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A new class of inhibitors of peptide sorption and acylation in PLGA

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Abstract

Acylation of peptides occurring within controlled-release depots prepared from copolymers of lactic and glycolic acid (PLGA) is a degradation reaction that may compromise product safety and efficacy. As peptide sorption to PLGA is believed to be a common precursor to peptide acylation, a new method to inhibit acylation is presented involving disruptors of peptide sorption, namely, inorganic divalent cations. Kinetics of sorption of a model peptide, octreotide acetate, to free-acid end-group PLGA was monitored in the presence and absence of water-soluble inorganic divalent cationic salts in HEPES buffer solution (pH 7.4, 37 °C). Sorption of cations and octreotide attained pseudo-equilibrium by 24 h. From 24-h sorption isotherms, all cations studied inhibited octreotide sorption to PLGA—the inhibiting effect of the cations increased in the order: $\text{Na}^+ < \text{Mg}^{2+} < \text{Ca}^{2+}$, $\text{Sr}^{2+} < \text{Ni}^{2+} < \text{Mn}^{2+}$. Long-term inhibition of octreotide sorption in the presence of 15 mM CaCl_2 and MnCl_2 translated to decreased acylated octreotide present in solution by greater than 50% at 21 days incubation, i.e., from 32% in the cation-free control to 14 and 13% for CaCl_2 and MnCl_2 , respectively. Over one month in vitro release, PLGA implants encapsulating octreotide acetate and CaCl_2 or MnCl_2 also showed substantial inhibition of acylation relative to no salt or NaCl controls, and similarly, strong inhibition of acylation upon divalent salt incorporation was observed during solvent extrusion of suspended peptide with polar organic carrier solvents. Hence, disrupting peptide sorption to PLGA with addition of inorganic divalent cations is a simple and viable strategy to inhibit acylation of peptides in PLGA delivery systems.

Keywords

acylation; octreotide acetate; sorption; divalent cations; PLGA

1. Introduction

Injectable biodegradable microspheres and implants control the release of peptide or protein drugs over the course of weeks to months, providing a distinct advantage over daily injections in terms of patient acceptability and compliance. The PLGA family of copolymers of lactic and glycolic acids is one of the few biodegradable polymers used in US FDA approved pharmaceutical products or medical devices [1], and is widely used in

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commercially available controlled release peptide delivery systems, including the Lupron Depot[®] (leuprolide acetate), Sandostatin LAR[®] (octreotide acetate), and Zoladex[®] implant (goserelin acetate).

A very significant challenge in the development of controlled release PLGA systems is the instability of peptides and proteins. For larger protein molecules numerous physical and chemical pathways of instability have been extensively reviewed [2–5]. Acylation was postulated [3] and later proven as a pathway of instability for peptides encapsulated in PLGA implants [6]. Nucleophilic primary amines, such as from the N-terminus and lysine side chain, can interact with solid PLGA and/or PLGA degradation products to form acylated peptide adducts [7]. Peptide acylation may potentially result in loss of activity [8], a change of receptor specificity, or immunogenicity (see [6] and citations therein). For the important, clinically used octreotide, acylation has been shown to occur in both linear PLGA and glucose-star PLGA copolymers [9, 10], and a mechanism has been proposed to involve an ionic interaction between a protonated amine and the carboxylate PLGA end-group, followed by a nucleophilic attack of another nucleophilic amine on the lactate or glycolate carbonyl carbon and subsequent polymer hydrolysis [11].

Several methods to minimize acylation within PLGA microparticles have been proposed, including: (a) increasing the microclimate pH from 2 to 6 [6, 9, 12], (b) reducing polymer hydrolysis rate by encapsulating with PLGAs of high lactic:glycolic ratio [6, 10], (c) facilitating the release of water soluble oligomers (for example by using PEG as a porosigen) [6], and (d) shielding the reactive amino-group on the peptide by PEGylation [11]. Unfortunately, these methods either: do not strongly inhibit acylation, limit formulation options, or involve chemically modifying the drug molecule. Additional approaches, particularly the use of additives, are needed to optimize PLGA delivery of peptides susceptible to this reaction.

