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Research Paper

Long-term Stability and *in vitro* Release of hPTH(1–34) from a Multi-reservoir Array

Elizabeth R. Proos,[1,2] James H. Prescott,[1] and Mark A. Staples[1]

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Purpose. Therapeutic peptides generally exhibit poor oral bioavailability and require alternative methods of delivery. Implanted microelectromechanical systems-based multi-reservoir devices enable programmable, chronic, pulsatile peptide delivery. This report describes parathyroid hormone fragment (hPTH (1–34)) formulations suitable for delivery from a multi-reservoir array.
Methods. The stability of hPTH(1–34) lyophilizates obtained from aqueous acidic solutions was assessed by reverse phase high pressure liquid chromatography. An *in vitro* test device measured *in vitro* release kinetics.
Results. Novel, highly concentrated (>50 mg/mL) hPTH(1–34) solutions were dispensed as bulk samples (1–3 mg peptide) in vials and as individual doses (13–21 μg peptide) in reservoir arrays. Bulk and array samples were lyophilized and stored at 37°C. Bulk lyophilizate hPTH(1–34) purity after lyophilization, after 8 weeks, and after 26 weeks exceeded 96%, 90%, and 80%, respectively. The hPTH(1–34) stored in multi-reservoir arrays exhibited similar purity over 29 weeks at 37°C. Initially and over 29 weeks, over half of the peptide was consistently released from arrays into neutral, isotonic solution in less than 30 min with quantitative recoveries (>95%) within 3 h.
Conclusions. Clinically relevant formulations of hPTH(1–34) for use with implantable multi-reservoir devices are achievable.

KEY WORDS: drug delivery; microchip; osteoporosis; parathyroid hormone; protein and peptide formulation.

INTRODUCTION

Osteoporosis is a silent disease which affects more than 75 million people in the USA, Europe, and Japan (1). While not immediately life threatening, the mortality rate for women 1 year after an osteoporotic hip fracture is one in five, similar to breast cancer mortality rates. The mortality rates for men are even worse (2). Even with such dire consequences for non-treatment, due to its asymptomatic nature, osteoporosis patients are often unwilling to comply with current treatment regimes (3,4).

Human parathyroid hormone, hPTH(1–84), and the 34-amino acid N-terminal fragment, hPTH(1–34), have a long history as biologics of clinical interest in osteoporosis treatment (5,6). The development of hPTH(1–34) for clinical use, ultimately resulting in the marketed product Forteo® [teriparatide (rDNA origin) injection], parallels the evolution of the biotechnology industry. Early work with parathyroid hormone (PTH) used extracts from mammalian parathyroid tissue (7). Subsequently, hPTH(1–34) was produced using solid-phase synthetic methods (8). The hPTH(1–34) currently used in Forteo® [teriparatide (rDNA origin) injection], is produced using recombinant methods (*E. coli*). The next step in this evolution is development of a novel delivery method for a full course of hPTH(1–34) therapy without the pain, inconvenience, and noncompliance associated with daily injections, addressing a significant unmet medical need.

Pulsatile delivery of hPTH(1–34) is required for the desired physiological effect (9). Daily subcutaneous injection of low doses of hPTH(1–34) result in an increase in bone density (10,11), while steady state delivery favors bone resorption and a decrease in bone density (12,13). Alternative hPTH(1–34) delivery methods that have been evaluated for osteoporosis treatment include oral (14), nasal (15), pulmonary (16), and transdermal (17), but none of these approaches has yielded an approved product. Although pulsatile kinetics have been reported using non-injected dose delivery, the bioavailabilities of hPTH(1–34) preparations have been lower than when injected. The implantable multi-dose device being developed can provide automated, chronic pulsatile peptide delivery with a corresponding high bioavailability (18) and provides an attractive alternative to the injectable hPTH(1–34) therapy.

Several challenges to achieving acceptable hPTH(1–34) release characteristics include the preparation of highly concentrated hPTH(1–34) filling solutions, development of formulations that are sufficiently stable at 37°C for the desired duration of therapy, and achievement of complete,

[1] MicroCHIPS, Inc., 6-B Preston Court, Bedford, Massachusetts 01730, USA.
[2] To whom correspondence should be addressed. (e-mail: eproos@mchips.com

rapid, pulsatile release from small (≤300 nL) reservoirs. This study reports how these challenges were successfully met using process steps that are controllable and scalable. Concentrated hPTH(1–34) solutions can be dispensed in implantable multi-reservoir arrays and subsequently lyophilized to yield 20 μg of hPTH(1–34) per reservoir, which is equivalent to the mass of hPTH(1–34) contained in a single daily dose of Forteo® [teriparatide (rDNA origin) injection]. The doses can be rapidly and completely released into a mimetic of physiological fluid. Formulations are described which exhibit sufficient stability at 37°C to enable the study of hPTH(1–34) pharmacokinetics and efficacy following chronic delivery from an implanted multi-reservoir device *in vivo*, over a period of 8–12 weeks.

