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Biodegradable microspheres as depot system for parenteral delivery of peptide drugs

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Synthetic biodegradable polymers of lactic and glycolic acid have been extensively investigated for sustained drug delivery. Drug release from lactide/glycolide polymers can be either diffusion controlled or matrix erosion controlled. Polymer degradation was dependent on the molecular weight of the polymer and matrix structure of the delivery system. High surface area, lower particle size and lower bulk density increased the rate of degradation. Gamma irradiation of the delivery system also increased the rate of matrix erosion by reduction of molecular weight. Microspheres containing peptide, salmon calcitonin (sCT), were prepared by solvent extraction techniques using temperature or dilution. sCT was also incorporated into pre-formed microspheres by adsorption. sCT has a strong hydrophobic region and hence can bind to the polymer by a combination of hydrophobic and ionic forces, resulting in high incorporation from an aqueous medium. Adsorption appeared to result in multiple layers of adsorbed peptide on the polymer surface. The microspheres with entrapped sCT exhibited in vivo release of sCT between days 5 and 9, whereas microspheres with adsorbed sCT showed low but detectable in vivo release for 3-4 days. Combination of microspheres with different release properties can be used to achieve various in vivo serum profiles.

Key words: Biodegradable microsphere; Peptide; Lactide/glycolide polymer; Salmom calcitonin; Drug delivery

Introduction

Synthetic biodegradable materials which are biocompatible are being utilized in medical applications as drug carriers, surgical sutures and various prostheses. Biodegradable polymers degrade either by homogeneous degradation, where random cleavage of the polymer chains in the polymer matrix results in degradation, or by heterogeneous degradation, where the degradation is restricted to the surface of the matrix. Biodegradable polymers can be found among various chemical classes. Polyorthoesters are stable in alkaline conditions but undergo heterogeneous degradation in acidic environment [1]. Polyanhydrides which can be prepared at different hydrophobicities to influence drug release as a function ofpH, hydrolytically degrade by a heterogeneous process [2]. Synthetic poly amides based on glutamic acid are highly stable and degrade by hydrolysis at glutamic add residues. The

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degradation is also influenced by non-specific amidases and is reported to be unreliable [3]. Polyalkylcyanoacrylates are formed by spontaneous polymerization of monomers in the presence of water and hence may be used for in situ polymer formation. The degradation rate is dependent on the monomer chain length and produces formaldehyde as a by-product creating toxicity concerns [4]. Polyesters are the most widely studied synthetic polymers for drug delivery. They degrade by random hydrolysis of ester bonds and produce organic acids and alcohols. Selection of appropriate monomers can result in a polymer devoid of any toxicity.

Polyesters of lactic acid and glycolic acid are the most commonly used synthetic biodegradable materials due to their low toxicity and excellent biocompatibility. The degradation products of these polymers are natural constituents of the body and are converted to carbon dioxide and water in the energy metabolism cycles. Poly(L-lactic acid) was the first biodegradable polyester to be used in drug delivery as implants containing cyclazocine [5]. The primary drawback of this device was the lengthy degradation time of several years. Polyglycolic acid has a more acceptable degradation time of several months [6] which makes it a good candidate as a carrier in drug delivery systems. Many different drugs, including anti-cancer agents, narcotic antagonists and steroids, have since been incorporated into devices made from the polymers of lactic acid and glycolic acid [7-12]. Recently the lactide/glycolide copolymers have been used more extensively because of their amorphous nature and shorter biodegradation times, in terms of several weeks.

Various methods can be used to incorporate drugs into the polymer matrix for delivery. Microspheres have been studied extensively as a carrier system for a variety of drugs. Traditional solvent-evaporation methods generally produce non-porous microspheres [13]. While suitable for small molecular weight water soluble drugs, non-porous microspheres are not as desirable for peptide and protein delivery. A porous matrix which would allow increased diffusion of large molecules is more suitable for peptides and proteins. The hydrophobic properties of polymers of lactide/glycolide series also offer the opportunity to bind hydrophobic peptides to the surface of microspheres for use in controlled delivery. This paper describes the in vitro degradation behavior of poly50:50(D,L-lactide-co-glycolide) (PLGA), some of the processing parameters that affect the structure of salmon calcitonin (sCT) microspheres, the adsorption of sCT to the surface of the microspheres and the in vivo release of the peptide from the microspheres.

