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In Vivo Release Kinetics of Octreotide Acetate From Experimental Polymeric Microsphere Formulations Using Oil/Water and Oil/Oil Processes

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Santos B. Murty,[1] Qui Wei,[2] B.C. Thanoo,[3] and Patrick P. DeLuca[2]

[1]Murty Pharmaceuticals Inc, Lexington, KY 40509
[2]Faculty of Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, KY 40536
[3]Oakwood Laboratories LLC, Oakwood, OH 44146

ABSTRACT

The purpose of the present study was to characterize the in vivo release kinetics of octreotide acetate from microsphere formulations designed to minimize peptide acylation and improve drug stability. Microspheres were prepared by a conventional oil/water (o/w) method or an experimental oil/oil (o/o) dispersion technique. The dosage forms were administered subcutaneously to a rat animal model, and serum samples were analyzed by radioimmunoassay over a 2-month period. An averaged kinetic profile from each treatment group, as a result, was treated with fractional differential equations. The results indicated that poly(l-lactide) microspheres prepared by the o/o dispersion technique provided lower area under the curve (AUC) values during the initial diffusion-controlled release phase, 7.79 ng×d/mL, versus 75.8 ng×d/mL for the o/w batch. During the subsequent erosion-controlled release phase, on the other hand, the o/o technique yielded higher AUC values, 123 ng×d/mL, versus 42.2 ng×d/mL for the o/w batch. The differences observed between the 2 techniques were attributed to the site of drug incorporation during the manufacturing process, given that microspheres contain both porous hydrophilic channels and dense hydrophobic matrix regions. An o/o dispersion technique was therefore expected to produce microspheres with lower incorporation in the aqueous channels, which are responsible for diffusion-mediated drug release.

KEYWORDS: in vivo modeling, octreotide acetate, PLA microspheres, PLGA microspheres, radioimmunoassay.

INTRODUCTION

Previously, investigation into the chemical stability of octreotide acetate formulated in poly(d,l-lactide-co-glycolide) (PLGA) and poly(l-lactide) (PLA) microspheres revealed the occurrence of peptide acylation reactions to form adduct compounds.[1] These hydrophobic-related adducts of parent octreotide were identified by Fourier transform-mass spectrometry (FT-MS) and liquid-chromatography-mass spectrometry (LC-MS/MS) to be glycoyl and lactoyl substituted compounds, or +58 m/z (mass/charge) and +72 m/z over parent m/z value, respectively. In fact, 9 separate compounds with various permutations of substitution were identified from the LC-MS/MS instrumental analysis, with the possibility of even a greater number of undetectable compounds present. The previous study also found a reduction in the extent of acylated products by altering the polymer:comonomer ratio from PLGA 50:50 to PLA 100. Lactic acid monomers, as opposed to glycolic acid monomers, were postulated to hinder nucleophilic attack by reactive functional groups on peptide amino acid side chains.

Consequently, microspheres composed of polymers with greater lactide content displayed a significantly lower percentage of impurities.[1] The commercial formulation of depot octreotide (Sandostatin LAR) uses a glucose star PLGA 50:50 copolymer and hence was found to form the above mentioned adducts (+58 m/z and +72 m/z) and behave similarly to high molecular weight (MW) PLGA 50:50 microspheres in terms of extent of impurity formation. As a result, the previous study sought to use polymers for depot formulations of octreotide acetate that minimized the formation of potential antigenic adduct compounds. This speculation on antigenicity arose from literature reports on compounds undergoing acylation, which were found to possess hapten-like properties in vivo. The classic example was the reactivity of aspirin with tissue proteins to form N-salicyloyl protein products, which could induce hypersensitivity reactions.[2]

Unfortunately, the drug release kinetics from octreotide PLA microspheres did not provide prolonged drug release when prepared by the solvent extraction evaporation technique used typically for the delivery of a bioactive peptide in monthly intervals.[3-5] In fact, in phosphate-buffered saline (PBS) media of physiological pH, the octreotide release profile for PLA microspheres resulted in a rapid diffusional burst release with subsequent minimal erosion-phase release kinetics.[1] Hence, alteration in formulation processing parameters and characteristics was required for the use of PLA polymer in octreotide depot formulations for prolonged delivery. In addition to the more conventional oil/water (o/w) emulsification method for manufacturing, Herrmann and Bodmeier[6] described the use of an oil/oil (o/o) nonaqueous method for solvent evaporation

Corresponding Author: Patrick P. DeLuca, Faculty of Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, KY 40536. Tel: (859) 257-1831. Fax: (606) 323-0242. Email: ppdelu1@uky.edu.

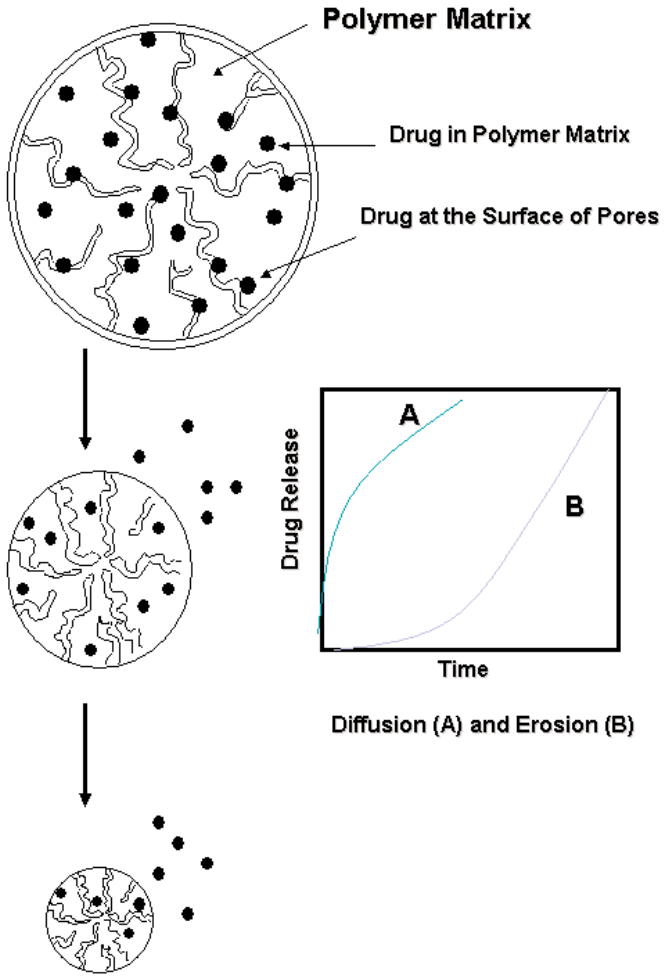

Figure 1. Theoretical modes of drug release (erosion vs diffusion) based on residence of drug molecules within the microsphere.

for the formulation of somatostatin acetate in PLGA polymers. The authors suggested that the use of an o/o method would be useful when peptide stability and solubility issues are inherent with aqueous-phase dispersion processes.

