AAPS PharmSciTech, Vol. 12, No. 1, March 2011 (© 2011)
DOI: 10.1208/s12249-011-9591-4

Research Article

Theme: Sterile Products: Advances and Challenges in Formulation, Manufacturing, Devices and Regulatory Aspects
Guest Editors: Lavinia Lewis, Jim Agalloco, Bill Lambert, Russell Madsen, and Mark Staples

Solution Formulation Development of a VEGF Inhibitor for Intravitreal Injection

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Received 18 June 2010; accepted 19 January 2011; published online 11 February 2011

Abstract. PF-00337210 is a potent, selective small molecule inhibitor of VEGFRs and has been under consideration for the treatment of age-related macular degeneration. An ophthalmic solution formulation intended for intravitreal injection was developed. This formulation was designed to maximize drug properties such that the formulation would precipitate upon injection into the vitreous for sustained delivery. As a parenteral formulation with additional constraints dictated by this specialized delivery route, multiple features were balanced in order to develop a successful formulation. Some of these considerations included low dosing volumes (≤0.1 mL), a limited repertoire of safe excipients for intravitreal injection, and the unique physical chemical properties of the drug. The aqueous solubility as a function of pH was characterized, buffer stressing studies to select the minimal amount of buffer were conducted, and both chemical and physical stability studies were executed. The selected formulation consisted of an isotonic solution comprised of PF-00337210 free base in a citrate-buffered vehicle containing NaCl for tonicity. The highest strength for regulatory toxicology studies was 60 mg/mL. The selected formulation exhibited sufficient chemical stability upon storage with no precipitation, and acceptable potency and recovery through an intravitreal dosing syringe. Formulation performance was simulated by precipitation experiments using extracted vitreous humor. In simulated injection experiments, PF-00337210 solutions reproducibly precipitated upon introduction to the vitreous so that a depot was formed. To our knowledge, this is the first time that a nonpolymeric *in situ*-forming depot formulation has been developed for intravitreal delivery, with the active ingredient as the precipitating agent.

KEY WORDS: AMD; depot; ophthalmic; parenteral; sustained delivery.

INTRODUCTION

Drug administration for the treatment of retinal diseases is challenging. The anatomical features of the eye present multiple barriers to any foreign substance. Main barriers to delivery include the cornea, the blood–retinal barrier, and the blood aqueous barrier (1). As a consequence, the drug levels achievable by more conventional delivery routes, such as topical ocular administration or oral administration, are severely limited. In general, invasive drug delivery strategies requiring injection directly into the vitreous are needed to deliver drugs to the retina.

The intravitreal injection route presents several unique challenges to the formulator. The eye is an extremely sensitive organ, and there is a limited collection of excipients acceptable for intravitreal injection compared with other delivery routes. As intravitreal injection is an invasive route, there is always a small but significant risk of infection with each new injection, thus, there is a drive to minimize the injection frequency (1–3). Moreover, the elimination of small molecules in solution from the vitreal space is quite rapid; it is well established that small molecules have an elimination half-life of <60 h from the vitreous (2,4–7). Consequently, a drug depot or some type of sustained release is desired. The injection volume is limited to less than 0.10 mL per eye. All these constraints present challenges that are not easily overcome.

Vascular endothelial growth factor (VEGF) is required for regulation of blood vessel growth for both tumors (8,9) and other conditions such as diabetic retinopathy (DME) and age-related macular degeneration (AMD) (10,11). AMD is the most common cause of severe and irreversible vision loss in the elderly in the developed world (12). Many VEGF inhibitors are under development or already approved for the treatment of retinal diseases, both AMD (13–19) and DME (20,21).

PF-00337210 is a VEGF receptor 2 tyrosine kinase inhibitor that was originally developed as an oncology drug and was later considered for the treatment of retinal diseases (Fig. 1, 22). The objective of the present work was to develop an ophthalmic intravitreal injection formulation of PF-