Methods

In vitro degradation of polymers

In vitro degradation of (sCT containing) PLGA microspheres was carried out in 0.1 M phosphate buffer (pH 7.4) made isotonic with Nad at 37°C. Fifty mg of microspheres were transferred to scintillation vials and 10 ml of the buffer medium added. The contents were agitated in a bath incubator shaker. At specific intervals, the microspheres were filtered through an HA 0.45-μ. membrane filter (Millipore) and washed with 10 ml of water. The microspheres were dried under vacuum overnight at room temperature. The initial (0 time) value was obtained from microspheres soaked for 5 min, filtered, washed and dried in the same way.

The molecular weight (M_w and M_n) of the PLGA microspheres was determined by GPC (Waters, 990) using two ultra styragel columns (10^4 and 500 A). Tetrahydrofuran was used as the elution solvent at a flow rate of 1 ml/min. Polystyrene standards from Polysciences were used as molecular weight calibration standards. From the GPC curve of the sample and the retention times of the standards the molecular weight was calculated using a Maxima 820 (Waters) program.

Preparation of sCT microspheres

The sCT PLGA microspheres were prepared by an aqueous emulsification-solvent removal technique [14]. Briefly, the technique involved

the dispersion of a solution ofPLGA and sCT in methylene chloride and methanol (dispersed phase (DP)) into an aqueous continuous phase (CP) containing 0.4% sodium oleate. Microspheres were then solidified by removal of the DP solvents. Solvent removal can be achieved in two ways: (i) a controlled temperature method, wherein the solvent is evaporated at its boiling point; or (ii) a controlled dilution method, wherein the solvent is extracted into a suitable medium. The former method involves heating and evaporation while the latter depends on solvent dilution and extraction.

Interaction of sCT with PLGA microspheres

Blank PLGA microspheres with mean diameters of 14.8 ± 1.3 /μm and specific surface area of 0.323 m^2/g were prepared by the controlled temperature method described earlier. Various amounts of microspheres were added to 1 ml of 1 mg/ml sCT solution and allowed to interact for a specified period of time. The supernatants were analysed for sCT concentration by reversed phase HPLC method using acetonitrile and trifluoroacetic acid as phase modifiers in water. The peptide was quantified by UV spectrophotometric detection. The effects of peptide and microsphere concentrations were examined on the adsorption of peptide to the polymeric microspheres.

In vivo evaluation of sCT microspheres

In vivo evaluation of the sCT PLGA microspheres was performed in female Sprage-Dawley rats. Microspheres suspended in a suitable vehicle or free drug equivalent to 40 U/kg were administered by a subcutanous injection and the serum samples were collected from a femoral catheter daily for 14 days. If catheter clogging occurred, the animals were re-catheterized and sampling was resumed after 24 h, resulting in an unequal number of samples for each day. sCT concentrations in the serum samples were determined by a double antibody radioimmuno assay with a sensitivity of 20 pg/ml.

Results and Discussion

In vitro degradation of polymers

Table 1 presents a summary of results obtained on in vitro degradation of three types of microspheres prepared from PLGA. Microspheres of Type I with high surface area and low bulk density (resulting in high porosity) show the shortest time for onset of mass loss (T_{onset}) whereas the microspheres of Type II, with lower surface area and higher bulk density (resulting in lower porosity) have a later onset. Type II microspheres were prepared from Type III microspheres by ^{60}Co gamma irradiation which resulted in lowering of the molecular weight. Type III microspheres, due to the higher molecular weight, have a longer T_{onset}. In addition to all these physico-chemical characteristics, cellular interactions at the site of administration may further influence the biodegradation process.

The polymers oflactide and glycolide series can be synthesized as homopolymers or co-polymers [15-19] with each type having different physicochemical properties and degradation rates. The 50:50 co-polymer of D,L-lactide and glycolide degrades the fastest, because it is the least likely to possess crystalline blocks of either the glycolide or lactide monomers.

The in vitro degradation of the D,L-lactide-co-glycolide polymers exhibit four distinguishable stages typical of the bulk (homogeneous) degradation. The process is depicted by Fig. 1 and includes:

(i) an initial lag period during which hydration of the polymer occurs;
(ii) random ester bond cleavage throughout the polymer matrix resulting in a decrease in molecular weight, M_w;
(iii) continued bond cleavage to a critical M_w resulting in the onset of polymer mass loss caused by solubilization of the low M_w polymer fractions;
(iv) Complete solubilization.

