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Research Paper

Parathyroid Hormone PTH(1-34) Formulation that Enables Uniform Coating on a Novel Transdermal Microprojection Delivery System

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Purpose. Assess formulation parameters to enable >24-h continuous accurate and uniform coating of PTH(1-34) on a novel transdermal microprojection array delivery system.
Methods. Surface activity and rheology of the liquid formulation was determined by contact angle measurement and cone-plate viscometry. The formulation's delivery performance was assessed *in vivo* using the hairless guinea pig model. Peptide gelation was investigated by rheological and viscoelastic behavior changes.
Results. Accurate and uniform coating was achieved by formulating the liquid formulation to a preferred contact angle range of 30–60° with a surfactant and by establishing a Newtonian fluid (defined as a fluid maintaining a constant viscosity with shear rate and time) with a viscosity of ≥20 cps via adjusting the peptide concentration and using an appropriate acidic counterion. A non-volatile acidic counterion was found critical to compensate for the loss of the volatile acetate counterion to maintain the peptide formulation's solubility upon rehydration in the skin. Finally, the 15.5% *w/w* PTH(1-34) concentration was found to be the most physically stable formulation (delayed gelation) in the roll-coating reservoir. With a properly designed coating reservoir for shear force reduction, the liquid formulation could last for more than 24 h without gelation.
Conclusions. The study successfully offered scientific rationales for developing an optimal liquid formulation for a novel titanium microprojection array coating process. The resultant formulation has an enduring physical stability (>24 h) in the coating reservoir and maintained good *in vivo* dissolution performance.

KEY WORDS: dip-coating; gelation; microneedles; parathyroid hormone; PTH(1-34); rheological; transdermal microprojection delivery system; viscoelastic.

INTRODUCTION

There are a variety of microneedle delivery devices in development, including hollow microneedles (syringe-mounted or on patches) for delivering liquid formulation, solid microneedles on which a drug/vaccine is dry-coated and solid microneedles made of a dried drug/vaccine formulation (1). A significant amount of development work on vaccine-coated microneedles has been published (2–6). Of these studies, some were dedicated to investigating formulation parameters to enable their particular coating process (5,6).

A transdermal patch coated with parathyroid hormone 1-34, i.e., ZP-PTH, has been proven safe and efficacious in a Phase 2 human clinical trial (7) and is currently entering a Phase 3 study. This small drug-coated patch is 5 cm^2 in area and seated in a patch retainer ring. The patch is applied to the skin 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 epidermal/dermal layers (50–150 micrometers in depth), where the drug formulation rapidly dissolves in the interstitial fluid and releases into the skin for microcapillary uptake and systemic absorption.

Manufacturing the ZP-PTH patch system requires a series of novel processes, including a dip coating technology by placing a minute amount of PTH(1-34) formulation (1.5×10^{-7} mL or 0.06 microgram of solid formulation after dehydration) on the tip of each microprojection. The microprojection tip features a small arrow-head (Fig. 1c) with a dimension of 100 μm in height, 115 μm in width (the surface area of ~5.75×10^{-5} cm^2), and 25 μm in thickness. A dip coating concept (8,9) evolved into a robust coating apparatus engineered to coat a uniform dose in a controlled fashion on the microprojection array (Fig. 2). It employs a rotating drum to create a liquid drug formulation film with a controlled thickness. Microprojection tips, moving in the same direction as the rotating drum, were dipped into the film at a controlled depth. Certainly, the mechanical designs

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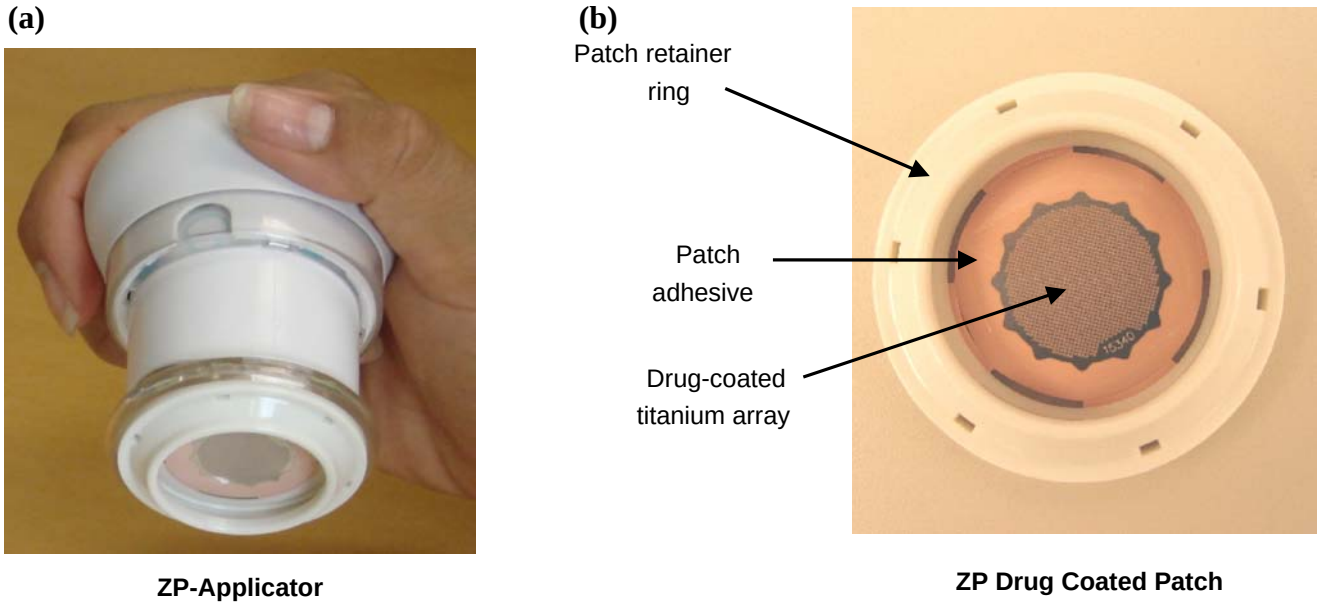

Fig. 1. Transdermal microprojection patch delivery system: **a** applicator; **b** drug-coated patch; **c** microprojection array and coating at microprojection tips.

and engineering controls and manipulations are essential for coating accuracy and uniformity. The liquid formulation, however, plays an equally critical role, if not more important. The liquid formulation must be chemically and physically stable during the coating process and should possess proper properties allowing the formulation to be effectively coated on the titanium microprojections. This study was to primarily investigate the physical stability and coating properties of the liquid formulation. The chemical stability of the liquid formulation, as reflected by the solid-state formulation in the final product, has been assessed and reported previously (10,11).