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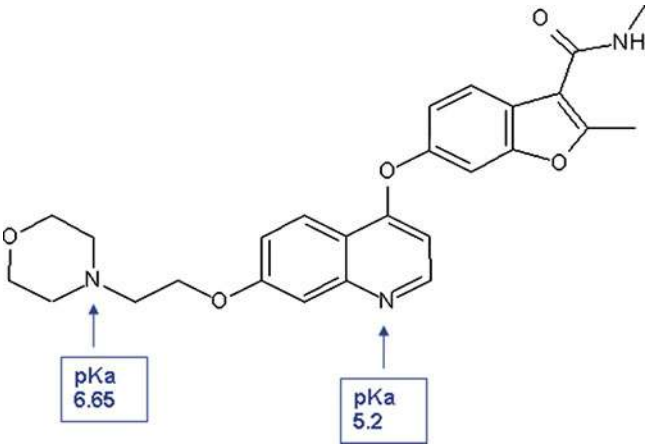

Fig. 1. Compound structure of PF-00337210, a VEGF inhibitor. PF-00337210 possesses two basic pKas

00337210 for use in regulatory toxicological and clinical studies covering doses up to 3.0 mg. The drug needed to be administered locally in order to reach the site of action and to avoid side effects associated with RTK inhibitors. The desired specific attributes for this formulation are summarized in Table I. A sterile, ready-to-use (RTU) solution or suspension formulation suitable for intravitreal injection was desired for the clinical formulation. The corresponding toxicological formulations needed to have the identical formulation components as the clinical formulation, although extemporaneous preparation was suitable for the toxicological formulations to maximize speed and flexibility. The present work describes how the multiple requirements of the delivery route and the physical chemical properties of the drug were balanced in order to develop a successful formulation.

MATERIALS AND METHODS

Materials

PF-00337210 free base crystalline solid was manufactured at Pfizer Inc, with a purity >99%. All formulation components met USP requirements for parenteral use. Citric acid monohydrate and sodium chloride were obtained from Merck. Hydrochloric acid was obtained from JT Baker.

Solubility Studies

PF-00337210's physical chemical properties were profiled in order to design the formulation. Equilibrium solubilities were characterized spanning a pH range from 3 to 11 and were determined using the shake-flask method. Samples were prepared in either water or NaCl solution. An excess amount of PF-00337210 was added. The pH was adjusted using HCl or NaOH. Samples were incubated with gentle agitation for 2 weeks at 5°C. Excess solids were extracted from each sample by centrifugation. Final equilibrium pH values were measured. Supernatant PF-00337210 concentrations were determined using a stability-indicating HPLC method.

Buffer Selection

Buffer stressing studies were conducted to select the minimal buffer amount needed to safely maintain the desired

Table I. Summary of Targeted and Final Attributes for PF-00337210 Intravitreal Formulations

Attribute	Target	Final formulation
Profile	Clinical formulation: sterile ready-to-use (RTU) solution or suspension formulation for intravitreal injection. Toxicological formulation: identical components as clinical formulation, extemporaneous preparation for tox formulation would be acceptable.	Clinical formulation: sterile RTU solution formulation for intravitreal injection. Toxicological formulation: identical components as clinical formulation, extemporaneous preparation for tox formulation.
Strength(s)	Clinical formulation: high strength of 30 mg/mL, dilution strategy for lower doses. Toxicological formulation: high strength of 60 mg/mL, dilution strategy for lower doses	Clinical formulation: high strength of 30 mg/mL, dilution strategy for lower doses Toxicological formulation: high strength of 60 mg/mL, dilution strategy for lower doses
pH	Within safe intravitreal limits: pH 3–8.	Acceptable manufacture pH range: pH 3.3–3.6 (for tox formulation, 3.2–3.4) Acceptable pH limits upon stability: pH 3.0–3.6 (for tox formulation, 3.2–3.5)
Buffer and buffer strength	As low as possible while still achieving suitable window for long-term storage.	10 mMol citrate
Tonicity	Isotonic	NaCl used for tonicity adjustment
Osmolality	300±30 mOsm	300±30 mOsm
Preservative	No; single use	No
Stability/shelf-life	Room temperature or refrigerated storage for ≥12 months	Refrigerated storage, ≥12 months.
Dose recovery from syringe ("syringability")	90–110%	90–110%
Duration	≥3 months	≥3 months

pH range. Minimum needed formulation buffer capacities (β) were calculated using the equation $\beta = \Delta B/\Delta pH$. ΔB is a small change in acid or base equivalents, and ΔpH represents the amount of acceptable pH shift (Table II). The following were assumed in calculating the minimum required buffer capacity: (1) Long-term formulation storage is the primary concern. Because the toxicological formulation (60 mg/mL) was intended to be prepared extemporaneously and only the clinical formulation would be subjected to long-term storage, the clinically relevant concentration of 30 mg/mL PF-00337210 was selected as the maximum concentration for this experiment, (2) the maximum allowed degradation was assumed to be 0.5% upon storage for the clinical drug product, (3) degradation converts completely to acid or base. This would have the potential to induce the maximal pH shift, (4) the drug degradation would be primarily responsible for any pH drift. The assumption is that this drift would be more significant than any shifts due to leachables from container/closure system or excipients, and (5) formulation stability pH target window is 3.0–3.6. This window was selected based on solubility studies and is discussed later.

