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Long acting injectable oxytetracycline-liposphere formulations

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Abstract

Oxytetracycline (OTC) was encapsulated in a liposphere delivery system composed of OTC, solid triglyceride, phospholipid, buffer solution and preservatives. The formulation is an injectable microdispersion containing 8–20 wt% OTC with an average particle of size of 30 μm. The in vitro $t_{1/2}$ release of OTC was in the range of 28 h. Serum OTC concentration after a single intramuscular injection of OTC-liposphere formulation in turkeys showed an effective extended release for 3–5 days as compared to about 1 day for the commercial OTC solution (Dabicycline, 10 wt% in acidic solution). A mixture of OTC in lipospheres and OTC solution in a 4:3 volume ratio resulted in initial high OTC serum levels that lasted for 5 days. The biodegradability of the formulation was demonstrated by observing the injection site for up to 28 days after injection. The blank formulation was readily eliminated from the site and no signs of residuals were observed 28 days after injection. OTC loaded formulations based on trilaurin were also degraded but at slower rate than the blank formulation that might be a result of the hydrophobicity of OTC which retards hydrolysis and biological elimination.

Keywords: Liposphere; Oxytetracycline; Drug delivery; Controlled release; Antibiotic

1. Introduction

Parenteral oxytetracycline (OTC) therapy in farm animals requires daily administration of drug over several days, usually 3–5 days, in order to provide prolonged therapeutic blood levels. Serum OTC concentration of potential clinical and therapeutic values in the treatment of OTC sensitive organisms are estimated in the range of 0.15–1.5 μg/ml. The minimum inhibitory concentration (MIC) in μg/ml of certain farm animals' pathogens are: *Pasteurella multocida*, 0.15; *Staphylococcus* and *P. anatipestifer*, 0.3; *Hemophyllis paragallinarum*, 0.4; *Mycoplasma gallisepticum*, 0.8; *E. coli*, 1.5. Blood levels of above 0.5 μg/ml are required for treatment of most bacterial infections.

Several long-acting oxytetracycline formulations have been recently reported (Kiorpes et al., 1989; Appleyard and Gilmour, 1990; Adawa et al., 1992; Landoni and Errecalde, 1992; Oukessou et al., 1992). These formulations have been tested in various farm animals and showed adequate

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blood levels for 72 h after a single injection at a dose of 20 mg/kg. Although these formulations were effective they are expensive for veterinary use.

The liposphere drug delivery system is an aqueous microdispersion of solid water insoluble spherical microparticles of a particle size between 0.2 and 100 μm. The lipospheres are made of solid hydrophobic triglycerides having a monolayer of phospholipids embedded on the surface of the particle. The solid core contains the bioactive compound dissolved or dispersed in a solid fat matrix. The system has been used for the controlled delivery of anti-inflammatory agents (Domb, 1993a), vaccines (Amselem et al., 1992a, b) and local anesthetics (Domb, 1993b).

This report describes the use of the liposphere system for the preparation of injectable controlled release formulations of oxytetracycline. In this study we compared serum oxytetracycline concentrations in turkeys after intramuscular injection of a oxytetracycline solution (Dabicycline-100) and a long acting liposphere preparation. Turkeys were used in this study as potential users of these formulations against bacterial infections.

2. Materials and methods

2.1. Materials

Tristearin, trilaurin, and tricaprin, 99% pure were a gift from Hulls (Hulls, NJ). Oxytetracycline powder (USP grade, micronized to particle size below 5 μm) and commercial oxytetracycline solution (Dabicycline-100, 10% OTC in acidic solution) were a gift from Abic LTD, Israel). Egg phosphatidylcholine (EPC, 99% pure) was purchased from Princeton Lipids (Princeton, NJ). Soybean phosphatidylcholine (SPC, 99% pure) was obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Food grade lecithin (SPC-FG, 96% acetone insoluble phospholipids) was purchased from Central Soya (Fort Wayne, IN). Phosphate-buffered solution (100 mM, pH 7.4) used as suspending medium for the liposphere formulations was prepared by dissolving monosodium phosphate (3.40 g) and dipotassium phosphate (14.44 g), both from EM Science (Cherry Hill, NJ), in 1 l distilled water. Methylparaben (0.1 w/w %) and propylparaben (0.05 w/w %) both from Napp Chemicals Inc. (Lodi, NJ) were added to the formulation as preservative. Turkeys were from a local supplier.

