Biodegradable Indium-111 Labeled Microspheres for *in Vivo* Evaluation of Distribution and Elimination

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INTRODUCTION

Microparticulate polymeric delivery systems are becoming increasingly popular for sustained and targeted delivery of biologically active substances. Sustained delivery is achieved primarily by controlling the physico-chemical characteristics of the delivery systems, whereas targeted delivery relies heavily on the interaction of delivery systems with the biological environment encountered after administration. While the mechanisms of sustained delivery from microparticulate polymeric delivery systems are well characterized, the mechanisms of altered deposition, distribution and clearance are little known. Well characterized radiolabelled microparticles can serve as a valuable tool in understanding the processes of deposition, distribution and clearance of microparticulate polymeric delivery systems.

Radiolabelled microparticles prepared from the polymers of natural and synthetic origin used extensively in medicine has been reviewed by Arshady [1]. Macroaggregated albumin labelled with Tc-99m has been the system of choice for cardiovascular studies. Recently, albumin microspheres of other gamma emitting isotopes such as I-131, In-111 and In-113m have been developed [2]. Larger particles or beads labelled with In-111 and Tc-99m have been used for understanding the gastro-intestinal transit times of delivery systems [3-6]. Nanoparticles of polymethylmethacrylate have been labelled with C-14 [7] and In-111 [8] for studying distribution and clearance following intravenous administration. Non-biodegradable polystyrene - divinyl benzene particles labelled with Ce-141 have been used in distribution and clearance studies for intravenous, intraarterial, and ophthalmic delivery systems [9,10].

Biodegradable microspheres of synthetic polymers such as polyglycolides have been labelled with In-111 for studying the tissue distribution following systemic administration [11]. In spite of all these developments, there is a need for radiolabelled microparticle systems that are well characterized, biodegradable and can retain the radiolabel under various physiological conditions.

The objective of this research was to develop biodegradable radiolabelled microspheres of defined properties to study the deposition, distribution and clearance following inhalation administration and for Peyer's patch uptake following oral administration. Indium-111 labelled microspheres of polylactide, polyglycolide and polylactide-co-glycolide were prepared and characterized.

MATERIALS AND METHODS

Materials

Poly-l-lactide (PLA, M.Wt. 50,000) polyglycolide (PGA, M.Wt. 60,000) and hexafluoracetone (sesquihydrate) were obtained from Polysciences (Warrington, Pennsylvania). Poly(d-l-lactide-co-glycolide) (PGL 50:50; M.Wt. 34,000) was obtained from Henley Chemicals (Boston, Massachusetts). Indium-111 chloride ($^{111}InCl_3$) was purchased from Mediphysics as a solution in 0.04M HCl at a specific activity of 10mCi/ml. All other chemicals were reagent grade and used as obtained.

Preparation of Indium-Oxine

$^{111}InCl_3$ was converted to ^{111}In-oxine by complexing with 8-hydroxyquinoline. The $^{111}InCl_3$ solution as received (0.5 ml for 5 mCi) was diluted to 2.5 ml with water and additional 7.5 ml of 0.1 M acetate buffer, pH 5, was added at room temperature. About 250 1μl of 8-hydroxyquinoline in 95% ethanol at a concentration of 1 mg/ml was added to the $^{111}InCl_3$ solution. The mixture was allowed to stand for 5 minutes at room temperature and the converted ^{111}In-oxine was extracted with two 5 ml fractions of chloroform. An additional 250 μl 8-hydroxyquinoline solution was added to the aqueous layer, allowed to react with residual $^{111}InCl_3$ for 5 minutes and extracted with two 5 ml fractions of chloroform. The chloroform extracts were combined and the solvent was removed under a stream of nitrogen to obtain dry ^{111}In-oxine.