Several potential buffer systems were then designed to satisfy the minimum buffer capacity requirements (Table III). Buffer systems that slightly exceeded the buffer capacity requirements were selected in the event that some of the original assumptions were somewhat optimistic and to account for impacts of pKa shift with ionic strength and temperature, which would in turn affect buffer capacity.

Selected test formulations at 30 mg/mL and corresponding placebo formulations were made containing the designed buffer systems. These formulations were stored and inverted in the container–closure system intended for clinical drug product storage. Arrhenius activation energies from historical solution stability data were used to estimate how long it would take to quickly induce ~0.5% degradation under high temperature storage conditions of 70°C. Time points were determined from this assessment.

Stability Studies

Both short-term (48 h) and longer-term accelerated (6 weeks) stability studies of prototype formulations were performed to support both the regulatory toxicology studies and the clinical formulation requirements, respectively. All formulations were prepared aseptically under conditions that closely simulated those of the extemporaneous preparation technique to be used for the regulatory toxicology studies, utilizing sterile and low endotoxin materials and supplies.

Stability studies of 2.5- and 60-mg/mL formulations were conducted at 5°C and 25°C for 48 h to support extemporaneously prepared formulations for toxicology studies. For the 60-mg/mL strength, an appropriate amount of sodium chloride, citric acid monohydrate, and PF-00337210 were added to sterile water for injection. A solution of 1.0 N hydrochloric acid was slowly added until the drug dissolved and the pH was between 3.20 and 3.40. The final osmolarity was within 300±30 mOsm. The final solution was sterile-filtered through a 0.22-μm polyvinylidene fluoride (PVDF) syringe filter unit prior to dispensing into 2-cc Flint Type I clear glass vials (Schott) equipped with 13-mm West B-2-coated stopper and flip-off seals (West).

Table II. Calculated Minimum Buffer Capacity Requirements for PF-00337210 Formulation

pH drift →	±0.2	±0.3
Maximum PF-00337210 degradation	Minimum needed buffer capacity, β	Minimum needed buffer capacity, β
0.5%	0.0016	0.0011
1.0%	0.0032	0.0022

Table III. Estimated Buffer Capacities for Potential Buffer Systems for PF-00337210 Formulation in Desired pH Range of 3.0 to 3.6

Buffer	pH 3.0	pH 3.2	pH 3.4	pH 3.6
10 mMol citrate	0.0033	0.0039	0.0065	0.0117
50 mMol phosphate	0.0095	0.0064	0.0042	0.0027
50 mMol sulfate	0.0088	0.0059	0.0039	0.0025

Buffer capacities were calculated from appropriate buffer capacity equations for mono- (35) and polybasic acids (36)

The 60-mg/mL PF-00337210 formulation was used as a stock solution for dilution to prepare the lower formulation strength of 2.5 mg/mL, using 0.9% NaCl solution as the diluent. The 2.5-mg/mL formulation was filtered using a 0.22-μm PVDF syringe filter before dispensing into the storage container–closure system. Placebo formulations consisted of 10 mM citrate in 0.9% NaCl, initial pH 3.3+0.1 adjusted with NaOH. The resulting osmolarity was within 300±30 mOsm. Physical, chemical, pH, and osmolarity stabilities were monitored under 5°C and 25°C storage.

Longer-term stability studies were conducted in support of a ready-to-use clinical formulation. A 30-mg/mL strength was stored for 6 weeks at 5°C, 25°C, and 40°C. Physical, chemical, pH, and osmolarity stabilities were monitored.

