Poly(L-lactic Acid) Microspheres Containing Neutron-Activatable Holmium-165: A Study of the Physical Characteristics of Microspheres Before and After Irradiation in a Nuclear Reactor

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The solvent evaporation technique was employed to prepare poly(L-lactic acid) (PLA) microspheres with ^{165}Ho acetylacetonate (Ho-AcAc). Particle size, percentage Ho-165, percent residual solvent, and retentive ability of the spheres were found to be strongly affected by preparatory conditions. Differential scanning calorimetry (DSC) thermograms suggested that the Ho-AcAc existed in the PLA matrix as a molecular dispersion. High neutron flux irradiations of the PLA spheres in a nuclear reactor produced Ho-166, a therapeutic radionuclide that emits high-energy negatrons (E_{max} = 1.84 MeV; half-life = 26.9 hr). The gamma radiation dose (53–75 Mrad) from the core of the reactor provided an overkill of all bioburdens in the PLA spheres. Gel permeation chromatography (GPC) analysis showed that these irradiations caused a reduction in PLA molecular weight. Infrared spectra, ^{13}C NMR spectra, ^{1}H NMR spectra, and DSC thermograms further confirmed the presence of lower molecular weight PLA but proved the overall maintenance of PLA structure.

KEY WORDS: acetylacetone; holmium; microspheres; nuclear reactor; poly-L-lactic acid.

INTRODUCTION

The overall objective of our work was to develop a biodegradable, radiotherapeutic microsphere intended for internal radiation therapy that provides an improved treatment for hepatic carcinomas. Hepatic carcinomas are relatively resistant to chemotherapeutic agents and respond poorly to external beam radiation (1,2). Stable Holmium-165 can be incorporated into the biodegradable microspheres under nonhazardous conditions and irradiated later in a nuclear reactor to convert the ^{165}Ho to ^{166}Ho, a high-energy negatron emitter (E_{max} = 1.84 MeV, half-life = 26.9 hr). The purpose of this paper is to report the effects of neutron irradiation on the PLA microspheres. Holmium-165 was found to be the best candidate for incorporation into PLA microspheres. It has a high neutron capture cross section of 64 barns (1 barn = 10^{-24} cm^2) and is easily activated to produce therapeutic amounts of ^{166}Ho activity with decreased irradiation times and has a natural abundance of 100%. Poly(L-lactic acid) (PLA), a relatively amorphous polymer, is an ideal polymer for incorporation of the stable ^{165}Ho based upon its high melting point, near plasma density of 1.25 g/cm^3 (which makes it easily suspended in aqueous media), and apparent ability to withstand neutron irradiation with maintenance of its favorable properties. The PLA spheres should not degrade or release the encapsulated radioactive ^{166}Ho until the isotope has fully decayed (10 half-lives or ~270 hr). In addition to thermal effects on the polylactides (3,4) the effects of the nuclear reactor environment and the effects of the negatron emission of the encapsulated ^{166}Ho also play a vital role in the ability of PLA microspheres to contain the ^{166}Ho until the isotope's complete decay. Much work has previously been reported on the effects of gamma-sterilization on PLA implant materials (5), PLA microspheres, and hollow fibers (6–10). Gamma radiation doses needed to kill the bioburden were of the order of a few megarad. PLA microspheres with ^{165}Ho, irradiated in a nuclear reactor for up to 3 hr, may receive up to 100 Mrad of gamma-radiation.

MATERIALS AND METHODS

Polymers. Poly(L-lactic acid) (PLA; 57,000 MW) was obtained from the Henley Company, Montvale, NJ. Polyvinyl alcohol (88% hydrolyzed; average MW 77,000–79,000) was supplied by Aldrich Chemical Company, Inc., Milwaukee, WI. Polystyrene standards (MW range, 1250–2.78 × 10^6; M_w/M_n ratios, less than 1.1) were purchased from Polymer Laboratories, LTD., Church Stretton, Shropshire, UK.