2.2. Preparation of lipospheres

Liposphere formulations containing 8–20% OTC were prepared by a melt technique. OTC incorporated in solid triglyceride particles were prepared by homogenizing a melted mixture of triglycerides and OTC in a hot buffer solution using phospholipids as the dispersing agent. The hot microdispersion was then rapidly cooled to room temperature to yield a uniform smooth injectable microdispersion. In a typical preparation, the solid triglyceride tricaprin (15.0 g) and OTC (8.0 g) were added to a 200 ml beaker. The flask was heated at about 75°C to melt the tristearin and OTC was uniformly dispersed in the melt. The hot 0.1 M phosphate buffer solution (75°C, to 100 ml) was then added at once along with the phospholipid powder (10.0 g). The hot mixture was homogenized for about 2–5 min using a Silverson L4 portable homogenizer with a 0.8 inch probe size (Silverson, MA), resulting in the formation of a uniform emulsion. The yellowish milky formulation was then rapidly cooled down to about 20°C by immersing the formulation vial in an acetone-dry ice bath while homogenization continued. A uniform dispersion was obtained which was stored at 4°C until use. If needed, the pH of the formulation was adjusted to 7.4 with a 1 N HCl solution. The compositions of the formulations are given in Table 1.

Particle size and size distribution of the liposphere formulations were determined using a Coulter LS100 particle size analyzer (Coulter Electronics, NJ) (Amselem et al., 1992b). OTC concentration in buffer solutions from the in vitro release experiment were determined by UV absorbance at 270 nm. Viscosity measurements of formulations were conducted using an RTV Brookfield viscometer (Brookfield Labs, Stourton, MA) with an RV spindle no. 4.

2.3. In vitro release experiments

Release studies were conducted in dialysis tubing with a molecular weight cut-off of 300 000 (Spectrum, CA) or without tubing. 1 ml of liposphere formulation was introduced into prewashed dialysis tubing and the tubing was placed in a jar containing 800 ml of 0.1 M phosphate buffer pH 4.5. Alternatively, 1 ml dispersion was added directly to the buffer solution. The jars were placed on an orbital shaker at 100 rpm, in an oven equilibrated at 37°C. Samples were taken at discrete times, centrifuged, and analyzed by UV spectrophotometry at 270 nm to determine the drug release rate from the formulations. As a control, OTC solution (Dabicycline-100) was placed in the dialysis tubing to determine whether the dialysis tubing was limiting the rate of release.

The storage stability of 8% OTC-liposphere formulations (10.0 g) packed under argon in sealed 10 ml amber glass containers and stored at 4 and 25°C in 60% humidity cabinets was followed with time. The particle size, OTC content, viscosity, and appearance of the formulations were monitored for 12 months. Samples were analyzed in triplicate at 0, 1, 3, 6, and 12 months of storage.

2.4. Oxytetracycline blood levels and elimination studies

2.4.1. OTC blood levels and evaluation of implant site after a single injection of several OTC-liposphere formulations

5-week-old turkeys of the large bred strain from a local source (weighing 1.0 ± 0.2 kg) randomly divided into six groups of six birds each group, were injected intramuscularly with 100 mg/kg of one of the formulations listed in Table 2. The dose selected in this trial, 100 mg/kg, was greater than the usual 20 mg/kg daily dose, in order to allow follow up of blood levels for a period of a few days. A blank liposphere formulation (injection volume 0.4 ml) was used as control. The birds were bled nine times during 8 days. The blood samples were allowed to clot for 2 h at room temperature, after which the samples were centrifuged and the serum was removed and saved for analysis. At 7, 11, and 28 days post-injection, two birds of each group were killed, the injection site was observed and the deposits were collected for composition analysis. The deposits were analyzed for OTC content by extraction with 0.1 N HCl solution and analysis by UV spectrophotometry at 270 nm.

2.4.2. Comparison between OTC-solution and OTC-liposphere formulations

3-week-old turkeys of the large bred strain from a local source (weighing 350 g ± 10%) were randomly divided into three groups of 10 birds each. The first group was injected intramuscularly in the pectorial muscle with 1.0 ml/kg of 10% OTC solution (Dabicycline-100). The second group was injected with 1.18 ml/kg of 8.0% oxytetracycline-liposphere formulation and the third group was injected with a 4:3 v/v mixture of Dabicycline-100 and 8.0% oxytetracycline-liposphere formulation. At each bleeding time all 10 birds of each group were bled. The blood samples were allowed to clot for 2 h at room temperature, after which the samples were centrifuged and the serum was removed and saved for analysis. On the 9th and 12th day, five birds from each group were killed and autopsied for histopathology evaluation of the injection site.