MATERIALS AND METHODS

Materials

All chemicals were used as received without further purification: hPTH(1–34) acetate lyophilizate (PolyPeptide Laboratories, Torrance, CA, ≥97% purity, 83%± 3% peptide content), glacial acetic acid (EMD, Gibbstown, NJ), histidine, polyethylene glycol (PEG) 3500, sodium phosphate monobasic, sodium phosphate dibasic, potassium chloride, and Polysorbate 20 (Sigma-Aldrich, St. Louis, MO), citric acid and sodium chloride (Mallinkrodt Baker, Phillipsburg, NJ) and trehalose (Ferro, Cleveland, OH). Isotonic, neutral (pH 7.4) phosphate buffer (PBS: 10 mM sodium phosphate, 140 mM sodium chloride, 3 mM potassium chloride) was prepared from mono- and di-basic sodium phosphate with sodium chloride and potassium chloride in reverse-osmosis deionized water and was filtered through a 0.2 μm filter before adjusting to 0.004% Tween 20 (w/v). Chromatographic analysis was performed with high pressure liquid chromatography (HPLC) grade acetonitrile and water (Mallinckrodt Baker, Phillipsburg, NJ). Trifluoroacetic acid (TFA, Pierce Biotechnology, Rockland, IL) was used to modify the pH of the HPLC mobile phase.

hPTH(1–34) Solution Preparation and Concentration Determination

Concentrated peptide solutions (>50 mg/mL) were prepared by the direct addition of aqueous solution(s) to solid hPTH(1–34) acetate at ambient temperature, followed by gentle mixing. The hPTH(1–34) concentration and purity were quantified by reverse phase-high pressure liquid chromatography (RP-HPLC). Chromatography was performed with an Agilent Series 1100 HPLC (Agilent Technologies, Wilmington, DE), using an octadecylsilane stationary phase (Vydac; part number 218TP5215; 5 μm silica; 0.21×15 cm column). Gradient mobile phase conditions were employed (82% water and 18% acetonitrile (v/v) with 0.2% TFA (v/v) from 0 to 3 min, linear gradient to 58% water and 42% acetonitrile (v/v) with 0.2% TFA (v/v) from 3 to 54 min), with a flow rate of 0.2 ml/min. Analyte detection was by absorbance at 215 nm. All concentrated peptide solutions required dilution prior to analysis.

Sample Preparation for Bulk Lyophilization and Stability Testing

Aliquots (20 μL) of the concentrated hPTH(1–34) solutions were dispensed into 2 mL borosilicate glass serum vials (Kimble, Vineland, NJ), and the masses of the delivered volumes were recorded. The samples were frozen on the pre-chilled shelf (−50°C) of a commercial freeze dryer (Virtis Inc., Advantage EL). Samples were lyophilized using a conservative, non-optimized cycle (freeze 12 h at −50°C, primary drying for 24 h at −50°C, secondary drying for 12 h at 5°C and 3 h at 25°C, all drying at 30 mTorr). After freeze-drying the vials were closed with butyl rubber stoppers, under argon, crimp-sealed with aluminum caps, and placed in a 37°C incubator (non-humidity controlled) for stability testing. Samples were protected from light.

Dissolution and Stability Testing

Stability testing included observations of dissolution properties and chromatographic determinations of peptide recovery and purity. Individual samples of the bulk hPTH (1–34) formulations were assessed for dissolution properties immediately after lyophilization and again after storage at 37°C, using PBS as the diluent. A known volume of PBS was placed on top of the lyophilized cake, taking care to minimize sample agitation. Dissolution time was based on visual inspection up to 1 h. After 1 h the sample was gently mixed to ensure solution homogeneity prior to sampling for chromatographic analysis. Peptide stability was determined by RP-HPLC using the previously described method. Three separate samples of lyophilized hPTH(1–34) were dissolved and analyzed for each formulation/time point combination.

Select stability samples were analyzed for the presence and content of non-covalent hPTH(1–34) multimer(s) by the method of Kamberi *et al*. (19). In brief, samples were dissolved in PBS as described previously and analyzed by size exclusion chromatography (SEC) using a TSK G2000-SW column (5 μm silica; 7.8 mm×30 cm column) and isocratic mobile phase conditions (80% 0.2 M sodium chloride and 20% acetonitrile (v/v) with 0.1% TFA (v/v)), with a flow rate of 0.5 ml/min. Analyte detection was by absorbance at 280 nm.

LC/MS Analysis

LC/MS analysis was used to assign molecular weight values to peaks observed in the RP-HPLC chromatography of select stability samples. The work was performed using a RP-HPLC method, similar to the one described above for separation, and an ABI 4000LT mass spectrometer with Turbo Spray source for all on-line LC/UV/MS analyses. Samples were also subjected to tryptic digest and analyzed by LC/MS/MS.

Reservoir Filling

Microchip arrays were fabricated as described previously (20) by Micralyne, Inc. (Edmonton, CANADA). Each array contains 24 reservoirs with a volumetric capacity of 300 nL per reservoir (21). Individual reservoirs of the multi-reservoir

Table I. hPTH(1–34) Formulation Compositions: Peptide Concentration Determined by RP-HPLC

Component	Formulation ID											
	A	B	C	D	E	F	G	H	I	J	K	L
Final hPTH(1–34) Concentration (mg/mL)	116	66	116	59	59	108	109	117	182	249	121	119
Composition of sample diluent												
Acetic acid[a] (M)	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Citric acid (mM)		200	200	200	200	200	200	200	200	200	200	200
Histidine (mg/mL)				20	60	20	20	40	20	20		
PEG 3500 (mg/mL)												1
Trehalose (mg/mL)											20	
Approximate total % solids (w/v)	12	11	15	12	16	17	17	20	24	31	18	16

[a] Note: acetic acid is removed during the lyophilization step

array were filled with 200 nL of the concentrated hPTH(1–34) solution(s) using an automated dispensing apparatus. The dispensing apparatus, which has been described previously, provides accurate and precise delivery of nanoliter volumes (21). After each reservoir array was filled, the contents were frozen and lyophilized, using the previously described lyophilization cycle. After lyophilization reservoir filling apertures were sealed with a moisture and solvent resistant adhesive film (Axygen Scientific, Union City, CA).