Preparation of Microspheres

Microspheres of PLA, PGA and PGL were prepared by an emulsion-solvent-extraction technique developed earlier [12-14]. Polymer and ^{111}In-oxine were dissolved in a dispersed phase solvent and added dropwise to a continuous phase solvent under agitation at 7,000 to 10,000 rpm. The dispersion was agitated for a predetermined time after which the dispersed phase solvents were removed either by extraction into a diluent phase or by extraction into the continuous phase followed by evaporation. For the preparation of PLA microspheres methylene chloride was used as the solvent for PLA (0.5 g) and indium oxine. Glycerine was used as the continuous phase. Polymer solution was added to the continuous phase and stirred for 5 minutes at 5°C and 10 minutes at 40°C. 5% isopropanol in water was used to extract the solvent by adding to the continuous phase and stirring for 5 minutes at room temperature. To prepare PGA microspheres same procedure was followed, however, hexafluoroacetone

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was used as the solvent and carbon tetrachloride as the continuous phase. Dioxan was used as the extraction medium.

PGL microspheres were prepared by solvent evaporation process. The polymer and indium oxine was dissolved in 1:1 volume mixture of methylene chloride and chloroform. The polymer solution was dispersed in 0.04% sodium oleate and stirred at 5°C for 60 minutes. The solvent was removed by continuing the stirring at 40°C for 120 minutes. The microspheres were isolated by filtration and dried in vacuum oven overnight.

Particle Size

The particle size distribution was obtained using a Malvern Lazer Diffraction particle sizer (series 2600C)(Malvern, England). About 2 mg microspheres were dispersed in 1 ml of 0.05% Tween 80 solution and added dropwise to the counting chamber until a desired defraction intensity was obtained. The instrument then measured the volume and number distribution of the particles. All the results reported here were based on the volume distribution.

Specific Activity

The specific activity of the microspheres was obtained by measuring the total activity of a known weight of dry microspheres in a Capintec radioisotope dose calibrator CRC-12 (Capintec, Inc.) or a Miniaxi Auto-Gamma 5000 counter (Packard Instrument Company, Downers Grove, Illinois) by calibrating the instrument with a known amount of $^{111}InCl_3$ and corrected for decay of In-111 isotope.

Label Permanency

The permanency of the incorporated ^{111}In-oxine was determined by performing a release study in various media selected on the basis of the intended application of the microspheres. The media selected were 0.1M acetate buffer at pH 3.6, 4.6 and 5.6, phosphate buffered saline at pH 7.4 with 0.1% Tween 80, simulated gastric fluid, U.S.P., simulated intestinal fluid, U.S.P. and rat lung homogenate. A known amount of microspheres was placed in a 50 ml centrifuge tube and 20 ml medium was added. The tubes were incubated at RT or 37°C on a shaking device and periodic samples were obtained over a 24 hour period. The samples were centrifuged and the radioactivity was measured using Miniaxi Auto-Gamma 5000 Counter and corrected for the decay of In-111 isotope.

RESULTS AND DISCUSSION

Microsphere Preparation

Although we had successfully prepared ^{111}In-labeled PGA microspheres earlier [11], the specific activity was only 0.09 μCi/mg. This was not suitable for extensive imaging or identification of a small fraction taken up by the Peyer's patches. Table I shows the properties of the microsphere prepared in the current study. All of the new processes resulted in microspheres free of aggregates and fibers. Current procedure provides spherical and smooth microspheres. PLA microspheres had a lower specific activity due to the limited solubility of the ^{111}In-oxine complex in methylene chloride and due to extraction of the incorporated complex during solvent extraction and washing stages. In spite of this, 66% labeling efficiency was obtained with a final specific activity of 1.74 μCi/mg. Higher specific activity resulted with PGA microspheres due to the higher solubility of the complex in the dispersed phase. However, the incorporation efficiency was similar to the PLA microsphere due to extraction of the complex in dioxane during washing. For the PGL microsphere system, incorporation efficiency similar to the PLA and PGA microspheres was obtained at low targeted incorporation (up to 8 μCi/mg). However, at higher targeted incorporation (~14 μCi/mg), over 90% of the label was incorporated into the microspheres. The microsphere yields of all the processes were in the range of 50 to 80%. The particle size distribution was narrow with a mean diameter ranging from 3.4 μm to 7.4 μm. The particle in this size range are suitable for uptake by Peyer's patch and for deposition in the tracheo-bronchial and alveolar regions of the lungs. SEM analysis show smooth, non-porous and spherical particles (Figure 1).

