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Impurity formation studies with peptide-loaded polymeric microspheres Part II. In vitro evaluation

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Abstract

Since acylated peptide impurities were isolated from octreotide microspheres following incubation in an in vivo environment, the present investigation was undertaken to determine the dosage form dynamics responsible for facilitating acylation. In particular, microsphere batches made with poly(L-lactide) (PLA) and poly(lactide-co-glycolide) (PLGA) 85:15 were studied for in vitro drug release, mass balance relationships, mass loss behavior, hydration uptake, and solid-state stability. Furthermore, native octreotide was incubated in a varying pH stability model (heat treated lactic acid solutions 42.5%, w/w) to determine the effects of acidity on impurity formation. From a review of the experimental results, the appearance of octreotide impurities or related substances occurred with the onset of polymeric mass loss. In fact, the significant formation of acylated peptide did not appear until >90% mass loss, which was observed at 14 days. It was surmised that because of water uptake, the hydrolytic cleavage of the polymeric backbone created an acidic microenvironment to facilitate the covalent coupling of peptide with polymer. The lactic acid solution stability model corroborated with greater evidence of acylation at pH 2.25 where the presence lactoyl (+72 *m*/*z*) derivatives of octreotide were confirmed by MALDI-TOF mass spectrometry.

Keywords: Somatostatin analogues; Octreotide acetate; PLA microspheres; PLGA microspheres; Peptide acylation; Peptide stability

1. Introduction

Octreotide acetate encapsulated in poly(lactide-co-glycolide) (PLGA) and poly(L-lactide) (PLA) microspheres was shown to form hydrophobic related substances during in vitro release testing and upon incubation in the in vivo subcutaneous tissue envi-

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ronment. The results of this study also indicated that adduct or impurity species were minimally present prior to the incubation (<1%) and hence the peptide was assumed to be stable through the manufacturing process (Murty, 2003; Murty et al., 2003). As a result, the appearance of related substances was thought to occur during the hydration process where the influx of water resulted in hydrolytic cleavage of the PLGA or PLA polymeric backbone. The resulting formation of glycolic and/or lactic acid monomers may have formed an acidic microenvironment to catalyze the formation of hydrophobic related substances by an acylation mechanism (Lucke et al., 2002; Lucke and Gopferich, 2003; Fu et al., 2000; Na et al., 2003a).

Water influx into the dosage form also could have potentiated acylation either by increasing the dynamic mobility of molecules (plasticizing effect), by participating as a medium solvent, or by directly participating as a reactant (i.e. hydrolysis) (Lai et al., 1999a,b). The plasticizing and solvent medium effects were reported with deamidation reactions in PVP and PVA formulations loaded with Asn-Hexapeptide (Lai et al., 1999a,b). These formulations, however, did not possess the hydrolytic cleavage and microenvironmental pH phenomena associated with PLGA and PLA polymers (Fu et al., 2000). Acylation of salmon calcitonin inside PLGA systems, for instance, was studied using a heat-treated lactic acid solution as a stability model. In pH 2 lactic acid solutions, enhanced acylation was reported as opposed to pH 5 solutions. The authors suggested the use of equilibrated lactic acid solutions at various concentrations to test the acylation potential for a particular drug molecule formulated inside polyester microspheres (Lucke et al., 2002; Lucke and Gopferich, 2003).

Important questions as to the physical factors involved in the formation of octreotide related substances (O.R.S.) during in vitro and in vivo incubation of formulated microspheres remain to be answered. Thus far, evidence suggests that water influx into polymeric microspheres could result in a solvent medium effect with subsequent hydrolytic cleavage of the polymer backbone resulting in acidic pH formation. With an in vivo model, however, the ability to perform invasive measures of microsphere activity was not feasible on a practical level. The purpose of the present study was therefore to study the dynamic attributes of microspheres during in vitro incubation at physiological pH and temperature (phosphate buffer and 37 °C) to support the in vivo findings. The changing characteristics that were studied included drug release, impurity formation, and mass balance determinations for selected batches of microspheres prepared by an oil/oil (O/O) dispersion technique. Concurrently, microspheres were independently studied for mass loss and hydration changes through the same time points to find correlations with the onset of impurity formation. Finally, the present study was aimed at developing a lactic acid stability model to test the effects of oligomer solution pH and concentration as a model for the formation of O.R.S. in microsphere formulations.

2. Materials and methods

2.1. 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). Poly(D,L-lactide-co-glycolide) 85:15 co-polymer (9 kDa) and poly(L-lactide) homopolymer (8 kDa) were purchased from Alkermes Inc. (Cincinnati, OH). All other chemicals used were of analytical reagent grade.