Chemicals. Inositol, chloroform (99.9+%, A.C.S. HPLC grade), and 2,4-pentanedione (99+%) were obtained from Aldrich Chemical Company. Holmium chloride hexahydrate, 99.9%, was supplied by Rare Earth Products, Chesire, WA. All other chemicals were of analytical reagent grade and were obtained from commercial sources.

Preparation of Ho-AcAc. Seven batches of Holmium-165 acetylacetonate (^{165}Ho-AcAc) were prepared according to the method of Brown *et al.* (11). Briefly, ammonium hydroxide (pH 11.88) was slowly added to solutions of holmium chloride and 2,4-pentanedionate (acetylacetone; AcAc) until the desired pH was obtained (range, 5.3–7.4).

Preparation of PLA Microspheres. PLA microspheres containing Ho-AcAc and blank (no Ho-AcAc) PLA microspheres were prepared by the solvent evaporation technique (12), whereupon 1.5 g PLA (57,000 MW) and prepared Ho-AcAc (0.9 or 0.0 g) were dissolved in 30.0 ml chloroform. This dispersed phase was then added to 240.0 ml of 1 or 3% polyvinyl alcohol (PVA) in deionized water. An agitation rate of 1140 or 2060 rpm was maintained for 15 min using a Caframo stirrer (Model No. RZR 1, Wiarton, Ontario). The solvent was then removed by either (a) transferring the oil-in-water emulsion to a 2000-ml round-bottom flask, diluting with 100 ml of deionized water, and rotoevaporating for 25 min (Model R110 Brinkman; vacuum pressure, 14.0 mm Hg; water bath, 60°C) or (b) placing the stirring oil-in-water emulsion in a fume hood (25°C at atmospheric pressure) and allowing the solvent to evaporate for up to 13 hr. Precipitated spheres were sonicated for 10 min and collected on a nylon

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filter. The spheres were resuspended in 800.0 ml of 0.1 *N* HCl for 2 min to remove the unincorporated Ho-AcAc, refiltered, washed with deionized water, collected, and stored in a desiccator. The particle size distribution of the microspheres was verified by optical microscopy. (Nikkon Optiphot, Scientific Instruments, Carpentersville, IL). Sphere density was measured by volume displacement.

Determination of Residual Solvent in the Spheres. Residual chloroform in the spheres was estimated from the chlorine content determinations. Chlorine analysis of spheres was performed by Atlantic Microlab, Inc., Norcross, GA, utilizing the Schoniger flask combustion method.

Determination of Ho-AcAc Content in the Microspheres. A ^{252}Cf source (1.25-mg source; neutron flux, 10^6 n/cm^2 sec; University of Kentucky Chemistry Department) was used to determine the percent incorporation of stable ^{165}Ho in the PLA microspheres. Irradiated samples and ^{166}Ho-AcAc standards were counted in a gamma counter (60- to 100-keV window; Packard Model No. 101750, Minaxi Auto-Gamma 5000 series).

Low-Neutron Flux Irradiations of Spheres. PLA microspheres were irradiated to saturation (~6 days) in the ^{252}Cf source for *in vitro* release studies. The low neutron flux of the ^{252}Cf source was capable of producing only very small amounts of ^{166}Ho activity (less than 0.1 μCi).

High-Neutron Flux Irradiations of Samples. PLA microspheres were irradiated for 114, 130, or 160 min in the TRIGA Reactor at the University of Illinois in Urbana to produce therapeutic amounts of ^{166}Ho. Inositol (m.p. = 225°C) was used as a diluent during irradiation to disperse the internal heat produced during sphere irradiation. In addition, PLA (two samples), PLA and inositol, PLA and Ho-AcAc, Ho-AcAc, and spore strips of *Bacillus pumilus* (STS, Inc.; 1.3×10^6 spores/strip) were irradiated for 160 min. For all irradiations a thermal neutron flux of 8.88×10^{12} n/cm^2 sec (with an added epithermal neutron flux of 7.10×10^{11} n/cm^2 sec) was used.