Label Permanency

In order to determine later in-vivo distribution and clearance, the microspheres should retain the radiolabel

Table I. Properties of the Indium Labelled Microspheres

Polymer	Specific Activity (μCi/mg) Target	Specific Activity (μCi/mg) Achieved	Incorp. efficiency (%)	Yield (%)	Particle size (volume distribution) (μm) 10% Under	50% Under	90% Under
PLA (M.Wt. 50,000)	2.62	1.74	66	69	—	—	—
PGA (M.Wt. 60,000)	5.93	4.30	73	49	—	—	—
	5.90	4.00	68	55	—	7.4	—
PGL (M.Wt. 30,000)	2.30	1.57	68	61	2.3	4.1	8.7
	7.53	5.09	68	66	2.3	3.6	7.8
	13.34	12.28	92	79	2.5	3.5	5.4
	13.91	12.29	88	79	2.4	3.5	6.2
	14.15	12.72	90	75	2.5	3.6	6.5

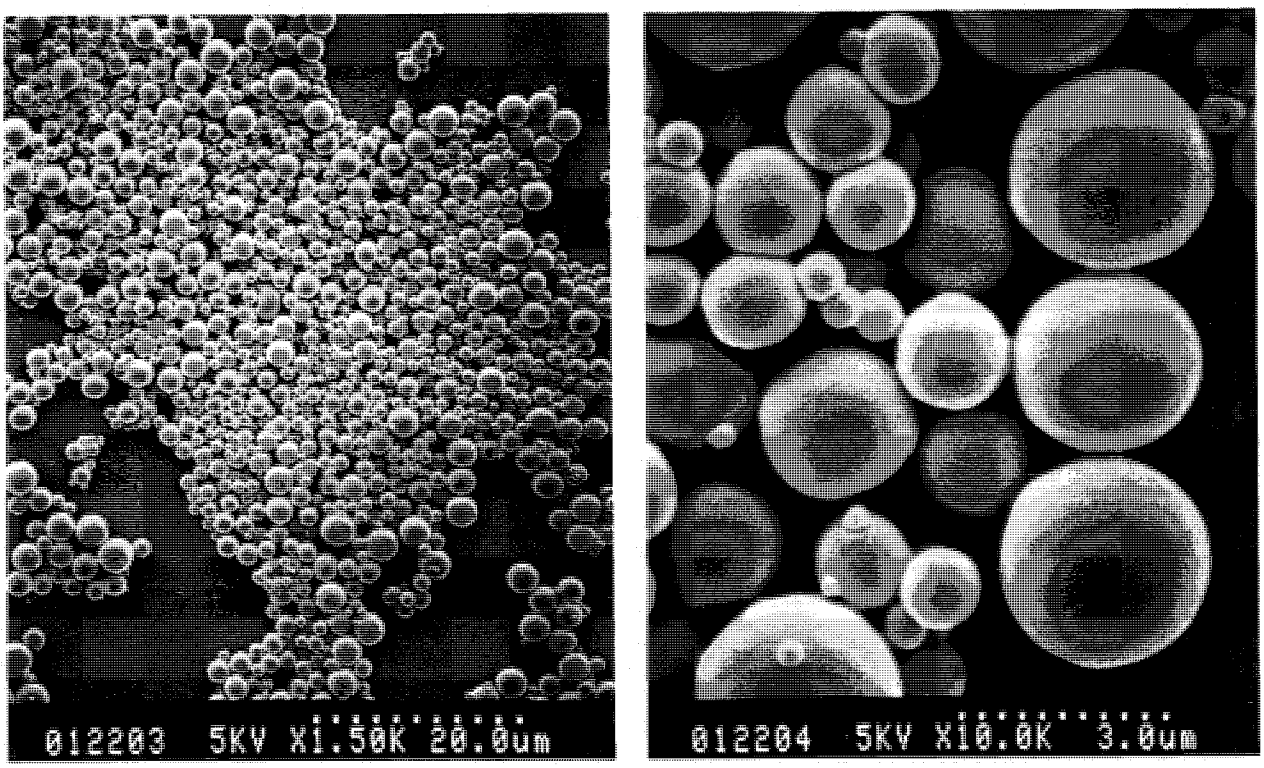

Fig. 1. Scanning electron micrograph of Indium-111 oxine labeled PGL microspheres.