Evidence for the use of o/o dispersion to address the peptide stability issue is seen with the significant formation of acylated products in microspheres prepared by o/w dispersion.[1] Although the reaction was reported to occur minimally during the manufacturing process, significant formation of acylated products resulted during incubation of microspheres in aqueous buffer media. Hence, water influx into the dosage form provided a solvent medium effect for chemical reaction to take place as seen in the case with asparagine (Asn)-hexapeptide formulated in poly(vinyl pyrrolidone) (PVP) polymeric formulations.[7,8] Hence, the o/o dispersion process may minimize acylation found during the microsphere manufacturing process.

Furthermore, with o/o dispersion, octreotide entrapment efficiency within the polymeric matrix may be enhanced. The rapid burst release of octreotide from low molecular weight PLA microspheres in PBS, pH 7.2, resulted in rapid drug depletion from microspheres.[1] This burst effect suggests that during the o/w dispersion method drug entrapment occurs predominantly in the hydrophilic porous regions as opposed to within the hydrophobic matrix of the microsphere. Figure 1 displays a diagram with drug molecules represented by circular points within spherical objects containing tortuous pathways. From this diagram, drug release can occur by either diffusion or erosion where each process is associated with drug entrapment within porous regions and matrix regions, respectively.

One could further hypothesize that during encapsulation the presence of an oil phase in substitution for an external aqueous phase may force peptide molecules to associate with the polymer matrix to a greater extent as opposed to the superficial porous regions. The greater entrapment within the matrix would allow for enhanced release during the erosion-controlled phase and minimized release during the diffusion-controlled burst phase. Consequently, the purpose of the present study was to evaluate octreotide microspheres prepared by both o/w and o/o dispersion methods for release kinetics. The Sprague-Dawley (S-D) rat animal model was used for accurate assessment of formulation activity in a biological system.

Materials and Methods

Materials

Octreotide acetate (H_2N-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol; MW 1018.4) was obtained from Bachem Inc (Torrance, CA). PLGA 8515DL1AP copolymer and PLA 100DL1AP homopolymer were purchased from Alkermes Inc (Cincinnati, OH). Sandostatin LAR kits were purchased commercially from Novartis Pharma (Basel, Switzerland). All other chemicals used were of analytical reagent grade.

Microsphere Preparation

Drug-loaded (octreotide acetate) PLGA and PLA microspheres were prepared by both o/w and o/o dispersion methods with solvent extraction/evaporation as shown in Figure 2.[3-6] For the o/w method, the dispersed phase included methanol/methylene chloride solvent system and the continuous phase included 0.35% poly(vinyl alcohol) (PVA) aqueous solution. Octreotide acetate was dissolved in methanol and combined with the polymer solution dissolved in methylene chloride. This constituted the disperse phase, which was slowly added to the continuous phase (0.35% wt/vol solution of PVA at pH 7.2). The mixture was stirred at 5500 rpm with a Silverson L4R homogenizer (Silverson Machines Ltd, Waterside, UK). Continuous stirring at 40°C for 1 hour resulted in the extraction/evaporation of the solvents. Finally, hardened microspheres were recovered by vacuum filtration and washed with water. To evaporate any residual water and solvent, microspheres were then placed under vacuum drying for 48 hours.

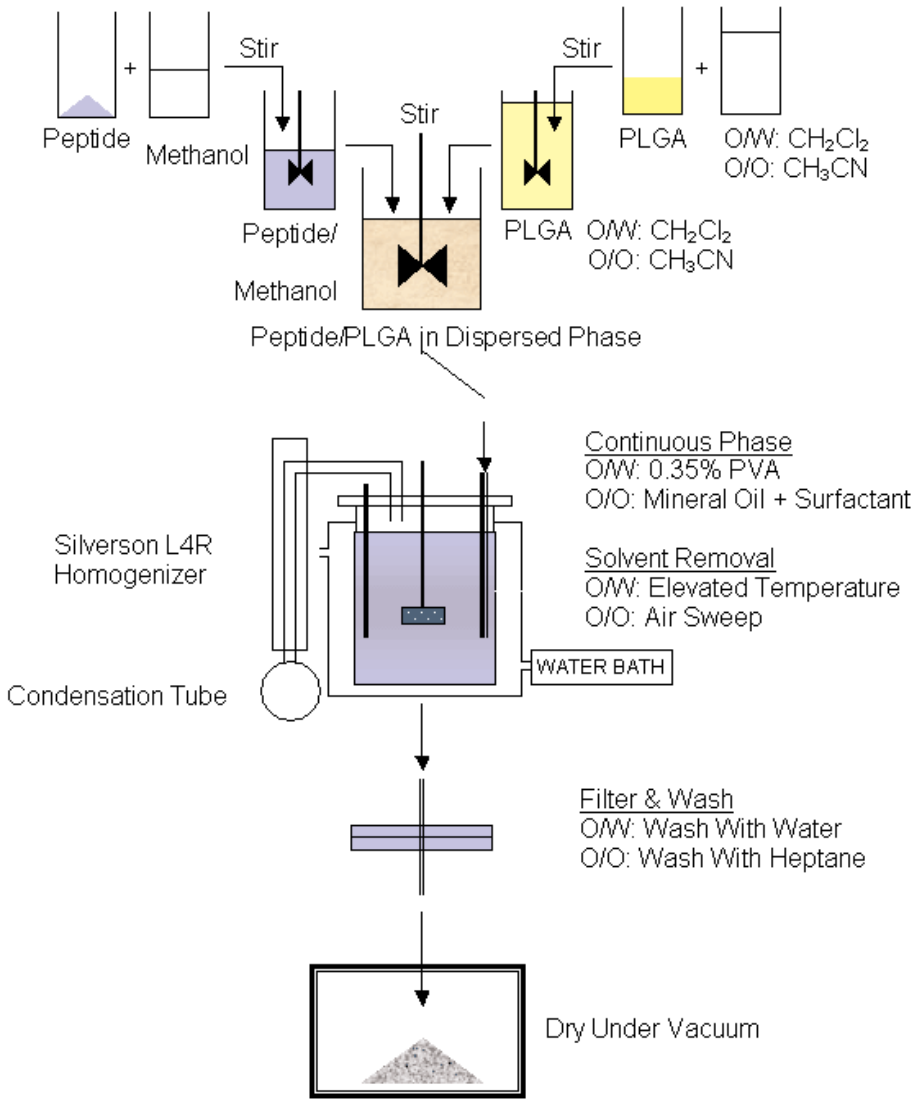

Figure 2. Preparation of PLGA microspheres by either o/w or o/o dispersion methods.