In Vitro *Release Studies of Irradiated Spheres.* Irradiated samples (both ^{252}Cf and reactor irradiated) were placed in 4-in. segments of pure regenerated natural cellulose dialysis membrane (Fisher, Spectra/Por 7 membranes, 50,000 MW cutoff) and submerged in 25 ml human plasma contained in 50-ml polypropylene tubes. The tubes were incubated in a 37°C water bath at 80 oscillations/min. At the end of the study, the whole volume of plasma and the membrane containing the spheres were counted for ^{166}Ho content. The membrane was then opened, washed with deionized water to remove spheres, and counted to determine the amount of released activity adsorbed to the membrane. The total plasma activity was then adjusted for the released activity adsorbed to the membrane.

Determination of Polymer Molecular Weights. One hundred microliters of polymer or sphere solutions dissolved in chloroform (0.2%) was injected into a Waters 501 HPLC pump with a PLgel 10-μm column (microstyragel; mixed pore size) obtained from Polymer Laboratories, LTD. Calibration of the column (flow rate of 1 ml/min) was made with polystyrene standards.

Infrared and NMR Analysis of Samples. Structural information of nonirradiated and irradiated materials was acquired by a 1430 ratio recording infrared spectrophotometer (Perkin-Elmer, Norwalk, CT) and a Varian NMR (Model VXR; 300 MHz).

Thermal Analysis of Samples. Thermal analysis of nonirradiated and reactor-irradiated samples was performed by differential scanning calorimetry (DSC) (Perkin–Elmer, Model DSC 7 with Model 7500 professional computer). The DSC was calibrated with indium (heat of fusion, 28.4 J/g) and was used to determine the glass transition temperature (T_g), the melting temperature (T_m), and the heat of fusion of the various materials investigated. The heat of fusion was calculated from the area enclosed by the DSC curve. Heating was performed under nitrogen at a flow rate of 10°C/min.

Sterility Tests of Spheres. Two microsphere samples (~50 mg of reactor-irradiated PLA spheres and nonirradiated PLA spheres) were transferred aseptically under laminar airflow conditions to vials containing 15 ml soybean-casein digest medium (SCDM). One hundred microliters of *Bacillus subtilus* (100 microorganisms/100 μl; BEC Laboratories, Toledo, OH) was transferred to an additional vial and was used as a positive control.

RESULTS AND DISCUSSION

Preparation and Characterization of Prepared Ho-AcAc. The extraction and complexation of ^{165}Ho from an aqueous solution by 2,4-pentanedione were found to be pH dependent, with maximum complexation occurring at approximately pH 7.4 (Fig. 1). Extrapolation of percentage extraction versus pH back to the *y* axis revealed maximum complexation of available ^{165}Ho slightly below 87%. Two Ho-AcAc products (both of which were prepared up to pH 7.36) showed reproducible complexation of 84.66 and 86.96%. These data were considered very near to the maximum extraction ability of 2,4-pentanedione since further additions of pH 11.88 ammonium hydroxide beyond pH 7.4 caused large precipitation of holmium hydroxide. All seven Ho-AcAc preparations easily dissolved in chloroform (260 ± 3.2 mg/ml) and were recrystallized and dried, leaving a free-flowing yellowish powder (m.p. = 116–121°C). The percentage extraction of ^{165}Ho by 2,4-pentanedione was considerably higher than the maximum of 57.5% (maximum pH 5.75) as reported by Brown. One explanation for the improved extraction percentages may be that the Ho-AcAc mixtures prepared in this study were proven to be anhydrous (from

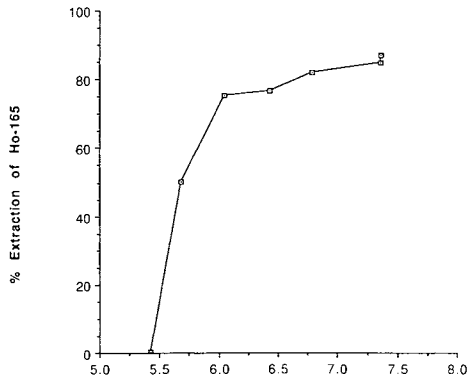

Fig. 1. Complexation of ^{165}Ho by 2,4-pentanedione at various pH's.