Dose Recovery Studies

The ability of the dosage form to effectively deliver the correct dose was assessed using a "syringability" test. The "syringability" is a simulated use evaluation for the overall formulation amount effectively dosed from an injection through a syringe, using the size and type employed for actual intravitreal administration. An overage of formulation of ~0.4 mL was withdrawn into a sterile 1-cc Luer-Lok syringe (Becton Dickinson) equipped with an 18 GA×1–1/2A PrecisionGlide Needle (Becton Dickinson). The syringe barrel was gently tapped to remove air bubbles. Excess sample was expunged from the syringe vial so that the 0.1-mL formulation remained in the syringe. The 18 GA×1–1/2A needle was then replaced with a sterile 27 GA×1–1/2A hypodermic needle (Kendall). Excess sample was expunged so that a typical toxicological dose of 0.05-mL formulation remained in the syringe. This 0.05 mL was dispensed into a volumetric flask and dissolved for HPLC analysis.

Both the withdrawal and release of contents during syringability studies were intended to mimic the procedure for administration to the eye. The initial withdrawal using a larger needle is used because it is sturdier and can easily puncture the septum of a serum vial. In addition, the withdrawal of drug into the syringe is smoother, introducing fewer air bubbles and less foaming into the formulation. The larger needle is then replaced

with the smaller needle to mimic drug administration into the eye. The smaller needle is used so that the wound is self-sealing and no stitch is required.

Simulated Intravitreal Injection Studies

Formulation performance was evaluated under simulated intravitreal injection conditions. *In situ* precipitation experiments were conducted using freshly harvested rabbit and dog vitreous humor (Bioreclamation Inc, Jericho, NY). The clinical dose volume was intended to be 100 μl into the human eye. A dosed volume of 100 μl of the 30-mg/mL formulation would result in a 3-mg total dose. In terms of the therapeutic concentrations in the eye, given the vitreous volume of a human eye (4 mL), the intended top dose of 3 mg would correspond to a concentration of 0.75 mg/mL in the vitreous. For the simulated performance studies using the excised vitreous humor of preclinical species, the delivered formulation to vitreous volume ratio mimicked prescribed *in vivo* usage conditions (1:40 dilution). Five microliters of formulated PF-00337210 solution at various preselected concentrations was delivered into 0.200 mL of either rabbit or dog vitreous stored in 1.5-mL test tubes and equilibrated to room temperature. The final diluted PF-00337210 concentrations in this experiment covered up to a final simulated dose of 0.45 mg. Vitreous samples containing PF-00337210 formulation were vortexed for 5–10 s and subsequently transferred into a 37-°C incubator for 12 h. Following incubation at body temperature under static conditions, sample tubes were centrifuged at 14,000 rpm for 60 min, and the supernatants were transferred into new tubes. Pellets were isolated and dissolved in 1.5 mL of ethanol for potency analysis by HPLC. Supernatants were also diluted 100-fold in ethanol for HPLC analysis. Seven-point calibration curves were constructed using a matrix matching approach in rabbit or dog vitreous and diluted by ethanol.

RESULTS AND DISCUSSION

Suspension *vs.* Solution Formulation Considerations

Small molecule half-life in the vitreal space is quite low; if the drug is in solution, it will be cleared within days (2,4–6). Moreover, frequent intravitreal dosing is undesirable. Each new injection presents a new increased risk of infection. Therefore, it was imperative to minimize dosing frequency for this drug, ideally down to every 3 months or less. A strategy to sustain the release of this small molecule drug was therefore required. Two possible ways of achieving this without the use of a drug delivery device were to create a suspension formulation, or a solution formulation that formed a depot upon injection. Both dosage form options were initially investigated. Many factors, both for this unique delivery route and for the specific drug properties in question, had to be managed in order to develop a successful formulation.

Each type of dosage form has advantages and disadvantages. One factor favoring the solution formulation over the suspension is the manufacturing process. Solution formulations are easier to manufacture in several respects. If the drug cannot withstand terminal sterilization, a solution formulation manufacture lends itself to the next best option of sterile filtration, whereas for a sterile suspension formulation, the manufacture becomes much more complex, usually requiring an entirely aseptic process (23). Aseptic manufacturing is a difficult process and can pose higher risks than for terminally sterilized products (24). The process becomes even more complex for ophthalmic formulations which have stringent low endotoxin and foreign particulate requirements.