The degradation of polymers also depends on the molecular weight, monomer sequencing and

TABLE 1
Initial properties of three types of microspheres and T_{onset} for mass loss during in vitro degradation

Type of microsphere	M_w	M_n	Specific Surface Area (m^2 g)	Bulk Density (g/ ml)	Particle Size (μm)	T_{onset} (Weeks)
I sCT/PGLA	29 049	15 903	5.2545	0.094	109	1.5
II[a] Blank PGLA (radiated, 2.5 Mrad)	25 643	13 496	0.3176	0.167	200	3.0
III Blank, PGLA (non-radiated)	33 092	17 016	0.3822	0.167	200	4.3

[a]II & III are from the same batch of M

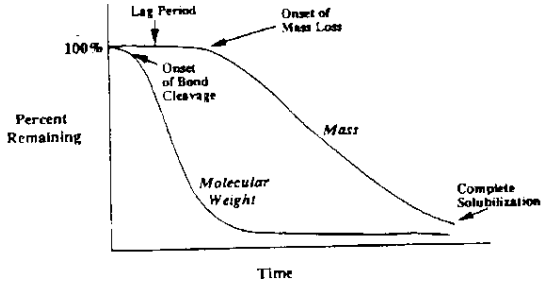


Fig. 1. Schematic representation of the in vitro degradation of the D,L-lactide-co-glycolide polymers.

cross-linking within the polymer backbone. In addition to the polymer degradation, the actual time of solubilization of the matrix depends on the surface area available, water penetration and matrix porosity for hydration.

PLGA microspheres containing salmon calcitonin

Previous studies with sCT-PLGA microspheres revealed that polymer molecular weight, co-solvent concentration, peptide concentration, DP/CP ratio and the solvent removal method influenced the matrix porosity and hence the surface area of the microspheres [20]. In this study the method and rate of solvent removal was found to influence the internal structure of the microspheres.

Controlled temperature technique

In the controlled temperature method removal of methylene chloride was achieved by ramping the temperature to 40 ± 1 °C to effect evaporation from the continuous phase and rapid extraction from the microspheres. This technique resulted in microspheres with a hollow core and a honey-comb like porous wall. The size of the core and the thickness of the wall were dependent on the gradient employed to raise the temperature from 15 to 40 °C. A rapid rise of temperature during solvent evaporation resulted in a thin wall and a large hollow core (Fig. 2). When the temperature was raised more gradually from 15 to 25° C, and then raised to 40 °C, the core size was reduced (Fig. 3).

During the microsphere preparation process, a large percentage of methylene chloride partitions into the CP immediately after the addition of DP to the CP. This results in a rapid solidification of the external wall of the microspheres entrapping the remaining methylene chloride. Subsequent removal of the residual methylene chloride (10-20% of initial) from the microspheres governs the formation of the internal structure. When the temperature is raised from 15 to 40° C, a rapid removal of methylene chloride (b.p. 39.75°C) takes place resulting in the formation of a hollow core, presumably due to rapid expansion of residual methylene chloride. Solidification of microspheres upon complete removal of methylene chloride formed a thin porous wall. However, the wall structure could be changed by changing the temperature gradient. During a gradual increase in temperature from 15 to 40 °C, methylene chloride was removed by