2.4.3. Biological assay for OTC in blood

The OTC concentrations in blood samples were determined by the disc diffusion assay technique. Antibiotic assay medium no. 2 (Difco) pH 5.6 was used. *Staphylococus aureus* test organism (ATCC 6538) (1 ml) of inoculum density of 1×10^4 CFU/ml was added to 100 ml medium and poured into a Nunc 53.0 cm^2 Bio-Assay dish. The standard OTC was prepared in blank turkey serum. The detection limit was 0.2 μg/ml.

3. Results and discussion

3.1. Preparation and characterization of OTC-loaded lipospheres

OTC-liposphere formulations were prepared from common inexpensive natural ingredients,

solid triglycerides and phospholipids, in one step without the use of solvents. The formulation was preserved by parabens, propylparaben in the oil phase and methylparaben in the aqueous phase.

A unimodal particle size distribution was observed. The average particle size was in the range of 15–40 μm with less than 2% of particles greater than 100 μm. The blank formulations were of average particle size of 10 μm. Oxytetracycline did not melt or dissolved in the triglyceride core material and it remained dispersed in the final formulation as drug particles of 5 μm which resulted in an increase in the final particle size of the formulation as compared to the blank formulations.

A study was conducted to determine the effect of liposphere composition on its physical properties, drug release rate, and injectability through a 22 gauge needle. The following parameters were investigated: triglyceride composition, phospholipid type, continous aqueous phase composition, drug loading, and the OTC/triglyceride/phopholipid ratio. Three triglycerides were used, tristearin (TL, m.p. 75°C), trilaurin (TL, m.p. 42°C), and tricaprin (TC, m.p. 33°C). Three types of lecithin were utilized, highly pure (99%) egg or soybean phospholipids and food grade lecithin (96% acetone insoluble). The continuous medium was either 0.1 M phosphate buffer or 5% dextrose solution. The results of this study are summarized in Table 1.

The formulations based on tricaprin regardless of the type of phospholipid or continuous medium composition, loaded with up to 15% OTC were stable and injectable and provided prolonged drug release rate in vitro (Fig. 1). The release was determined from either adding the formulation in a dialysis tubing or directly dispersed in a large volume of buffer. The in vitro release from dialysis tubing was significantly slower than OTC solution which was released within 1 h. Direct dispersion into buffer solution resulted in faster release from lipospheres. This can be explained by the difference in the OTC solubilization conditions, the solubilization of the drug from the concentrated dispersion in the bag being less effective as

Table 1
Physical properties of oxytetracycline (OTC) formulations of various compositions

Code	Triglyceride (mg/ml)	Phospholipid (mg/ml)	OTC (mg/ml)	Aqueous medium	Particle size (μm)	Injectability	In vitro $t_{1/2}$ (h)
1	TS-150	EPC-100	80	phosphate buffer	34	+	30
2	TL-150	EPC-100	80	phosphate buffer	30	+ +	26
3	TC-150	EPC-100	80	phosphate buffer	31	+ +	24
4	TC-150	EPC-50	100	phosphate buffer	35	+ +	26
5	TC-150	EPC-150	100	phosphate buffer	27	+ +	29
6	TC-150	EPC-100	100	dextrose	26	+ +	26
7	TC-150	SPC-100	100	dextrose	30	+ +	28
8	TC-150	SPC-100	100	phosphate buffer	35	+ +	27
9	TC-150	SPC-FG-100	100	phosphate buffer	33	+ +	28
10	TC-150	SPC-FG-100	100	phosphate buffer	30	+ +	29
11	TC-150	EPC-50	80	phosphate buffer	35	+ +	28
12	TC-150	EPC-50	150	phosphate buffer	28	+ +	28
13	TC-150	EPC-50	200	phosphate buffer	46	–	–
14	TC-0	EPC-50	150	phosphate buffer	32	+ + +	15
15	TC-150	EPC-50	0	phosphate buffer	13	+ + +	–

The formulations were prepared by a melt process using tristearin (TS), trilaurin (TL), and tricaprin (TC) as solid core triglycerides and egg phospholipid (EPC, 99% pure), soybean phospholipid (SPC, 99%), and food grade lecithin (SPC-FG, 96% acetone insoluble) as dispersing agent. The particle size was determined by a Coulter LS100 particle size analyzer. The ease of injectability was graded as very easy (+ + +), easy (+ +), formulation with some clogging (+) and formulation was too viscous to be used (–). The $t_{1/2}$ was determined as the time for 50% release of OTC using a dialysis tubing system.