For suspension formulations, the starting drug particle size and solid form are known and can generally be better controlled compared with that of the solution formulation; for the solution formulation, there is essentially no control over the precipitation process *in vivo* and the resulting particle sizes. At the same time, suspension formulation drug product development also has the added intricacies of physical stability and its implications. Stabilizing agents are needed, and there are limited excipients qualified as safe for intravitreal injection (25). In addition, suspensions suffer from lack of predictability of the long-term physical stability shelf-life. Whereas solution formulation shelf-life can be initially qualified by accelerated stability studies, suspension formulations cannot and require more up-front development for a robust formulation. The dosing of a suspension formulation is also more complex: achieving an acceptable and reproducible dose through an intravitreal dosing syringe can be tricky.

PF-00337210's physical chemical properties were such that both solution and suspension formulations could be considered. PF-00337210 possesses two ionizable basic groups with approximate pKas of 6.5 and 5.2. As such, at low pH values of ~pH 3, PF-00337210 is soluble, and high solution concentrations are achievable. Upon injection in the vitreous, which has a neutral pH environment, PF-00337210 solution formulations could then theoretically precipitate to form a drug depot. A defining factor for the selection of the solution formulation over the suspension, however, was the existence of an anhydrate-to-hydrate conversion of the solid state PF-00337210. When attempts were made to make a suspension formulation starting with the anhydrate drug substance, the anhydrate converted to the hydrate form once it was in an aqueous environment. Upon conversion to the hydrate, substantial aggregation and particle size changes occurred, to the extent that the suspension formulation could no longer be pushed through a dosing syringe. The mean particle size for the anhydrate starting material was on the order of 30 μm or less, a size that has had no trouble with syringability for other similar suspension formulations. The post-conversion particle size was not measured as it was a gross agglomeration of solid. An alternate strategy of formulating using the hydrate form drug substance as the starting material was also attempted. Whenever the hydrate drug substance was dried, however, it reverted back to the anhydrous form. Further attempts to stabilize the hydrate in the solid state failed. The suspension formulations were unmanageable from the standpoint of dosing through a syringe, and the usual advantage of particle size control was lost. Therefore, a solution formulation was ultimately easier to develop and contend with over the suspension formulation.

Solubility Behavior and Selection of Formulation Components

PF-00337210 possesses two ionizable basic groups with approximate pKas of 6.5 and 5.2 (Advanced Chemistry

Development (ACD) lab software prediction), and therefore, the solubility is highly pH-dependent. As such, at low pH, PF-00337210 is soluble, and high solution concentrations are achievable. This strategy was used to achieve the desired solution formulation concentration.

Solubility as a function of pH is shown in Table IV for solubilities in pH-adjusted water and 0.7% NaCl. Studies were performed at 5°C, the most likely long-term formulation storage condition for this drug product. Little to no drug degradation (<<1%) was observed for solubility samples over the course of this experiment. Solubilities in 0.7% NaCl were somewhat greater than those at the corresponding pH in water. This could perhaps be due to the influence of ionic strength on pKa values.

The highest dose required for toxicological studies was 3.0 mg. In order to provide an intravitreal injection volume of 50 μL to preclinical species, a formulation concentration of 60 mg/mL was required. Likewise, a maximal clinical formulation concentration of 30 mg/mL was desired. The solubility data suggest that the desired solution concentrations can be achieved below pH 4. In line with current practice, only injections with pH >3 may be injected into the eye. Therefore, a balance between the two pH extremes had to be achieved. Due to the two close pKas on the molecule, the solubility in the pH 3–4 region changes greatly with small pH shifts. Solubility simulations were run to delineate the pH range more precisely and with more surety.

With the two basic pKas, the pH-dependent solubility of PF-00337210 free base should exhibit the following equation:

$$S = \mathrm{So}[1 + [H^+]/\mathrm{Ka2} + [H^+]2/(\mathrm{Ka1} \times \mathrm{Ka2})],$$

Where S is the solubility as a function of pH, So is the intrinsic solubility, $[H^+]$ is the hydrogen ion concentration, and Ka1 and Ka2 are the dissociation constants for the two ionization sites on PF-00337210.