2.2. Microsphere preparation and characterization

Microspheres were manufactured by the previously described oil/oil dispersion technique followed by solvent extraction/evaporation (Murty, 2003). The microspheres were subsequently analyzed for drug content and initial impurity content by organic phase dissolution (methylene chloride) of microspheres and buffer phase extraction of peptide. After dissolution of microparticulates with methylene chloride and the addition of 0.1 M acetate buffer pH 4.0, each vial was placed on a wrist-shaker apparatus for 30 min and centrifuged for 20 min at 2000 rpm. The drug content determinations allowed for subsequent calculations of drug release and mass balance relationships. One batch of PLA microspheres and three batches of PLGA 85:15 microspheres of varying drug loads were selected for further incubation studies. The summary of microsphere characteristics was presented previously (Murty, 2003).

2.3. Incubation of microspheres in phosphate buffered saline

For impurity formation, drug release, and mass balance determinations, 15 mg of microspheres were placed in 15 mL polypropylene tubes ($n = 10$ vials for each batch). After addition of 4 mL of 0.02 M phosphate buffered saline to each vial, the samples were continuously agitated at 100 rpm in a shaking air bath set at 37 °C. At weekly intervals, all tubes were centrifuged to facilitate the removal of buffer for subsequent peptide analysis by HPLC. The microspheres were then resuspended with 4 mL of fresh buffer by vortexing and were placed onto the shaking air bath.

At specified time points (i.e. days 7, 14, 35, and 56), two tubes from each batch were removed for impurity content inside microspheres as well as mass balance determinations. The vial contents were treated with a 1:1 mixture of dimethylsulfoxide/methlyene chloride for polymer dissolution followed with the addition of 0.1 M acetate buffer (pH 4.0) for peptide extraction. The supernatant samples and microsphere extracts were analyzed by HPLC by two separate gradient elution methods to account for the large dimethylsulfoxide solvent front peak observed.

2.4. Mass loss and hydration studies

A gravimetric study was concurrently initiated where 50 mg of microspheres were placed in each vial ($n = 12$ vials per each batch). At specified time points (i.e. days 7, 14, 35, and 56), three tubes were removed for gravimetric analysis. The microspheres were recovered by vacuum filtration (0.45 μm filter) and weighed to obtain a value designated as M_w (wet mass). After drying the microspheres for 24 h under vacuum conditions, the M_d (dry mass) value was determined. The following equation allowed for the calculation of degree of hydration.

$$\text{degree of hydration} = \left(\frac{M_w - M_d}{M_d}\right)$$

In addition, the % mass loss was calculated based on the M_o value (initial mass prior to incubation using the following equation:

$$\text{mass remaining \%} = \left(\frac{M_d}{M_o}\right) \times 100$$

The degree of hydration and mass loss % values were plotted as a function of time to correlate with changes in impurity formation or mass balance determinations.

2.5. Solid state incubation of microspheres

The microspheres were also subjected to incubation under anhydrous conditions at 37 °C to serve as a control for the formation of impurities in the presence of aqueous buffer. Briefly, 15 mg of freeze-dried microspheres were placed in separate vials under tightly sealed conditions ($n = 12$ per batch). At designated intervals, three vials were removed and treated with a 1:1 mixture of dimethylsulfoxide/methylene chloride. Subsequent extraction with acetate buffer allowed for determination of drug content and drug purity for HPLC analysis.

2.6. Acylation stability model in concentrated lactic acid solutions

Lucke et al. described the preparation steps for treating lactic acid solutions for acylation stability experiments (Lucke et al., 2002; Lucke and Gopferich, 2003). The reaction model was designed around heat-treated lactic acid solutions at varying concentrations of lactic acid (1%, 5%, 10%, and 50% (w/w)) with pH adjustments. For the present experiment, concentrated lactic acid (85%, w/w) was diluted to 42.5% (w/w) and subsequently pH was adjusted to four different values between 2 and 6. The solutions were further equilibrated at 90 °C for 24 h, which allowed for the stabilization of oligomer content. This procedure was performed to account for the dynamic equilibrium that exists between monomeric lactic acid and esterified lactic acid (dimers, trimers, etc.). Equilibration would take months at room temperature and hence, the accelerated approach was implemented at elevated temperatures to obtain stable oligomer content in solution (Lucke et al., 2002; Lucke and Gopferich, 2003). With each of the lactic acid solutions (pH 2.25, 3.11, 4.23, and 6.09), 100 μg/mL of octreotide acetate was added ($n = 3$ per pH). At regular intervals, each vial was sampled by HPLC for percent purity.