Another objective of the study was to illustrate the effect of acetate, as a volatile counterion to the peptide, on the coating formulation's *in vivo* performance.

MATERIAL AND METHODS

Materials

Synthetic PTH(1-34) acetate was supplied by Bachem Americas (Torrance, CA) with an initial acetate content of 5.7% for API Lot # FPTH0501 and of 3.4% for API Lot #T-34591. Sucrose NF (High Purity Low Endotoxin Grade) was

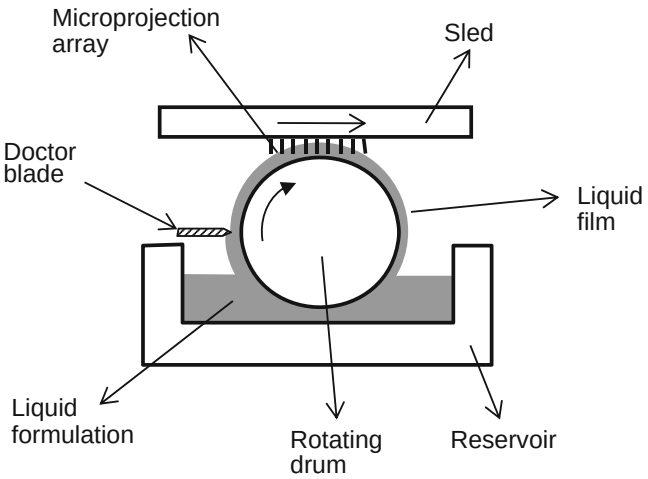

Fig. 2. Coating apparatus and concept.

obtained from Pfanstiehl Laboratories (Waukegan, IL). Polysorbate 20 (Crillet 1 HP, high purity, low peroxide) was sourced from Croda (Edison, NJ). Malic acid, tartaric acid, citric acid, glycolic acid, HCl, EDTA, and acetonitrile, all USP Grade, were sourced from Sigma Chemical Company (St. Louis, MO). Titanium metal sheets (Commercially pure Grade 2, 25 μm in thickness) were 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. 1c). Other patch components include the polycarbonate ring (Jatco, Union City, CA), adhesive patch (Medical Tape 1523, 3M, 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) for Acetate Quantification

RP-HPLC was used to quantify acetate in the PTH(1-34) drug formulation. The acetate in the formulation was fully protonated with phosphoric acid and then separated from PTH(1-34) using a Luna C18(2) column (4.6 mm ID × 250 mm, 5 um) (Phenomenex CA, USA) at ambient temperature. The eluted acetate was detected by UV absorption at 210 nm. Elution was isocratic at a flow rate of 1.3 mL/minute using a mobile phase composed of a mixture of ammonium hydroxide (0.14%) (Product# 338818, Sigma-Aldrich Corp., MO, USA) and phosphoric acid (0.60%) (Fluka Product #79606, Sigma-Aldrich, MO, USA) with 2% methanol (JT Baker Product #9093, Mallinckrodt Baker, NJ, USA) for the separation segment, followed by a stepped washing segment using the same buffer with 70% methanol.

Chromatography was performed using an HPLC system (1100 series, Agilent Technologies, Inc., CA, USA) provided with a binary pump, a thermostatted autosampler, 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).

UV-Visible for PTH(1-34) Quantification

PTH (1-34) content was measured by absorbance at 275 nm using an Agilent 8354 UV/Visible Spectrophotometer (Agilent, Wilmington, DE). The samples were measured in quartz micro-cuvettes without dilution after the extraction of the coated microprojection arrays with ultra-pure water (Milli-Q, Millipore Corporation, Billerica, MA) and blanked against the water. Quantitation was done based on a three-point standard curve (typically 80, 120 and 200-μg/mL) prepared from PTH (1-34) reference standard (in-house qualification) in water.

Microprojection Arrays and Coating

Titanium microprojection arrays were fabricated by a photo/chemical etching and formed using a controlled manufacturing process (12).

Drug formulation coating on the microprojection array 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 drug formulation film of a controlled thickness of ~100 μm (8,9). Microprojection tips on the array are dipped into the thin film. The amount of coating is controlled by the number of dips (passes) through the drug film. The time between each dip is only a few seconds, which is sufficient to dry the coated liquid formulation under the ambient condition. The reservoir was circulated with coolant to maintain a temperature of 1°C. Since the reservoir is open to the ambient air, the coating apparatus was positioned inside a dew-point control system. Dew-point control minimizes moisture condensation into or evaporation from the liquid formulation during coating.

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).

Contact Angle Measurement

Static contact angle of drug solution formulations on titanium surface was determined using a contact angle meter (Model OCA15, FDS Corp., Garden City, NY) employing an optical contact angle method called “Sessile drop.” For static contact angle measurement, a photo snapshot is taken once a drop of the solution (5 mcL) is dispensed from the syringe and laid on a clean titanium foil surface. The angle between the baseline of the drop and the tangent at the drop boundary is measured on both sides. Complete measurement was obtained by averaging the two numbers. A minimum of five readings were recorded for each sample.

Hairless Guinea Pigs (HGP) PK Study

HGPs were anesthetized by intramuscular injection of xylazine (8 mg/kg) and ketamine HCl (44 mg/kg). Anesthetized HGPs were catheterized through carotid artery. The catheter was flushed with heparinized saline (20 IU/mL) to prevent clotting. Animals were maintained under anaesthesia throughout the experiment via injection of sodium pentobarbital (32 mg/mL) directly into the catheter (0.1 mL/injection). Before application, blood samples were taken into heparinized vials (final concentration of heparin at 15 IU/mL), which served as baseline samples.