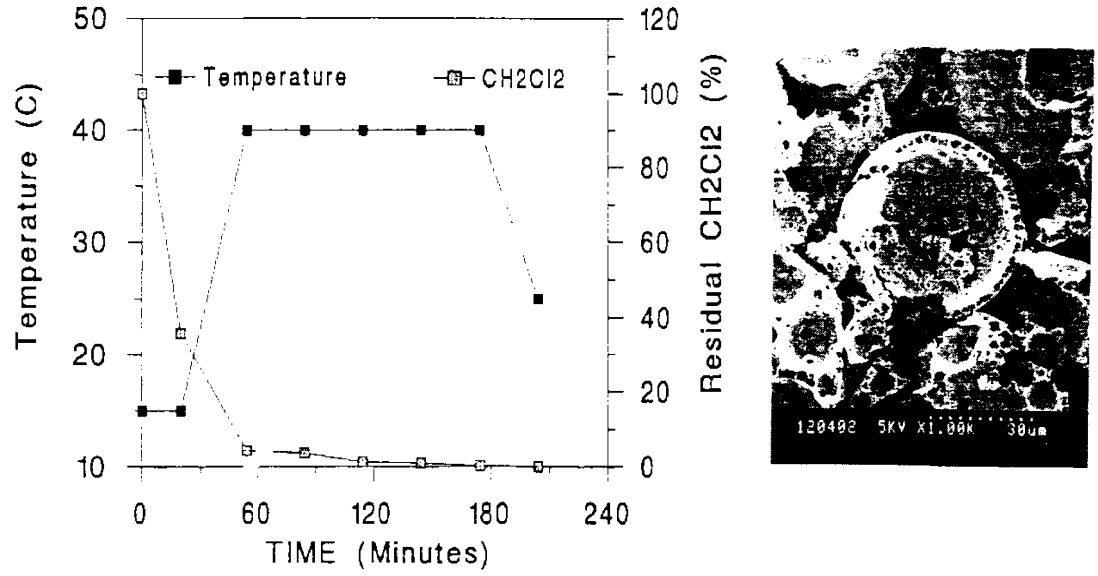


Fig. 2. Solvent removal profile (a) and scanning electron micrograph of the cross section of sCT-PGL microspheres (b) prepared by the controlled temperature method with fast temperature change.

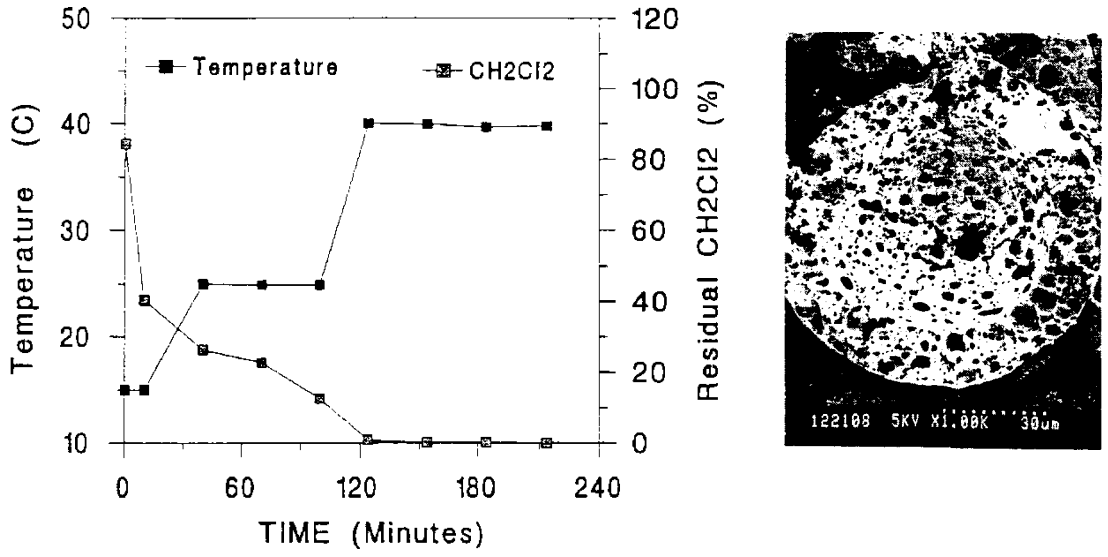


Fig. 3. Solvent removal profile (a) and canning electron micrograph of the cross section of sCT-PGL microspheres (b) prepared by the controlled temperature method with slow temperature change.

slow diffusion out of the microsphere matrix which remained soft until the residual methylene chloride was 4-6% of initial concentration. The size of the core could be altered by the rate of heating but in all cases when the temperature approached 40°C, rapid removal of CH_2Cl_2 oc-