MS, NMR, and IR analysis), while those prepared by Brown's method were monohydrated. The monohydrates of Ho-AcAc (composed of a lower percentage of Holmium-165) would have greater solubility in the aqueous media, thereby reducing the overall extraction of ^{165}Ho.

Preparation and Characterization of PLA Spheres. Table I contains information pertaining to the preparation and characterization of the sphere batches. All six preparations of PLA microspheres resulted in the formation of spherical and relatively uniform particles (density, 1.4 g/cm^3). Batches B-F, prepared by removing the solvent in the hood at 25°C at atmospheric pressure, were free-flowing powders. However, batch A, prepared by removing the solvent at an elevated temperature and high vacuum, left microspheres that appeared wet and tended to adhere to the glass vial even after being dried to constant weight. Increasing concentrations of PVA in the continuous phase (from 1 to 3%) resulted in the formation of smaller spheres. This can be attributed to the higher viscosity in the continuous phase, leading to smaller droplet size in the dispersed phase. A similar effect of percentage PVA on PLA particle size was also reported by Lin *et al.* (13). An increased stirring rate (from 1140 to 2060 rpm) also led to the precipitation of smaller microspheres. The effect of centrifugation (to remove the highly viscous 3% PVA solution for facilitated filtration) on the retained particle size was also evident. Prolonged filtration of batch D due to the high viscosity of the 3% PVA led to the loss of smaller particles. Microsieving batch A for 7 hr (and collection of the sphere fraction on a 20-μm sieve) resulted in a very small particle size range, while microsieving batch B for only 1.5 hr led to the retention of a much larger sphere diameter range. Increased solvent removal time (from 25 min to 13 hr) allowed more Ho-AcAc to partition into the continuous phase, and thus, the percentage incorporation of ^{165}Ho was reduced. However, there was a trend in the data to suggest that increased solvent removal time also led to a lower percentage residual solvent in the spheres. The percentage residual solvent in the spheres was calculated from the chlorine analysis data using the assumption that all chlorine present was in the form of chloroform. The percentage chloroform retained in the spheres ranged from 0.8 to 5.3% and is in agreement with other reported studies (14). The chloroform content of all microspheres stored in a desiccator was found to decrease over time.

Reactor-Irradiated Samples. PLA sphere samples were irradiated in high-density polyethylene vials in the TRIGA reactor at the University of Illinois to produce 33.3–47.8 mCi ^{166}Ho. Irradiations were completed in the central thimble of the reactor (reactor power, 600 kW). The absorbed radiation dose rate in the central thimble was 7×10^7 rad/hr (reactor power, 1500 kW) as measured by General Atomics. Therefore, the total absorbed radiation dose for a given reactor power can be calculated by the following equation:

$$\underset{\text{(rad)}}{\text{adsorbed dose}} = (7 \times 10^7 \text{ rad/hr}) * \frac{\text{Rp}}{1500 \text{ kW}} * T_i$$

where Rp is reactor power in kilowatts and T_i is time of irradiation in hours.