The coated microprojection arrays were applied on the flank of the anesthetized animals ($n=5$) with an applicator (total energy = 0.4 Joules), and the patches were worn on the skin for 1 h. A group of animals ($n=5$) received an intravenous injection (IV) of 22 mcg PTH(1-34) as the control. Blood samples were collected through the carotid catheter at time intervals following patch application. All blood samples were centrifuged immediately to collect plasma samples, which were stored at −80°C until analysis. Plasma PTH(1-34) was determined by enzyme immunoassay using a commercial kit for PTH(1-34) obtained from the Peninsula Lab (San Carlos, CA). The sensitivity of the assay is 0.5 ng/mL with a coefficient of variation of <10%. The PTH (1-34) dose delivered by the patch was extrapolated based on the area under the curve (AUC) calculation compared to the IV control.

Rheology

Rheological characterization of the liquid PTH(1-34) formulations was conducted utilizing a rheometer (model CVOR150, Bohlin Instrument, Cranbury, NJ) configured with a cone and plate geometry (a cone angle of 1° and radius 10 mm). Seventy μL of the PTH(1-34) liquid formulation was utilized for each experiment. To determine the gel point of a particular PTH liquid formulation, the sample was sheared at 1,334 s^{-1}, and viscosities were recorded every 30 s. Gelation point was noted at the inflection of the viscosity *versus* time curve, i.e. at the point where a rapid increase in viscosity was observed. To measure complex modulus and phase angle, the mode of oscillatory stress sweeps, i.e., applying a sinusoidal increasing stress, was used at a constant frequency of 0.1 Hz. To obtain elastic modulus (G′) and viscous modulus (G″), the mode of oscillatory frequency sweeps was utilized, i.e., cycling the frequency in the range of 0.01 to 10 Hz at a constant stress of 0.05 Pa. All measurements were made 10°C±0.2°C. Although 1°C is a preferred temperature for rheological measurement (because it is also the temperature for coating), the liquid sample tended to freeze at 1°C during measurement. The viscosity data for temperatures of <5°C could be extrapolated from the viscosity-temperature curve established using 5, 10, and 20°C.

RESULTS AND DISCUSSION

The development of a PTH(1-34) liquid formulation is primarily catering to the unique coating process to produce the drug-coated patch. In addition, the liquid formulation is the prerequisite to a stable solid-state drug formulation after post-coating dehydration. We previously reported how we overcame various challenges to achieve ≥2 year ambient temperature storage shelf life for ZP-PTH based on a liquid formulation consisting of 15.5% *w/w* PTH(1-34), 16.6% *w/w* sucrose, 0.2% *w/w* polysorbate 20, 0.4% *w/w* HCl, 0.3% *w/w* NaOH, 0.03% *w/w* EDTA, 65% *w/w* WFI at pH 5 (10, 11). Arriving at this formulation composition was partially driven by chemical stability considerations. Sucrose was the primary peptide stabilizer, and its content was limited to *ca.* 1:1 sucrose:peptide wt/wt ratio because increasing sucrose content would add more solid to the coating of the same peptide dose on the microprojection tips, which would eventually blunt the microprojection's sharpness and hinder skin penetration. EDTA served as an antioxidant. The solution pH was adjusted to 5, which was reported to be the pH of optimal solution stability (13).

This study considers the physical stability of the formulation used in the microprojection array coating process and particularly favourable coating properties and sustained physical stability (preventing gelation) during coating, as well as evaluates the solubility and dissolution properties of the dried solid coating.

The coating process must provide a uniform amount of coating not only on different arrays but also on microprojection tips of the same array. This process requires precision engineering designs of the coater which allowed accurate control in the depth of the microprojection tip dipping into the liquid film (Fig. 2). The properties of the liquid formulation would certainly dictate how uniform and how fast the liquid can be applied to the titanium substrate. In each dip the microprojection tip could pick up sufficient volume of liquid for drying and could achieve the desired drug dose with a minimum number of dips. Three formulation parameters—surface activity, peptide concentration, and viscosity—were found to be the most important for coating uniformity.

Surface Activity

Surface activity, or wettability, determines the ability of the liquid formulation to attach, adhere, and spread over the substrate surface (titanium) to be coated. Both the liquid formulation and the substrate surface can separately affect wettability. Contact angle measurements of liquid droplets on substrate surfaces are commonly used to characterize surface wettability, and generally the lower the contact angle, the greater the wettability.

Surface Activity of Titanium Substrate

Titanium is a successful biocompatible material and has been extensively used in medical devices (14,15). Titanium is known to passivate; an oxide film grows spontaneously when titanium is in contact with air, contributing to the excellent chemical inertness and corrosion resistance of titanium and its alloys (15). The titanium oxide layer is hydrophilic but is particularly sensitive to contamination from organic and inorganic species in ambient air. Contact angle data in Table I demonstrates the sensitive nature of the titanium surface activity. The titanium surface without treatment (Control in Table I) established a contact angle of 90° from

a pure water drop, suggesting relatively poor surface activity. After rinsing with acetone, the contact angle decreased to 61°, indicating that acetone removed some organic contaminations. Plasma cleaning is most efficient in removing organic contaminations and exhibited a very low contact angle with pure water, 0°. However, the clean titanium surface after plasma treatment reabsorbed organic materials during ambient storage. The surface became increasingly contaminated (more hydrophobic) with storage time. For example, the contact angle with pure water increased to ~69° after 6-month storage in a metal tray.

Thus, the dynamic nature of titanium surface activity makes it difficult to maintain a "controlled" titanium surface during the manufacturing process. It is preferred to control the surface activity of the liquid formulation.

Surface Activity of PTH(1-34) Liquid Formulation

The unformulated PTH(1-34) acetate solution (15.5%) exhibited a contact angle of 59° on an untreated titanium substrate (Table I), suggesting that the peptide itself is somewhat surface active. However, due to the non-uniform and variable surface energy of the titanium, a surfactant was added to the formulation. With 0.2% polysorbate 20 in the peptide formulation, the contact angle was reduced to 42° on the untreated titanium surface.

