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Research Paper

Demonstrated Solid-State Stability of Parathyroid Hormone PTH(1–34) Coated on a Novel Transdermal Microprojection Delivery System

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Purpose. This study assessed conditions necessary for at least a 2-year, ambient temperature storage stability of the peptide parathyroid hormone 1–34, or PTH(1–34), coated on a novel transdermal microprojection delivery system, or ZP-PTH.
Methods. Liquid coating characterization of high concentration PTH(1–34) formulations (>20% *w/w*) was assessed by viscosity and contact angle measurements along with RP-HPLC and SEC-HPLC. Solid-state coating morphology of PTH(1–34) on microprojection arrays was determined by SEM, and stability on storage was assessed after dissolution and testing with stability indicating assays. Internal vapor analysis was performed to detect and quantify volatile organics released by patch components into the headspace inside the final package.
Results. Aggregation and oxidation were the primary degradation mechanisms for solid-state PTH(1–34) in this transdermal delivery system. Although these two degradation pathways can be retarded by appropriate stabilizers and use of foil pouch packaging (nitrogen purged and desiccant), the solid-state drug formulation's compatibility with patch components, particularly the plastic retainer ring, surprisingly dictated PTH(1–34) stability. Internal vapor analysis demonstrated that PTH(1–34) was particularly vulnerable to vapors such as moisture, oxygen, and outgassed formaldehyde, and each of these volatiles played a unique and significant role in PTH(1–34)'s degradation mechanism.
Conclusions. Identifying degradation mechanisms of volatile compounds on solid-state PTH(1–34) peptide stability allowed for the rationale for selection of final formulation, system components and packaging conditions. A >2-yr, ambient temperature storage stability was demonstrated for solid-state drug coated on a novel transdermal microprojection delivery system. This system was successfully tested in a Phase 2 clinical trial for the treatment of post-menopausal women with osteoporosis.

KEY WORDS: aggregation; drug-coating; formulation compatibility; internal vapor analysis; oxidation; parathyroid hormone; PTH(1–34); transdermal microprojection delivery system.

INTRODUCTION

Recombinant human PTH (1–34), Forteo®, is currently the only anabolic therapy approved for treatment of osteoporosis. This peptide hormone is unique in its bone-building effect (1), ability to promote bone strength (2,3), and ability to reduce long-term bone fracture risk (4) relative to bisphosphonates (anti-resorptive agents). Despite these clinical benefits, the use of PTH(1–34) therapy is limited by practical factors (5,6), with one of the most important being mode of administration. As PTH(1–34) is a peptide hormone, it cannot be given orally and currently can only be administered as a daily subcutaneous injection. Many patients that could benefit from this treatment choose not be treated with this medication due to the daily injection requirement (7). In addition, the marketed PTH(1–34) liquid injectable formulation has to be stored under refrigeration, i.e., 2–8°C, which causes inconvenience to users during travel. The need to develop a more user-friendly alternative delivery method led to the development of a transdermal microprojection array patch delivery system for PTH(1–34) (8,9) i.e., ZP-PTH.

This transdermal microprojection delivery system is capable of penetrating the superficial skin barrier to provide expanded drug delivery opportunities without pain or inconvenience (10–13). The small drug-coated patch is 5 cm^2 in area and seated in a patch retainer ring. The patch is applied with a hand-held reusable applicator (Fig. 1a). The patch consists of a titanium microprojection array (~1,300 microprojections per 2 cm^2 and 190 μm in length in Fig. 1b) attached to the center of an adhesive backing. Drug formulation is coated on the tip of each microprojection. When the patch is applied onto the skin, the drug-coated microprojections penetrate through the superficial skin barrier layer into the

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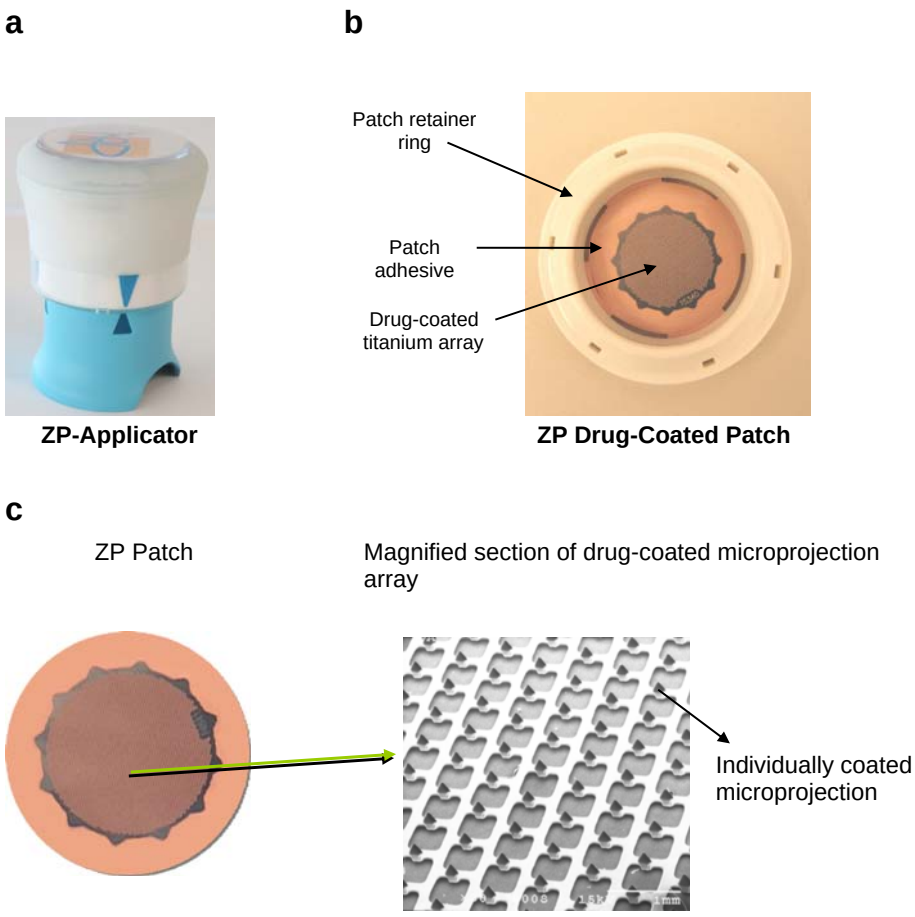