In contrast, the o/o method employed acetonitrile for solubilization of the polymer phase. Thereafter, the primary phase was dispersed in mineral oil containing a surfactant. Solvent removal occurred at temperatures greater than 30°C with continuous air sweeping for 12 to 24 hours. The microspheres were recovered by vacuum filtration and washed with heptane solvent to remove mineral oil containing surfactant.

Microsphere Characterization

Microspheres were dissolved in methylene chloride, and the peptide was subsequently extracted using 0.1 M acetate buffer (pH 4.0). Peptide concentration was determined by high-performance liquid chromatography (HPLC). Microspheres were also characterized by laser diffractometry using a Malvern 2600 laser sizer (Malvern, UK). Average particle sizes were reported in micrometers. Further, microspheres were characterized for bulk density (g/mL) by the tapping method as described by Hausberger et al.[9]

High-Performance Liquid Chromatography Method for Drug Content Assay

Octreotide Acetate was analyzed by reverse-phase (RP)-HPLC using a C18 column, 250 mm × 4.60 mm (Alltech Associates, Deerfield, IL). A gradient elution method was used with mobile phase A (0.1% [vol/vol] trifluoroacetic acid in water) and mobile phase B (0.1% [vol/vol] trifluoroacetic acid in acetonitrile). The gradient was 80:20 (A:B) to 40:60 (A:B) over 25 minutes, with a flow rate of 1.5 mL/min. UV absorbance was measured at 220 nm. The generated concentration values were subsequently used to calculate actual drug-load values.

Administration of Microspheres to Sprague-Dawley Male Rats

Following the University Institutional Animal Care Committee approval, male rats weighing ~300 g were injected with both o/w and o/o formulations of low MW PLGA 85:15 and low MW PLA 100 microspheres (n = 5 per treatment group). The microsphere batches were reconstituted

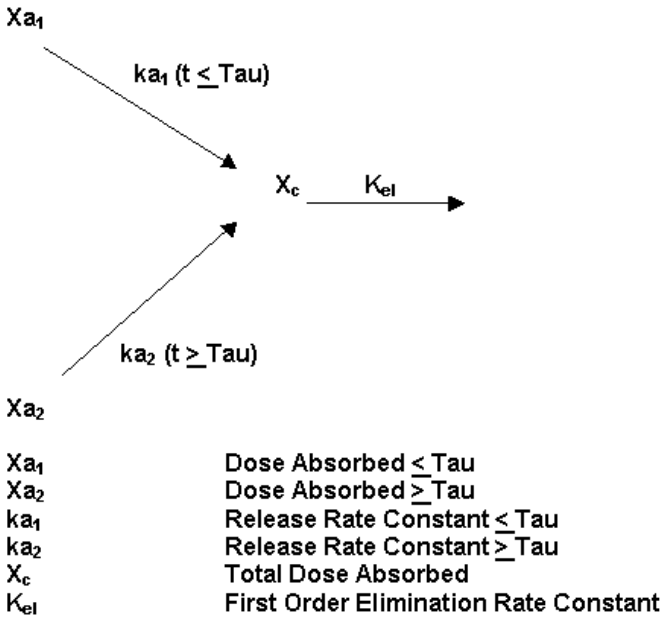

Figure 3. Flowchart depicting modes of drug release.

with a solution containing Tween 80, mannitol, and hydroxypropyl methylcellulose (HPMC) to create a uniform suspension for subcutaneous administration. Six groups were injected with microsphere formulations, behind the neck region, with a dose of 5 mg of octreotide encapsulated in either 1 of 2 polymers (PLA or PLGA 85:15). A PLA batch prepared by the o/w process, however, was delivered with a lower dose (3 mg/rat) owing to an anticipated 10-fold higher burst level of octreotide. In addition, to serve as a reference formulation, the commercial octreotide formulation Sandostatin LAR was administered to one group. For the purposes of the present study, the marketed microspheres were treated with additional steps: (1) washing cycle, (2) reconstitution in vehicle (HPMC, mannitol, and Tween 80), and (3) freeze-drying cycle. This allowed for better reconstitution before administration to S-D rats. Finally, vehicle alone was separately injected into the last group of rats to detect the presence of any interfering substances in the subsequent radioimmunoassay (RIA).

At specified time points, 0.5-mL blood samples were taken via the tail vein and centrifuged in Microtainer tubes (Becton Dickinson, Franklin Lakes, NJ). Sampling was performed at 0, 0.25, 1, 4, 8, 15, 22, 30, 40, and 50 days including 60-day sampling for selected batches. The resultant serum samples were collected and stored at -20°C until analysis was performed with octreotide RIA kits (Bachem).

Radioimmunoassay Procedures

The commercial kit allowed for quantification of peptide within the 1- to 125-pg range, and hence only 10 μL of serum sample was required for each tube in the analysis. The 3-day RIA procedure involved the following: day 1, addition of primary rabbit anti-octreotide antibody to all tubes; day 2, addition of radiolabeled octreotide (I-125 iodinated tracer); and day 3, addition of secondary antibody with normal rabbit serum. A precipitate was allowed to form in each tube and was subsequently collected by centrifugation and isolated by aspiration. A standard curve was constructed using a sigmoidal competitive binding equation provided by Graph Pad Prism software (GraphPad Software Inc, San Diego, CA).

Model-dependent Analysis of In Vivo Release Kinetics

Although nonparametric approaches using deconvolution have been performed to analyze in vivo release data, a model-dependent approach was chosen for the present experiment.[10-12] For PLA 100, PLGA 85:15, and Sandostatin LAR microspheres, a proposed model was developed as an adaptation of the pharmacokinetic analysis described by Murata et al[11] for the characterization of irregular absorption profiles. The model centers on a successive fractional release method to convey the phases of drug release from a dosage form (ie, burst release, diffusion-controlled release, and erosion-controlled release for microspheres).

A 1-compartment model was identified (rat serum concentration) and depicted by the letters "X_c" with "ka_1" and "ka_2" as the 2 absorption rate constants for $t \leq Tau$ and $t \geq Tau$, respectively. For this particular scenario, the time point, Tau, represents a change-point in the model, where the drug-release mechanism changes from phase-1 control (eg, diffusional burst) to phase-2 control (eg, matrix erosion). Murata et al[11] further described this scenario as shown in Figure 3.