As to the effect of surface activity on coating, it was found that a contact angle in the range of 30°–60° could provide a consistent coating condition where the coating is located uniformly on the tip of the microprojection (Fig. 3A). A contact angle too low (<30°) would create a surface too active and encourage the liquid formulation to "wick" beyond the tip area (arrow-head) onto the shaft, or even the base of the microprojection (Fig. 3B). On the contrary, a contact angle too high (>60°) would discourage the liquid from being uniformly distributed on the arrow-head area (Fig. 3C).

Peptide Concentration

A high PTH(1-34) concentration is needed to increase the total solid content of formulation and to enhance solution viscosity. A liquid formulation with a higher solid content and sufficient viscosity would allow a greater amount of solid coated from each dip.

PTH(1-34)'s solubility depends on its charge status as a function of pH (Fig. 4A), which was calculated according to its amino acid sequence (16). PTH(1-34) exhibited 9 positive charges at low pHs and 5 negative charges at high pHs. From the charge profile, the isoelectric point (pI with net zero charge) of PTH(1-34) is *ca.* pH 9 and the charge changes markedly within pH 3–12. Since the peptide charge is a distribution at a specific pH, it is important to calculate the mole fraction of insoluble species (carrying net zero charge) as function of pH, as given in Fig. 4 (16). The distribution having a net charge of zero has peak at *ca.* pH 9, indicating that the greatest amount of insoluble material is at that pH; however, between the pH ranges of 1–6 and 11.5–14, there is no mole fraction of insoluble material, indicating that the peptide is fully soluble at those pH ranges, Thus, formulating PTH(1-34) at pH 5 (*ca.* + 4 charges if fully ionized) satisfies not only optimal chemical stability (10) but also good solubility.

Under this condition, PTH(1-34) is very soluble, >250 mg/mL. Thus, the total solid content of the liquid formulation could be greater than 500 mg/mL (together with sucrose, another predominant component in the formulation). However, peptide concentration was found to affect viscosity and physical stability of the liquid formulation and was assessed separately.

Effect of Peptide Concentration on Viscosity and Coating

A liquid formulation with a viscosity of >20 cps is preferred to ensure that each dip of microprojections can pick up sufficient volume of the liquid, which will not quickly drip back into the reservoir after dipping and before drying. The viscosity of a liquid formulation is often dictated by the concentration and the molecular weight of the major ingredients in the formulation, i.e., PTH(1-34) and sucrose in this case. Sucrose, however, contributed little to the viscosity of the liquid formulation due to its low molecular weight (342.3 Dalton), e.g., 20% aqueous sucrose solution has a water-like viscosity (~2 cps at 20°C). Thus, PTH(1-34) is expected to be the major contributor to solution viscosity. The viscosity of liquid formulations containing PTH(1-34) at a concentration of 17%, 15.5%, and 12% is 38, 21, and 12 cps, respectively, at 1°C (also the coating temperature). These three formulations were coated on microprojection arrays, and the coated PTH (1-34) amount is plotted as a function of the number of dips (Fig. 5). The relationship between the coated amount and the number of dips is linear for all three formulations. To reach the 40-mcg PTH(1-34) dose, it required 3, 4, and 5 dips for the 17%, 15.5%, and 12% peptide formulation, respectively. Minimizing the number of dips to achieve the desired dose will benefit production efficiency (output) during large-scale manufacturing. However, high viscosity (i.e., 17% formula-

Table I. Contact Angles Between a Titanium Substrate and a PTH(1-34) Formulation or Pure Water (Mili-Q)

ID	Titanium Surface Treatment	Liquid	Contact Angle (degrees)
Control	no	pure water	90.0±1.7
A	Rinse with acetone	pure water	61.3±1.2
B	Rinse with acetone and cleaned by 120W plasma	pure water	0.0±0.8
C	B after 1 month stored in metal tray	pure water	44.0±2.1
D	B after 3 months stored in metal tray	pure water	59.5±2.2
E	B after 6 months stored in metal tray	pure water	68.7±2.2
F	no	PTH(1-34)	59.0±1.8
G	no	17% PTH, 20% sucrose, 0.2% Polysorbate 20	42.0±1.2

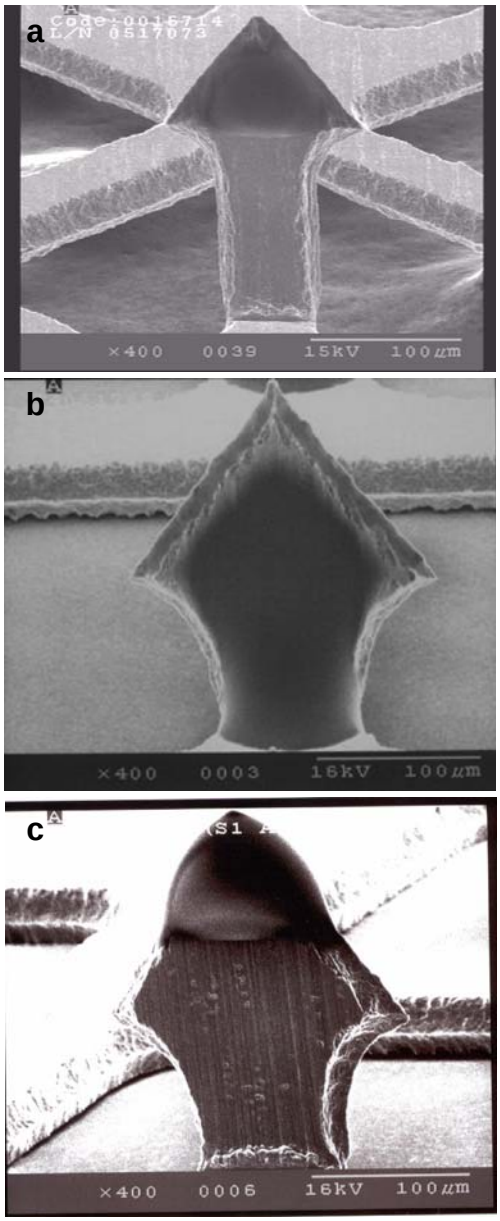

Fig. 3. Scanning electron monographs showing different degrees of coating uniformity on the arrow-head of the microprojection with a PTH(1-34) formulation of different contact angles: (**A**) uniformly distributed on the arrow-head for a contact angle 50°; (**B**) coating beyond the arrow-head into the shaft of the microprojection for a contact angle of 20°; (**C**) coating only at the top portion of the arrow-head for a contact angle of 80°.

tion) seemed to cause greater coating variations (greater error bars in Fig. 5). Furthermore, it can be expected that small changes in environmental conditions, particularly variations in temperature and humidity (resulting in water evaporation or condensation), during coating will have a bigger impact on viscosity to formulations of high peptide concentrations. Thus, using PTH(1-34) formulations with concentrations greater than 17% has no obvious advantage. As to which concentration (among 17%, 15.5%, and 12%) is optimal, it will be determined by their physical stability (to be discussed later).