Fig. 1. Transdermal microprojection patch delivery system—(**a**) applicator; (**b**) drug-coated patch; (**c**) microprojection array.

epidermal/dermal layers (50–150 μm in depth), where the drug formulation rapidly dissolves and releases into the skin for microcapillary uptake and systemic absorption. The ZP-PTH patch has been demonstrated to be safe and efficacious in a Phase 2 human trial (9) and is entering into Phase 3 clinical studies.

To provide additional user-friendly benefits over the current PTH(1–34) liquid injectable formulation which has a 2-year shelf life under refrigeration, ZP-PTH formulation is targeted for cold-chain free storage, i.e., >25°C, with a shelf life of at least 2 years. As a solid-state formulation, ZP-PTH can be theoretically more stable than the respective liquid formulation. However, it's reported that the purity of lyophilized PTH(1–34) formulations decreased significantly to ~80% under the accelerated storage conditions, 37°C for 26 weeks (14), which may present a challenge for this targeted shelf-life with the purity specification of ≥95% at the end of the shelf-life. In addition, the unique patch configuration may present additional stability challenges—coexisting with multiple patch components such as titanium metal surface interactions and potentially organic outgassing of patch components in the sealed pouch packaging.

This paper identifies prospective solid-state drug degradation mechanisms and a rationale for selection of patch system components to aid in the development of a stable transdermal microprojection patch delivery system.

MATERIAL AND METHODS

Materials

Synthetic PTH(1–34) acetate was supplied by Bachem Americas (Torrance, CA) with an initial purity of 96.5% for API Lot #FPTH2290301 and of 99.5% for API Lot #FPTH0401. Sucrose NF (High Purity Low Endotoxin Grade) was obtained from Pfanstiehl Laboratories (Waukegan, IL). Polysorbate 20 (Crillet 1 HP, high purity, low peroxide) was sourced from Croda (Edison, NJ). Methionine, tartaric acid, citric acid, glycolic acid, HCl, EDTA, and acetonitrile, all USP Grade, were sourced from Sigma Chemical Company (St. Louis, MO). Formaldehyde was obtained from VWR Scientific (West Chester, PA). Nitrogen gas with 1% and 4% oxygen were sourced from MESA Specialty Gases & Equipment (Santa Ana, CA). Titanium metal sheet (25 μm in thickness) was obtained from Hamilton Precision Metals (Lancaster, PA).

The ZP-PTH system consists of a 2 cm^2 titanium array of 1,300 microprojections with a length of 190 μm (Kemac, Azusa, CA). The length and the width of the microprojection

head is 100 μm and 115 μm, respectively, with a tip angle of 60° (see Fig. 2). Other patch components include polycarbonate ring (Jatco, Union City, CA), adhesive patch (Medical Tape 1523, 3 M, St. Paul, MN), 3 Å molecular sieve desiccant sachet (3.5 g Minipax, Multisorb, Buffalo, NY) and an aluminium foil pouch (Mangar, New Britain, PA).

Methods

Reverse-Phase HPLC (RP-HPLC) and Size-Exclusion HPLC (SEC-HPLC)

RP-HPLC was used to quantify PTH(1–34) oxidation. Oxidized PTH(1–34) products were separated from native PTH(1–34) using a Zorbax 300 SB-C8 reversed phase column (4.6 mm ID×150 mm, 3.5 um) (Agilent Technologies, Inc. CA, USA) maintained at 55°C. The eluted PTH(1–34) was detected by UV at 215 nm. The mobile phase involved a gradient elution, with solvent A: 0.1% trifluoroacetic acid in water, and solvent B: 0.09% trifluoroacetic acid (Product # 28904, Piece/Thermo Fisher Scientific, Rockford, IL) in acetonitrile (HPLC-Grade, >99.9%, Sigma-Aldrich), and was pumped at the flow rate of 1 mL/min.

Soluble aggregates were determined by SEC-HPLC (UV detection at 214 nm) using a TCK-gel G2000 SWXL column (7.8 mm ID×300 mm, 5 μm) (Toso Haas, Japan) with an isocratic mobile phase consisting of 0.1% trifluoroacetic acid in 0.2 M NaCl and acetonitrile (70/30 by volume), at a flow rate of 0.5 mL/min.

Chromatography for both assays was performed with an HPLC system (1100 series, Agilent Technologies, Inc., CA, USA) provided with a binary pump, a thermostatted auto-sampler, a thermostatted column compartment, and a multiple wavelength DAD/UV detector. Data were collected and analyzed using a Turbochrom Client Server Software, version 6.2 (Perkin Elmer, Inc).