Na and Deluca have shown that the interaction of octreotide with PLGA was attenuated when the polymer was end-capped and when octreotide was PEGylated, resulting in an inhibition of acylation [11]. This important finding strongly suggests a critical precursor role of the ionic interaction between dicationic octreotide and the carboxylic acid end-groups of PLGA in acylation. This peptide-polymer interaction is the basis for our first effort to identify new acylation inhibitors.

PEGylating polypeptide therapeutics is an attractive option for molecules that would benefit from improved physical stability, resistance to proteases, reduced immunogenicity, or increased half-life. In some cases, PEGylation may be a useful strategy for extending an existing product's patent-life by applying for regulatory approval of the new chemical entity. Unfortunately, there are many documented cases where PEGylation has resulted in a substantial decrease in receptor affinity [13] or may not be a feasible strategy because of financial concerns with gaining approval of a new drug entity.

The interaction of cations in the diffuse side of electric double layers of negatively charged surfaces is well documented [14]. Similarly, the binding mechanism of divalent cations to surfaces such as emulsion droplets [15] and phospholipid membranes [16–19] has been the

topic of extensive research. The specific interaction of divalent cations with biological membranes is essential for several cellular processes, including endo- and exo-cytosis, signal transduction, transport of molecules, and binding of proteins. This body of work motivated us to investigate the effect of water-soluble inorganic divalent cationic salts on the interaction between octreotide and PLGA. Indeed, we found for certain cations, such as Ca^{2+} and Mn^{2+} , which are significantly present in living systems, a very strong inhibition of both peptide sorption to PLGA and peptide acylation using octreotide as a model peptide.

2. Materials and methods

2.1. Materials

Octreotide acetate was obtained from Novartis (Basel, Switzerland). PLGA 50:50 (Resomer 502H, I.V. 0.2 dl/g) was purchased from Boehringer Ingelheim GmbH (Ingelheim, Germany). (Hydroxyethyl)-piperazine-(ethanesulfonic acid) (HEPES), calcium chloride (CaCl_2), magnesium chloride (MgCl_2), manganese chloride (MnCl_2), and sodium chloride (NaCl) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Nickel chloride (NiCl_2) and strontium chloride (SrCl_2) were purchased from Fisher Scientific. Standard solutions for inductively coupled plasma-optical emission spectroscopy (ICP-OES) were purchased from GFS Chemicals (Columbus, OH). All other reagents used were of analytical grade or purer and purchased from commercial suppliers.

2.2. Analysis of octreotide by HPLC and HPLC-MS

The concentration of native and acylated octreotide was determined by HPLC and the octreotide degradation products were identified as acylated octreotide by HPLC-MS, similarly as described by Murty, et al. [10]. See Supplementary Information for details regarding the LC-MS analysis, including reference chromatograms and table of peak identifications. Injection volumes of 20 μL were loaded onto a Nova Pak C-18 column (3.9 $\times$ 150 mm, Waters) for RP-HPLC (Waters Alliance[®]) analysis using UV detection (280 nm). Solvent A was 0.1% TFA in acetonitrile and Solvent B was 0.1% TFA in water. A linear gradient of 25 to 35% A in 10 min, with a flowrate of 1.0 mL/min was used. The formation of acylated octreotide was verified by HPLC-MS, where a linear gradient of 15 to 45% A in 30 min was used, with a flowrate of 1.0 mL/min, followed by infusion into an electrospray ionization mass spectrometer with ion-trap detection in positive ion mode (ThermoFinnigan Surveyor HPLC and LCQ MS, San Jose, CA). Solutions of octreotide (0.9 mM) before and after the addition of CaCl_2 and MnCl_2 (50 mM) were analyzed by HPLC after 24 hr incubation to validate that the divalent cations do not interfere with the HPLC analysis or degrade octreotide. The addition of CaCl_2 or MnCl_2 did not affect the concentration of octreotide detected (data not shown).