Additionally, maintaining a constant viscosity over the entire duriation of the coating process is important to the consistency of the coated dose. It means that the liquid formulation should exhibit Newtonian behavior.

Effect of Counterions/Buffers on Rheological Properties

The rheological behavior of the peptide solution may also be affected by the counterion/salt in the formulation for pH control. Several weak acid buffers, including one triacid (citric acid), two diacids (malic acid and tartaric acid), a monoacid (glycolic acid), and a strong acid (HCl) were tested. The viscosity profiles of formulations including these acids were measured as a function of time. Citric and malic acid buffered formulations exhibited thixotropic behavior, i.e., a decrease in viscosity as a function of time, while Newtonian behavior was observed for formulations buffered by tartaric and glycolic acid. Interestingly, PTH(1-34) in the absence of

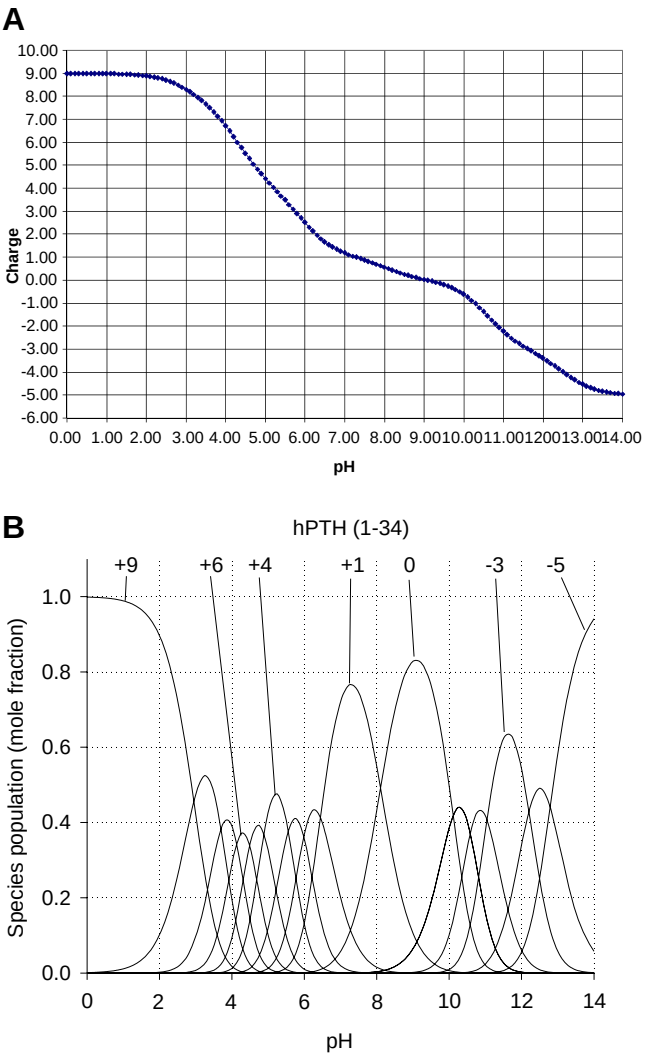

Fig. 4. **A** PTH(1-34) charge profile as a function of pH calculated according to its amino acid sequence (Reference 10); **B** PTH(1-34) charge distribution as a function of charge species and pH also calculated per Reference 10.

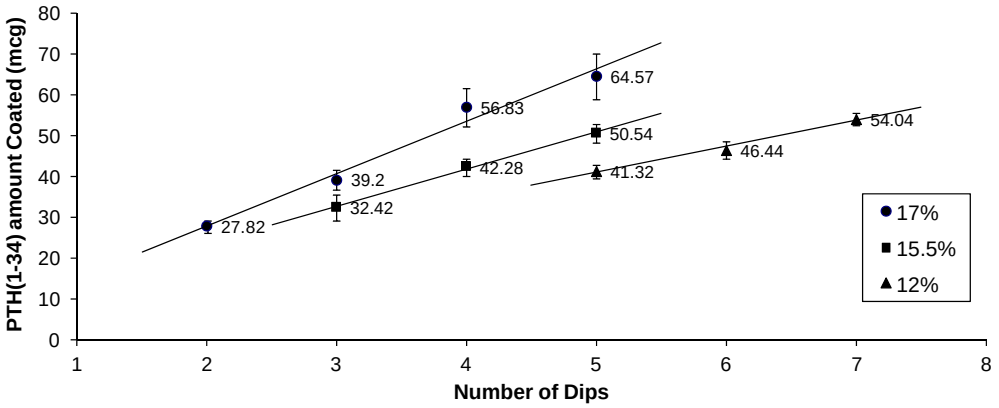

Fig. 5. The PTH(1-34) amount coated on a microprojection array as a function of number of dips for formulations of three PTH(1-34) concentrations, 17% (●), 15.5% (■), and 12% (▲).

buffers or other counterions (i.e., only the existing acetate in the API) exhibited rheopectic properties, the contrary of thixotropy. In addition, these counterions/salts affected formulation viscosity significantly. For example, for the 20% PTH(1-34) acetate formulation (containing no other counterions/salts), its viscosity at 1°C is 65 cps. When counterions were added, the viscosity changed following the decreasing order of citric acid (170 cps) > malic acid (120 cps) > tartaric acid (80 cps) > glycolic acid (55 cps), which appeared to follow the trend of triacid > diacid > monoacid for viscosity enhancement. Presumably, viscosity enhancement of the weak acid buffers is achieved by the interaction of the weak acid anion with the positively charged peptide and/or by hydrogen-bonding via the carboxyl/hydroxyl groups of these weak acid buffers. Adding HCl enhanced the viscosity to 90 cps and also made the peptide formulation Newtonian. The same trend was observed for lower PTH(1-34) acetate concentrations (data not shown).