For 600-kW reactor power, the absorbed radiation dose rate was 2.8×10^7 rad/hr or 28 Mrad/hr. For irradiation times of 114, 130, and 160 min, the total radiation absorbed dose to the samples was 53.2, 60.7, and 74.4 Mrad, respectively. All irradiated sphere batches consisted of 50.0 mg PLA spheres and 150.0 mg inositol. PLA sphere size was not affected by high-neutron flux irradiation. Irradiated PLA sphere batch A showed an average particle size of 23.1 ± 7.1 μm, while batch B was 28.7 ± 8.7 μm. Visual inspection of the irradiated samples showed that the inositol was browned by irradiation; however, the spheres remained intact and maintained their structural integrity. The two irradiated PLA samples (57,000 MW) appeared to have undergone some physical changes (i.e., nonirradiated PLA was a powder, while irradiated PLA became a solid mass). Ho-AcAc samples that were irradiated also did not retain their initial powdery nature. Thin-layer chromatography (silica gel plates; 250-μm layer) was performed on the irradiated Ho-AcAc using chloroform (with 0.75% ethanol) as the mobile solvent. R_f values for irradiated Ho-AcAc in chloroform were calculated and compared to the R_f values of nonirradiated Ho-AcAc. The R_f value for irradiated Ho-AcAc was 0.636 and the R_f value for nonirradiated Ho-AcAc was 0.641. The developed TLC plate for irradiated Ho-AcAc was cut into six

Table I. Summary of the Preparatory Conditions and Characterization of PLA Microspheres with Ho-AcAc

	Batch					
	A	B	C	D	E	F
% PVA	1	1	1	3	3	1
Stirring rate (rpm)	2060	1140	1140	1140	1140	1140
Particle size (μm) (SD)	20.2 (5.7)	30.9 (12.1)	22.8 (10.3)	6.1 (2.6)	3.4[a] (2.0)	ND
Microsieving time (hr)[b]	7	1.5	0	0	0	0
% ^{165}Ho	10.3	9.0	10.0	9.1	7.1	0.0
Solvent removal time (hr)	0.4	3	3	8	13	5.5
% residual solvent (days)	2.7 (17)	4.8 (8)	4.0 (8)	1.4 (10)	1.6 (10)	5.3 (29)
	2.4 (41)	3.6 (26)	3.1 (26)	0.8 (42)	0.8 (42)	
			1.8 (90)			

[a] Centrifuged (10 min at 2500 rpm) so that PVA could be decanted from settled spheres to facilitate filtration.

[b] Desired diameter ranges of spheres (20–45 μm) were obtained by selective sizing using a mechanical microsiever (Model SS-5, Gilson Company Inc., Worthington, Ohio) and a series of microsieves.

regions and assayed in a gamma counter for ^{166}Ho to determine the distribution of activity on the plate. It was found that 99.7% of the activity remained at the origin even though both developed plates appeared identical under UV light. One explanation for this outcome may have originated from the physical phenomena of neutron absorption. In this process, less than 1% of the activatable element actually absorbs neutrons. Thus, greater than 99% of the atoms remain unchanged. The neutron was absorbed by ^{165}Ho (converting it to ^{166}Ho) with enough kinetic energy to result in the recoil of ^{166}Ho. The recoiled ^{166}Ho could then complex with a more hydrophilic chemical species or form Ho_2O_3 which may have precipitated.