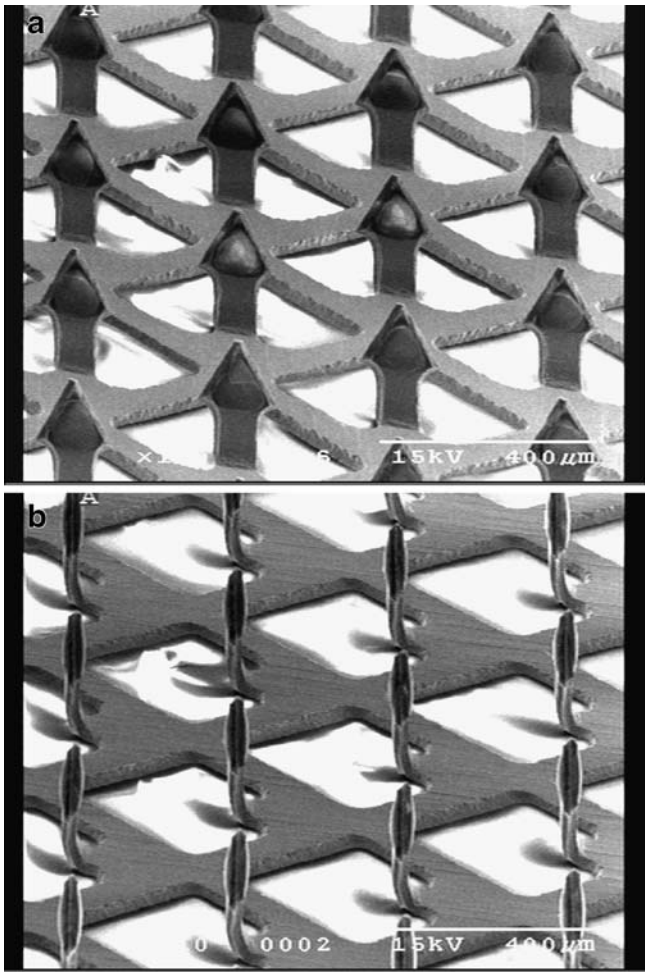

Fig. 2. SEM of solid-state coated PTH(1–34) formulation: (**a**) Front view; (**b**) Side view where the microprojection array coated with 40 mcg of PTH(1–34), equivalent to 80 mcg of total formulation solids. The length of microprojection is 190 μm, the length and the width of the microprojection head is 100 μm and 115 μm, respectively, with a tip angle of 60°.

Formulation Screening Experiments

A convenient experimental design was utilized to understand the peptide's degradation mechanisms and screen formulation excipients/stabilizers. Briefly, one cm^2 titanium (Ti) discs were punched with the aid of a die from a sheet of Ti and were subsequently washed with an alkaline detergent (CIP-100) and rinsed in de-ionized water. On each Ti disc 3 μL of formulation was dispensed; the droplet was subsequently spread to ~1 cm^2 area over the surface of the Ti disc to aid drying. Each formulation was assigned four Ti discs per time point. The discs were then placed in glass vials and placed under vacuum for 24 h to facilitate drying. The glass vials were subsequently placed in desiccators with calcium chloride desiccant to maintain the %relative humidity (%RH) to <10% for the entire course of the study. The desiccator was placed in a temperature-controlled environment, which was maintained either at 2–8°C or 40°C. For analysis at each time-point, each disc was placed in a vial containing 0.5 mL of glycolic acid (pH 4.1) and mixed for a period of 5 min. The resulting solution was used for HPLC analysis.

Stability Experiments on Drug-Coated Delivery Systems

The PTH(1–34)-coated patch, assembled per Fig. 1b or its variation, was placed inside a foil pouch with or without a desiccant sachet, which was subsequently purged with nitrogen (6 s purge) and heat sealed with a Van der Stahl heat sealer (model MFG-18). The pouch was then stored in a stability chamber controlled at 2–8, 25, or 40°C. At each time-point, eight pouch samples per group were pulled from the stability chamber for HPLC analysis. To extract PTH(1–34) from the coated array, the array was first separated from the adhesive by exposing to liquid nitrogen vapor and then peeled from the adhesive. The coated array was then placed in a vial containing 0.5 mL of glycolic acid (pH 4.1) and mixed for a period of 5 min, of which 250 μL of the resulting solution was transferred into a secondary vial for HPLC analysis

Microprojection Arrays and Coating

Titanium microprojection arrays were fabricated by a photo/chemical etching and formed using a controlled manufacturing process (15).

Coating was conducted at ambient temperature utilizing a roller drum, rotating at 50 rpm, in a drug formulation reservoir (2 mL in volume) to produce a thin film of controlled thickness of ~100 μm in thickness (16,17). Microprojection arrays are dipped into the thin film, and the amount of coating is controlled by the number of dips (passes) through the drug film. The time between each dip is approximately 5 s, which is sufficient to dry the coated liquid formulation under the ambient condition.

Headspace Analysis (Internal Vapor Analysis)

A headspace gas sample was taken and subjected to quadrupole mass spectroscopy analysis for the identification and quantitation of low molecular weight volatile compounds, including moisture, oxygen, nitrogen, and volatile organics. Briefly, the pouch sample was placed in a test chamber sealed against a Viton™ O-ring. A pin pierced the pouch through the center of the O-ring to take a headspace sample, which was then subjected to a mass spectrometer (Model IVA-110, Oneida Research Services, Whitesboro, NY).

Moisture Sorption Analysis

The moisture sorption/desorption profile of the solid sample was established using a vapor sorption analyzer (SGA-100, VTI) over a range 5–60% relative humidity (RH) at 10% RH intervals. Equilibrium criteria used for analysis was less than 0.01% weight change in 2 h, with a maximum equilibration time of 3 h if the weight criterion was not met.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to determine the morphology and distribution of the coating on the microprojections. The coated titanium arrays were adhered to aluminum studs with carbon double-stick tape and placed in the vacuum chamber of a Hitachi scanning electron microscope (Model S-2460N).

Determination of the Relative Biopotency of PTH (1–34) in MC4 Cells

Human PTH (1–34) activity was measured in a cell-based assay by detecting the levels of 3,5 cyclic adenosine monophosphate (cAMP) in a responsive cell line (MC4) via a competitive immunoassay for cAMP detection. The cAMP induced by PTH (1–34) is released by lysing the cells and captured with an anti-cAMP antibody. PTH(1–34)-induced cAMP competes for binding with HRP (horse radish peroxidise)-conjugated cAMP. In the absence of PTH(1–34)-induced cAMP, most of the HRP-cAMP is bound to the antibody, resulting in high relative fluorescence units (RFU) readings. Increasing levels of PTH (1–34)-specific cAMP competitively decrease the amount of HRP-cAMP-Ab conjugate in a dose-dependent manner, resulting in decreasing RFU readings.