Given the overall rheological effect, tartaric acid, glycolic acid, and HCl are the preferred counterions for pH adjustment in the PTH(1-34) coating formulation. Unlike acetic acid, which is volatile (a vapour pressure of 17.5 mmHg at 20°C), tartaric acid and glycolic acid are not volatile with negligible vapor pressure at 20°C. HCl is a strong gaseous acid and is often supplied as an aqueous solution. In water, HCl almost fully dissociates into Cl^- and H^+ (pKa = −7) and is not volatile (vapour pressure of 0.014 mmHg in 10% aqueous solution). The volatility of the acid was found to play a critical role in *in vivo* dissolution of the dry-coated formulation.

Effect of Acetate Depletion on Dissolution of Coated Formulation

After solid-phase synthesis, the crude peptide was dissolved in acetic acid for purification by preparative HPLC and then desalted with ammonium acetate via another chromatography elution to convert PTH(1-34) into the acetate salt form. The purified peptide acetate was lyophilized into the final bulk. According to the Henderson–Hasselbalch equation (17), a weak acid such as acetic acid will establish an equilibration between its neutral form (AcOH) and ionized species (AcO^-) depending on pKa and pH. At pH 5, 65% of acetic acid would dissociate into AcO^- and H^+, which could ionize the peptide to positively charged molecules. However, the remaining acidic acid in the solution can volatize and be removed during any drying process, including the coating process.

Between each dip, the coated liquid was dehydrated via air drying under the ambient condition of ~30% relative humidity. The coated liquid is estimated to be dried within a few seconds in comparison with the drying time and conditions associated with spray-dried particles (e.g., 20 μm in diameter), primarily because both cases have comparable specific surface area, i.e., surface area per unit volume (calculations not shown). Please note that although the film temperature is ~1°C, drying actually took place at ambient temperature, ~20°C. Due to the extremely tiny volume and large surface area, heat transfer is very fast for the liquid pick-up during coating to reach the ambient temperature. Additionally, although temperature affected the drying rate, the major driving force for water removal during drying is the %RH difference between the liquid surface and the surrounding air. Overall, the effect of the film temperature on drying rate may not be significant in this case. Indeed, the dry-coated formulation was found to contain less acetate than the original API due most likely to acetic acid evaporation, from 5.7% acetate in the API (Lot #FPTH0501) to 4.6% acetate in the coating and from 3.4% acetate in the API (Lot #T-32591) to 2.1% in the coating. Acetate content reduction continued when vacuum drying was applied to the coated arrays. For example, the solid-state formulation with 4.6% acetate content after coating decreased to 3.9% after 10 days and to 0.8% after 27 days of vacuum drying. Such a reduction in acetate level is expected to drive the peptide molecules to lower charged species, thus decreasing solubility on rehydration. This effect might be more prominent at the outer surface of the coated formulation. When the acetate-depleted coating surface is in contact with the interstitial fluid after penetrating into the skin, it may take a longer time for the formulation to be dissolved by the fluid and result in less PTH(1-34) delivered into the skin to be absorbed systemically.

This hypothesis was tested in an animal study where the ZP-PTH systems were applied to hairless guinea pigs for PK analysis (Table II). The amount of PTH(1-34) absorbed into the blood was calculated from the area-under-the-curve (AUC) of the PK profile relative to intravenous injection. PTH(1-34) in the formulation containing only the volatile

Table II. Effect of Non-volatile Counterions on *In Vivo* Dissolution and Systemic Absorption of the Coated PTH(1-34) Formulation (Coated Dose of 40 mcg) in the Hairless Guinea Pigs

PTH liquid formulation[a]				
PTH(1-34) (wt%)	Acetic acid (wt%)	Non-volatile counterion (wt%)	Molar ratio of PTH:acetate: non-volatile counterion)	% of PTH absorbed (relative to coated dose)
25.3	1.1	no	1:3:0	4.3±4.9
22.4	0.7	HCl (0.4)	1:2:2	16.8±5.6
18.1	0.8	HCl (0.5)	1:3:3	20.3±13.0
8.9	0.4	Glycolic acid (2.1)	1:3:13	24.4±5.9
18.1	0.8	Tartaric acid (1.2)	1:3:2	14.5±5.3

[a] Containing sucrose, EDTA, and Polysorbate 20 in the same molar ratio (relative to PTH) throughout the study

acetate counterion (at the 1:3 molar ratio of PTH:acetate as the control) was absorbed poorly, <5% of the coated dose, suggesting the coated formulation was not dissolved effectively. The results of % PTH absorbed are statistically significant based on one way ANOVA analysis on data in Table II ($F_{4,17}$=4.2, P<0.05). According to Tukey test, the formulation containing only the acetic acid counterion is significantly different from the formulations containing non-volatile counterions.

Please note that the bioavailability (BA) of PTH delivered through the skin route is animal species dependent. The use of hairless guinea pig (with BA of <25%) is primarily for its skin structural similarity to human. In Phase 1 and Phase 2 human trials, the BA was found to be ~40% (1) and the application sites (abdomen *vs*. arm *vs*. thigh) have a profound effect on bioavailability due to different level of metabolism. Although skin metabolism is the major factor, there are some PTH residues on the microneedles and on the skin surface after application, which accounts for ~20% of the coated dose.

Effect of Non-volatile Counterion/Salt on Dissolution of Coated Formulation

Glycolic acid, tartaric acid, or HCl was added to the liquid formulation (Table II) to compensate for the loss of volatile acetic acid and maintain peptide molecules' charge state. These non-volatile counterions/salts were indeed effective in enhancing PTH(1-34)'s systemic absorption—a three- to six-fold increase in the amount of PTH(1-34) absorbed into the blood relative to the formulation containing only acetic acid.