Table IV. PF-00337210 Solubility Data Plotted in Fig. 2

PF-00337210 aqueous solubility as a function of pH at 5°C	
pH	Measured solubility (mg/mL) in water
3.94	33.3
4.03	10.9
4.07	21.7
4.18	14.6
4.86	0.581
7.20	0.0012
7.22	0.0027
9.70	0.0027
11.20	0.0010
PF-00337210 solubility in 0.7% NaCl as a function of pH at 5°C	
pH	Measured solubility (mg/mL) in 0.7% NaCl
3.98	63.7
3.99	89.4
4.02	40.5
4.12	26.6
4.12	26.6
4.55	2.42
4.59	4.32
4.79	0.60
5.98	0.0018
8.64	0.0012
11.07	0.0017

All pH values were measured after final equilibrium was achieved

Table V. Projected PF-00337210 Free Base Solubility as a Function of pH at 5°C

pH	Projected solubility in using ACD lab estimates[a] of pKas (mg/mL)	Projected solubility using measured values[b] of pKas (mg/mL)
3.0	1,425	839
3.3	360	212
3.5	144	85
3.6	92	54
3.7	58	34
4.0	15	8.9
5.0	0.23	0.14
6.0	0.012	0.0087
7.0	0.0029	0.0026
8.0	0.0021	0.0021

[a] ACD lab estimates of pKas: pKa1=5.2, pKa2=6.65. So=0.002 mg/mL for the projections

[b] Experimental values of pKas (capillary electrophoresis method): pKa1=5.15, pKa2=6.47. So=0.002 mg/mL for the projections

Scientist 3.0 software (Micromath) was used to project solubility in the low pH range of interest where the solubility was too high to obtain a saturated solution (Table V). The projections are based on Table IV solubility data for approximate So value (0.002 mg/mL) and ACD estimates of pKa (pKas of 5.2 and 6.65) as well as experimentally determined pKa values (pKas of 5.15 and 6.47, capillary electrophoresis method). Though the experimentally determined pKas closely matched the ACD predictions, the simulations shown in Table V illustrate how sensitive the solubility is to subtle changes in pKa.

Solubility changes of twofold are expected in the pH 3–4 region with as little as 0.15 units of pH drift. From the combination of solubility data and the projections, the desired clinical dosage strength of 30 mg/mL can be supported as a solution formulation below pH 3.6 (Fig. 2). The desired toxicological dosage strength of 60 mg/mL can be supported as a solution formulation below pH 3.5. These would represent the upper pH limits for physical stability upon storage; the initial manufacturing specification had to be set somewhat lower than this to accommodate pH shifts upon storage which are discussed in the subsequent Section on "Buffer Selection".

The solubility projections assumed that no salt solubility product Ksp was reached. This risk was assessed further. In order to evaluate the possibility of reaching a Ksp in the desired formulation, several 60-mg/mL solutions containing formulation components of potential buffers and sodium chloride were set up and stored under refrigerated conditions bracketing a pH range 3.0 to 4.0. After 2 months of storage under refrigeration and at 25°C, no precipitation was observed, suggesting that the desired 60-mg/mL formulation concentration did not pose a risk of exceeding a Ksp in the formulation vehicle.

Buffer Selection

Based on the PF-00337210 pH-solubility profile and safety considerations, a formulation pH window of 3.0 to 3.6 was set for long-term storage conditions to support the

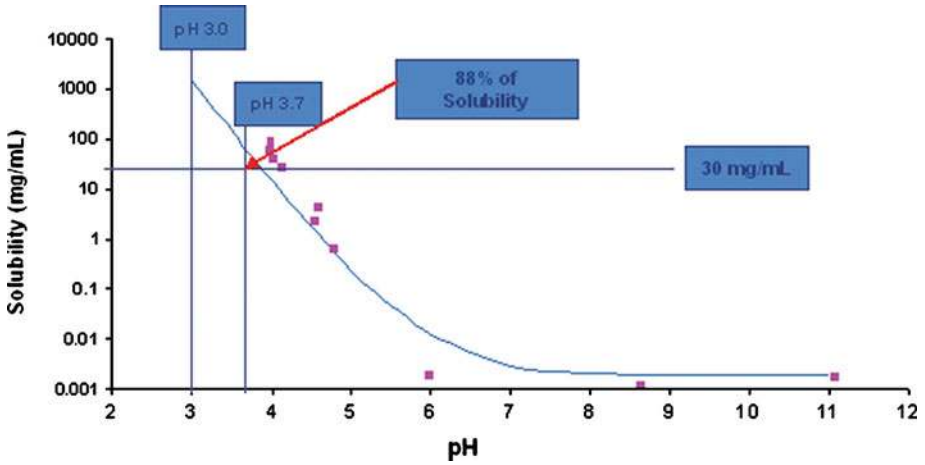

Fig. 2. PF-00337210 solubility as a function of pH at 5°C. The optimal window for the clinical formulation at the target of 30 mg/mL was within a bracket of 3.0 and 3.6—the limits between the minimum safe pH for intravitreal injection (pH 3.0) and the highest pH that safely stayed below the solubility