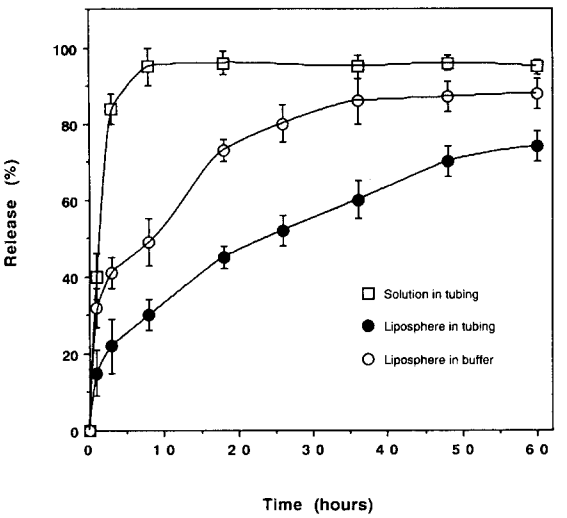

Fig. 1. In vitro release of oxytetracycline from liposphere formulations. 1 ml of liposphere formulation was placed into dialysis tubing (300000 Mol. Wt cut-off) or inroduced directly into 800 ml of phosphate buffer pH 4.5 at 37°C. Samples were taken at discrete times and analyzed by UV at 270 nm to determine the OTC release rate from the formulations. As a control, the release of OTC from commercial OTC formulation (Dabicycline-100, 10% OTC in acidic solution) was determined.

compared to the dispersed formulation where each liposphere is exposed to a large volume of buffer. Formulations containing less than 5% phospholipid or more than 15% OTC were viscous, less physically stable and often caused clogging of the needle and therefore were discarded. The food grade phospholipid was as effective as the highly pure phospholipids. All formulations were fluid enough to be taken and injected using a 22 gauge needle without any clogging. Formulations produced in a scale of 10–500 ml batch size yielded preparations with essentially the same characteristics with high reproducibility.

The OTC liposphere formulations were stable for at least 1 year at 4°C with essentially no change in the OTC content, and physical characteristics of the formulations. At 25°C, a gradual change from yellow to brown was recognized with a slight increase in particle size and viscosity. A decrease in OTC content of 7 and 12% was found at 6 and 12 months of storage, respectively.

3.2. *In vivo studies*

Two studies were conducted to evaluate the controlled release effect of the liposphere formulations by following the OTC blood levels and the elimination of the injected dose from the implantation site. In the first study, four OTC-loaded liposphere compositions were compared with OTC solution (Dabicycline-100) used as reference and a blank liposphere formulation used as control (Table 2). The formulations were injected into groups of six birds and the OTC blood levels were determined. The injection sites were observed for residuals at 7, 11 and 28 days post-injection.

The average OTC blood levels for the six formulations are given in Table 3. Typical data for

Table 2
Characteristics of oxytetracycline (OTC) formulations used in first turkey study

Animal group	Formulation code	OTC (mg/ml)	Triglyceride		Phospholipid (mg/ml)	Particle size (μm)	Dose (ml/kg)
			Type	mg/ml			
1	A	80	tristearin	100	100	36	1.3
2	B	80	trilaurin	100	100	40	1.3
3	C	120	trilaurin	150	150	34	0.8
4	D	blank	trilaurin	100	100	16	1.3
5	E	80	trilaurin	100	50	42	1.3
6	Dabicycline-100	100	aqueous solution	–	–	–	1.0

The formulations were prepared by a melt process using triglycerides and egg phospholipid (EPC, 99% pure). The particle size was determined by a Coulter LS100 particle size analyzer. Dabicycline-100 is a 10% commercial aqueous solution of OTC. A dose of 100 mg/kg OTC was administered intramuscularly to all animals in this study.