RESULTS AND DISCUSSION

There are two processes, as defined by the coating operation, to produce the drug-coated patch—dip coating of microprojections into a high concentration liquid drug formulation as well as drying and packaging of the solid-state drug formulation.

Liquid Formulation

The focus of this study is to evaluate the solid-state formulation, but the liquid formulation is the prerequisite to the understanding of the formulation strategy and critical to the coating process. Briefly, a liquid formulation is prepared to primarily satisfy three key coating formulation parameters—drug concentration, viscosity, and surface activity.

A liquid formulation with PTH(1–34) concentration of $\geq 20\% w/w$ and a viscosity of ≥20 cP is often required to ensure that each dip of microprojections into the liquid formulation can pick up sufficient volume of liquid for drying, which can achieve the desired drug dose with a minimum number of dips. The viscosity of the coating solution has to be high enough so that the coated liquid will not quickly drip back after dipping but before drying. Also important is the Newtonian behavior of the liquid formulation in the drug-coating reservoir, i.e., constant viscosity over shear rate, because the coating process involves a certain level of shear with the roller drum. The rheological behaviour of the protein/peptide solution is often affected by the counterion/salt in the formulation for pH control. Since the pH of the unformulated drug, PTH(1–34) acetate, in water is ~6, an acidic counterion is needed to reduce the pH to 5, which was reported to be the pH of optimal solution stability (18). Surface activity is paramount to establishing a hydrophilic interface between the liquid formulation and the titanium surface, which can be quantified by contact angle measurement. The preferred contact angle is 40°–60° (referenced to the contact angle of 70°–80° between water and the titanium surface). A surfactant, polysorbate 20, (0.2%) is added to the liquid PTH(1–34) formulation to decrease contact angle to 55° and improve coating on the titanium surface.

Other than a surfactant and a counterion required for coating, other excipients were screened for the purpose of peptide stabilization in the solid state.

Formulation Screening for Solid-State PTH(1–34) Degradation Pathways

Major degradation pathways for solid-state PTH(1–34) were investigated to allow selection of the appropriate stabilizing excipients. Among several degradation mechanisms (including deamidation and acid-catalyzed hydrolysis), oxidation and aggregation were found to be the major degradation pathways in the ZP-PTH patch system. For convenient screening, samples tested were not coated on microprojections but on titanium discs, i.e., drying liquid drops on discs (see Method Section).

PTH(1–34) is prone to methionine oxidation (19) at positions 8 & 18, denoted as 8Met[O]-PTH(1–34) and 18Met[O]-PTH(1–34). Thus, we first screened two antioxidants, methionine and EDTA, both being parenterally approved

excipients, to select formulation(s) that can inhibit or minimize oxidation. EDTA and methionine prevent / retard oxidation by different mechanisms. EDTA is a chelating agent; thus, it sequesters the trace amount of metals that may catalyze auto-oxidation reactions, while methionine is a free radical scavenger.

Table I summarizes oxidation results, at the end of 3-month storage at 2–8°C, for PTH(1–34) formulations (25%*w*/*w*) containing only EDTA or methionine at different concentrations. In all formulations, both 8Met[O]-PTH(1–34) and 18Met[O]-PTH(1–34) were detected with 18Met[O]-PTH (1–34) being the predominant species. Control formulation, devoid of any antioxidant, yielded the highest percentage of total oxidized product, ~1%, after 3 months. The results showed that methionine retards oxidation in a concentration-dependent manner, while EDTA is less concentration-dependent in inhibiting oxidation. Addition of 0.03% EDTA to the formulation was as effective as 0.18% EDTA in retarding oxidation. Overall, EDTA is more effective than methionine in retardation of oxidation, suggesting that oxidation might be metal catalyzed from trace metal contamination from excipients and/or the titanium itself.

Another screening experiment was performed to investigate PTH(1–34) aggregation and oxidation in relation to formulations containing multiple excipients, including sucrose, an acidic counterion, and an antioxidant EDTA. Six formulations (Table II) were dried on titanium discs, and their chemical stability at 40°C for a period of 60 days was monitored. All PTH(1–34) formulations were adjusted to pH 5 with an acidic counterion. The stability data suggests that the main mechanism of degradation of PTH(1–34) in the solid state is *via* an aggregation process. The addition of sucrose decreased aggregation from ~7% to <0.5%. Oxidation in this case is not a major mechanism of degradation even in the absence of antioxidants.

Although all sucrose-containing formulations, A-E in Table II, offered acceptable stability, Formulations B (HCl) & C (tartaric acid) demonstrated the best stability. Given the fact that the liquid formulation will be stored frozen (≤ −20°C) prior to coating, Formulation C was not selected for further studies because it tended to gel upon three freeze-thaw cycles (data not shown). Therefore, Formulation B (20% PTH(1–34), 20% sucrose, 0.6% HCl, 0.03% EDTA and 0.2% polysorbate 20) was coated on the microprojection array for formal stability investigation, primarily for assessing compatibility with patch and packaging components (below).

Coating Morphology on Microprojection Array

Fig. 2 shows uniform formulation coating on the tip of each 190 μm microprojection within the 2 cm^2 array as demonstrated by scanning electron microscopy (SEM) (front view 2a and side view 2b). The coating covers the entire tip head with a coating height of approximately 100 μm, which is consistent with the thickness of the film established by the roller drum. The coating surface morphology is smooth, and the thickness of the coating is between 10 and 25 μm on the side-view. A coating that is too bulky, or too close to the microprojection tip, may blunt the microprojection and hinder its ability to penetrate the skin.