2.3. Analysis of divalent cations by ICP-OES

The concentration of divalent cations were analyzed by ICP-OES (Perkin-Elmer Optima 2000 DV with Winlab software). Solutions containing divalent cations were diluted with water prior to analysis. Ca^{2+} was detected at 396.85 nm in the radial plasma mode; Mn^{2+} was detected at 257.61 nm in the axial plasma mode. Each measurement was an average of three scans.

2.4. Octreotide sorption studies

Solutions of octreotide (0.2–3.0 mM, 1 mL) in HEPES buffer (0.1 M, pH 7.4) were added to PLGA particles (10 mg, as received) and incubated (37°C) on a rotary shaker (320 rpm) (IKA KS 130 basic). For sorption inhibition studies, chloride salts of divalent cations (1–50 mM) or NaCl (50 mM) were added to octreotide solutions prior to incubation. HEPES buffer was necessary to solubilize the divalent cations, which can precipitate with conventionally used phosphate buffer ions. The amount of octreotide sorbed was determined by the loss of octreotide from solution. Samples were removed from the incubator, centrifuged (2 min at 9.0 rcf) (Eppendorf 5415 D), and the supernatant was analyzed by HPLC. To validate this method of sorption quantification, an octreotide mass balance was performed at 1 and 24 hr during the sorption experiment by recovering the sorbed octreotide from the polymer via two-phase extraction (see Section 2.6 below). The total amount of octreotide recoverable at 1 and 24 hr was 99.1% and 92.1%, respectively. Therefore, during sorption studies, virtually all sorbed octreotide was non-covalently bound to PLGA, with the likelihood that a small fraction had become covalently bound to the polymer or otherwise decomposed.

2.5. Preparation of PLGA millicylinders

Sieved octreotide acetate powder, or octreotide acetate and salt powder (sodium chloride, calcium chloride or manganese chloride, <90µm), both at 5% theoretical loading (see Table 1), were suspended in 62.5% (w/w) PLGA/acetone or PLGA/ methylene chloride solution. The suspension was then loaded into a 3 mL syringe and extruded into a silicone rubber tubing (0.8 mm) by a syringe pump (Harvard Apparatus, Holliston, MA) at approximately 0.01 mL/min. The silicone tubing loaded with suspension was dried at room temperature for 24 h followed by further drying in a vacuum oven at 40 °C for another 48 h.

2.6. Recovery of octreotide and divalent cation from PLGA via two-phase extraction

Millicylinders or PLGA particles, which were loaded or sorbed with octreotide and/or salt, were dissolved in 1 mL methylene chloride and the octreotide and salt were extracted with 2 mL acetate buffer (0.1 M, pH 4.0) three times. Octreotide concentration was determined by HPLC. The encapsulation efficiency of octreotide or divalent cation was determined by dividing the actual loading of octreotide, determined by HPLC, or divalent cation, determined by ICP-OES, by their theoretical loading.

2.7. Octreotide release from PLGA millicylinders

PLGA millicylinders (3–4 mg) loaded with octreotide acetate and no salt, NaCl, CaCl₂, or MnCl₂ (Table 1) were incubated in 1 mL PBS + 0.02% Tween 80, pH 7.4 (PBST) at 37 °C for various preset times. To determine the rate of acylation and release of encapsulated octreotide, the acylated fraction of peptide was measured in both the extract and in the release media. For assessment in the release media, at each time point, the release media was removed and replaced with fresh buffer, and the native and acylated octreotide content was measured by HPLC. For assessment by extraction, release media was similarly replaced, and peptide extraction was determined similarly as for peptide loading in 2.6.