2.7. High performance liquid chromatography

Two LC analytical assays were employed during the course of the study. The drug content or mass balance assay used an LC method with a longer run time since DMSO was present from drug extractions. The second method used for assay of PBS supernatant and lactic acid solutions utilized a shorter run time since only a buffer solvent front was present. Both methods utilized a C-18 column (250 mm × 4.6 mm by Alltech, Deerfield, IL) and a flow rate of 1.0 mL/min. In addition, a binary mobile phase was used where solution A contained water + 0.1% TFA and solution B contained acetonitrile + 0.1% TFA. For the drug content LC method, a 75:25 (A:B) to 65:35 (A:B) gradient over 25 min was used. For the solution assay, a 72.5:27.5 (A:B) to 62.5:37.5 (A:B) gradient over 17 min was used.

2.8. Mass spectrometry

The lactic acid solutions were analyzed by MALDI-TOF Mass Spectrometry (Bruker Daltronics). For the mass spectral analyses, all samples were placed under rotary vacuum conditions to dry off aqueous portions. Subsequently, the residual solid material was incorporated into a α-cyanohydroxy cinnamic acid (α-CHCA) matrix for crystallization.

3. Results

3.1. Analysis of peptide release into supernatant

Although the main purpose was to understand O.R.S. formation by studying the dynamic characteristics of microspheres incubated in PBS, drug release into the surrounding aqueous environment was inevitable. In fact, with the PLA batch, approximately 60% cumulative drug release was observed by the termination of the experiment (56 days), which is consistent with data previously obtained (Murty et al., 2003). Fig. 1 shows the % octreotide released into supernatant over the course of the experiment. The PLA batch displays the fastest release rate and the lowest percentage of detectable impurities (∼10% by 56 days). With the PLGA 85:15 batches with 10% and 12% target loads (T.L.), the release profiles are similar with 46% and 50% cumulative release by day 56, respectively (12% T.L. data not shown in Fig. 1). Approximately 24% of the total mass release (peptide + impurities) is in the form of O.R.S. for both batches. Finally, with the low target load PLGA 85:15 batch, release is nominal by 56 days with only 22.2% cumulative release (intact peptide) and 31.6% total release (peptide + impurities).

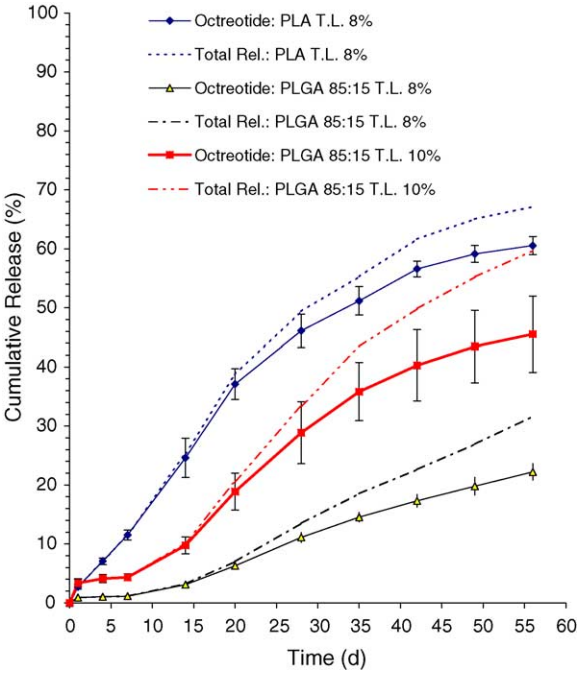

Fig. 1. % Octreotide in supernatant (0.02 M PBS pH 7.25).

The appearance of O.R.S. under in vitro release testing conditions was previously observed where the co-monomer ratio and the polymer molecular weights influenced the extent of impurity formation (Murty et al., 2003). With the present study, mass balance relationships were also determined by extracting vials of incubated microspheres at specified time points ($N = 2$ sacrificed at days 7, 14, 35, and 56). The buffer extract of the microspheres from the specific vial was analyzed by HPLC for both drug content and impurity content. Subsequently, the total peptide content from the extract was added to the total % release (peptide + impurities) for the specific vial. Fig. 2 shows the mass balance recoveries for each batch of microspheres at all time points.

In Fig. 2, the mass balance values for the PLA batch are ∼98% and 107% for days 7 and 14, respectively.