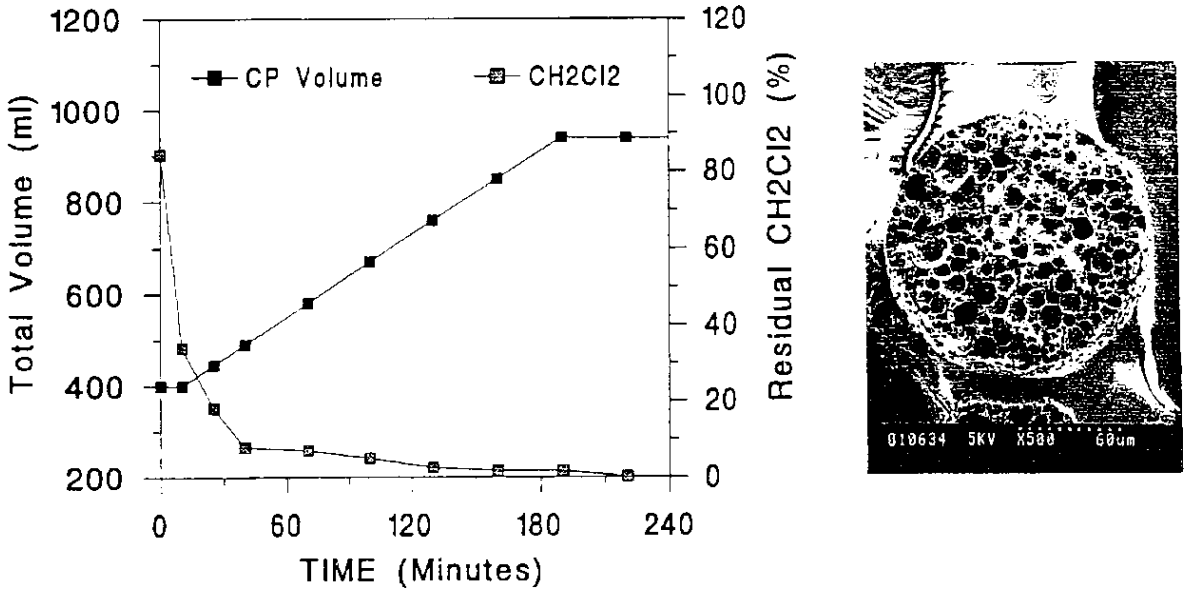


Fig. 4. Solvent removal profile (a) and scanning electron micrograph of the cross section of sCT-PGL microspheres (b) prepared by the controlled dilution method.

curred and the core would result. The gradual removal of solvent seemed to favor the formation of a honeycomb-like internal structure.

Controlled dilution technique

In the controlled temperature method, maintaining the system at 25°C for a sufficient amount of time essentially results in solvent extraction from the dispersed phase rather than solvent evaporation. Thus, the internal structure formed in the controlled temperature method at slower temperature gradient is a result of solvent extraction rather than solvent evaporation.

When sCT microspheres were prepared by the controlled dilution technique, the matrix seemed to be uniformly honey-comb like without any hollow core. Figure 4 shows the solvent removal profile and the internal structure of sCT microspheres prepared by Um method. In this case the CP volume used for dilution was 1.5x the initial volume. Similar internal structure was seen with dilution volumes of 2.4x the initial CP volume. Thus, irrespective of the CP volume used for the dilution, the microsphcrc matrix was honeycomb like without any hollow core.

In the controlled dilution method, a solvent gradient was employed instead of a temperature gradient. Upon the initial addition of the dispersed phase, there was only a partial partitioning of methylene chloride into the CP. Subsequent removal of methylene chloride was then accomplished by controlled dilution of the CP. During this controlled dilution, since the methylene chloride was removed slowly and gradually, microspheres remained soft for a longer period resulting in the formation of a more uniform, honeycomb like internal structure without a hollow core. Further dilution of the CP did not alter the structure.

Interaction of sCT with PLGA microspheres

Although the peptide was incorporated into the PLGA matrix there was evidence of binding of the peptide to the polymer. The interaction of sCT and PLGA was evaluated to quantitate the extent and type of adsorption of the peptide to the polymer. Figure 5 shows the adsorption of sCT to PLGA microspheres at various microsphere concentrations. With 10-mg microspheres