In vitro Release Testing

In vitro release experiments were performed as described previously (21). Multi-reservoir arrays containing discrete doses of hPTH(1–34) were mounted in *in vitro* test devices and stored at 37°C. Releases were performed into an aqueous recovery solution, and effluent fractions from the test device were collected and analyzed for peptide content by RP-HPLC. The recovery solution for the release experiments was either PBS or diluted acetic acid. Four reservoirs were opened per release event to permit determinations of hPTH (1–34) purity in the more concentrated fractions. At the time of reservoir opening the recovery solution was held static over the array and periodically pumped across the array, using a low flow rate (<2 mL/min), to collect the timed fractions. Between testing time points, arrays were stored in a 37°C incubator (non-humidity controlled) in a desiccant chamber and were light-protected.

RESULTS

Quantitation of High Concentration hPTH(1–34) Solutions

Highly concentrated (>67 mg/mL) solutions of hPTH(1–34) must be prepared in order to provide a clinically relevant (20 μg) hPTH(1–34) dose in a small volume (<300 nL). A series of concentrated hPTH(1–34) solutions, 59–249 mg/mL, were prepared in aqueous acetic acid, with or without other formulation components such as citric acid and histidine, and were lyophilized. RP-HPLC was employed to measure the starting concentration of these hPTH(1–34) formulation solutions and to determine the peptide purity of each starting solution. Formulation components of select formulations are presented in Table I. Nominal concentrations were within 20% of target and retained the starting material purity (>97%).

Dissolution of Bulk hPTH(1–34) Lyophilized Formulations

hPTH(1–34) exhibits significantly less solubility in neutral buffer than acidic solution. Highly concentrated acidic hPTH(1–34) solutions may be prepared containing acetic acid (as shown above), but subsequent lyophilization removes the volatile acetic acid. The resulting lyophilizate will not reconstitute rapidly in PBS, creating a challenge for high recovery and rapid release of the hPTH(1–34) dose from a reservoir into physiological fluid. Inclusion of a non-volatile acid component, such as citric acid, in the starting solution, yields an acidic lyophilizate, which creates a solubility promoting acidic microenvironment within the reservoir upon exposure to PBS. Lyophilized samples of the above solutions were reconstituted in PBS immediately after lyophilization and at two time points after storage at 37°C. The observed sample dissolution times are presented in Table II.

hPTH(1–34) lyophilized from acetic acid alone, Formulation A, is poorly soluble in PBS, while formulations

Table II. Solubility Observations of Bulk Lyophilized hPTH(1–34) Formulations in PBS pH 7.4 with Minimal Agitation (Gentle Swirling)

Source Formulation of Lyophilizate	Observed Time to Complete Dissolution in PBS		
	Post-lyophilization	8 weeks at 37°C	26 weeks at 37°C
A	Incomplete in 1 h	ND	Incomplete in 1 week
B	<1 min	3 min	1 h
C	<1 min	<1 min	1 h
D	<1 min	3 min	<1 min
E	<1 min	<1 min	<1 min
F	<1 min	<1 min	1 h
G	<1 min	3 min	20 min
H	<1 min	3 min	1 h
I	5 min	1 h	Incomplete in 1 h
J	30 min	Incomplete in 1 h	Incomplete in 1 h
K	<1 min	<1 min	20 min
L	<1 min	1 h	Incomplete in 1 h

ND = not determined

containing the non-volatile citric acid dissolve rapidly in the neutral buffer (Formulations B-H, K and L) immediately after lyophilization. Differences in time to complete dissolution after lyophilization were noted between formulations containing the same amount of citric acid but that differed in solids content and other formulation component ratios (Formulations I and J). The same lyophilization cycle was used for all formulations but may not be sufficiently optimized to exhibit reproducible physical characteristics for all lyophilizate compositions tested. Use of a non-optimized lyophilization cycle may also contribute to the changing dissolution properties observed over time for some formulations. Most formulations containing citric acid dissolve rapidly at 8 weeks storage at 37°C (with the exceptions of I, J, and L), and Formulations D and E still dissolve rapidly after 26 weeks storage at 37°C.

Stability of Bulk hPTH(1–34) Lyophilized Formulations

Stability of the lyophilized hPTH(1–34) formulations was assessed chromatographically by determining the percent (peptide) purity retained and percent (mass) recovery following dissolution testing post-lyophilization and at two timepoints after storage at 37°C. The percent purity retained is defined as the area of the hPTH(1–34) peak relative to the summed area of all relevant peaks compared to the purity of the concentrated starting solution. The percent recovery is defined as the summed area of all relevant peaks relative to the theoretical value, based on the known concentration and volume of the starting solution. Calculations were performed for individual sample vials (N=3 means that three separate vials were sampled and analyzed) for each formulation tested and averaged to produce the results presented in Table III.