3. Results and Discussion

3.1. Kinetics of octreotide sorption to PLGA

Before evaluating cation inhibition of octreotide acylation, the baseline kinetics of octreotide sorption was determined for an initial octreotide concentration of 0.8 mM. As shown in Fig. 1, the sorption of octreotide to PLGA was rapid up to 6 h. Subsequently, the increase in sorption with time was attenuated occurring at a very low and roughly constant rate. Although sorption never entirely reached equilibrium, possibly due to the generation of carboxylates by polymer hydrolysis, the rapid initial sorption was complete by 24 h. For reference, the sorption kinetics of one of the potential inhibitory divalent cations, Ca^{2+} , was found to follow a similar trend as that with octreotide (Fig. 1), indicating the usefulness of the 24-hour time point to characterize the binding behavior of the potential peptide acylation inhibitors. Therefore, this time point was designated as time to pseudo-equilibrium.

3.2. Effect of divalent cations on octreotide sorption

To determine the effect of the physical properties of divalent cations on their ability to inhibit octreotide sorption, 24-hour sorption isotherms were obtained using various concentrations of different cations in HEPES buffer (0.1 M, pH 7.4). Octreotide sorption isotherms containing 0, 1, 15, and 50 mM MgCl_2 or CaCl_2 are shown in Figure 2. Langmuir-like behavior was observed in the absence of salt and only for 1 mM divalent salt concentration; for higher concentrations of divalent salt, the amount of sorbed peptide appeared to increase sigmoidally with equilibrium octreotide concentration. Ca^{2+} was more effective at inhibiting sorption than Mg^{2+} at all concentrations tested.

Next, the effect of chloride salts of other divalent (Sr^{2+} , Ni^{2+} , Mn^{2+}) and monovalent (Na^+) cations were investigated to determine if such cations could inhibit sorption more effectively than Ca^{2+} (Fig. 3). The octreotide adsorption was very strongly inhibited in the presence of Mn^{2+} , and in the presence of 15 mM divalent cations, inhibition decreased in the order: $\text{Mn}^{2+} > \text{Ni}^{2+} > \text{Sr}^{2+}$, $\text{Ca}^{2+} > \text{Mg}^{2+}$. When 50 mM NaCl was added to the HEPES buffer, the amount of octreotide sorbed decreased slightly compared to the salt-free octreotide isotherm (Fig. 2), but was greater than in the presence of 15 mM divalent chlorides. For example, the amount of sorption from an initial concentration of 2 mM octreotide was reduced from 70 $\mu\text{mol/gPLGA}$ for 50 mM Na^+ to 66, 32, and 2 for 15 mM Mg^{2+} , Ca^{2+} , and Mn^{2+} , respectively.

The reduction in the amount of octreotide sorbed upon the addition of salt suggests that ionic strength may play a role in the interaction of octreotide with PLGA at pH 7.4. The ionic strength of octreotide solutions in HEPES buffer without salts is 50 mM, while the addition of 15 mM divalent cation chloride salt or 50 mM NaCl increased the ionic strength to approximately 100 mM. However, the increased ionic strength cannot account for the decreased sorption of octreotide to PLGA in the presence of divalent cations relative to Na^+ .

The overall binding strength of an ion with its ligand results from the balance of the intrinsic binding affinity of complexation (exothermic) and differences in hydration resulting from the liberation of water upon binding (endothermic) [20, 21]. The intrinsic binding affinity of divalent cations may change in solution for cations susceptible to oxidation, such as Mn^{2+} ,

which may react to form ions with increased positive charge [22]. Furthermore, hydrolysis of hydrated aquo-cations, $X[H_2O]_n^{2+}$, where X is a cation in solution, may result in the reduction of their positive charge, (e.g. $X[H_2O]_{n-1}[OH]^+$) [23]. The order of effectiveness for the group II cations suggests that with increasing size, the higher hydration energy of Ca^{2+} may compensate for the loss of intrinsic binding affinity present at lower charge densities (e.g., as for Mg^{2+}). Thus, the relatively poor inhibition of Mg^{2+} may be due to a higher ratio of bound cation to PLGA carboxylates at quasi-equilibrium for Ca^{2+} than for Mg^{2+} .