Compatibility of PTH(1–34) Formulation with Patch Components

The drug-coated microprojection array (2 cm^2) coexists with a 5 cm^2 adhesive patch and a plastic retainer ring (see patch configuration in Fig. 1b) inside a nitrogen-purged, heat-sealed foil pouch. Although the formulation is not in direct contact with these components, the volatile compounds released by these components may negatively affect peptide stability over long-term storage. One acrylic-based adhesive (3 M Medical Tape #1523) and three types of plastic retainer ring, based on polycarbonate (PC), polyformaldehyde (Delrin®), and acrylonitrile butadiene styrene (ABS), were evaluated for aggregation and oxidation effects after 5-week storage at 40°C (Table III).

Compared to the control (coated array stored in the absence of patch components), none of the plastic materials are compatible with PTH(1–34) formulation: PC and ABS induced substantial oxidation (>30%) and Delrin® resulted in significant aggregation (~10%). Although the effect of adhesive alone was not assessed in this study, it is postulated that such incompatibility may arise from volatile compound(s) released from the plastic material. To verify this hypothesis, internal vapor analysis was performed to quantify gaseous compounds inside the pouch.

Table I. PTH(1–34) Formulation Screening for Oxidation Stability—Formulations Containing 25%*w*/*w* PTH(1–34) and Only Different Concentrations of Methionine or EDTA as Antioxidant Stored at 2–8°C for 3 Months

		%Oxidized PTH(1–34)		
Formulation ID	Antioxidant (% *w*/*w*)	8Met[O]-PTH(1–34)	18Met[O]- PTH(1–34)	Total
A (control)	none	0.33±0.08	0.73±0.20	1.06±0.28
B	0.5% methionine	0.31±0.03	0.67±0.10	0.98±0.13
C	1.0% methionine	0.26±0.01	0.61±0.02	0.89±0.05
D	3.0% methionine	0.18±0.02	0.50±0.04	0.68±0.06
E	0.03% EDTA	0.17±0.01	0.39±0.01	0.57±0.02
F	0.06% EDTA	0.17±0.04	0.41±0.07	0.58±0.11
G	0.18% EDTA	0.17±0.06	0.41±0.09	0.59±0.15

Table II. Solid-State PTH(1–34) Formulation Screening—Formulations Containing 20%*w/w* PTH(1–34), 20% Sucrose, 0.2% Polysorbate 20, and/or 0.03% EDTA with Different Acidic Counterions (Adjusted the Liquid Formulation to pH 5) at 40°C for Two Months

ID	Acidic counterion for pH adjustment	Antioxidant (%*w/w*)	*T*=0 aggregation (%)	*T*=0 oxidation (%)	%Aggregation	%Oxidation
Control	0.6% HCl	0	0.10±0.01	0.22±0.02	6.93±0.37	0.38±0.03
A	0.6% HCl	0	0.12±0.01	0.45±0.01	0.88±0.53	0.57±0.01
B	0.6% HCl	0.03% EDTA	0.14±0.01	0.44±0.01	0.38±0.04	0.47±0.01
C	1.2% Tartaric acid	0.03% EDTA	0.12±0.01	0.35±0.04	0.36±0.11	0.41±0.02
D	1.2% Glycolic acid	0.03% EDTA	0.15±0.02	0.40±0.02	0.82±0.46	0.42±0.01
E	1.7% Citric acid	0.03% EDTA	0.11±0.01	0.37±0.01	2.14±1.85	0.33±0.03

Internal Vapor Analysis (Headspace Analysis)

Four different patch configurations, packaged in nitrogen-purged, heat sealed foil pouches, were subjected to internal vapor analysis to detect and quantify low molecular-weight gases in the part-per-million (ppm) level (Table IV). Volatile compounds of interest included moisture (H_2O), oxygen, and formaldehyde. The results confirmed that the patch components can have a major impact on the equilibrium vapor composition in the headspace. Without adhesive and the ring (i.e., coated microprojection array alone), both moisture and oxygen levels were low: 2,212 ppm (equivalent to 9% RH) and 246 ppm, respectively. However, when the array was assembled with the adhesive patch and a PC ring, the headspace became rich in moisture and oxygen, increasing to 18,100 ppm (66% RH) and 5,100 ppm, respectively, suggesting that the combination of the PC ring and the adhesive released these two gases into the headspace. When the PC ring was replaced with a Delrin® ring, despite some increase in oxygen (1,120 ppm), the moisture level actually decreased to 650 ppm (2.6% RH) compared to the array-alone configuration, and formaldehyde vapor was detected (84 ppm). In the final configuration, where a 3.5 g molecular sieve desiccant sachet was co-packaged with the PC ring and patch, the moisture level reduced to 41 ppm (0.12% RH) within 10 min, although the oxygen level remained the same.

After headspace analysis, two additional experiments were performed to verify the effect of formaldehyde and moisture/oxygen on the stability of solid-state PTH(1–34) coating.

Effect of Formaldehyde on PTH(1–34) Aggregation

The coated microprojection arrays were incubated with formaldehyde vapor for 24 h at 40°C. Under this condition, the headspace concentration of formaldehyde was estimated to be ~124,000 ppm, according to the vapor pressure of 93.6 mmHg for formaldehyde at 38°C (data cited from Instant Chemical Hazards & Safety Data). After incubation the coated PTH(1–34) was rendered completely insoluble, suggesting that formaldehyde facilitated peptide cross-linking. The same cross-linking phenomenon, although at a much slower rate due to a lower level of formaldehyde vapor pressure (84 ppm of formaldehyde detected in the headspace by internal vapor analysis), was observed with PTH(1–34) in the Delrin® ring patch system being stored for 5 years at 25°C (data not shown). Thus, the Delrin® ring is highly incompatible with the peptide even at very low vapor concentrations of formaldehyde in the headspace.