The fraction of the hPTH(1–34) lyophilizate obtained from aqueous acetic acid (Formulation A) that is soluble in neutral buffer after 26 weeks storage at 37°C retains a high purity but exhibits a low mass recovery (13%) after 1 h dissolution in PBS at room temperature. It was visually observed that while a portion of the lyophilized material was soluble, a much larger portion remained as a clear gel stuck to the base of the vial. In an effort to determine the nature of this gel, the sample was left to sit at room temperature (light protected) for 1 week and was reassayed. Recovery results were much higher (78%), indicating that the material may be either very slowly soluble under neutral buffer conditions or consisted of a reversible aggregate. In contrast, multiple citric acid containing formulations (Formulations C, D–G, I) remain soluble after 26 weeks at 37°C, with total mass recoveries exceeding 90%.

Immediately post lyophilization all of the citric acid containing formulations (Formulations B–L) exhibit high purity. Sample purities are comparable to the starting material (≥ 97%). After 8 weeks storage at 37°C the formulations containing only citric acid (Formulations B and C) show a significant reduction in peptide purity compared to formulations that contain both citric acid and histidine (Formulations D–I). The exception is Formulation J, which contained the highest total solids content of the formulations tested. Formulation J was also the only formulation to exhibit a physical change over time, with portions of the lyophilizate appearing sticky or melted at 8 and 26 weeks post-lyophilization. This change in appearance may be due to use of a non-optimized lyophilization cycle, as mentioned above. After 26 weeks storage at 37°C histidine containing formulations are more stable than formulations without histidine. Neither the trehalose nor PEG 3500 containing formulations were as stable as histidine containing formulations at either time point.

A range of hPTH(1–34) concentrations were considered in the preparation of concentrated hPTH(1–34) solutions (Table I) that were used to obtain the lyophilized formulations of Table III. While histidine appears to promote hPTH (1–34) stability, a range of histidine to hPTH(1–34) ratios was observed to give comparably high yields and purity on dissolution. The most stable formulation (highest purity and mass recovery), Formulation E, contains the highest ratio of histidine to hPTH(1–34) tested. Formulations D, F, G, H, and

Table III. Purity and Recovery Data for Bulk hPTH(1–34) Formulations, Recovered in PBS pH 7.4

	Post Lyophilization		8 weeks at 37°C		26 weeks at 37°C	
Source Formulation for Lyophilizate	Average % Purity Retained	Average % Recovery	Average % Purity Retained	Average % Recovery	Average % Purity Retained	Average % Recovery
A[a]	99	79	Not performed		96	13
B	98 (<1% RSD)	113 (2% RSD)	55 (15% RSD)	96 (1% RSD)	35 (6% RSD)	72 (1% RSD)
C	98 (<1% RSD)	93 (1% RSD)	81 (4% RSD)	94 (1% RSD)	61 (4% RSD)	90 (2% RSD)
D[b]	98	117	89 (2% RSD)	106 (5% RSD)	80 (4% RSD)	96 (1% RSD)
E[b]	98	115	95 (2% RSD)	99 (8% RSD)	86 (3% RSD)	97 (3% RSD)
F[b]	98	100	90 (2% RSD)	105 (3% RSD)	83 (1% RSD)	99 (6% RSD)
G	97 (<1% RSD)	101 (3% RSD)	89 (1% RSD)	100 (1% RSD)	80 (1% RSD)	92 (6% RSD)
H	97 (<1% RSD)	99 (2% RSD)	92 (<1% RSD)	97 (5% RSD)	88 (1% RSD)	88 (3% RSD)
I	98 (<1% RSD)	109 (2% RSD)	88 (2% RSD)	110 (7% RSD)	80 (<1% RSD)	102 (5% RSD)
J	98 (<1% RSD)	105 (3% RSD)	85 (2% RSD)	94 (5% RSD)	79 (5% RSD)	82 (5% RSD)
K[b]	98	87	79 (5% RSD)	91 (2% RSD)	67 (3% RSD)	87 (4% RSD)
L[b]	98	87	70 (10% RSD)	89 (2% RSD)	53 (5% RSD)	83 (5% RSD)

N=3 for each formulation at each time point, except where noted
RSD = relative standard deviation
[a] N=2 for post lyophilization recovery and 26 week recovery
[b] N=2 for post-lyophilization recovery

Table IV. Non-covalent Aggregate Presence in Select hPTH(1–34) Formulations as Detected by Size Exclusion Chromatography

Formulation	Timepoint	% Non-covalent Aggregate
E	58 weeks at 37°C	1.7%
F	57 weeks at 37°C	2.1%
Starting material	As supplied by manufacturer	0.2%

I exhibit similar purity and mass recovery values at 8 weeks storage at 37°C. The starting solutions for these samples contained 20 mg histidine/mL, while the hPTH(1–34) concentrations increased from 59 mg/mL to 182 mg/mL across the series.

Retained stability samples of Formulations E and F were examined for the presence of non-covalent hPTH(1–34) multimers using the SEC method of Kamberi *et al.* (19). At the time of the analysis the samples had been stored at 37°C for 57 weeks and 58 weeks respectively. The results of the analysis are summarized in Table IV. The mass fraction of hPTH(1–34) multimer (apparent dimer) was 1.7% in the 58 week old sample of Formulation E, 2.2% in the 57 week old sample of Formulation F, and 0.2% in the hPTH(1–34) starting material as received. Quantitative mass recoveries were observed for all samples.