The terms "Xa_1" and "Xa_2" represent the fraction of total drug absorbed during the respective phase of drug release. Further, after the X_c value, the K_{el} parameter represents the first-order elimination rate constant, which was calculated from the model as depicted by the following differential equations:

(1) R_1 phase (diffusion-controlled)

$$\frac{dXa_1}{dt} = -ka_1 \times Xa_1 \quad (1)$$

Upon integration of Equation 1, a first-order expression was created, where α represents the fraction of drug release, X represents the total dose, and ka_1 represents the burst release rate constant:

$$Xa_1 = \alpha \times X\left(e^{-ka_1 \times t}\right) \quad (2)$$

(2) R_2 phase (erosion-controlled)

$$\frac{dXa_2}{dt} = -ka_2 \times Xa_2 \quad (3)$$

Upon integration of Equation 3, another first-order expression was created with Xa_2 representing the amount of dose

Table 1. Characteristics of Conventional Microspheres Prepared by an Oil/Water Dispersion Method*

Batch	PLA	PLGA 85:15
Polymer	**100DLCA4**	**85:15DL1AP**
Molecular weight	8 kd	10 kd
Target load, %	10	11.7
Actual load, %	8.47	9.70
Drug encapsulation efficiency, %	84.7	82.9
Particle size, μm		
10% under	4.80	2.20
25% under	8.40	9.20
50% under	15.4	22.5
90% under	29.9	41.1
Bulk density, g/mL	0.26	0.74

*PLA indicates poly(l-lactide); and PLGA, poly(d,l-lactide-co-glycolide).

absorbed in the second phase and ka_2 representing the second phase absorption constant.

$$Xa_2 = (1-\alpha)\times X(e^{-ka_2\times t}) \quad (4)$$

Furthermore, for PLA microspheres, Equations 1 and 2 still applied to characterize the burst phase release. For the erosion phase release, however, a zero-order model was constructed, since in vitro drug release was observed to be linear with respect to time.[1] As a result, Equation 4 reduces to Equation 5:

$$Xa_2 = ka_2 \times t \quad (5)$$

Finally, an elimination factor was introduced to account for the disappearance of peptide after absorption from the subcutaneous injection site. K_{el} represents the elimination constant, and V_d represents the volume of distribution of the peptide in rats. A V_d estimate of 0.4 L/kg was obtained from the literature, where octreotide disposition was measured after IV administration to male rats.[13]

$$\frac{dC}{dt} = -K_{el} \times V_d \times C \quad (6)$$

In summary, an overall differential equation to describe the entire release profile can be the additive terms of each phase of release minus the elimination equation.

$$V_d \times \frac{dC}{dt} = Xa_1 \times ka_1 + Xa_2 \times ka_2 - K_{el} \times V_d \times C \quad (7)$$

Further, for the PLGA 85:15 microspheres, the model can be integrated to the form of Equation 8.

$$V_d \times \frac{dC}{dt} = \alpha \times X(e^{-ka_1\times(t<Tau)}) \times ka_1 + (1-\alpha)\times X(e^{-ka_2\times(t>Tau)})\times ka_2 - K_{el}\times V_d \times C \quad (8)$$

Finally, for the PLA 100 batches, Equation 9 was used to account for the reduction of the first-order process to a zero-order process.

$$V_d \times \frac{dC}{dt} = \alpha \times X(e^{-ka_1\times(t<tau)}) \times ka_1 + ka_2 - K_{el} \times V_d \times C \quad (9)$$

Differential equations were subsequently programmed into the Scientist® software package (MicroMath Research, St. Louis, MS) to model the average concentration versus time profile obtained within each particular group of animals in the experiment.

Results and Discussion

Microsphere Characterization

Tables 1 and 2 display the microsphere characteristics for o/w and o/o batches, respectively. The key characteristics described include drug load, encapsulation efficiency, particle size ranges (μm), and bulk density (g/mL). For the o/w method, 2 batches were selected after optimization of manu-

Table 2. Characteristics of Experimental Microspheres Prepared by an Oil/Oil Dispersion Method*

Batch	PLA (Low TL)*	PLA (Mid TL)	PLGA 85:15 (Low TL)	PLGA 85:15 (Mid TL)	PLGA 85:15 (High TL)
Polymer	**100DLCA4**	**100DLCA4**	**85:15DL1AP**	**85:15DL1AP**	**85:15DL1AP**
Molecular weight	8 kd	8 kd	10 kd	10 kd	10 kd
Target load, %	8	10	8	10	12
Actual load, %	8.36	8.46	6.98	8.65	9.82
Drug encapsulation efficiency, %	>100	84.6	87.3	86.5	81.8
Particle size, μm					
10% under	13.15	13.42	13.25	9.92	3.19
25% under	25.0	22.14	22.63	18.72	17.57
50% under	32.29	28.70	34.43	24.28	23.39
75% under	39.87	35.83	49.75	30.27	29.23
90% under	48.74	43.53	73.81	37.58	34.92
Bulk density, g/mL	0.65	0.70	0.78	0.78	0.78

*PLA indicates poly(l-lactide); PLGA, poly(d,l-lactide-co-glycolide); and TL, target load.

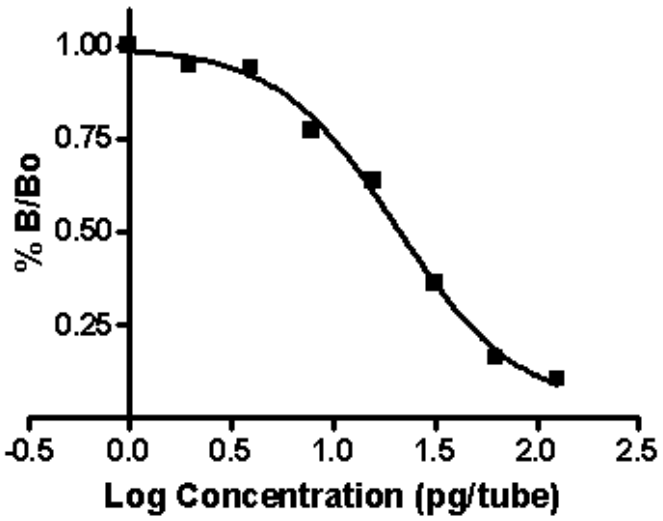

Figure 4. RIA binding curve generated from standards.

facturing methods for PLA and PLGA 85:15 microspheres. For the o/o method, on the other hand, 5 experimental batches were selected for further evaluation including 2 PLA batches and 3 PLGA 85:15 batches, with varying target drug loads (ie, 8%, 10%, and 12%).

When comparing PLA batches produced by the 2 methods, the o/w method produced microspheres with smaller average particles size values and lower bulk density values. This may have been owing to the low MW of the polymer, resulting in higher porosity during the aqueous dispersion process. The PLGA 85:15 microspheres, on the other hand, did not appear different in either process with respect to encapsulation efficiency or average particle size. Although the PLA appeared to be the polymer of choice to minimize chemical reactivity, PLGA 85:15 was used as an alternative polymer for studying and optimizing release kinetics.[1]

Radioimmunoassay Binding Curve

The range for the assay was between 1 and 125 pg/tube, where a sigmoidal 1 site competitive binding curve fit the experimental standards used in the RIA procedure. Figure 4 shows a typical standard curve generated for each assay. The goodness-of-fit statistics for the curve includes R^2 and the absolute sum of squares, which were determined to be 0.996 and 0.004 07, respectively. For each generated curve, an EC-50 (ie, effective concentration at 50% of maximum response) value is also determined, which in this particular curve was determined to be 20.38. The value agrees with the EC-50 generated by the manufacturer (~19).