Effect of Headspace Moisture/Oxygen on PTH(1–34) Oxidation

(a) PC Ring Can Easily Release Moisture

The weighing method was applied to measure the water content of the PC ring. Four rings were dried to a constant weight utilizing a vacuum oven operated at 60°C (under vacuum <1 in. Hg) for a period of five days, and their masses were recorded. These rings were then stored at 40°C / 75%

Table III. Compatibility of Solid-State PTH(1–34) Formulation Coated on Titanium Array with Patch Components—An Adhesive and One of Three Plastic Retainer Rings: Polycarbonate, Polyformaldehyde (Delrin®), and Acrylonitrile Butadiene Styrene (ABS)—Stored Inside a Nitrogen-purged, Heat Sealed Foil Pouch at 40°C for 5 Weeks

System ID	Adhesive	Plastic retainer ring			%Aggregation	%Oxidation
		Polycarbonate	Delrin®()	ABS		
Control	X	X	X	X	0.4	4.6±1.6
A	√	√	X	X	3.3	37.3±10.7
B	√	X	√	X	9.8	5.6±3.2
C	√	X	X	√	1.6	33.0±10.3

√=presence of components
X=absence of components

RH for a period of four days, and the mass change was recorded daily. The calculated % increase in mass from the initial time point was 0.26%, which corresponds to a water content of 8.9 mg (based on weight of the ring at 3.4 g). This water content is substantially in excess of the weight of coated PTH(1–34) formulation, which is <0.1 mg. This minute amount of peptide formulation could be overwhelmed by moisture even if the PC ring releases a small fraction of its water into the headspace.

(b) High Headspace %RH Resulting in High Equilibrium Water Content in the Coated PTH(1–34) Formulation

Although the effect of environmental moisture on PTH (1–34) oxidation mechanism is not clear at this time, maintaining the water content in the dry coating formulation at low levels is a well-perceived strategy to preserve the peptide's long-term stability from the concept of molecular mobility and glass transition temperature (Tg). Unfortunately, it's difficult to directly measure the water content of coated PTH(1–34) formulation due to its unique array configuration where the <0.1 mg formulation solid is evenly distributed over the tips of more than 1,000 microprojections. Therefore, we construed a method to estimate the water content in the coated PTH(1–34) formulation.

The PTH(1–34) liquid formulation was lyophilized, and the lyophilized powder was subjected to moisture sorption/desorption analysis. In this analysis, the powdered sample absorbs or desorbs water as the %RH in headspace is increased or decreased. This phenomenon is universal as the water content of the solid formulation will always be in equilibrium with the moisture level of the ambient air, i.e., water vapor pressure or %RH as long as sufficient time is given to reach such an equilibration. In the vapor sorption analyzer, the lyophilized powder was equilibrated for 2 h between each %RH condition. Therefore, it's reasonable and reliable to extract the water content information from the relationship of headspace %RH *vs*. water content established in Fig. 3.

With 66% headspace RH for the patch with PC ring (Table IV), the extrapolated water content of the coated formulation could reach as high as 13–14%. When a 3.5 g desiccant sachet was included in the pouch, the %RH decreased to 0.12% (Table IV), and the corresponding equilibrium water content was reduced to below 1%. Such a low water content level should greatly improve the stability of coated PTH(1–34).

(c) High Water Content Caused Significant Oxidation of Coated PTH(1–34)

Coated PTH(1–34) stability in three patch configurations shown in Table IV was monitored up to 12-month storage at 25°C, and the %oxidation results are shown in Fig. 4. The level of peptide oxidation is consistent with the moisture level in the headspace. The patch with the PC ring in the absence of desiccant created a highly humid environment, 66% RH (13% water content), which resulted in significant PTH(1–34) oxidation, 22% over 12 months. However, oxidation was reduced to 2.5% in the same configuration containing a 3.5 g desiccant sachet shown to reduce RH to 0.12%. In addition, maintaining a dry headspace with the array-alone configuration (9% RH) similarly demonstrated improved oxidation protection (4.1% oxidation after 12-month storage).

(d) Oxygen Caused Oxidation of Coated PTH(1–34) Only at Very High Headspace Concentrations

Both the plastic ring and the desiccant sachet released oxygen into the headspace, as demonstrated by the higher oxygen levels for the patch with a PC ring (5,099 ppm or 0.5%), and the patch with a PC ring + a desiccant sachet (4,788 ppm or 0.48%) compared to the array-alone condition (246 ppm or 0.02%). Two observations may help in understanding the role of oxygen on coated PTH(1–34). Firstly,

Table IV. Headspace Analysis on Different Patch Configurations—All Packaged in a Nitrogen-Purged, Heat-sealed Foil Pouch

Residual gas analysis	Array only	Patch with polycarbonate ring	Patch with Delrin® ring	Patch with PC ring + 3.5 g desiccant
Moisture* (ppm)	2,212	18,100	650	41
Oxygen (ppm)	246	5,099	1,120	4,788
Formaldehyde (ppm)	ND	ND	84	ND
*Calculated %RH (at 22°C)	9.00%	66.00%	2.60%	0.12%

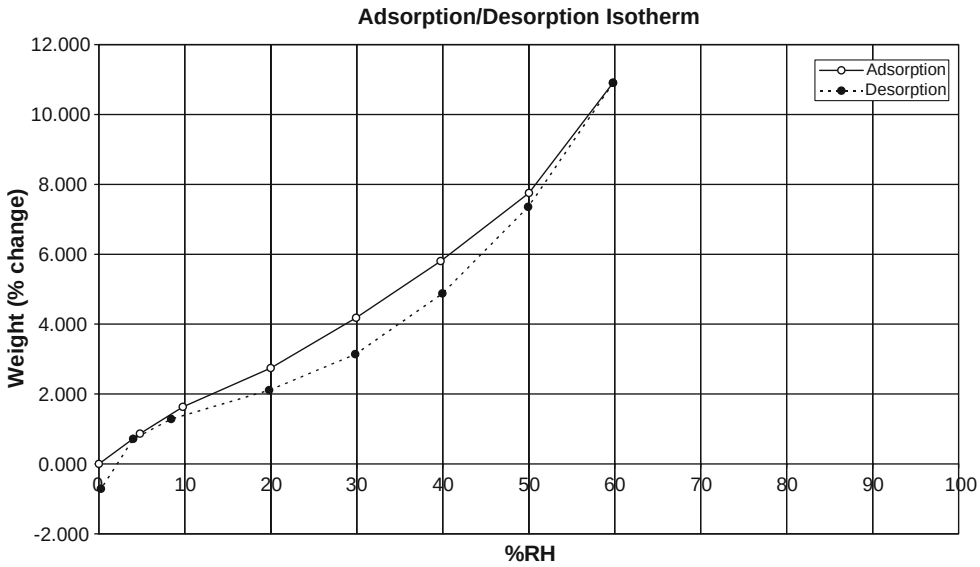

Fig. 3. Moisture sorption/desorption profile for lyophilized PTH(1–34) formulation (Formulation B in Table II).