LC/MS Data

Overlaid reverse phase chromatographs of the soluble fractions obtained from Formulations B and E after 26 weeks at 37°C are presented in Fig. 1. For comparison the chromatographic data obtained from Formulations B and E before lyophilization and of the soluble fraction recovered in neutral buffer immediately post-lyophilization (Fig. 2A and B) as well as a representative chromatogram of the starting material in PBS solution (Fig. 2C) are presented. The theoretical peptide concentration is the same in all samples (25 μg/mL) except for the reference standard which is 30 μg/mL. Formulation E, which was the most stable lyophilizate at the 26 week time point, contained histidine, and Formulation B did not. A number of peaks are present at the 26 week time point that were either not observed or were present in much lesser quantities immediately post lyophilization. The inclusion of histidine in Formulation E has significantly reduced the magnitude of these peaks upon storage, and there is an unresolved doublet immediately preceding the main hPTH (1–34) peak in the Fig. 1 chromatograph of Formulation B that is absent in the data for Formulation E.

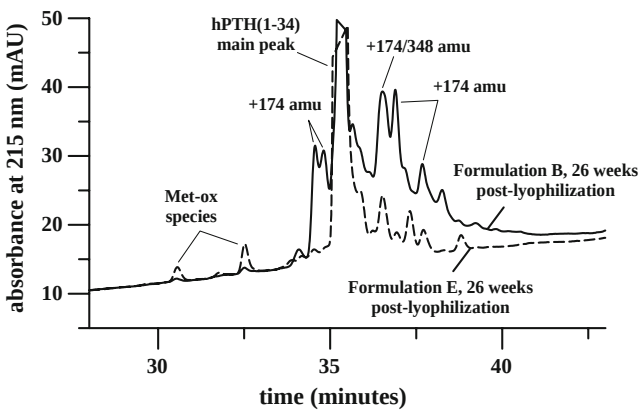

Fig. 1. Overlaid chromatographs of reconstituted hPTH(1–34) lyophilizates after 26 weeks storage at 37°C: illustration of histidine benefit—Formulation E contains histidine and Formulation B does not.

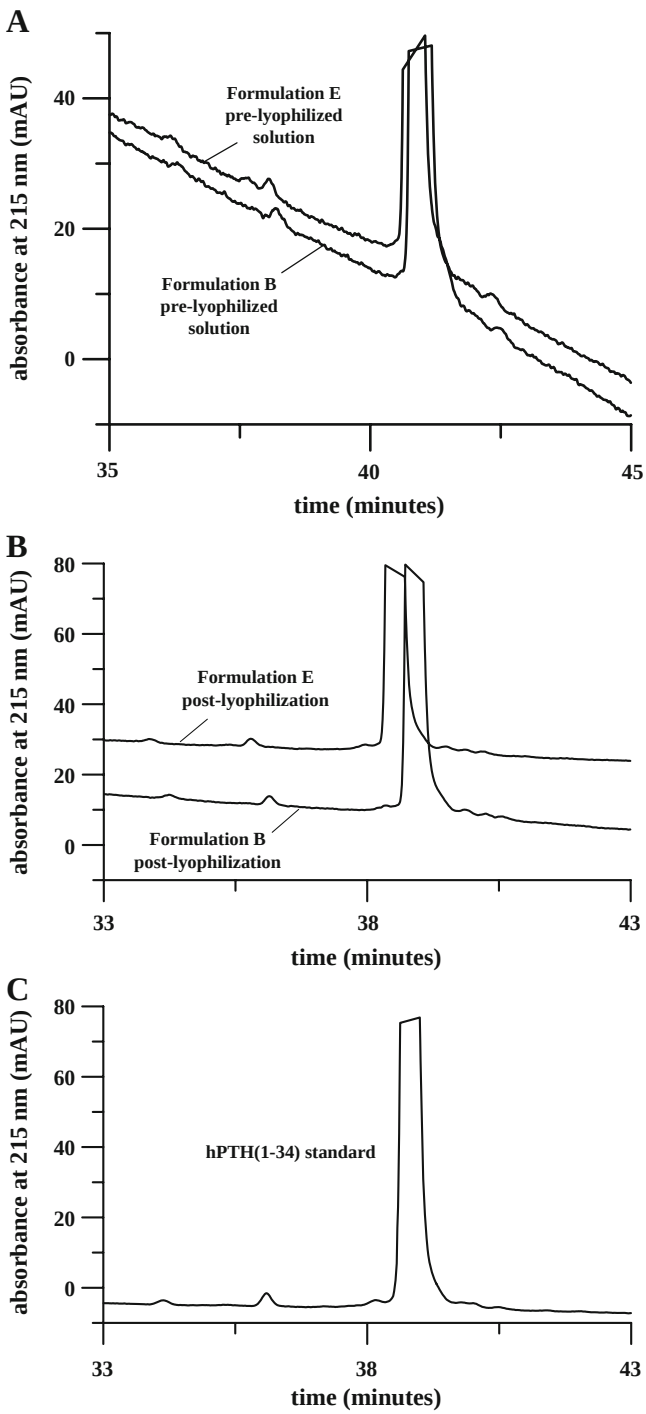

Fig. 2. Overlaid chromatographs of starting hPTH(1–34) concentrated solutions (**A**), reconstituted hPTH(1–34) lyophilizates immediately after lyophilization (**B**) and an hPTH(1–34) reference standard (**C**).