With this particular assay, the question arose on the sensitivity of the assay to the parent octreotide compound with the hydrophobic-related substances previously described.[1] RIA methods inherently possess high degrees of intraassay and interassay variability. In fact, for the RIA method developed

Table 3. RIA Determination of Spiked Octreotide Samples Into Serum*

Theoretical Amount of Octreotide/Tube (pg)†	RIA Determination (pg)	% Theoretical
20	23.6	118.2%
26	31.4	120.7%
19.25	22.1	114.6%
Average		117.8%

*RIA indicates radioimmunoassay.

†As determined by high-performance liquid chromatography.

for RC-160, somatostatin analog, the interassay coefficient of variation was reported to be between 14% and 30%.[13] In addition to the variability within the assay, cross-reactivity existed for peptide structurally similar to RC-160. Mason-Garcia et al,[14] for instance, reported 15% cross-reactivity between octreotide and RC-160.

To test cross-reactivity, extracts from PLGA 50:50 microspheres incubated in PBS were assayed by HPLC for the concentration of octreotide and related peptides, assuming similar molar absorptivity coefficients for all eluting molecules.[1] Further, the extracts were diluted down to a concentration close to the EC-50 of the RIA assay (picogram range) for the most accurate experimental determinations. Table 3 summarizes the results for 3 independent determinations of microsphere extracts containing ~50% parent peptide and 50% adduct. According to the results, the percentage theoretical range is between 114.6% and 120.7% of theoretical as quantified by HPLC. If the assay were to detect all adduct species, then approximately 200% of theoretical values (for samples containing 50% native peptide and 50% modified peptide) would be expected. Consequently, a small degree of cross-reactivity may exist for the hydrophobic-related substances or the intraassay and interassay variability factors predominate in the observed deviations shown in Table 3. For the purpose of subsequent mathematical modeling, the concentration versus time profiles were assumed to depict the release pattern of parent octreotide alone.

In Vivo Release Kinetics

Based on preliminary in vitro release data in PBS (pH 7.4), the o/o batches were expected to provide prolonged or enhanced release profiles with steadier concentration levels. In previous investigations, the PLA microspheres prepared by the o/w dispersion method displayed a high initial burst with a minimal release phase thereafter.[1] With o/o-based PLA microspheres, however, under the same in vitro testing conditions, the burst was much lower with a subsequent pseudo-zero-order release phase over a 2-month period (data not shown). Figures 5, 6, 7 and 8 display the in vivo release kinetics for all batches of microspheres.

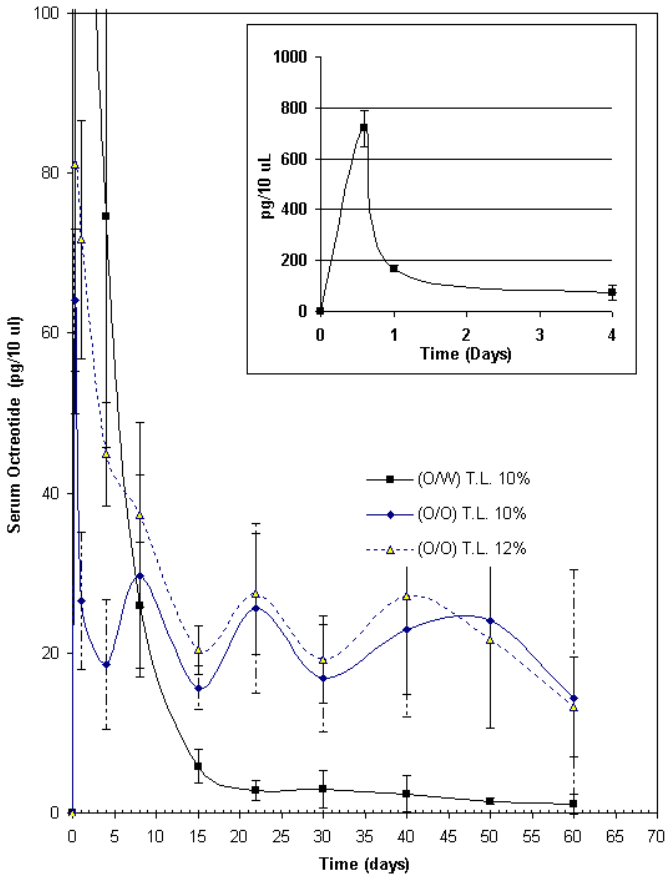

Figure 5. In vivo release profiles for PLA microspheres (n = 5).

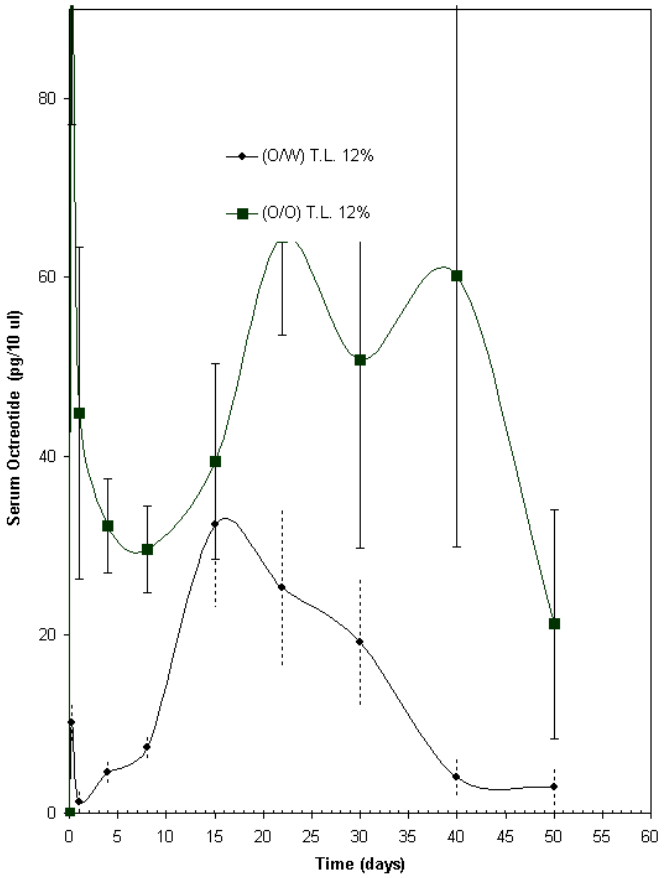

Figure 6. In vivo release profiles for PLGA 85:15 microspheres at high target load of 12% (n = 5).