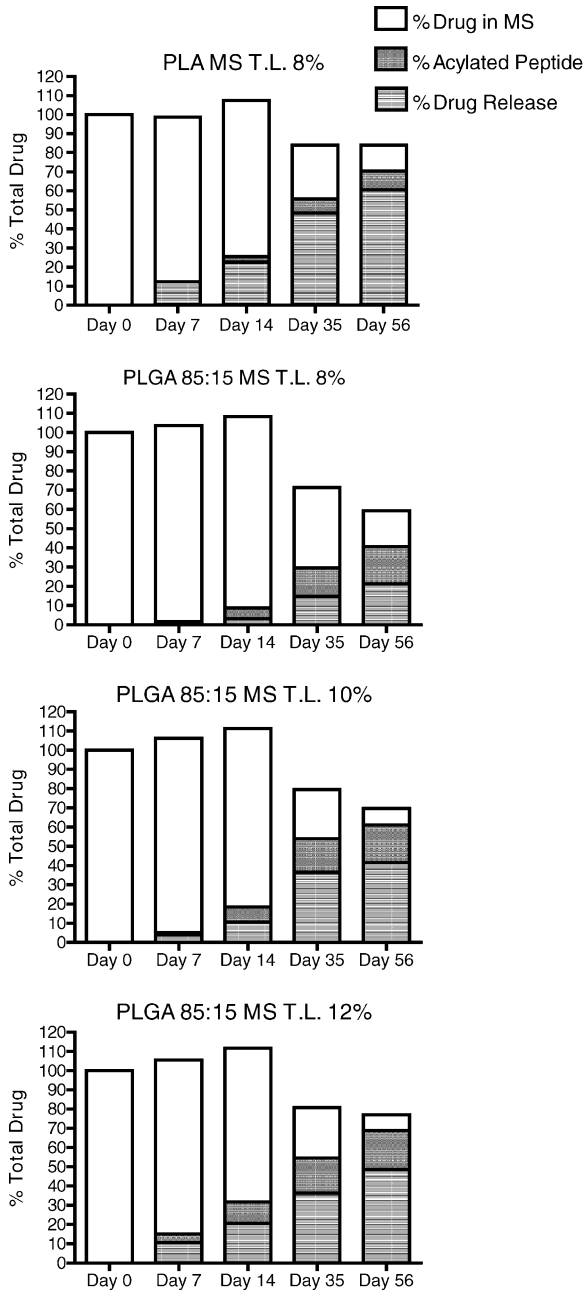

Fig. 2. Mass balance relationships.

By day 7, minimal drug release is observed (<5%) and hence the mass balance determination is logical for this time point. By day 14, however, the higher mass balance value (107%) may indicate an analytical error within the acceptable 10% variation range or may indicate the presence of O.R.S. not detectable in the zero time drug content assay. A similar result was observed during in vitro release testing in 0.1 M acetate buffer (pH 4.0) for octreotide microsphere batches prepared by the oil/water dispersion technique. In that particular scenario for three microsphere batches, cumulative drug release was 100% by termination of the release experiment and with the superimposition of O.R.S., the total % release was between 107% and 110% (Murty et al., 2003). It was postulated from Fig. 3 (proposed acylation mechanism) that step 1 of O.R.S. formation could occur during manufacturing and step 2 could occur with the influx of water media to liberate glycoyl and lactoyl adduct species from the polymer backbone. For the other time points, days 35 and 56, approximately 84% of the total mass is recovered from the extraction procedure and the supernatant release. Hence, 16% of the theoretical peptide mass (parent peptide and O.R.S.) cannot be accounted for from the analytical procedures.

With the other batches in the present study, similar mass balance profiles at days 7 and 14 were obtained where the determined values were higher than 100%. By days 35 and 56, the mass balance determinations again resulted in unaccounted losses of peptide mass (parent peptide + O.R.S.). For instance, for PLGA 85:15 T.L. (Target Load) 8%, mass balance at day 35 was ∼70% and by day 56, the value was ∼60%. In addition, the mass balance values for PLGA 85:15 T.L. 10% are ∼80 and ∼70% by days 35 and 56, respectively. Finally, for the high target load PLGA 85:15 batch, the calculated values were similar to the one obtained for PLA T.L. 8%.

One could postulate several explanations for peptide loss, which cannot be accounted for by quantitation of parent peptide and O.R.S. from the present HPLC separation techniques; hence, one could argue for alternative degradation or reaction mechanisms. One possibility includes the presence of polymer-conjugated peptide where step 1 of the reaction mechanism shown in Fig. 3 occurred and trapped peptide molecules onto the polymer backbone. As a result, step 2 of the mechanism was required in order for O.R.S. detection by HPLC or mass spectrometric instruments. Another possible explanation includes the loss of peptide by a disulfide exchange mechanism where octreotide molecules and/or O.R.S. chemically conjugated to each other by an SN_2 nucleophilic attack. This mechanism has been