LC/MS analysis was performed on the samples from Fig. 1, as described. Multiple masses equivalent to the hPTH(1–34) molecular ion with the addition of either 174 or 348 atomic mass units (amu) were detected in Formulation B (without histidine) (data not shown). These are consistent with the addition of one or two citrate molecules accompanied by the loss of a single water molecule for each addition. The elution positions, relative to hPTH(1–34), of specific mass additions among the detected peaks are posted in Fig. 1. A tryptic digest, performed on Formulation B and followed by LC/MS/MS analysis, identified serine residues as the primary point for mass addition, possibly through formation of an ester (hPTH(1–34) has 3 serine residues) (data not shown). The mechanism by which histidine stabilized the hPTH(1–34) is not clear, although it does appear to have either blocked or retarded reaction(s) between hPTH(1–34) and citrate. A literature search failed to indicate precedent for strong citrate–polypeptide interaction in more dilute preparations. Two peaks in Fig. 1 were also identified as the methionine oxidized species. These peaks are present in the starting material as received and do not increase in amount during storage at 37°C under the conditions utilized for this study (sealed under argon).

In vitro Release Data

The solubility of hPTH(1–34) acetate in an isotonic neutral PBS, pH 7.4, was observed to be less than 20 mg/mL. Above its solubility limit, hPTH(1–34) formed an uncharacterized gel/precipitate. The limited solubility of hPTH(1–34) in neutral aqueous solutions presents a challenge when attempting to release hPTH(1–34) from reservoir devices into a mimetic of physiological fluid, such as PBS, because the hPTH(1–34) may fail to dissolve completely or may precipitate and either block or retard release. Such phenomenon can be avoided by including an acidic component to the hPTH(1–34) formulation which, upon contact with neutral buffer, dissolves and creates a low pH microenvironment inside the reservoir, allowing the hPTH(1–34) to dissolve completely and remain soluble while diffusing from the reservoir.

In vitro release experiments were performed with reservoir arrays containing either hPTH(1–34) acetate, obtained by lyophilization from an hPTH(1–34) solution containing acetic acid (same composition as Formulation A), or a novel hPTH(1–34) formulation, obtained by lyophilization from an hPTH(1–34) solution containing acetic acid, citric acid and histidine (same composition as Formulation F). Releases were performed using an *in vitro* test device. Initially the recovery solution was PBS. The formulation containing citric acid released from the chip quickly and completely, while the hPTH(1–34) acetate released slowly. After 24 h the recovery solution was changed from PBS to 10% acetic acid. No further hPTH(1–34) was recovered from the novel formulation, indicating a complete recovery had been obtained in PBS. Using acidic recovery solution, the hPTH(1–34) acetate formulation also released quickly and completely, suggesting that pH dependent solubility limits the release of hPTH(1–34) into neutral solution (Fig. 3).

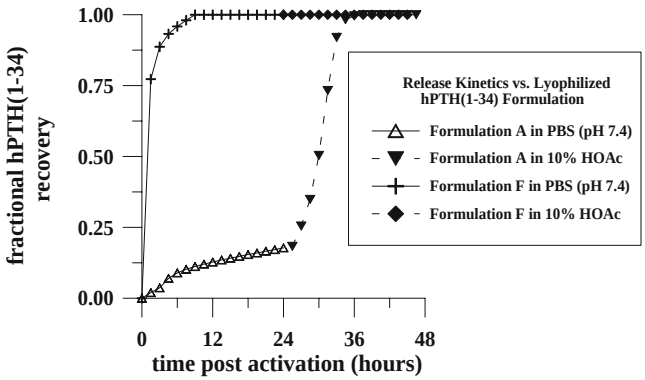

Fig. 3. pH dependence of hPTH(1–34) formulation solubilization: rapid release of Formulation F (hPTH(1–34), citric acid, and histidine) compared to Formulation A (hPTH(1–34) acetate) in PBS.

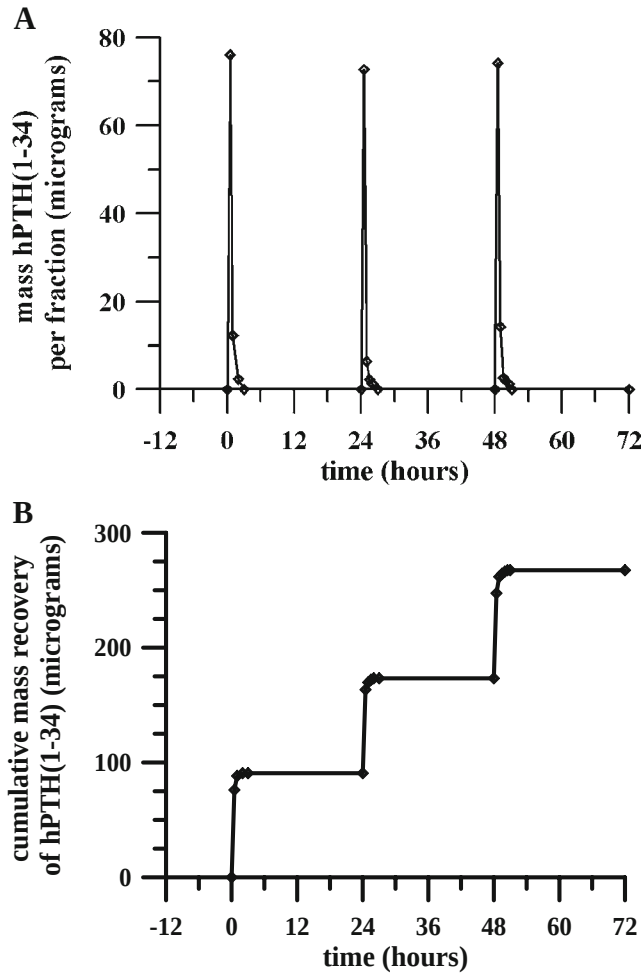

Fig. 4. Three independent release events show the reproducible pulsatile release of hPTH(1–34) (**A**) and cumulative recovery of hPTH(1–34) (**B**) from a single multi-reservoir array.