For transition metal cations, complexation with a ligand causes field splitting of the d orbitals, resulting in crystal field stabilization. In the high-spin (weak field) electron configuration, Mn^{2+} is not stabilized by crystal field splitting, whereas Ni^{2+} is stabilized [22]. Thus, one would expect Ni^{2+} to be a more effective inhibitor of octreotide adsorption if the crystal field stabilization energy were the most significant component affecting cation binding. One possibility for the increased effectiveness of Mn^{2+} is the difference in rate constant for the exchange of water, which is three orders of magnitude higher for $Mn(H_2O)^{2+}$ than $Ni(H_2O)^{2+}$ [23]. There also exists a potential for Mn^{2+} oxidation to Mn^{3+} or other oxidized manganese species. In support of this notion, the Pourbaix diagram for manganese shows that the stability of Mn^{2+} decreases with increasing pH and is unstable above pH 7.6 [22].

To test the stability of Mn^{2+} in the chloride salt solution containing octreotide acetate in the presence and absence of PLGA, the hyperfine splitting of Mn^{2+} was evaluated using EPR spectroscopy. The typical hyperfine splitting of Mn^{2+} was observed for all spectra. No significant difference in the peak shape or intensity of the EPR spectra was observed for the incubated samples in the absence or presence of PLGA, relative to the non-incubated control (see Fig. S.2 in Supplementary Information). Therefore, the inherent inhibitory potential of Mn^{2+} in the presence of octreotide in the 24-h sorption isotherms was unlikely caused by other oxidized Mn-species, such as Mn^{3+} , which is EPR silent [24].

3.3. Long-term interaction of octreotide with PLGA

Long-term sorption studies were performed to determine the effect of 15 mM $CaCl_2$ and $MnCl_2$ on the sorption and acylation of octreotide (Fig. 4). In the absence of salt, the onset of octreotide sorption occurred rapidly (1 day), followed by a continuous increase in the total amount of octreotide in solution after 3 days. Octreotide acylation products appeared at 3 days and continuously increased to 32% of total octreotide in solution by 21 days. In the presence of modest concentrations of $CaCl_2$ or $MnCl_2$, the peak amount of sorption occurred later (14 days). Exhibiting a similar delay, octreotide acylation products did not appear until 7 days and increased to 14% of total octreotide in solution by 21 days in the presence of $CaCl_2$. While the onset of acylation was not delayed in the presence of $MnCl_2$, the amount of acylation at 21 days was 13%, similar to that found with $CaCl_2$ and less than half that of the salt-free value. The similar effectiveness of Ca^{2+} and Mn^{2+} is consistent with our suggestion that several mechanisms may contribute to the inhibition of sorption by divalent cations. The oxidation of Mn^{2+} to form $MnO(s)$ may contribute to the reduced effectiveness of $MnCl_2$ in long-term studies. Solutions of $MnCl_2$ in HEPES buffer displayed visible signs of a solid brown precipitate, typical of MnO , after 2–3 weeks at room temperature (see Fig.

S3 in Supplementary Information). Another possibility is that differences between the microclimate environment within the pores and the HEPES buffer solution altered the water-exchange rates or the pKa of hydrolysis of the aquo-cations. Nevertheless, these results strongly support the previously formed hypothesis that some type of sorption to PLGA is involved in the acylation mechanism [11], and that inhibiting adsorption is a useful strategy to minimize octreotide acylation.