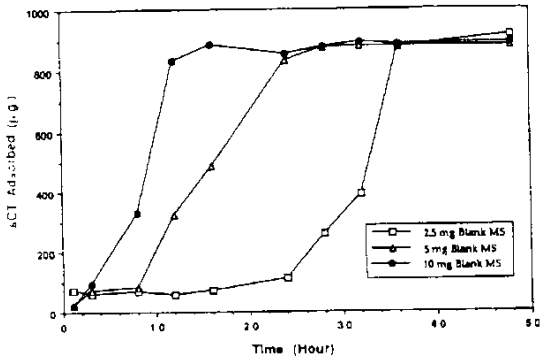
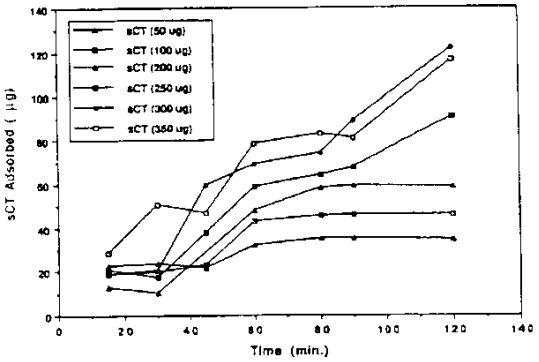


Fig. 5. Kinetics of sCT adsorption to different amounts of PLGA microspheres suspended in 1 ml of solution containing 1 mg sCT.



Time (min.)

Fig. 6. Kinetics of sCT adsorption to 10 mg PGLA microspheres suspended in 1 ml of solution containing different amounts of sCT.

maximum adsorption was reached in 12 h. With 5-mg microspheres this took 24 h while, for 2.5-mg microspheres, 48 h were required to reach maximum adsorption. More than 90% of the peptide was depleted from the solution at the end of the study irrespective of the microsphere concentration. If more sCT were available in the adsorption medium, the samples with 5- and 10-mg microspheres would adsorb the same amount of sCT per unit surface area as that adsorbed by 2.5-mg microspheres. At high PLGA concentrations the adsorption would be greater due to the availability of a larger surface. However, when the PLGA concentration is low the intermolecular hydrophobic interaction between sCT molecules may be greater resulting in a delayed adsorption.

The concentration of peptide in solution is one of the most important parameters that determines the adsorption characteristics. Adsorption kinetics of sCT onto PLGA microspheres at six different concentrations (0.05-0.35 mg/ml) were evaluated in 0.01 M, pH 7.4, phosphate buffer (Fig. 6). At sCT concentrations below 200 μg/ml, a lag period of 45 min for 50 and 100 μg was seen in the adsorption. Following the lag period, adsorption occurs and reaches a plateau between 1 and 2 h. As the concentration of sCT in adsorption medium was increased, the lag period reduced and at 300/ig/ml, no lag period was evident. Also, at concentrations above 200 μg/ml, a transient equilibrium occurred between 60 and 80 min. After the equilibrium, adsorption continued. The second phase of adsorption at higher sCT concentrations may be attributed to formation of multiple layers of the peptide on the surface of polymer. This high adsorption capacity of sCT to hydrophobic polyesters can be successfully exploited for controlled drug delivery.

The interaction ofpeptides and proteins at interfaces is of great biological, medical and technical significance. Adsorption of peptides to polymer surfaces appears to involve a series of complex interactions which are neither easily described nor accurately predicted. Due to the advances in biotechnology and genetic engineering peptides and proteins have become very important therapeutic agents. Adsorption of therapeutic peptides onto polymeric surfaces such as the polyesters can be used favorably in drug delivery system development as the release rate of the peptide can be altered by peptide-polymer interaction.

In vivo evaluation of sCT microspheres

The in vivo characterization of peptide incorporated polyester microspheres has generally been focused in two areas: bioavailability and tissue tolerability. This was achieved in a rat model where sCT microspheres were injected subcutaneously and daily serum samples were collected for 14 days.

Serum sCT concentrations following administration ofsCT-PLGA microspheres containing 3.5% sCT prepared by the controlled temperature technique are presented in Fig. 7. Low levels of sCT in serum for the first 4 days followed by a rapid increase on day 5 with higher levels maintained through day 9. The sCT levels on day 10 return to baseline levels. This suggests that the interaction between sCT and PLGA was substantially reduced at a definite time resulting in an abrupt release of peptide and increase in serum sCT followed by maintenance at higher levels for 4-5 days.

Serum sCT concentrations resulting from PLGA microspheres with an additional 1% sCT adsorbed on the surface (total load=4.5%), exhibited an initial rapid release of sCT followed by a slow release with low but detectable levels ofsCT for about 3-4 days (Fig. 8). This release profile is considerably different from that obtained following administration of free sCT in which sCT concentration returned to baseline levels 2 h after administration due to its short serum half-life.