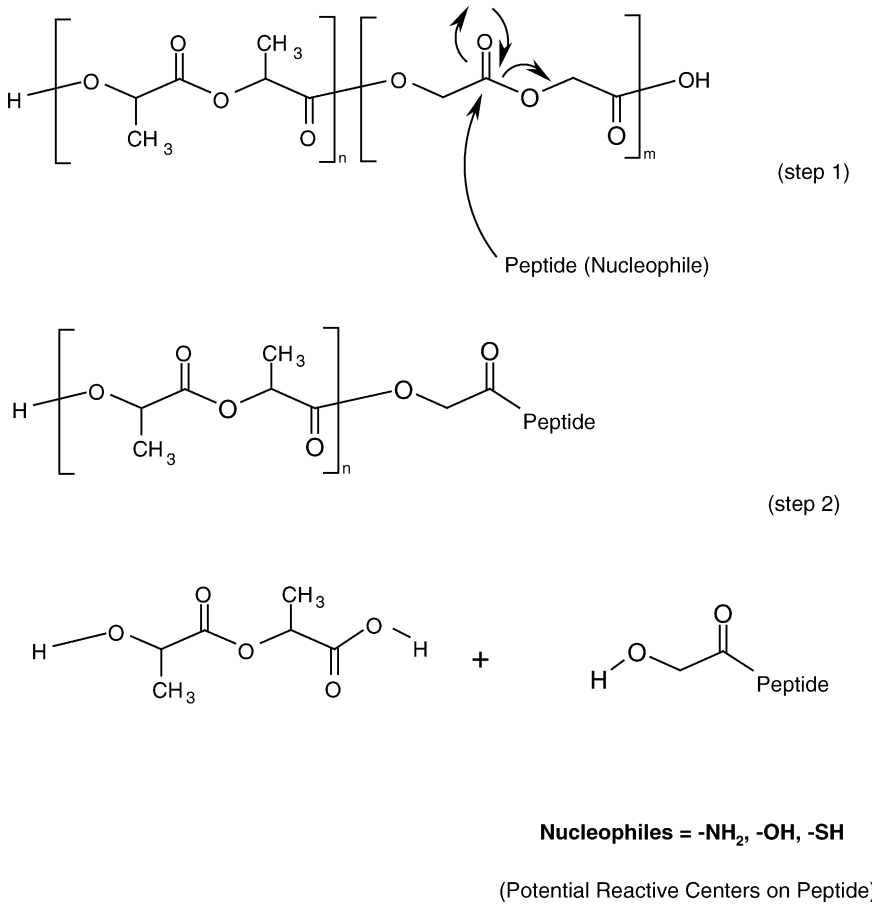

Fig. 3. Proposed mechanism of reaction between peptide and polymer (Lucke et al., 2002; Lucke and Gopferich, 2003).

reported with RC-160, a somatostatin analogue formulated in freeze-dried cakes where a solid-state reaction took place under accelerated stability testing conditions (Pourrat et al., 1995; Barthomeuf et al., 1996). In fact, the disulfide reaction has also been reported for freeze-dried ANP (atrial natriuretic peptide) where the authors postulated moisture induced aggregation because of a phase change (amorphous to crystalline) in the freeze-dried formulation (Wu et al., 2000).

In addition, the disulfide exchange mechanism between molecules could be catalyzed by acidic pH values (Manning et al., 1989). Hence, one could argue that the peptide mass unaccounted for by days 35 and 56 as shown in Fig. 2, was a result of acid catalyzed and moisture induced aggregation of peptide molecules. These proposed chemical entities, however, were not detected in previous mass spectral analyses of buffer extracts from incubated microspheres (Murty, 2003; Murty et al., 2003). The possibility of such a reaction mechanism, however, should not be discounted during the hydration processes, which occurs during incubation of the PLGA and PLA dosage forms in buffer media.

3.2. Hydration and mass loss

Fig. 4 displays the hydration profiles for all batches of microspheres utilized in the study. The high target load PLGA 85:15 batch appeared to hydrate to the greatest degree (∼16 by day 56) possibly due to greater attractive forces for the influx of water as shown pre-

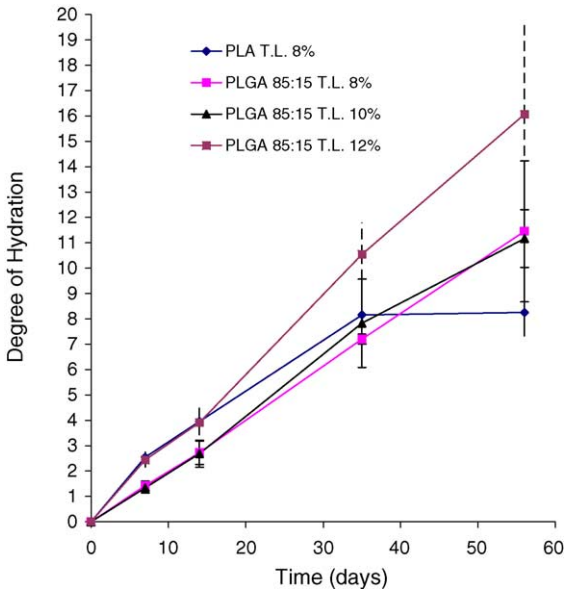

Fig. 4. Degree of hydration for octreotide microspheres ($N = 3$) (0.02 M PBS pH 7.25).