3.4. Effect of salts on peptide acylation when encapsulated in PLGA

The microclimate pH within PLGA controlled-release depots has been reported to be acidic [25], protonating the carboxylate groups responsible for the peptide interaction and making them unavailable for sorption. However, the polymer carboxylates can form a salt with the peptide during drying. During release, the microclimate pH is often acidic but variable [26]. Furthermore, the monomer and oligomers of lactic and glycolic acid have pKa's that are very low (e.g., 3.1–3.6), so a significant fraction of carboxylates will be ionized within the common microclimate pH range (e.g., 2.5 – 5) [27].

To determine if the divalent cations could inhibit acylation of encapsulated peptide, PLGA millicylinders containing octreotide acetate and either no salt, NaCl, CaCl₂, or MnCl₂ (Table 1) were prepared by a solvent extrusion method [28], except that methylene chloride was used in place of acetone as a carrier solvent. In control studies, where extrusion was performed with an end-capped polymer (PLGA 50/50, i.v.=0.6 dl/g) and acetone, we observed strong acylation of 5% loaded octreotide (65 ± 3 % acylation) following solvent extrusion. This acylation was strongly inhibited by 5% loaded divalent salts (e.g., CaCl₂: 14 ± 1 % acylation and MnCl₂: 23 ± 1 % acylation) relative to 5% loaded NaCl control (57 ± 3 % acylation) (see Table S.2 in Supplementary Information).

Following encapsulation with methylene chloride, no peptide acylation was observed although encapsulation efficiency of both peptide and encapsulated salts was slightly lower, i.e., ~60–80% (Table 1), than traditionally with acetone [28]. The resulting millicylinders with initially native octreotide were then incubated in PBST at 37 °C for 28 days. As encapsulated salts may modulate porosity, osmotic pressure, water uptake, transport of soluble oligomers, and pH, and therefore peptide acylation rate in the PLGA-peptide system [1], the addition of NaCl was used as an additional control. For the peptide release study, we recorded the fraction of extractable peptide remaining in the polymer, as octreotide is known to be unstable at physiological pH [29]. To assess acylation kinetics, we resolved the acylated peptide fraction from the total peptide in the release media (presuming similar decomposition rates between acylated and native peptide species, Table 2), as well as the acylated fraction appearing in the polymer extract (see Table S.3 in Supplementary Information).

The total extractable octreotide remaining within the millicylinders decreased steadily to approximately 25% over the course of 4 weeks for all formulations. Relative to the no-salt and NaCl controls, the addition of the divalent chlorides of Ca²⁺ or Mn²⁺ reduced the fraction of acylated octreotide extractable from the millicylinders at 7 days by approximately 75% from 8% to under 2%. The amount of acylation within the millicylinders formulated with divalent chlorides remained lower than the no-salt and NaCl control at all

time-points, although the benefit from the addition of divalent chlorides decreased with time, likely due to the release of the salts during incubation. Future studies will focus on maximizing salt content, distribution, and retention to maintain the highest competitive advantage for the anionic binding sites on the polymer.

4. Conclusions

A new class of inhibitors of the sorption and acylation of a model peptide, octreotide acetate, has been described. At neutral pH, all cations studied inhibited octreotide sorption to PLGA—the inhibiting effect of the cations increased in the order: $\text{Na}^+ < \text{Mg}^{2+} < \text{Ca}^{2+}$, $\text{Sr}^{2+} < \text{Ni}^{2+} < \text{Mn}^{2+}$. Long-term sorption studies in the presence of CaCl_2 and MnCl_2 indicated that disrupting peptide sorption to PLGA with the inorganic divalent cation inhibitors translates to inhibition of peptide acylation. Acylation of octreotide encapsulated in PLGA millicylinders containing equivalent weight ratio CaCl_2 or MnCl_2 relative to peptide was also inhibited relative to no salt or NaCl controls during encapsulation or release incubation. In a future report we will demonstrate the strong inhibition of octreotide acylation afforded by divalent cations during release of octreotide from PLGA microspheres.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Kinetics of octreotide acetate sorption to PLGA 50/50 in the presence of 15 mM CaCl_2 (◆), MnCl_2 (▲), no salt (●), and kinetics of Ca^{2+} (○) sorption to PLGA during 24 h incubation in HEPES buffer solution (0.1 M, pH 7.4) at 37 °C. PLGA was 10 mg in 1 mL of buffer solution. Dotted trendlines shown for clarity.