Table 3
Oxytetracycline (OTC) serum level (μg/ml) in 5-week-old turkeys following a single intramuscular dose of formulations described in Table 2

Group	Formulation	Mean OTC serum level (μg/ml) at hours post-injection								
		2	5	24	48	72	96	120	168	192
1	A	0.3	0.8	1.6	1.5	0.9	0.6	0.5	0.06	0.0
2	B	0.5	1.5	1.3	0.9	0.4	0.08	0.03	0.0	0.0
3	C	0.7	1.2	1.9	1.8	1.3	0.7	0.4	0.1	0.0
4	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	E	0.7	1.0	2.3	1.7	1.0	0.5	0.4	0.2	0.0
6	Dabicycline-100	15.2	10.4	2.3	0.9	0.3	0.0	0.0	0.0	0.0

The formulations described in Table 2 were injected (100 mg OTC/kg) in the muscle of 1 kg turkeys. OTC blood levels were determined by a bacterial inhibition assay.

the blood levels of individual birds within each group are given in Fig. 2. All four liposphere formulations show OTC levels above MIC for at least 3 days. Formulation E composed of OTC (80 mg/ml) and trilaurin and phospholipid in a ratio of 2:1 showed similar OTC blood levels to the formulations based on tristearin (formulation A) and the more concentrated (120 mg/ml) trilaurin based formulation. The formulation composed of trilaurin/phospholipid in a 1:1 ratio (formulation B) was less effective. These results indicate that lower phospholipid to triglyceride ratio improves the duration of drug release and that higher drug loading (formulation C) does not affect the duration of drug relase. Although tristearin showed good results, it is not preferred because it is less susceptible to elimination from the injection site as described below.

The residual amount at the injection site evaluated at 7 and 11 days (Table 4) was maximal (about 90% of the original dose) for the tristearin-based formulation (formulation A), about 50% for the trilaurin based formulations (B, C and E), and about 20% for the blank formulation. The deposits contained less than 10% OTC of the original dose. After 28 days, only the tristearin based formulation (formulation A) had a significant amount of deposits at the injection site which was mostly tristearin (Table 4). No OTC was detected in any of the deposits retrieved from the animals after 28 days. These results indicate that formulations based on TL, a semi-solid fat at body temperature (m.p. = 44°C), are more readily eliminated from the injection site and thus they were selected for further studies.

Based on these results a second animal study was conducted in order to identify an optimal formulation which would provide maximal OTC blood levels after a single injection. The composition of formulation E (Tables 2 and 3) was tested in comparison with a mixture of formulation E and Dabicycline-100 in a 4:3 volume ratio. Dabicycline-100 commercial OTC solution was used as

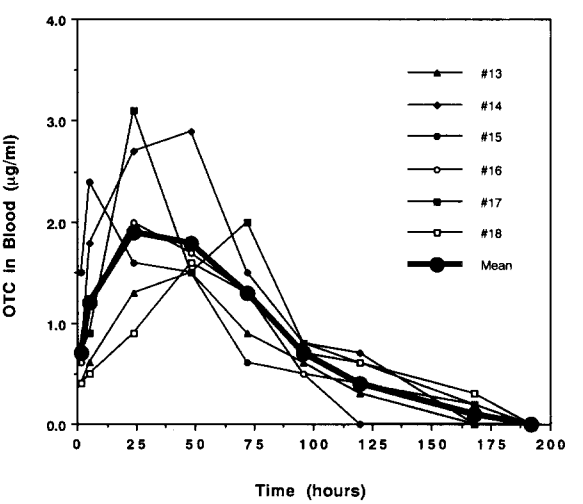

Fig. 2. Oxytetracycline (OTC) serum level (μg/ml) of individual 5-week-old turkeys following a single intramuscular dose of 100 mg OTC/kg. OTC-liposphere formulations composed of OTC (12%), trilaurin (15%), egg phospholipid (15%) in buffer pH 7.4, were used. OTC blood levels were determined by a bacterial inhibition assay with a detection limit of 0.2 μg/ml.

Table 4
Inspection of injection site 7, 11 and 28 days post-injection in turkeys of study described in Tables 2 and 3

Group	Formulation	OTC (mg/ml)	Injection site histopathology		
			7 days ($n = 2$)	11 days ($n = 4$)	28 days ($n = 4$)
1	A	80	heavy green localized thick encapsulation	heavy green localized thick deposits	moderate localized deposits
2	B	80	thin spreading deposit extended over a large area	less deposits than at 7 days	slight localized deposits
3	C	120	moderate green localized deposits	localized thin deposits less deposits than at 7 days	slight localized deposits
4	D	blank	very thin spreading white deposit large area	localized thin deposits less deposits than at 7 days	no deposits found
5	E	80	light localized yellow deposits	light localized deposits deposits	slight localized
Dabicycline-100		100	traces deposits observed	no deposits observed	no deposits found