without releasing it under physiological conditions. The in-vitro release thus should be minimal or should stop after a small initial burst release. A pH dependent release of ^{111}In-oxine from PGL microspheres with a specific activity of 12.28 μCi/mg was seen in acetate buffer at RT with greater release at lower pH. The initial burst release (in 10 minutes) was more which account for 9% at pH 3.6, 7% at pH 4.6 and 2% at pH 5.6. The greater release can be attributed to larger burst release at lower pH as a result of solubilization of surface bound ^{111}In-oxine, which is more soluble at lower pH. No subsequent release of entrapped ^{111}In-oxine was observed. These microspheres are suitable for studies above pH 5.0. For applications encountering low physiological pH, the microspheres can be rinsed free of surface bound radioactivity with low pH buffer to give microsphere with non-leaching radiolabel.

In phosphate buffered saline at pH 7.4 with 0.1% Tween 80, PLA and PGA microspheres exhibited a burst of about 5% radioactivity followed by a slow release of incorporated ^{111}In-oxine with about 17 to 20% total release in 24 hours (Figure 2). The leaching may be due to porous structure of these microsphere resulting from the solvent-extraction precipitation process [14]. The vehicle can easily enter the pores and dissolve incorporated but pore-lined indium-oxine. Tween-80 would aid in wetting the polymer surface simulating the surface tension effect of plasma and other body fluids. The amount of activity remaining at the end of 24 hours should be sufficient to permit scintigraphy or direct tissue radioactivity measurements to determine distribution of microspheres after administration.

PGL microspheres with 12.7 μCi/mg specific radioactivity were also evaluated for in-vitro release in simulated fluids. Figure 3 shows the release of radioactivity in SIF and in SGF followed by SIF. After 2 hours the SGF medium is changed to SIF to simulate the oral pathway. About 6% burst is seen in SIF with very slow release thereafter. The burst in SGF is about 12% with an additional 3 - 4% release in 2 hours and a negligible release thereafter upon changing the medium to SIF. The burst in both SIF and SGF was significantly reduced when the microspheres were washed with SGF during the final rinsing step. A total of 2% radioactivity is released in SIF in 48 hours and a total of 4% (2.5% in SGF and 1.5% in SIF) is released in SGF followed by SIF. The small amount of activity released should distribute in a manner similar to free $^{111}In\ Cl_3$ and hence can be separated from the small fraction taken up as microspheres. The microspheres are well suited for studying the oral delivery of microparticulate carriers, especially with respect to intestinal uptake by Peyer's patches. The high specific activity should permit easy identification and quantitation of a very small fraction of microspheres taken-up in various body tissue for up to 7 days after administration.

Another potential route of microparticulate delivery is via inhalation into the lungs. PLA microspheres were evaluated for label permanency by performing release tests in a porcine lung homogenate. As seen in Figure 2, only 5% of incorporated radioactivity was released in-vitro in lung homogenate at 37°C, indicating that these microspheres could be employed in the evaluation of pulmonary delivery of microspheres. The specific activity is sufficient to permit scintigraphic observation and direct tissue radioactivity measurement.