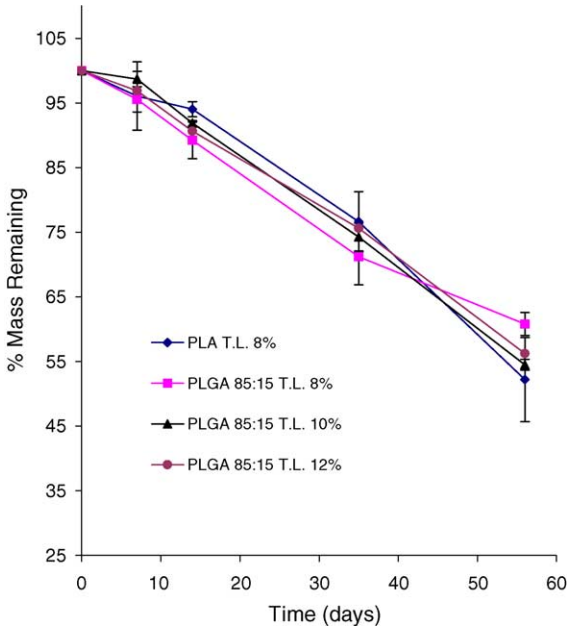

Fig. 5. Mass loss for octreotide microspheres ($N = 3$) (0.02 M PBS pH 7.25).

viously for blank and drug loaded PLA microspheres (Woo et al., 2001). The PLGA 85:15 T.L. 8% and 10% batches displayed similar hydration profiles where by day 56, the degree of hydration (D.H.) was approximately 11. For the PLA batch, however, the D.H. appeared to level off at a value of 8 after 35 days.

The hydration profiles for all batches were linear with respect to time. There, however, was no direct correlation between D.H. values and the onset or extent of O.R.S. formation. The data in Fig. 4, however, provided evidence for the high influx of water into the dosage form since a D.H. value of only 1 represents 100% hydration. At this point, one could not discriminate between the effects of water as a plasticizer, solvent medium, or a direct participant in the acylation reaction mechanism shown in Fig. 3 (Lai et al., 1999a,b). Regardless, without the influx of water into the dosage form, the encapsulated peptide appeared stable when microspheres were incubated under anhydrous conditions at 37 °C. In Table 1, the % drug content values indicated that there was no degradation associated with solid-state incubation for 45 days. For instance, for the PLA batch, the recovery of peptide after extraction of microspheres was between 8.1% and 8.5%, which was consistent with the original drug load at zero time (8.36%). The minor deviation in the recovery values with the original drug content value was the result of anticipated variation in extraction or analytical procedures. In addition, minor deviations in content uniformity were observed for microspheres manufactured under the experimental oil/oil dispersion process.

As for the mass loss behavior, Fig. 5 displays linear profiles with respect to time for all formulations. There appeared to be no significant difference in mass loss behavior between formulations. By day 56, the total polymer mass remaining was around 55%. Further, when corroborating with mass balance data shown in Fig. 2, the mass loss data was found to correlate with the onset of O.R.S. For instance, prior to the onset of significant mass loss observed at day 14, the detection of O.R.S. was negligible in the in vitro release

Table 1
Microsphere drug content values (%) after solid-state incubation in tightly sealed containers at 37 °C ($n = 2$)

Time (days)	PLA 8% T.L.	PLGA 85:15 8% T.L.	PLGA 85:15 10% T.L.
0	8.36	6.98	8.65
14	8.14	6.54	8.64
28	8.56	6.76	8.22
45	8.30	6.80	8.64

experiment (Fig. 1). With the gradual hydrolysis of the polyester and subsequent degradation into oligomers and monomers, an acidic microenvironment could have formed in the highly localized environment within the microsphere (Fu et al., 2000; Makino et al., 1985; Brunner et al., 1999; Mader et al., 1998).

3.3. Acylation in lactic acid oligomer solutions

In some situations, the microevironment within the PLGA microsphere may enhance stability of therapeutic moieties as in the case observed with camptothecin analogues (Shenderova et al., 1999). With octreotide microspheres, insight into the role of the microenvironment pH on acylation requires further understanding. In addition, a solution model to predict stability of octreotide within the extreme conditions was determined to be a useful tool for developing strategies that block the formation of O.R.S. (Lucke et al., 2002; Lucke and Gopferich, 2003).