A mixture of sCT entrapped and sCT adsorbed PLGA microspheres produced bimodal release with an initial rapid release followed by low but detected release and finally sustained release at higher sCT levels. The release profile obtained was similar to a summation of the individual entrapped and adsorbed sCT-PLGA microspheres.

sCT-PLGA microspheres prepared by the controlled dilution technique with a drug load of 3.5% displayed an initial burst release, as seen from the serum sCT concentrations in Fig. 9, which was similar to the microspheres with adsorbed sCT. This initial burst was followed by a lag period lasting 3-6 days, when a second peak was detected. Thereafter sCT levels returned to baseline. The samples in the shaded area could not be analysed due to some unknown interference in the RIA analysis. Because of the higher surface area and porosity of these microspheres, the sCT may have been more accessable and therefore the release or diffusion from the matrix was more rapid when compared to microspheres of lower surface area and porosity.

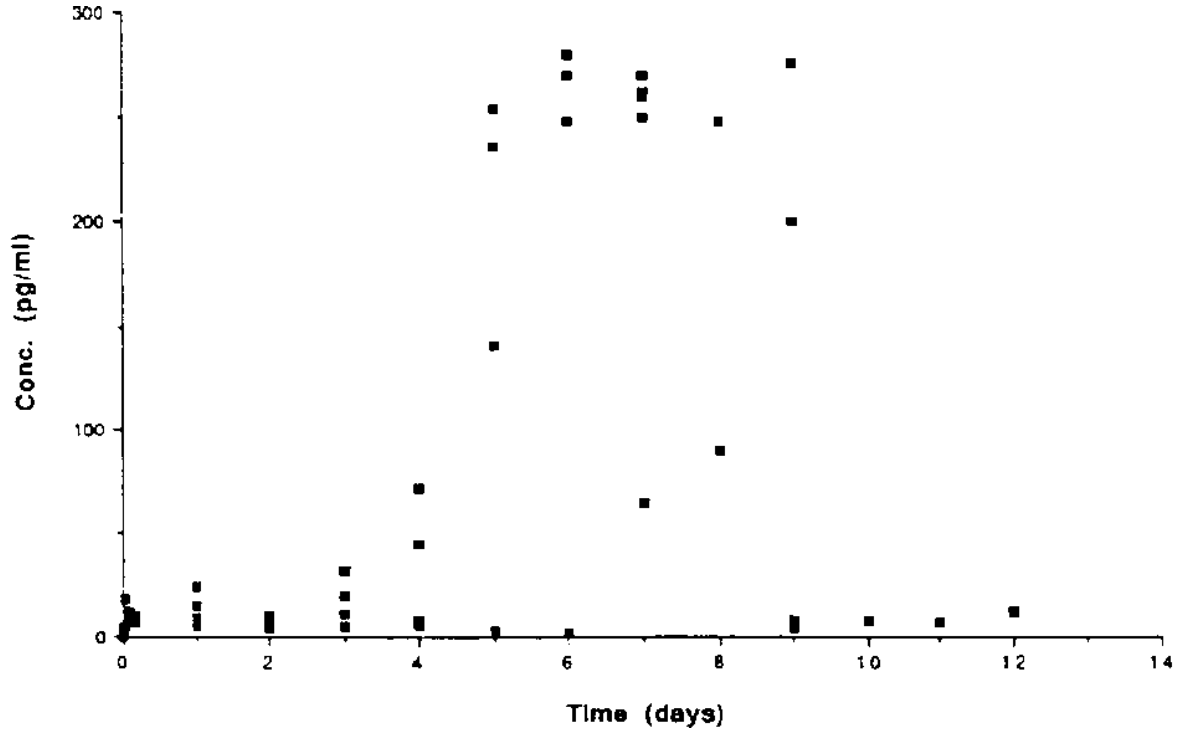


Fig. 7. Serum sCT levels following administration of 40 U/kg sCT entrapped in PLGA microspheres prepared by controlled temperature technique. Each square represents serum levels from a single animal.