Birds were injected with 100 mg/kg OTC formulations and the birds (two of each group at each time point) were killed at 7 and 11 days post-injection. The injection site was observed and the deposits were collected for analysis. The deposits at 7 and 11 days contained small amount of OTC and were mostly the triglyceride component.

reference. Dabicycline-100 was added to formulation E by simply mixing the two formulations, in order to increase the initial blood levels which were too low for the first 5 h after injection. The three formulations were administered intramuscularly to turkeys (10 birds for each formulation). The mean serum concentrations expressed in μg/ml of OTC are given in Fig. 3. OTC solution (Dabicycline-100) resulted in very high serum levels at 3 and 6 h, 19.9 and 15.2 μg/ml, respectively, and drug levels of 2.1, 0.4 and < 0.1 μg/ml were found at 24, 48 and 72 h, respectively. The mixture of Dabicycline-100 and liposphere formulation gave intermediate blood levels during the first day and higher levels than lipospheres alone for the next 3 days. Evaluation of the injection sites after 11 days showed localized residues which were estimated as 40–50 and 30–40% of the injected dose for the liposphere and the Dabicycline-100-liposphere mixture, respectively. The residue was mostly the triglyceride and phospholipid and a negligible amount of OTC. No residuals were found in the injection site of OTC solution (Dabicycline-100).

All animals in these studies were healthy and

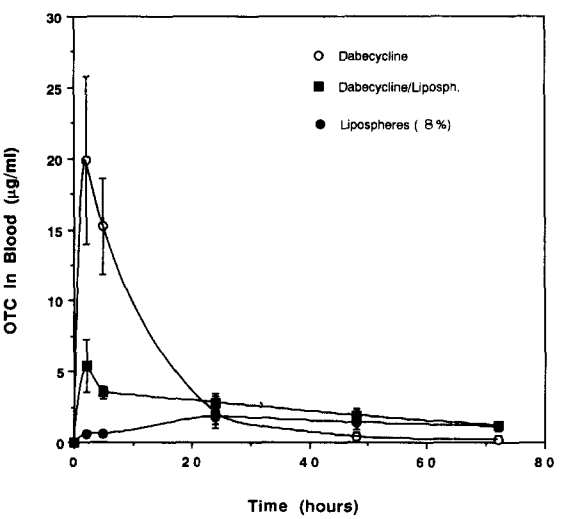

Fig. 3. Oxytetracycline (OTC) serum level (μg/ml) in turkeys following a single intramuscular dose of liposphere and liposphere-Dabicycline-100 mixture. Turkeys (weighing 350 g, $n = 10$) were injected intramuscularly (100 mg OTC/kg) with either 10% OTC acidic solution (Dabicycline-100), 8.0% oxytetracycline-liposphere formulation, and a 4:3 mixture of Dabicycline-100 and 8.0% oxytetracycline-liposphere formulation. At each bleeding time all 10 birds of each group were bled and OTC concentration in serum was determined by antibacterial-cell culture test.

gained weight as the non-treated animals with no pathological signs. In all injection sites there were no signs of damage, swelling or inflammation. All injection sites did not show any necrosis or encapsulation even when precipitates were observed.

4. Conclusions

This work demonstrates the usefulness of the liposphere encapsulation system for extending drug release after a single injection. Similar results were obtained for other liposphere drug formulations (Domb, 1993a). The particle sizes of the OTC formulation were larger than those obtained for vaccines (Amselem et al., 1992a, b), local anesthetics (Domb, 1993b), and DEET insect repellent (Domb, 1995). This difference is probably due to the insolubility of OTC in the triglyceride core, as the drug particles are dispersed in the solid core material resulting in larger lipospheres. An effective extended release of 3–5 days was obtained for OTC which is similar to the results reported for local anesthetics and steroids.

The biodegradability of the formulation was demonstrated by observing the injection site for up to 28 days after injection. The blank formulation was readily eliminated from the site and no signs of residuals were observed 28 days after injection. OTC-loaded formulations based on trilaurin were also degraded but at slower rate than the blank formulation which might be a result of the hydrophobicity of OTC which retards hydrolysis and biological elimination. Because of the encapsulation efficiency of OTC, inadequate initial levels of OTC, essential for effective killing of bacteria, were observed. To increase the initial OTC blood levels, the liposphere formulation was mixed with commercial OTC solution and indeed this combination provided initial high blood concentrations and higher blood levels throughout the study.

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