In Figure 5, the release data for PLA microspheres show a tremendous burst phase with the o/w batch (10% target load). In fact, 6 hours after administration, the concentration was ~720 ng/mL. This finding is in contrast to the highest concentration provided by the innovator formulation Sandostatin LAR (Figure 8), which was obtained 22 days after subcutaneous administration in rats. Consequently, the o/w process for the desired PLA polymer could have resulted in minimal matrix loading, with drug molecules primarily associating with hydrophilic porous regions of the microsphere (Figure 1). The PLA microspheres manufactured via the o/w process dramatically differ from the product produced via the o/o process. In Figure 5, the burst for the o/o batches with 10% and 12% target loads resulted in average concentrations of ~64 ng/mL and ~82 ng/mL, respectively. Therefore, an approximate 10-fold difference exists for the observed burst phenomenon between formulation processes for PLA microspheres. This occurred in spite of the lower dose administered with the o/w batch (3 mg).

With the PLGA 85:15 microspheres, a different scenario exists where the burst phenomenon is not reduced with the change in formulation process. In fact, with the higher drug loaded o/o microspheres (eg, 12% target load microspheres as shown in Figure 6), the burst is significantly increased with an average concentration of 105 ng/mL after 6 hours. This compares with minimal burst obtained with PLGA 85:15 o/w microspheres with 12% target load, where the average concentration after 6 hours only reached ~10 ng/mL. The data presented in Figure 6 counter the starting hypothesis that the o/o process allows for greater entrapment in the matrix as opposed to the hydrophilic porous regions. The original assumptions include that drug entrapped within aqueous pores will result in burst release and the drug associated with the matrix will release during the erosion phase. With the former assumption, the discrepancy in Figures 5 and 6 requires further investigation to explain any relationship between drug-polymer interactions and dispersion technique (eg, o/o and o/w processes).

For the latter assumption, however, starting theory may still hold validity where erosion phase release is enhanced with the o/o process. In Figures 6 and 7, it could be suggested that the total area under the curve (AUC) values from the average concentration time profiles for the o/o batches are all higher than the o/w batch throughout the erosion phase (R2 phase) of the drug release experiment. To provide more mathematical and statistical interpretation of the visual data shown in Figures 5, 6, 7 and 8, the mathematical model developed in the Methods section was applied for further insight. The model, therefore, provided a method of comparison of the average concentration versus time profiles for the microsphere batches manufactured under different processes and ini-

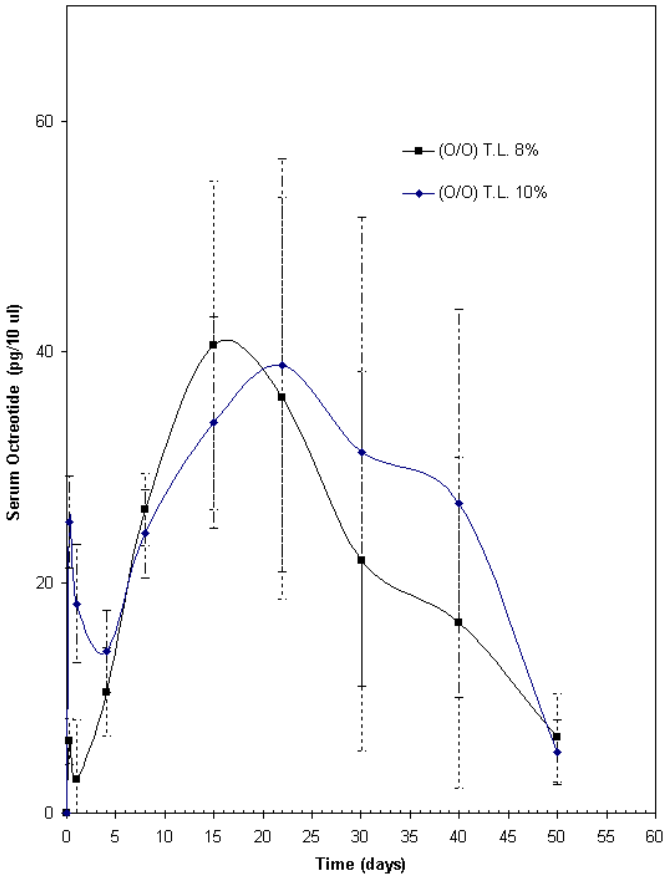

Figure 7. In vivo release profiles for PLGA 85:15 microspheres at low target load of 12% (n = 5).

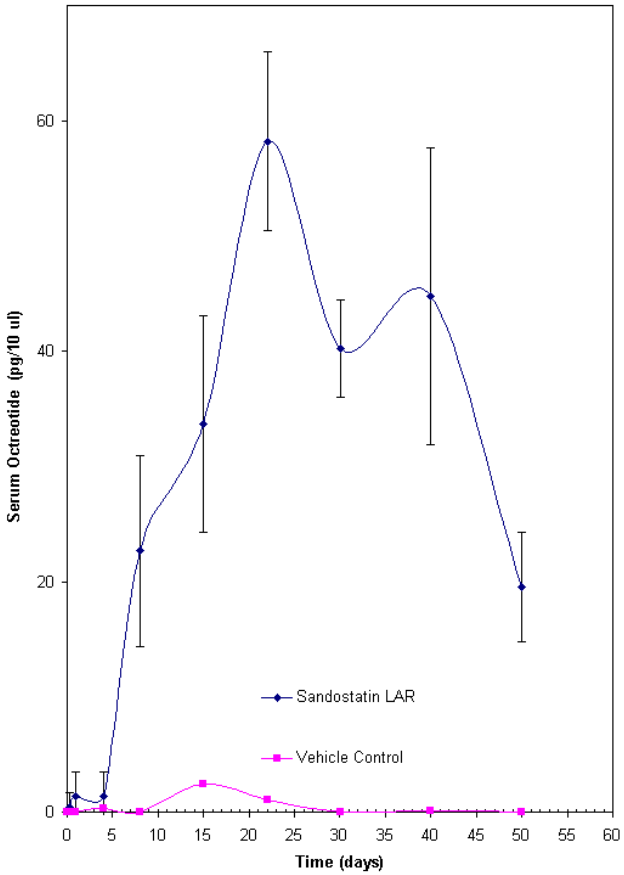

Figure 8. In vivo release profiles for Sandostatin LAR and vehicle control (n = 5).

tial target parameters. As a disclaimer, the subsequent model was limited, however, in interpretation of kinetic parameters (ka_1, ka_2, and K_{el}) for each treatment group owing to the high variability observed between individual rat subjects.