Three successive releases (four 21 μg doses per release) of a citric acid-histidine hPTH(1–34) formulation (same composition as Formulation F) into PBS from the multi-reservoir array show the reproducibility of release achievable using this device/ formulation combination *in vitro*. Releases were performed with the same *in vitro* test device as above, with initiation of the first release within 72 h of lyophilization. Fluid fractions were collected for a period of 24 h and each fraction was analyzed for hPTH(1–34) concentration. The data is presented as a mass (micrograms) of hPTH(1–34) collected in each fraction and reveals that all releases are

complete in less than 3 h (Fig. 4A). The average recovery was 89 μg per release event (nominally 84 μg) which equates to 106% recovery (5% RSD) (Fig. 4B). The data was normalized to calculate the time to 50% recovery for each release, yielding an average of 18±1 min. The average percent purity of the highest concentration fraction collected was 96% (3% RSD). Assay sensitivity would permit detection of single impurities representing 1% of the total peptide in the highest concentration samples.

In preparation for *in vivo* pharmacokinetic and efficacy studies, two hPTH(1–34) formulations (either 13 or 21 μg per reservoir, same composition as Formulations E and F, respectively) were prepared in multi-reservoir arrays, and releases were performed in PBS. Sample arrays were stored as long as 29 weeks at 37°C to mimic implantation at body temperature. Four reservoirs were opened simultaneously at each time point. Release solution fractions were collected for 24 h, per release event, and analyzed by RP-HPLC. Dosage purity was determined from the most concentrated *in vitro* release fraction. Results are presented in Table V. Both formulations yielded high quantitative recoveries and rapid time to 50% release. Purity retention over time was comparable to the bulk formulations, indicating that storage in a sealed array is comparable to a sealed vial. Consistent release kinetics were observed over the entire length of the study (Fig. 5A and B).

DISCUSSION

A successful hPTH(1–34)-delivery device for treating osteoporosis must provide a clinically relevant dose of hPTH (1–34) in a pulsatile manner with an acceptably narrow pulse width. In addition, the formulation must have acceptable chemical and physical stability until the time of release and during the release event. The feasibility of each requirement has been demonstrated by the data presented here.

Novel 59–249 mg/mL hPTH(1–34) solutions have been prepared for subsequent lyophilization. These acidic filling solutions enable sufficiently high hPTH(1–34) concentrations to load the size reservoirs used in this study with up to 75 μg of peptide in a single 300 nL reservoir; the current therapeutic dose is 20 μg. Such concentrations greatly exceed the peptide concentration of 0.25 mg/mL in the Forteo® [teriparatide (rDNA origin) injection] dosing solution. However the concentration and volume requirements discussed here apply only to administration of hPTH(1–34) from the microchip design used in this study. The flexibility of microelectromechanical systems (MEMS) technology allows design of numerous device configurations that will be driven by factors involving the optimal way to address unmet medical needs for a particular patient population.

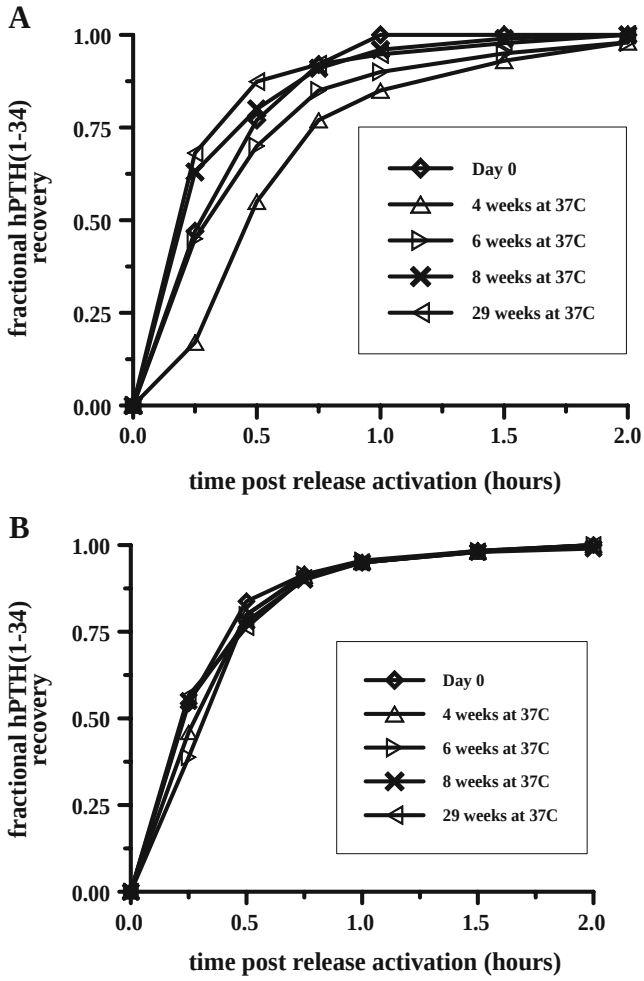

Fig. 5. Normalized release kinetic plots for 2 hPTH(1–34) formulations, followed over 29 weeks at 37°C: Formulation E—13 μg per reservoir (**A**) and Formulation F—21 μg per reservoir (**B**).