Figure 2.

Effect of (A) MgCl₂ and (B) CaCl₂ on octreotide acetate sorption to PLGA 50/50 at 0 (○), 1 (◆), 15 (▲), and 50 (●) mM salt concentrations after 24 hr incubation in HEPES buffer solution (0.1 M, pH 7.4) at 37 °C. PLGA was 10 mg in 1 mL of buffer solution.

Figure 3.

Effect of 15 mM Mg²⁺ (■), Ca²⁺ (△), Sr²⁺ (▲), Ni²⁺ (○), and Mn²⁺ (●) chloride and 50 mM NaCl (□) on octreotide acetate sorption to PLGA 50/50 after 24 hr incubation in HEPES buffer solution (0.1 M, pH 7.4) at 37 °C. PLGA was 10 mg in 1 mL of buffer solution.

Figure 4.

Octreotide sorption and formation of acylated products, native (●), acylated (○), and total (▼) octreotide, during incubation of PLGA at 37 °C in HEPES buffer solution (0.1 M, pH 7.4) containing (A) no additional salt, (B) 15 mM CaCl₂, and (C) 15 mM MnCl₂. Initial octreotide concentration was 0.2 mM and PLGA was 10 mg in 1 mL of buffer solution.

Table 1

Summary of encapsulation of octreotide and salt in PLGA millicylinder formulations.

Formulation	Theoretical Salt Loading (wt%)	Actual Salt Loading (wt%)	Salt Encapsulation Efficiency (%)	Theoretical Octreotide Loading (wt%)	Actual Octreotide Loading (wt%)	Octreotide Encapsulation Efficiency (%)
No Salt	--	--	--	5.07	3.1 ± 0.2 ^a	61 ± 3
NaCl	5.2	3.1 ± 0.1	59.7 ± 0.1	5.20	3.6 ± 0.2	69 ± 4
CaCl ₂	5.1	3.4 ± 1.0	67.5 ± 19	5.08	4.1 ± 0.2	81 ± 4
MnCl ₂	5.1	3.8 ± 0.1	74.7 ± 0.9	4.94	4.0 ± 0.1	81 ± 2

^a All values represent mean ± SEM (n = 3).

Table 2

Long-term release of native and acylated octreotide from PLGA millicylinders.

Formulation	Time (day)	Native (%) ^a	Acylated (%) ^a	Total (%) ^a
No Salt	7	0.8 ± 0	0	0.8 ± 0
	14	4.6 ± 0	0.8 ± 0	5.6 ± 0
	21	32.0 ± 3	7.6 ± 1	39.6 ± 4
	28	42.7 ± 6	14.7 ± 3	57.4 ± 9
NaCl	7	1.7 ± 1	0	1.7 ± 1
	14	16.8 ± 8	3.1 ± 1	19.9 ± 7
	21	37.0 ± 12	12.6 ± 3	49.6 ± 14
	28	43.8 ± 9	19.2 ± 2	63.0 ± 10
CaCl ₂	7	8.8 ± 1	0	8.8 ± 1
	14	17.7 ± 1	0.9 ± 0	18.6 ± 1
	21	38.8 ± 1	4.2 ± 0	43.0 ± 1
	28	43.7 ± 2	7.2 ± 1	50.9 ± 3
MnCl ₂	7	11.7 ± 2	0	11.7 ± 2
	14	23.8 ± 2	1.7 ± 0	25.5 ± 2
	21	32.2 ± 2	4.8 ± 2	37.0 ± 0
	28	39.6 ± 2	11.2 ± 2	50.8 ± 1

Values are extracted peptide normalized for initially loaded peptide; mean ± SEM (n = 3).