Results of the Parametric Analysis

Figures 9 and 10 display 2 examples of fitted curves for the PLA o/o (target load [TL] 10%) and the PLGA 85:15 o/o (TL 10%) batches, respectively. For the PLA microspheres, a first-order absorption equation was used to describe the burst release followed by a nonconcentration-dependent equation (pseudo-zero-order). In fact, the PLA model used for in vivo data corroborated with the in vitro release data, in which a linear concentration-time profile was observed during the secondary phase of release. For the PLGA 85:15 microspheres, on the other hand, the preliminary first-order absorption equation to characterize burst was followed with another first-order absorption equation to characterize the erosion-controlled phase. This model-dependent approach differs from previous attempts to characterize in vivo release data from octreotide microspheres injected intramuscularly in rabbit species.[10] Comets et al[10] injected PLGA 50:50 microspheres and used a first-order model to characterize burst release followed by a non-Fickian diffusion equation to characterize a transient release phase prior to erosion. Further, to characterize the erosion phase of release, a Weibull model, an adaptation of the first-order equation, was used to account for the sigmoidicity of the tertiary phase. In the present situation, due to the sampling procedures, limited data points were obtained through the initial phases of release, and hence the simplified models presented in the Methods section were used instead.

By the method of successive iterations, the best-fitted parameters were calculated by the Scientist software program. Table 4 shows the estimates for each of the parameters obtained. Although the burst release phase was included in the modeling, the parameter ka_1 (burst-phase absorption constant) could not be accurately determined owing to the lack of sufficient data points in the first 6 hours after administration. In fact, the first sample time point performed after drug administration at 6 hours was the maximal value for the burst phase of release (R1 phase). Further, for the PLA o/w batch shown in Figure 5, successful fitting could not be performed since an extreme burst phase was observed with subsequent minimal erosion-controlled phase. Hence, the Tau change-point time point was arbitrarily chosen at 4 days to allow for fractional determinations of AUC in both the R1 and R2 phases of release.

For all other batches, the α (fraction of drug absorbed in the burst phase, R1), ka_2 (R2 absorption-rate constant), Tau

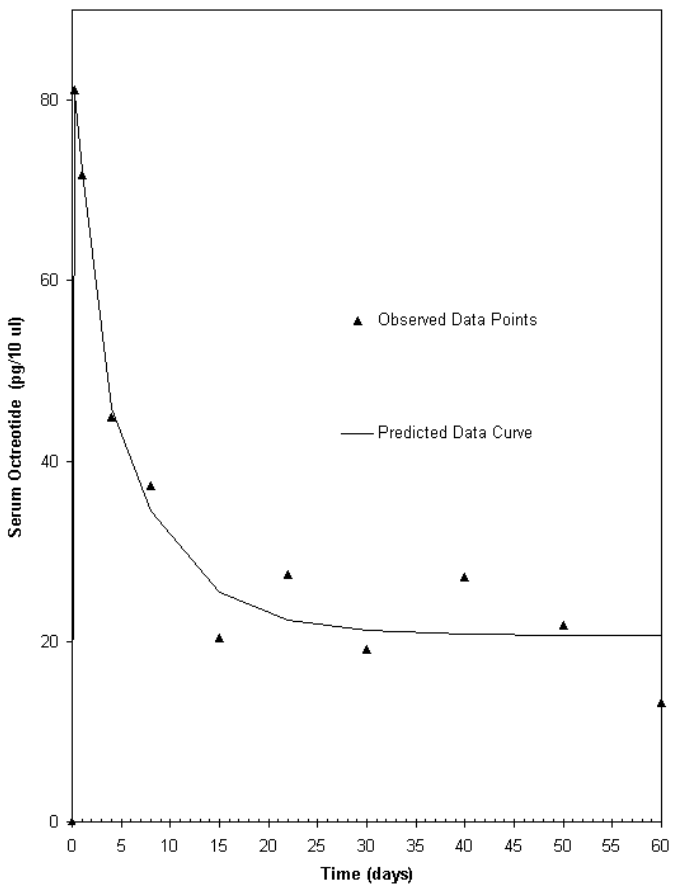

Figure 9. Model predicted in vivo release profile for PLA microspheres (o/o), TL 10%.

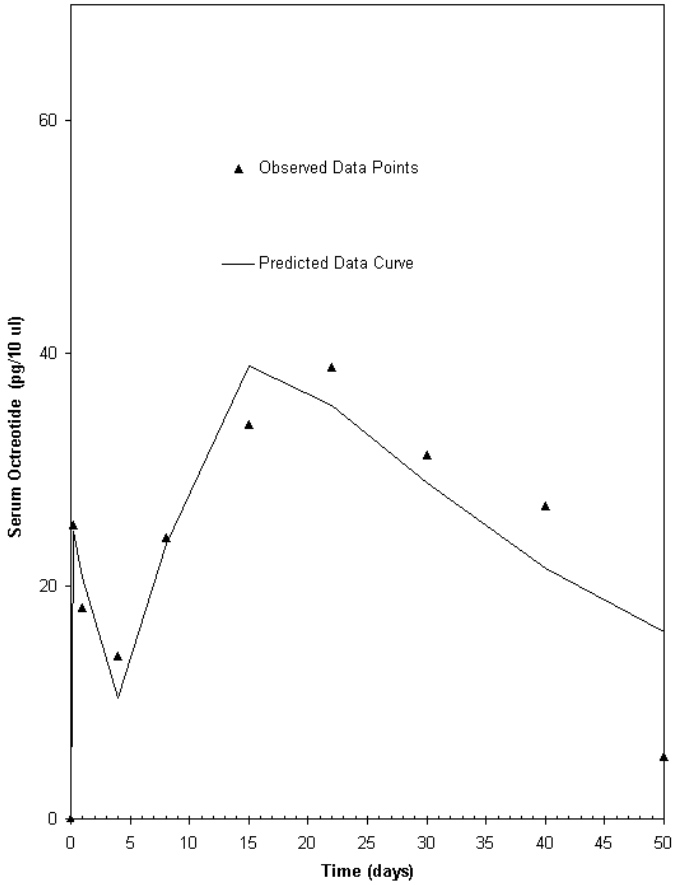

Figure 10. Model predicted in vivo release profile for PLA 85:15 microspheres (o/o), TL 10%.

(change-point between R1 and R2), K_{el} (elimination-rate constant), and the R^2 (goodness-of-fit statistic from model application) were obtained from fitting the equations to the average concentration versus time profiles. In addition, for each profile, manual calculations were performed for total AUC (ng×d/mL) and the AUC values associated with R1 (≤Tau) and R2 (≥Tau). Finally, the ratio of AUC 1 to total AUC was calculated for fractional AUC determination during R1 and as a method of comparison with the software determination of the α parameter.

When comparing the 3 PLA batches, the o/o process results in greater erosion-phase release with lower AUC 1 values (7.79 and 24.2 ng×d/mL for TL 10% and 12% batches, respectively) when compared with the o/w process (75.8 ng×d/mL when t < 4 days). Consequently, the mathematical approach further confirms the visual interpretations previously performed in Figure 5. The fractional AUC calculations also support the original hypothesis, in which the o/w batch shows 64.1% of total AUC released before 4 days and the o/o batches show 5.97% and 15% of total AUC released before Tau. The exaggerated differences between processes exist despite the lower dose of the o/w batch administered to rats (3 mg) in contrast to the o/o batches (5 mg).