Table V. Percent Recovery, Time to 50% Release, and % Purity Data for Two hPTH(1–34) *In Vivo* Candidate Formulations

Micrograms per Reservoir	Time Point	% Recovery[a]	Calculated Time to 50% Recovery (minutes)	% Purity of Highest Concentration Fraction
13 (Formulation E)	Day 0	99	18	97
	4 weeks	102	29	95
	6 weeks	103	20	94
	8 weeks	104	12	94
	29 weeks	99	11	82
21 (Formulation F)	Day 0	105	16	96
	4 weeks	97	18	96
	6 weeks	97	19	94
	8 weeks	96	17	94
	29 weeks	97	13	81

[a] Includes hPTH(1–34) and all detected degradation products

These highly concentrated hPTH(1–34) solutions are sufficiently stable at room temperature to allow processing within a day, but use of these solutions would be restricted to formulation and filling operations of limited duration. The issue of longer-term stability was addressed according to the most prevalent method for preservation of polypeptides, lyophilization (22). Accordingly, the novel dosage forms developed here focused on lyophilization of highly concentrated hPTH(1–34) solutions to create preparations potentially stable for extended periods of implantation *in vivo* at 37°C. Screening experiments demonstrate addition of histidine to acetic and citric acid solutions of hPTH(1–34) significantly improves the stability of the hPTH(1–34) at 37°C when lyophilized from high concentration solutions. LC/MS analysis will allow further characterization of impurity formation pathways in these formulations, to provide the basis for additional improvements in dosage form stability. Preliminary size exclusion data for a select number of samples reveals the presence of non-covalent multimer to be low after over 1 year storage at 37°C. This low level of hPTH(1–34) multimer is not stability limiting and will not affect the use of Formulation E or F in planned pre-clinical studies.

Due to the high hPTH(1–34) concentration solution required to obtain a single equivalent clinical dose per reservoir, excipient combinations with unusually low excipient: polypeptide ratios were screened in an effort to maintain conventional total solids content in the starting solution (less than 25%) prior to lyophilization. Although higher ratios generally enhance polypeptide stability (23), the data presented here shows that high protein concentrations may offer other stabilization mechanisms. Although the positive stabilization impact of high polypeptide concentration has not been extensively examined, Stratton *et al.* reported a deamidation rate decrease when a model peptide concentration was increased from 0.1 to 100 mg/mL (24). Given the increasing number of instances where highly concentrated (>10 mg/mL) polypeptide formulations are required for therapeutic products (25,26), a greater understanding of stabilization mechanisms for such systems would be generally useful.

An equally important challenge for delivery of hPTH(1–34) from implanted reservoir arrays is dose recovery from reservoirs. Given the solubility limitations of hPTH(1–34) in physiologic (*i.e.* neutral buffer) conditions, non-volatile organic acids were included in the formulation to provide a local low pH microenvironment during re-dissolution and release of the dose. Upon dose release to the subcutaneous space, dose distribution will be primarily diffusion-dependent. Without modification of the local environment upon reservoir opening, the formation of an hPTH(1–34) gel at the physiological fluid–lyophilizate interface may block or significantly retard the rate of release. Delay or widening of the pulse of hPTH(1–34) has critical therapeutic implications, because a predominantly catabolic rather than the desired anabolic effect could be induced. As evidenced by the data presented here, the formulation design can overcome the solubility limitations of a polypeptide such as hPTH(1–34) and allow for a rapid, pulsatile release profile *in vitro*.

In previous studies, a high leuprolide concentration formulation used in combination with a similar multi-reservoir device demonstrated very good *in vitro–in vivo* correlation, indicating that the rate limitation for delivery is the solubility and diffusion of the formulation from the reservoir (21). The rapid, pulsatile *in vitro* release data presented here for hPTH (1–34) suggests similar (desirable) pharmacokinetics may be observed *in vivo*, but *in vivo* experiments have not yet been performed.

Although the methods used to formulate, dispense, and process hPTH(1–34) were performed at small capacity, all are scalable unit operations. Manufacturing could be automated and performed at larger capacity for commercial production. Filling the multi-reservoir device and post-fill processing are nano-scale equivalents of traditional pharmaceutical fill and seal operations. Process versatility will permit a wide range of compounds (for example, peptides, proteins, and biosensing reagents) to be incorporated into similar process flows.

CONCLUSIONS

This work demonstrates the feasibility of developing an implantable multi-reservoir device product that provides an alternative hPTH(1–34) delivery route for the treatment of osteoporosis. Implanted devices enable courses of therapy where user convenience and compliance are significant issues. The described microchip device is especially advantageous where successful treatment requires precise control over dosing timing and kinetics. These studies show resolution of two technical hurdles: achievement of high concentration stable hPTH(1–34) dosage forms and consistent, pulsatile *in vitro* release kinetics in support of forthcoming *in vivo* pharmacokinetic and efficacy studies.

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