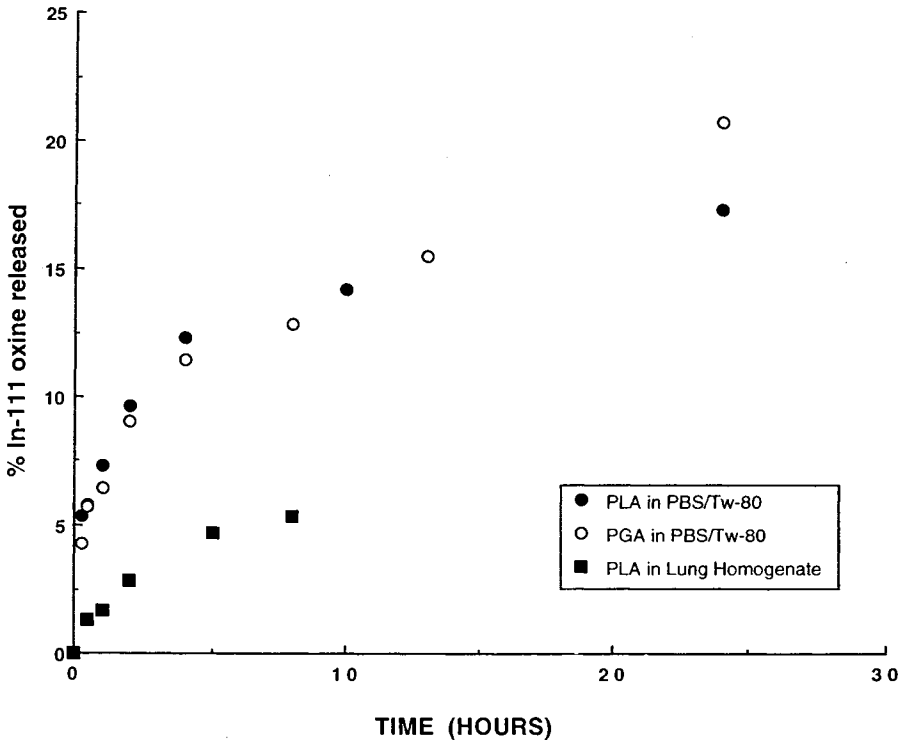

Fig. 2. Release of Indium-111 from PLA and PGA in phosphate buffered saline (pH 7.4) containing 0.1% Tween 80 and PLA microspheres in rat lung homogenate.

CONCLUSIONS

Short-lived gamma emitting radioisotopes can be incorporated into polylactide/glycolide polymeric microspheres with various specific activities for possible use in understanding the in-vivo deposition, distribution and clearance of microparticulate drug carrier systems. The incorporated radiolabel is stable with negligible leaching out of the microspheres. These microspheres are suitable for studying the oral uptake of particles, lung distribution after inhalation de-

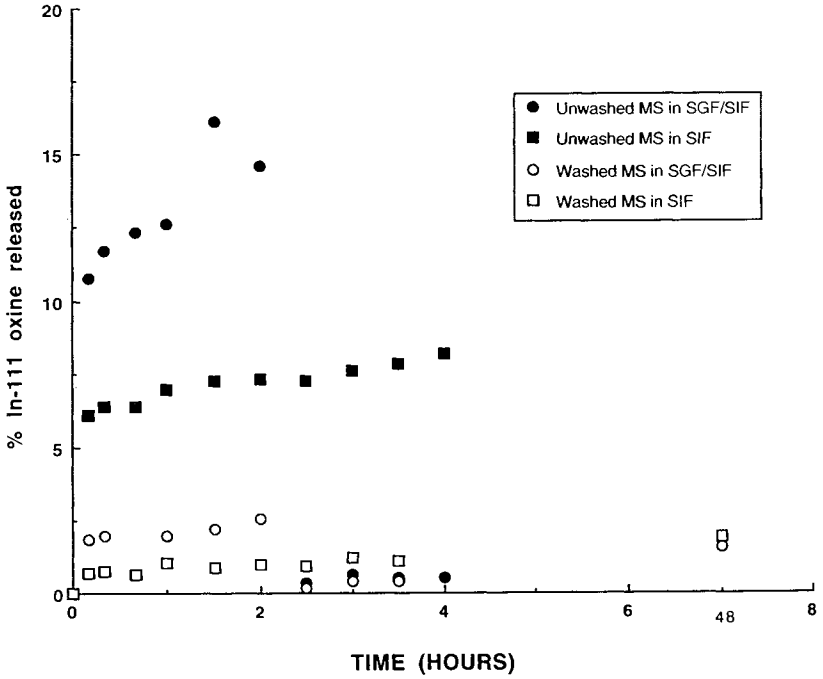

Fig. 3. Release of Indium-111 from unwashed and washed microspheres in simulated gastric (SGF) and intestinal fluids (SIF).

livery and evaluation of in-vivo fate following parenteral administration in systemic circulation or in specific tissue compartments.

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