For the PLGA 85:15 microspheres, as mentioned before, a different scenario exists, in which the fraction of drug release prior to Tau is greater with the higher target load o/o batches than the single o/w batch. For instance, the o/o batch with 12% TL has an α value of ~0.186 and a fractional AUC value (AUC 1/Total AUC) of ~0.196, which is significantly higher than the 0.0203 and the 0.0511 values obtained for the single o/w batch. During the R2 phase, the AUC 2 values after Tau (Total AUC - AUC 1) are higher with all the o/o batches. From Table 4, the AUC 2 for the o/w batch is ~67.0 ng×d/mL, whereas the AUC 2 values for the o/o batches are ~109, 124, and 194 ng×d/mL for the 3 successive target loads. As a result, for PLGA 85:15 polymer, both the burst and the erosion phases of release appear higher with respect to AUC when changing the formulation processing technique.

CONCLUSION

An o/o emulsification procedure resulted in significant changes in in vivo release characteristics. The PLA microspheres prepared by the new technique allowed for an enhanced sustained release effect while minimizing burst release. For PLGA 85:15 microspheres, the o/o technique allowed for significantly higher AUC values through the course of the release experiment (~50 days). These observations were performed with mathematical modeling of the in vivo release data.

Table 4. Results of Parametric Analysis of In Vivo Release Profiles*

Batch	PLA o/w	PLA o/o 1	PLA o/o 2	PLGA 85:15 o/w	PLGA 85:15 o/o 1	PLGA 85:15 o/o 2	PLGA 85:15 o/o 3	Sandostatin LAR
Target load	**10%**	**10%**	**12%**	**12%**	**8%**	**10%**	**12%**	**5%**
α	ND	ND	0.193 (0.0183)	0.0203 (0.0099)	0.0156 (0.0092)	0.0607 (0.0123)	0.186 (0.0367)	0.00260 (0.0145)
ka^2 (ng/day or $days^{-1}$)†	ND	0.293 (0.100)	0.0360 (0.0219)	0.0570 (0.0121)	0.0450 (0.0090)	0.0296 (0.0091)	0.0424 (0.0275)	0.0218 (0.0073)
Tau (days)	4‡	2.45 (1.65)	3.99 (4.50)	7.68 (0.208)	6.09 (0.696)	5.86 (1.22)	12.6 (2.76)	5.43 (1.90)
K_{el} ($days^{-1}$)	ND	1.18 (0.391)	0.151 (0.0802)	0.520 (0.0631)	0.319 (0.0380)	0.231 (0.0442)	0.141 (0.0468)	0.144 (0.0362)
R^2	ND	0.977	0.991	0.965	0.976	0.966	0.920	0.954
AUC Total (ng × d/mL)	118	131	161	70.5	114	134	240	171
AUC 1 (ng × d/mL)	75.8	7.79	24.2	3.61	5.25	9.85	46.0	1.21
AUC 2 (ng × d/mL)	42.2	123	137	67.0	109	124	194	170
AUC1/AUC Total	0.641	0.0597	0.150	0.0511	0.0462	0.0738	0.196	0.0070

* PLA indicates poly(l-lactide); PLGA, poly(d,l-lactide-co-glycolide); o/o, oil/oil; o/w, oil/water; and AUC, area under the curve. SD provided in parentheses where applicable. SD generated by Scientist software indicates the goodness-of-fit and is represented by the formula $\sigma=[(\sum^{n}(w_i(Ycal_i-Yobs_i))^2/DOF]^{1/2}$, where w_i represents the weights applied to each point and degrees of freedom (DOF) represents the number of data points minus the number of fitted parameters.

†Units vary with the zero or first processes for PLA and PLGA 85:15 formulations, respectively.

‡Value arbitrarily chosen based on burst release effect

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REFERENCES

1. Murty SB, Goodman J, Thanoo BC, DeLuca PP. Identification of chemically modified peptide from poly(D,L-lactide-co-glycolide) microspheres under in vitro release conditions. *AAPS PharmSciTech*. 2003;4:E50.

2. De Weck AL. Immunological effects of aspirin anhydride, a contaminant of commercial acetylsalicylic acid preparations. *Int Arch Allergy Appl Immunol*. 1971;41:393-418.

3. Jeyanthi R, Thanoo BC, Mehta RC, DeLuca PP. Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery. *J Control Release*. 1996;38:235-244.

4. Jeyanthi R, Mehta RC, Thanoo BC, DeLuca PP. Effect of processing parameters on the properties of peptide-containing PLGA microspheres. *J Microencapsul*. 1997;14:163-174.

5. Kostanski JW, Thanoo BC, DeLuca PP. Preparation, characterization, and in vitro evaluation of 1- and 4-month controlled release orntide PLA and PLGA microspheres. *Pharm Dev Technol*. 2000;5:585-596.

6. Herrmann J, Bodmeier R. Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. *Eur J Pharm Biopharm*. 1998;45:75-82.

7. Lai MC, Hageman MJ, Schowen RL, Borchardt RT, Topp EM. Chemical stability of peptides in polymers. 1. Effect of water on peptide deamidation in poly(vinyl alcohol) and poly(vinyl pyrrolidone) matrixes. *J Pharm Sci*. 1999;88:1073-1080.

8. Lai MC, Hageman MJ, Schowen RL, Borchardt RT, Laird BB, Topp EM. Chemical stability of peptides in polymers. 2. Discriminating between solvent and plasticizing effects of water on peptide deamidation in poly(vinylpyrrolidone). *J Pharm Sci*. 1999;88:1081-1089.

9. Hausberger AG, DeLuca PP. Characterization of biodegradable poly(D,L-lactide-co-glycolide) polymers and microspheres. *J Pharm Biomed Anal*. 1995;13:747-760.

10. Comets E, Mentre F, Kawai R, Nimmerfall F, Marbach P, Vonderscher J. Modeling the kinetics of release of octreotide from long-acting formulations injected intramuscularly in rabbits. *J Pharm Sci*. 2000;89:1123-1133.

11. Murata K, Noda K, Kohno K, Samejima M. Pharmacokinetic analysis of concentration data of drugs with irregular absorption profiles using multi-fraction absorption models. *J Pharm Sci*. 1987;76:109-113.

12. Hwang SS, Bayne W, Theeuwes F. *In vivo* evaluation of controlled-release products. *J Pharm Sci*. 1993;82:1145-1150.

13. Lemaire M, Azria M, Dannecker R, Marbach P, Schweitzer A, Maurer G. Disposition of sandostatin, a new synthetic somatostatin analogue, in rats. *Drug Metab Dispos*. 1989;17:699-703.

14. Mason-Garcia M, Vaccarella M, Horvath J, et al. Radioimmunoassay for octapeptide analogs of somatostatin: measurement of serum levels after administration of long-acting microcapsule formulations. *Proc Natl Acad Sci U S A*. 1988;85:5688-5692.