In Vitro *Plasma Release Studies.* Human plasma release profiles are plotted as a function of time in Fig. 2. A comparison of the release profiles of reactor irradiated PLA spheres, batches A and B, revealed an interesting contrast. Whereas batch B showed no burst effect (or initial release of ^{166}Ho), batch A, prepared by removing chloroform at an increased temperature and vacuum pressure, displayed an initial release of 1.5% of the ^{166}Ho. Since the shelf time of the PLA spheres before irradiation and the activity produced during irradiation for both samples were similar, the burst observed for batch A could be related to preparatory conditions. It was possible that the solvent removal conditions of batch A resulted in some physical alteration in PLA's mechanical strength that led to decreased stability during irradiation. Another explanation for the increased release rate for batch A may be that rapid chloroform removal from the dispersed phase caused a disruption in the PLA sphere matrix (15). However, Kishida *et al.* found that the solvent evaporation rate had little effect on PLA sphere morphology when PVA was used as the surfactant (16). An extended release study was carried out for irradiated sphere batch B since a much greater amount of activity, 419 μCi ^{166}Ho, was used. After 406 hr (>15 half-lives of ^{166}Ho), 0.01 μCi of the original ^{166}Ho remained. It was found that 99.0% of the activity in the spheres was retained after 191.5 hr (99.3% of the initial ^{166}Ho activity had decayed) and that the onset of rapid degradation and release of the activity occurred significantly later than with batch A. Analysis of the release profile for ^{252}Cf-irradiated PLA spheres should be approached very carefully. Although, it appeared that sphere batch D released the encapsulated material at a faster rate than reactor irradiated samples, the data points were subject to significant probability for error because of their low activity.

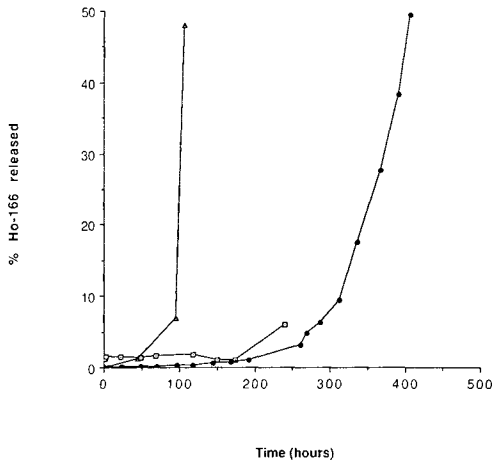

Fig. 2. *In vitro* human plasma release of ^{166}Ho. (□) PLA sphere batch A, shelf time before irradiation = 15 days; activity used in study = 75.7 μCi. (●) PLA sphere batch B, shelf time before irradiation = 9 days; activity used in study = 419.0 μCi. (△) PLA sphere batch D, ^{252}Cf irradiated; activity used in study = 1570.0 cpm.

Polymer Molecular Weights. Table II contains the weight averaged molecular weights (M_w) obtained by gel permeation chromatography. The refractometer and UV detector (247 nm) showed that nonirradiated PLA from the Henley Company had a molecular weight of 77,600 (mean value), which was slightly higher than the company's reported value of 57,000 MW. The difference in these values may have been due to the wide range of molecular weight polystyrene standards used to calibrate the column. Analysis of irradiated PLA and PLA spheres revealed a very large reduction in polymer molecular weight (Table II). The data collected at 21, 27, and 39 days after irradiation showed a continuous decline in polymer molecular weight, from 3500 to 1700 to 1600 daltons, respectively. Lower molecular weight PLA provided evidence that polymer scission occurred in PLA that was probably initiated by radicals formed during irradiation. Similar reductions in the molecular weight of gamma-sterilized PLA fibers and spheres have been reported and were attributed to production of free radicals during irradiation (7,10). The presence of free radicals in the irradiated PLA should not be considered a significant problem since our aim was to produce high levels of negatron-emitting radioisotopes, which can produce large amounts of free radicals in biological systems. In fact, the existence of even a small number of free radicals in the polymer may increase the biodegradability of the irradiated polymer by reducing the molecular weight. This can be advantageous as long as a reduction in molecular weight does not lead to increased release of ^{166}Ho.

Infrared Analysis. A comparison of the IR spectra of nonirradiated PLA spheres and reactor irradiated PLA spheres is shown in Fig. 3. Although major peaks were retained, the two peaks that were between 1600 and 1500 cm^{-1} were merged in the irradiated PLA sphere spectrum. In addition, the peak that was present at 930 cm^{-1} was significantly shorter in the irradiated PLA sphere spectrum. These differences in the spectra may have reflected the existence of

Table II. Molecular Weight Analysis of PLA and PLA Spheres by Gel Permeation Chromatography

Sample (n = 3)	Irradiation time (min)	Time after irradiation (days)	M_w
PLA	0	—	76,800[a]
PLA	0	—	78,400[b]
			1,300
PLA sphere batch E	160	21	3,500[a]
PLA sphere batch E	160	27	1,700[b]
PLA	160	35	1,600[b]

[a] MW obtained from refractive index (sensitivity = 2).
[b] MW obtained from UV absorption (247 nm).