Interestingly, the acidic counterions used to adjust the solution pH played a critical role in overall performance of the liquid formulation, particularly in rheological properties and dissolution performance. HCl was eventually selected over glycolic acid and tartaric acid because it's the most commonly used acid in parenteral pharmaceutical formulations. In addition, the liquid formulation is stored under the frozen condition (−20°C), and the HCl formulation is least susceptible to freeze/thaw stress than the glycolic acid and tartaric acid formulations (data not shown).

With all of these findings, the final consideration to liquid formulation development, particularly on peptide concentration selection, is then focused on the physical stability of the formulation in the coating reservoir over the entire 24-h coating time for a production lot.

Liquid Formulation Physical Stability (or Pot Life) During Coating

The high-concentration PTH(1-34) liquid formulation has to be physically stable, e.g., maintain its rheological behaviour for at least 24 h (the target coating run time), which can be a challenging task as the formulation is subjected to continuous shear force (with the drum rotating at 50 rpm) in the coater reservoir. Under this condition, the challenge for robust physical stability of the liquid formulation is to prevent gelation from occurring. Concentrated protein/peptide solutions are known to gel over time, induced by various parameters, such as temperature, shear, salt concentration (ionic strength), solvents, etc. Although gelation can be reversible and the effect on the peptide's chemical stability can be insignificant (18), liquid formulation gelation during coating is not acceptable as the viscosity increases substantially, making uniform coating impossible.

Temperature Effect on Gelation

Indeed, the PTH(1-34) liquid formulation at 15.5% *w*/*w* PTH(1-34), 16.6% *w*/*w* sucrose, 0.2% *w*/*w* polysorbate 20, 0.4% *w*/*w* HCl, 0.3% *w*/*w* NaOH, 0.03% *w*/*w* EDTA, 65% *w*/*w* WFI at pH 5 has a tendency to gel. This tendency is particularly sensitive to temperature. For this reason, the preparation of the formulation has to be conducted under refrigerated conditions (2–8°C). As observed, mixing and dissolving the peptide to this concentration under ambient temperature in a vial rotating at 8 rpm resulted in gelation within an hour. The gelled PTH(1-34) could be reversed by >500-fold dilution with water, which showed no increased soluble aggregates by SEC-HPLC (data not shown). To mitigate the temperature effect, coating was performed at a low temperature by controlling the coater reservoir temperature to ~1°C.

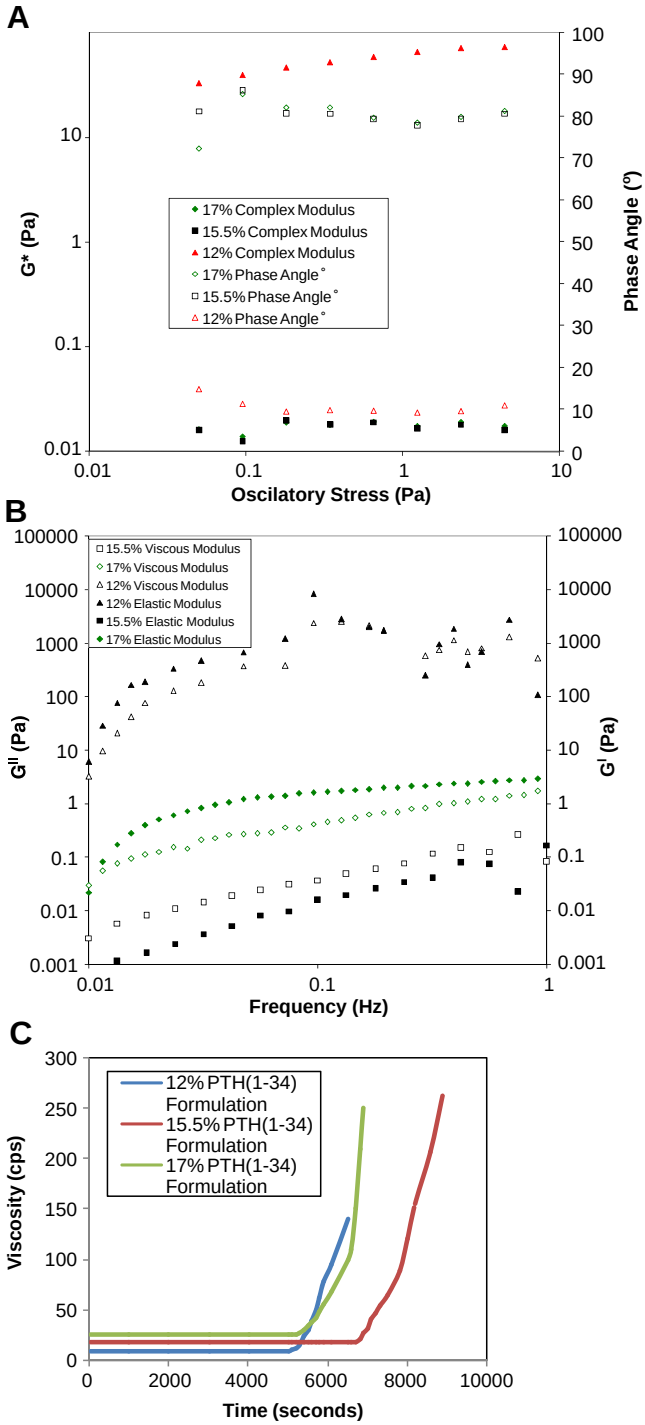

Fig. 6. **A** Measurement of complex modulus and phase angle as a function of oscillatory stress for formulations of three PTH(1-34) concentrations, 17% (◆ for complex modulus and ◇ for phase angle), 15.5% (■ for complex modulus and □ for phase angle), and 12% (▲ for complex modulus and Δ for phase angle); **B** Measurement of elastic modulus (G′) and viscous modulus (G″) as a function of oscillatory stress for formulations of three PTH(1-34) concentrations, 17% (◆ for G′ and ◇ for G″), 15.5% (■ for G′ and □ for G″), and 12% (▲ for G′ and Δ for G″); **C** Measurement of viscosity as a function of time for formulations of three PTH(1-34) concentrations, 17% (*green line*), 15.5% (*red line*), and 12% (*blue line*).

Effect of Peptide Concentration and Shear on Gelation

Shear stress is another well-known factor which causes protein/peptide solutions to gel. Viscoelastic and rheological properties of three PTH(1-34) concentrations (12%, 15.5%, and 17%) were determined to evaluate the effect of shear on gelation.