Peptide incubation (100 μg/mL) in concentrated (42.5%, w/w) and heat-treated lactic acid solutions was performed at varying pH values (i.e. 2.25, 3.11, 4.23, 6.09). The conditions for the lactic acid stability model were chosen based on experimental results provided by Lucke et al. In fact, the previous investigators reported increased acylation of salmon calcitonin (sCT) with lactic acid solutions of higher concentration (50%, w/w) as opposed to solutions with only 10% (w/w) concentration where the area percent of peptide related substances was <10% after 28 days of incubation. In addition, increased acylation of sCT was observed at pH 2 as opposed to pH 5 when measuring the area percent of related substances (Lucke et al., 2002).

Fig. 6 shows the area percent profiles for O.R.S., which eluted after the parent octreotide peak on each chromatogram. Fig. 7 displays representative chromatograms obtained from 42.5% (w/w) lactic solutions with octreotide at time zero and after 30 days of incubation. These hydrophobic peaks eluting after the parent octreotide peak were previously identified by LC–MS/MS as glycoyl and lactoyl substitutions (Murty et al., 2003). In fact, the impurities in the present experiment were also identified by mass spectrometry as lactoyl and lactoyl–lactyl adducts (+72 *m/z* and +144 *m/z*) of octreotide or variations thereof (Table 2) (Na et al., 2003b).

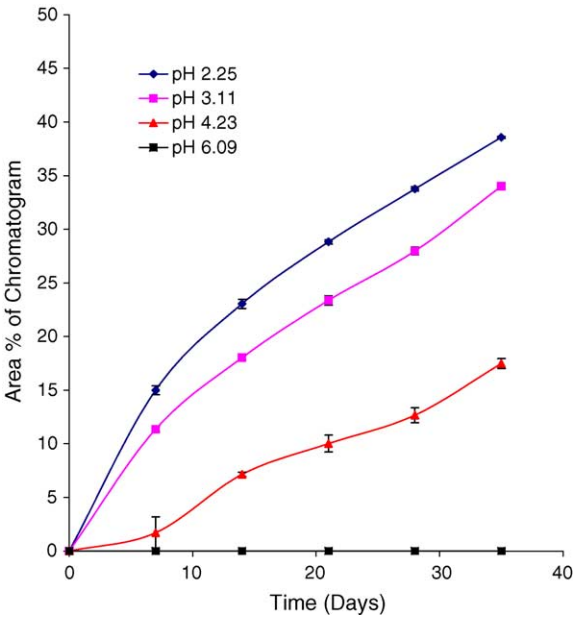

Fig. 6. Area percent of impurities from oligomer solutions of varying pH.

As for a comparison of solutions of differing pH values, at pH 2.25, the area percent of O.R.S. is ∼39% and at pH 3.11, the area percent is ∼34% at termination of the incubation experiment. For the pH 4.23 solution, on the other hand, the area percent is significantly lower with a value of 17.5% by day 35. Finally, at pH 6.09, the presence of acylated products was not detected. The data from Fig. 6 suggest a potential pH effect whereby the acidic microenvironment may catalyze the formation of O.R.S. The data, however, may be misleading since at the higher pH lactic acid solutions (especially pH 6.09), viscosity of the solutions interfered with the HPLC assay. In addition, a potential complexation phenomenon could have been observed with the pH 4.23 and pH 6.09 solutions. This may have occurred due to binding between ionized oligomers carboxylic end groups ($pK_a \sim 3.8$) and positively charged amine groups present on the lysine residue and the N-terminus.

Previously, a similar complexation phenomenon was proposed with orntide acetate, an LHRH antagonist and hydrophobic decapeptide, formulated in PLA microspheres. Under accelerated in vitro release testing conditions in 0.1 M acetate buffer (pH 4.0), release plateaus were observed with the onset of polymeric