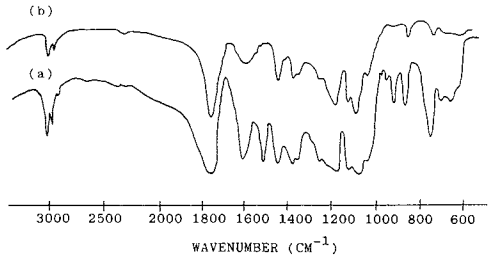

Fig. 3. Infrared spectra: (a) PLA sphere batch B; (b) irradiated PLA sphere batch E (time since irradiated, 28 days).

the lower molecular weight PLA as seen with GPC analysis. The retention of major peaks associated with PLA after irradiation (i.e., carbonyl at 1750 cm^{-1}, oxygen–carbon stretching at 1200 and 1100 cm^{-1}, and C–H stretching at 3000 cm^{-1}) provided further proof that irradiated PLA retained its chemical identity while undergoing a reduction in molecular weight.

NMR Analysis. The maintenance of PLA carbon shifts in ^{13}C NMR spectra (other than the splitting at 68 and 169 ppm) provided further evidence that PLA does maintain its chemical identity after irradiation. ^{1}H NMR spectra showed that reactor-irradiated PLA retained hydrogen shifts at 1.58 and 5.17 ppm. In addition to these explained shifts, the spectra contained five unassigned groups of shifts between 1.30 and 4.20 ppm, all of which had comparatively small integration values. Again, it was possible that these shifts were due to the existence of structures indicative of lower molecular weight species initiated by free radical scissions.

Thermal Analysis of Samples. DSC thermograms (Figs. 4 and 5) and supporting data (Table III) are shown. Evidence for low molecular weight polymer was provided in the thermogram for nonirradiated PLA. This melting endotherm at 164.4°C was absent in the DSC thermograms of sphere preparations. Most likely, low molecular weight PLA partitioned into the continuous phase during preparation of the spheres due to its high water solubility. Reactor-irradiated PLA and PLA spheres showed decreased T_g and heat of fusion, which was indicative of loss of crystallinity in the polymer. In addition, these lower values of T_g and heat of fusion reflect polymers with lower molecular weights (3). PLA sphere

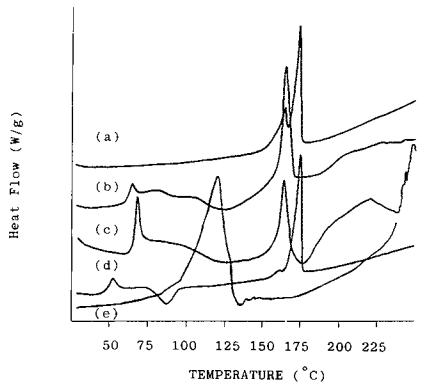

Fig. 4. DSC thermograms of nonirradiated samples: (a) PLA; (b) PLA sphere batch D, shelf time = 32 days; (c) PLA sphere batch A, shelf time = 254 days; (d) PLA sphere batch F; shelf time = 18 days; (e) Ho-AcAc.