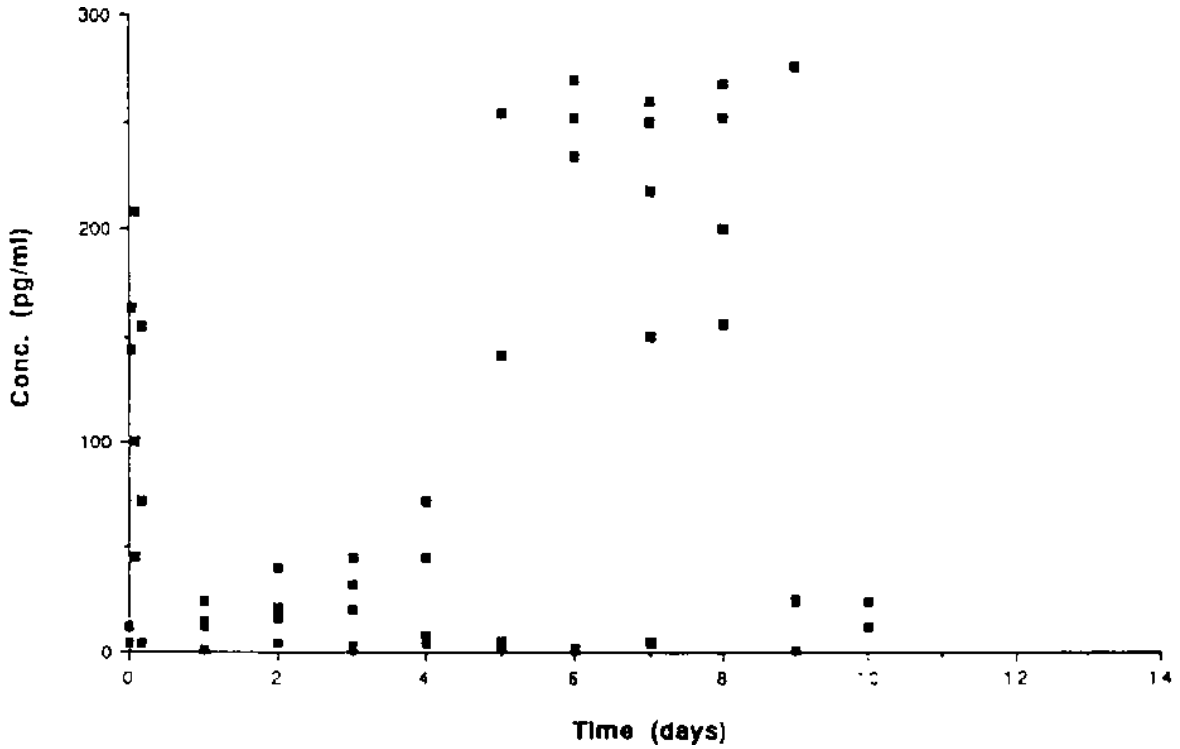


Fig. 8. Serum sCT levels following administration of a mixture of 40 U/kg sCT in entrapped in PLGA microspheres and 40 U/kg sCT adsorbed onto the microspheres.

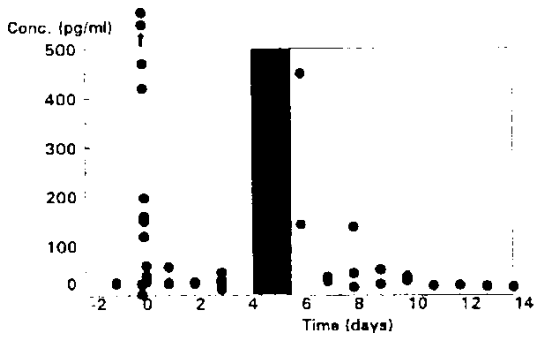


Fig. 9. Serum sCT levels following administration of 40 U/kg sCT entrapped in PLGA microspheres prepared by controlled dilution method. Arrows indicate number of levels above 500 pg/ml.

Conclusions

Biodegradable microspheres for delivery of peptide drugs can be prepared from synthetic polymers of lactic and glycolic acids. The rate of polymer degradation can be controlled by selection of appropriate polymer composition, molecular weight and matrix structure. Microspheres may be prepared from these polymers by solvent extraction techniques where the solvents may be extracted by temperature or dilution. Processing conditions affect the properties of microspheres and may be used to modify the in vitro and in vivo behavior of the microspheres. Adsorption can be used as an alternative method of loading peptides into the microspheres either during or after microsphere preparation depending on the stability of the peptide in solution.

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