oxygen may have an insignificant effect on PTH(1–34) oxidation if the moisture level is low, which can be demonstrated by comparing the two patch configurations—array alone and the patch with a PC ring containing a desiccant sachet. The latter, with a much higher level of oxygen (0.48%) but less moisture (0.12% RH), presented better stability than the array alone configuration with oxygen at 0.025% and moisture at 9% RH.

Secondly, under a very low level of moisture, high levels of oxygen can still cause PTH(1–34) oxidation. In a separate stability experiment, coated PTH(1–34) (API Lot #FPTH0401) array was assembled with a PC ring, packaged with a 3.5 g desiccant sachet, and purged with a gas containing four different levels of oxygen—0% (pure nitrogen), 1%, 4%, and 21% (ambient air) before the pouches were sealed. PTH(1–34) purity was analyzed for oxidation after the pouches were stored at 40°C for 1 month and 3 months, respectively. The results (Fig. 5) show that, under a very low level of moisture (<1% RH due to the desiccant), the amount of oxygen in the headspace has little effect on PTH(1–34) for patches stored for 1 month at 40°C but begins to exert obvious effects after 3-month storage when the headspace contained >1% oxygen. Thus, the packaging approaches of including a desiccant sachet and purging with nitrogen are essential to protect PTH(1–34) from oxidation.

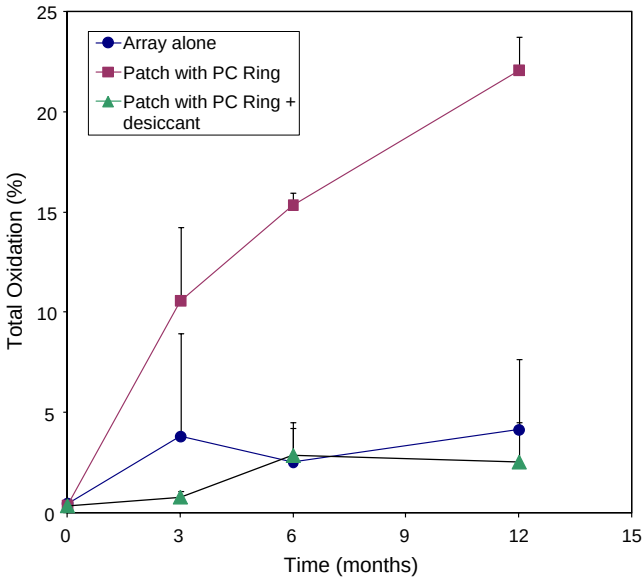

Fig. 4. Stability comparison of three PTH(1–34)-coated patch configurations: array alone (●), patch with PC ring (■), and patch with PC ring and a 3.5 g molecular sieve desiccant sachet (▲)—All systems stored at 25°C for up to 12 months.

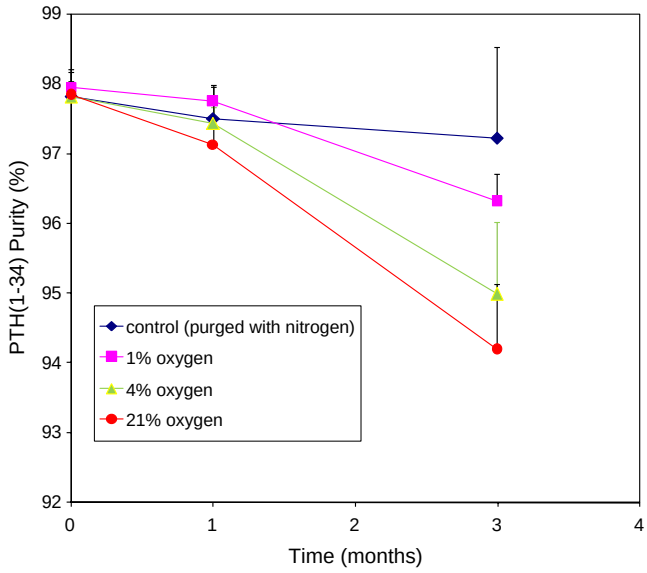

Fig. 5. Effect of headspace oxygen levels on %purity of coated PTH (1–34) array patches assembled with a PC ring and a 3.5 g desiccant sachet and purged with different levels of oxygen—0% (♦), 1% (■), 4% (▲), and 21% (●) prior to pouch seal.

(e) High Moisture/Oxygen Level has Little Effect on Sucrose-Formulated PTH(1–34) Aggregation

None of these three configurations, however, resulted in significant peptide aggregation, with all showing <0.2% aggregation even after 6-month storage at 40°C despite the marked difference in moisture level (aggregation data not shown). Therefore, aggregation is no longer a major degradation mechanism as long as the peptide is formulated with sucrose as the stabilizer.