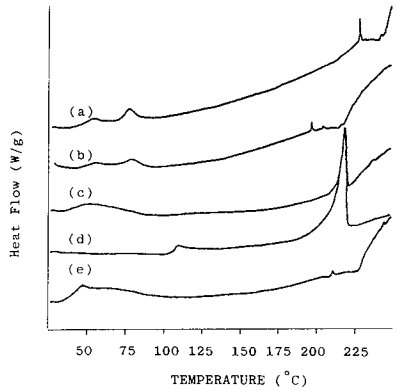

Fig. 5. DSC thermograms of reactor irradiated samples: (a) PLA (sample 1; time since irradiated, 37 days); (b) PLA (sample 2; time since irradiated, 37 days); (c) PLA sphere batch E with inositol (time since irradiated, 25 days); (d) PLA with inositol (time since irradiated, 25 days); (e) PLA sphere batch E (time since irradiated, 25 days).

batch A, prepared by removing the solvent at an increased temperature and vacuum pressure, also showed a loss of crystallinity (heat of fusion, 14.5 J/g) and a lower crystallization exotherm at 86.7°C. Insight as to the physical state of Ho-AcAc in the sphere matrix was also obtained by the DSC thermograms. Theoretically, Ho-AcAc could crystallize in the PLA matrix as the chloroform volatilizes to form a dispersion of crystalline drug, or it may exist as a molecular dispersion in the PLA wall, reflecting its inability to crystallize as the chloroform is removed. If the Ho-AcAc forms a molecular dispersion in the PLA wall, it may act as a plasticizer for the PLA or, alternatively, the PLA may be an inert carrier for the encapsulated material (14). Absence of a crystallization exotherm for Ho-AcAc in the DSC thermogram for PLA spheres proved that Ho-AcAc was not encapsulated in its crystalline form. It was possible that crystalline PLA could have dissolved in PLA during heating and not be detected, but this would be dependent on its miscibility with PLA. An attempt was made to determine the solubility of Ho-AcAc in chloroform with dissolved PLA. Increasing amounts of Ho-AcAc were added to fixed volumes of chloroform that contained 5% PLA (w/v). Results showed that Ho-AcAc had a lower solubility in chloroform (~200 mg/ml)

Table III. Differential Scanning Calorimetry Thermogram Data for PLA and PLA Spheres

Figure	T_g (°C)	T_c (°C)	T_m (°C)	Heat of fusion (J/g)
4a	65.3	—	173.4	38.3
4b	61.0	124.6	165.9	38.4
4c	63.9	119.7	163.5	14.5
4d	46.9	86.7	173.0	50.9
4e	—	—	121.3	103.9
5a	49.5	—	78.9	4.3
5b	49.2	—	79.7	2.4
5c	42.3	—	—	—
5d	—	—	110.9	8.5
5e	44.4	—	—	—

in the presence of PLA, providing evidence that Ho-AcAc most likely does not dissolve in PLA upon heating and behave as a plasticizer for PLA. The T_g for PLA sphere samples with Ho-AcAc was lowered, indicating that Ho-AcAc exists as a metastable molecular dispersion in the PLA, with little miscibility in PLA. The ability of PLA spheres to retain Ho-AcAc in the *in vitro* study suggests that this was the case. Residual chloroform in the spheres may explain some variation in T_g of the PLA, but to what extent remains unknown.

Sterility and Population Determination Tests. PLA sphere batch B irradiated for 160 min showed no growth in SCDM after 40 days at 35°C, while nonirradiated PLA sphere batch A and the *Bacillus subtilus* culture had large biological growth in SCDM even after 3 days.

Ten spore strips irradiated at Illinois receiving 74.7 Mrad of gamma-radiation were aseptically transferred to 7-ml tubes of SCDM, incubated at 32–35°C for 7 days, and showed no positive tests for biological growth. Population determination tests of the irradiated spore strips showed no surviving *Bacillus pumilus* spores. This was an anticipated result since *Bacillus pumilus* is known to have a *D* value (90% kill value) of 0.16 Mrad. Irradiation in the Illinois reactor to produce therapeutic amounts of ^{166}Ho can be considered a terminal sterilization method for the PLA spheres, providing an overkill of any bioburden.

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