug Concentration—In an attempt to increase the progesterone content of the microspheres, a number of experiments were performed in which increasing amounts of progesterone were added to a fixed weight (927 mg) of poly(*d,l*-lactide). The comments in Table IV indicate that microspheres containing ≤31.7% progesterone could be prepared without appearance of free drug crystals in the aqueous phase, provided the emulsifier was removed from the system before CH_2Cl_2 evaporation was complete. Above 31.7%, free progesterone crystals appeared in the aqueous phase whether or not the emulsifier was removed. Moreover, crystals embedded in the microsphere surfaces could be distinguished. When the progesterone payload was raised to 68.3%, the microspheres remained basically spherical, but surface defects were visible (Fig. 6).

Stability Studies—Four progesterone-loaded microsphere samples were stored in a desiccator at room temperature for 16 months. They were prepared while avoiding formation of free progesterone crystals in the aqueous phase. The microspheres containing 31.7% progesterone became yellowish whereas the color of the 23.2% progesterone microspheres remained unchanged after a storage period of 16 months, even though progesterone experienced significant degradation during this period (Table V).

Lomustine microspheres, prepared by the standard procedure avoiding crystallization in the aqueous phase, were stored under different temperatures, in desiccators, and protected from light. The stability experiment results are

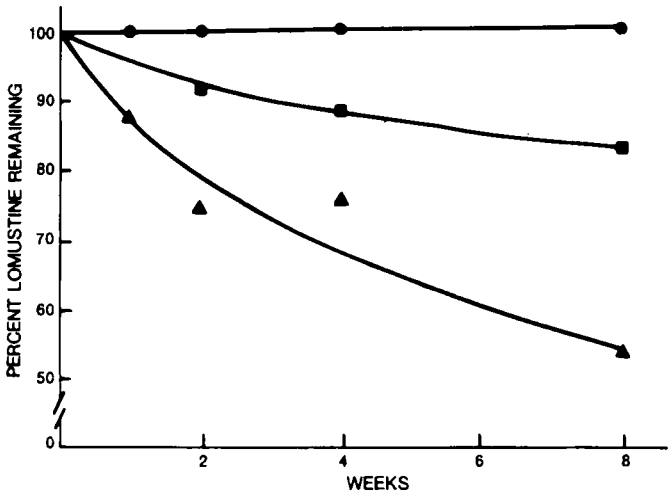

Figure 7—*Influence of storage temperature on desiccated shelf-life stability of lomustine-loaded (22.0%, w/w initially) poly(d,l-lactide) microspheres prepared by the interrupted evaporation process at 250 rpm with 0.27% (w/w) polyvinyl alcohol as emulsifier. Key, microsphere storage conditions: (●) 3° C; (■) 22° C; (▲) 37° C.*

shown in Fig. 7. At 3°C, lomustine incorporated into the microspheres remained stable for at least 8 weeks. However, degradation occurred within 8 weeks upon storage at 25° C and 37°C. The decomposition rate increased with increasing temperature. Lomustine crystals were stable over a period of 8 months at room temperature in a desiccator protected from light. Dispersing the drugs in the microspheres accelerated the degradation, probably as a result of their internal dilution within the coating polymer and chemical exposure to the polymer molecules.

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