Effect of Headspace Moisture on Physical Stability of the Coating

Robust physical stability of the coating, i.e., maintaining coating morphology during storage inside the pouch and during patch administration after the pouch is opened, needs to be demonstrated, because the coated formulation can be quite hygroscopic due to the presence of sucrose. For this purpose, the coated arrays were exposed to environments of high %RH, namely, 75% RH created by NaCl and 85% RH established by KCl. After exposure, coating morphology was monitored under SEM. The physical stability of the coating is indeed robust. After 24-hour exposure to 75% RH at 25°C, the coating remained intact (SEM not shown). It suggests that the coating can be physically stable over long-term storage even without inserting a desiccant sachet inside the pouch (<70% RH). However, after one-hour exposure to 85% RH at 25°C, deliquescence of the coating, i.e., coating drifting down the microprojection, was observed. Despite the fact that the indoor environment of 85%, or greater, RH humidity is rare, it's advisable to instruct the users to administer the patch within 30 min after the pouch is opened.

Overall, the degradation mechanism of the coated PTH (1–34) formulation varied pending its compatibility with co-existing patch components. The incompatibility with the polycarbonate ring as the result of increased moisture/oxygen vapors can be easily ameliorated by desiccation using a desiccant sachet. However, it's difficult to correct the incompatibility posed by the Delrin® ring which released a trace amount of formaldehyde vapor capable of promoting aggregation/cross-linking of PTH(1–34) over long-term storage.

Long-Term Stability of Phase 2 ZP-PTH Systems

With the identification of critical formulation parameters discussed above, ZP-PTH systems were manufactured for Phase 2 human clinical studies per Formulation B (Table II), coated on the titanium array (Fig. 2) with 40 μg PTH(1–34) dose (equivalent to 80 μg total solids), using patch components involving a polycarbonate retainer ring and a 5 cm^2 adhesive patch. The coated patch was heat sealed in a nitrogen-purged foil pouch containing a 3.5 g molecular sieve desiccant sachet. The final ZP-PTH systems were stored under two conditions—25°C/60% RH and 40°C/75% RH.

Fig. 6 summarizes PTH(1–34) %purity data for 25°C/60% RH up to 18 months and 40°C/75% RH up to 6 months. Under the accelerated conditions of 40°C/75% RH, PTH(1–34) stability is trending down slightly from ~99% to 97.7% over 6 months. However, ZP-PTH systems held excellent stability when stored at 25°C/60% RH; PTH(1–34) %purity at the 18-month time point is identical to that at $T=0$ (~99%) and showed no obvious trend of decreasing.

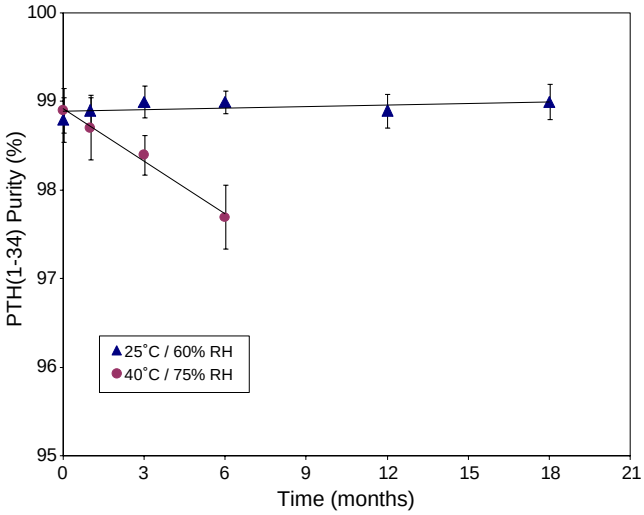

Fig. 6. Long-term stability of Phase 2 ZP-PTH systems where the titanium array was coated with 40 μg PTH(1–34) dose, assembled with a polycarbonate retainer ring and a 5 cm^2 adhesive patch, and heat sealed in a nitrogen-purged foil pouch containing a 3.5 g molecular sieve desiccant sachet. The final ZP-PTH systems were stored under two conditions—25°C/60% RH (▲) and 40°C/75% RH (●). Systems of $n=5$ were analyzed by RH-HPLC at each time point.

Confirming the Preservation of PTH(1–34) Bioactivity

Typically, bioactivity or potency assay is needed to evaluate the stability of a biomolecule because its tertiary structures/conformations may change during manufacturing and during storage. However, PTH(1–34), with 34 amino acids and a molar mass of 4117.8 Da, has no well-defined tertiary structure. There is ample evidence in the literature that reduction in bioactivity of PTH is only linked with secondary structure changes primarily associated with oxidation of methionine residues (position 8 or 18 or both) (20–22) because these methionine residues are known to be important for the biological activity of this hormone. Other significant changes to PTH(1–34), such as deletion of the first two, or even first six, amino acid residues have neither detectable alterations to secondary structure nor reduction in biological activity (22). Given these literature references, it's not anticipated that PTH (1–34) in this delivery system would suffer bioactivity loss in addition to the identified oxidized degradants. The reverse-phase HPLC method used here could effectively separate these three oxidized PTH species and accurately quantify their amount. Despite this, a potency assay was developed to verify the bioactivity of the coated PTH formulation stored at 25°C for 2 years (from the Phase 2 systems above). Indeed, given the wider variations associated with bioactivity assay (≥±20%), PTH(1–34) extracted from the 2-year storage sample showed a biopotency comparable to that of the API, confirming that the bioactivity is maintained.

CONCLUSIONS

The unique configuration of the transdermal microprojection delivery system presents new challenges to PTH

(1–34) formulation stability. The coexisting patch components were shown to contribute volatile compounds which are chemically and/or physically incompatible with the coated PTH(1–34) formulation. The volatile compounds, moisture, oxygen, and formaldehyde are detrimental to peptide's long-term stability via different degradation mechanisms. By recognizing the degradation pathways and the source of these volatile compounds, system components and packaging conditions were optimized for long-term stability. In this study the commercially viable polycarbonate ring was made compatible with the coated PTH(1–34) formulation by conveniently inserting a desiccant sachet in the package. With 95% being the PTH(1–34) purity specification at the end of shelf life for ZP-PTH systems, the long-term Phase 2 stability data suggest that the target of >2-year shelf life at ambient temperature storage can be achieved.

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