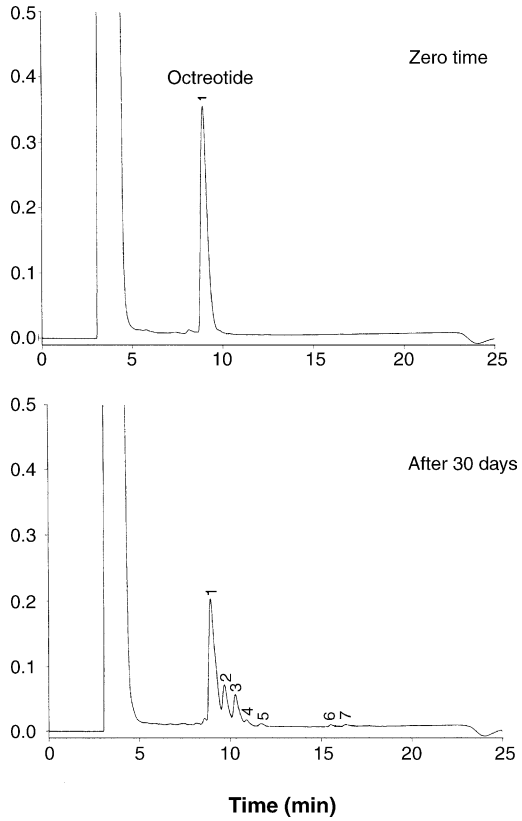

Fig. 7. Chromatogram from lactic acid stability solution at time zero and after 30 days of incubation.

mass loss (Murty et al., 2001; Gavini et al., 2002). With orntide acetate, the onset of mass loss resulted in the formation of negatively charged oligomers, which paired with positively charged peptide molecules at pH 4.0.

Regardless of the complications associated with the stability model at higher pH values, the lower pH solutions (2.25 and 3.11) could serve as a useful reaction model allowing for predictions of theoretical reaction rates and predictions of the effects of stabilizing agents within the microsphere dosage form. Such predictions would reflect conditions (i.e. pH and local concentration), which would occur only in the highly localized microenvironment of the microsphere. An extreme pH value of 1.5 was determined inside microspheres for a definitive period (Fu et al., 2000). Hence, while a local microenvironment pH could be achieved, buffer exchange with surrounding aqueous media eventually occurs with the creation of hydrophilic pores within the matrix as a result of erosive processes. Hence, rate constants generated may only apply to peptide acylation pathways for a transient phase where drug is entrapped in the hydrolytically generated microenvironment with extreme pH values and high local concentrations. With the pH 2.23 and 3.11 lactic acid solutions, for instance, 50% of initial peptide amount was depleted by the end of 1 month. As a result, if a pseudo first-order model were to fit to the experimental data, then under transient microenvironment conditions, a 1-month half-life would be expected for octreotide acetate.

4. Discussion

The physical factors responsible for the formation of octreotide related substances (O.R.S.) in PLGA and PLA microspheres were investigated in phosphate buffered saline. The in vitro incubation of microspheres allowed for quantitation of peptide release, impurity formation, and mass balance at selected time points. Concurrently, mass loss and hydration properties of the microspheres were determined for a better understanding of the onset of formation of O.R.S. Without the presence of moisture, the loss of peptide mass did not

Table 2
Characterization of acylation products of octreotide by MALDI-TOF MS (Na et al., 2003b)

HPLC peak[a]	Observed *m/z*	Expected structure
1	1019, 1041	Octreotide, octreotide-Na
2	1091, 1113	Octreotide-1LA, octreotide-Na-1LA
3	1113, 1129	Octreotide-Na-1LA, octreotide-Na-OH-1LA
4	1091, 1113, 1129	Octreotide-1LA, octreotide-Na-1LA, octreotide-Na-OH-1LA
5	1091, 1113, 1129	Octreotide-1LA, octreotide-Na-1LA, octreotide-Na-OH-1LA
6	1091, 1162	Octreotide-1A, octreotide-2LA
7	1091, 1162	Octreotide-1A, octreotide-2LA

[a] See Fig. 7.

occur and hence it was determined that water influx is necessary. Finally, a lactic acid stability model was implemented in this investigation to understand the role of acidic microenvironment in the formation of O.R.S.

The results of this investigation may provide insight into the acylation potential of several classes of bioactive peptides considered for PLGA or PLA delivery. In addition, stabilizing or blocking agents designed to minimize peptide degradation could be assessed within solution models designed to simulate the microenvironment. For instance, a stability-testing model may provide information on the utility of PEGylation of octreotide acetate before encapsulation within polymeric microspheres. The PEGylation of RC-160, a somatostatin analogue, has already been demonstrated where the investigators suggested enhanced pharmacological properties with the chemically conjugated moiety (Morpurgo et al., 2002; Na et al., 2003b).

These challenges with respect to peptide stability address the needs for proper in vitro and in vivo analytical tools. The overall aim should be for the safe and effective delivery of bioactive peptides using extended release dosage forms. Although several microsphere dosage forms are currently approved for the United States and European markets (e.g. Sandostatin LAR Depot), the formation of related peptide substances requires serious attention. For octreotide, the formation of such chemical species occurs in both in vivo injection sites and in vitro incubation conditions over extended time-periods. These reactions would not be detected under normal ICH stability testing guidelines where sealed formulation vials are incubated in control humidity and temperature conditions. Hence, the findings of this research article provide urgent issues to be addressed by regulatory agencies.

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