Viscoelastic characterization of a fluid can be a useful tool for predicting the fluid's gelation tendency (19). Measurement of viscoelasticity (i.e., elastic and viscous components) in a viscometer is based on a complex, theoretical model. Briefly, subjecting the material to an oscillatory stress or strain, whose value is small enough not to destroy the material's structure, produces the output of complex modulus (stress/strain) and phase angle. The complex modulus represents "rigidity" of the material and is the "sum" of elastic component (elastic modulus G′) and viscous component (viscous modulus G″). Fluids with a higher elastic modulus and a lower viscous modulus signify a greater level of rigidity and more ordered structure and may have a greater tendency to gel. Phase angle also correlates with relative importance of the elastic and viscous components of the fluid. When the phase angle is zero, i.e., the strain response being in phase with the applied stress, the fluid is mostly elastic, suggesting a more rigid, ordered structure. If the phase angle is 90°, the fluid is mostly viscous, suggesting a less ordered structure less prone to gelation.

The complex modulus and phase angle as a function of oscillatory stress for the three PTH(1-34) formulations (12%, 15.5%, and 17%) are presented in Fig. 6A. Their elastic modulus and viscous modulus as a function of oscillatory frequency are plotted in Fig. 6B. Based on complex modulus, the formulation's rigidity follows the order of 12% > 15.5% ≈ 17%. From the phase angle standpoint, the 15.5% and 17% formulations exhibited the similarly high phase angle (~80°), while the 12% displayed a much smaller phase angle, ~10°. The combined results of the complex modulus and the phase angle clearly suggested that the 12% formulation has the most rigid structure and might be most prone to gelation, while 15.5% and 17% formulations behave similarly. In the measurement for the elastic modulus (G′) and viscous modulus (G″) (Fig. 6B), both 12% and 17% formulations showed larger G′ than G″, while the 15.5% formulation has higher G″ than G′ and overall smallest G″. This analysis indicated that the 15.5% is more viscous, less rigid, and maybe less prone to gelation.

The third analysis is a simple viscosity measurement as a function of time. For the Newtonian liquid under a fixed shear rate, its viscosity should be constant with time initially and then increase quickly, indicating the point of gelation. The longer it takes to gel, the lower the gelling tendency of a formulation. The viscosity profiles for these three formulations as a function of time under a shear rate of 1,334 s^{-1} at 10°C are presented in Fig. 6C. The inflection point (where the viscosity begins to increase) is 5,000 s (1.4 h) for both the 12% and 17% peptide formulations and 6,800 s (1.9 h) for the 15.5% formulation. It again suggests that the 15.5% formulation is less prone to gelation, although it is not clear

why the gelation time did not correlate with peptide concentration.

Effect of Reservoir Design on Gelation

The coating run time is targeted for 24 h. It is difficult to predict if the selected formulation can last for at least 24 h before it gels in the coater. Prior to real-time testing, it is useful to understand the shear condition in the reservoir from a theoretical perspective. The liquid formulation experiences shear forces in the coater reservoir as the drum rotates (Fig. 2), primarily originating from the thin film of formulation created on the drum. The film thickness is defined by the gap between the drum and the doctor blade. The shear stress/force can be approximated by focusing on the thin film using a coaxial-cylinders model. This model is also the theoretical basis of a coaxial cylindrical viscometer where the liquid is filled between the two cylinders with the inner cylinder rotating at certain speed (20). By adopting the physical parameters of the coater −0.789 cm for the radius of the drum (representing the interior cylinder) and 100 μm (e.g., 0.01 cm) for the film thickness (the gap between the two cylinders), the maximum shear rate (at the drum surface) was calculated to be 67 s^{-1} when the drum rotates at 50 rpm, using Eq. 2 in Ref. 12 (for a Newtonian fluid). Please note that the shear force can be calculated by multiplying the shear rate, the viscosity, and the surface area.

However, unlike shearing the same volume of the liquid in the viscometer, at any given time during coating the film on the drum required only ~18% of the liquid formulation volume (1 mL), or 9% relative to 2 mL in the reservoir. The remainder of the formulation not used in creating the film would sit in the reservoir and be subjected to much smaller shear force. Thus, the averaged shear rate experienced by the liquid in the coater should be at least 5- or 10-fold lower than the calculated value, i.e., <15 or <8 s^{-1}, respectively. These lower shear rates may translate into >24-h physical stability for coating.

Two reservoir designs were assessed to coat the 15.5% PTH(1-34) formulation with drum rotation at 50 rpm and the drum temperature controlled at 1°C. Design A has a 1-mL reservoir and a narrow gap (~0.1 cm) between the drum and the reservoir at both ends of the drum. Design B has a 2-mL reservoir and a much wider gap (~0.5 cm) at both ends of the drum. With Design B, the formulation is stable for >24 h without gelation, while gelation occurred after 8 h of coating using the Design A reservoir. Higher stability in reservoir B can be attributed to lower overall stress acting on the formulation due to the larger volume and the lack of the narrow, stress-inducing gaps.

CONCLUSIONS

This study assessed and optimized several key parameters of the PTH(1-34) liquid formulation to allow >24-h continuous accurate and uniform coating. The effect of surface activity, viscosity, and peptide concentration on the coating process was elucidated, and optimization of these variables provided a process that was capable of reproducibly and accurately placing an extremely small volume of liquid formulation (1.5×10^{-7} mL) onto a very small surface area (5.75×10^{-5} cm^2) of each microprojection of the array (containing 1,300 microprojections per 2 cm^2). It is interesting to find that the acidic counterions for pH adjustment played a critical role in not only the rheological behavior of the liquid formulation (Newtonian *vs*. thixotropic *vs*. rheopectic) but also the solubility of the solid-state coated formulation. The non-volatile acidic counterions were needed to compensate for the loss of volatile acetic acid and to maintain the peptide's solubility for effective delivery into the skin. Finally, understanding the liquid formulation's tendency of gelation due to the influence of temperature, PTH(1-34) concentration, and shear (reservoir design) effects helped achieve the target physical stability of >24-h continuous coating without gelation.

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