clinical drug product strength of 30 mg/mL. Above pH 3.6, there was a high risk of PF-00337210 free base precipitation. Several potential buffer systems were designed based on required buffer capacity and were stress-tested.

Results are shown in Table VI for formulations data stress-tested at 70°C. The results showed that when ~0.7% PF-00337210 degradation was induced, which slightly exceed the degradation that would be allowed for drug product storage, the tested buffering systems hold the pH within ±0.3 pH units. In addition, pH drifts only downward, never upward, for these potential PF-00337210 formulations. Taken together, these data suggest that if the pH specification for clinical formulation manufacture starts at 3.30–3.60, the designated pH window of 3.00–3.60 during long-term storage will be maintained. In order to enable the higher PF-00337210 concentration for toxicology studies, a slightly lower pH window of pH 3.20–3.40 was targeted. Based on safety studies, 10 mM citrate was selected as the preferred buffer for the formulation over phosphate. Other anions (data not shown) were eliminated as buffering agents and/or *in situ* salt-forming agents based on safety considerations (26).

There were several additional valuable observations that could be made from the buffer stressing results. One was that the pH drift was miniscule for the placebo test samples—those buffered solutions containing no drug that were stored in the same container–closure system intended for the clinical drug product. Thus, the original assumption that the PF-00337210 degradation is primarily responsible for pH drift was correct, and any leachables from container/closure system were not introducing significant pH drift. The second observation was that there was little difference in percent degradation in comparing samples in the pH range of interest (0.74% *vs.* 0.73% degradation for initial formulation pH values of 3.19 and 3.69, respectively). Therefore, in the formulation pH region of 3.0–3.6, PF-00337210 degradation rate constants are relatively insensitive to pH.

Solubility with Dilution Scheme

Lower desired doses could be achieved by dilution of the highest formulation concentration into 0.9% NaCl. As expected, the dilution was accompanied by an increase in pH. To evaluate if the resulting diluted formulations might be supersaturated upon dilution, a 30-mg/mL formulation was diluted in 0.9% NaCl (Table VII). The concentration of PF-00337210 in every sample was below the solubility limit at the resulting pH. Therefore, dilutions of the concentrated formulation using normal saline should be physically stable because their concentrations remain below solubility limits at the resulting pH values. In all cases, osmolality was well within the target range of 300±30 mOsm.

Final Formulation Composition

The final toxicological formulation consisted of 10 mM citrate buffer, pH 3.3, at a maximum strength of 60 mg/mL (Table VIII). The formulation nominated for toxicological

Table VI. Results of Buffer Stressing Studies for Samples Stored at 70°C

PF-00337210 concentration (mg/mL)	Buffer	Initial pH	Final pH (t=19 h)	Change in pH	% degradation (t=19 h)
30	10 mMol citrate	3.19	2.94	−0.25	0.74
30	10 mMol citrate	3.69	3.53	−0.16	0.73
30	50 mMol phosphate	3.20	2.95	−0.25	0.78
0 (control)	10 mMol citrate	3.22	3.23	0.01	NA
0 (control)	10 mMol citrate	3.67	3.71	0.04	NA
0 (control)	50 mMol phosphate	3.23	3.24	0.01	NA

Samples were stored inverted in the container–closure system intended for the clinical drug product
NA not applicable

Table VII. Osmolality and pH Measurements Performed on Dilutions of 30 mg/mL PF-00337210 Formulation

Diluted concentration of PF-00337210 (mg/mL)	Osmolality (mOsm) average of three measurements	pH
30	305±2	3.40
20	305±2	3.48
12	302±0	3.55
6	295±1	3.68
3	290±1	3.80

0.9% NaCl was used as the diluent

studies was a buffered, isotonic aqueous solution prepared extemporaneously by aseptic technique using low endotoxin supplies. The drug product was a clear, light yellow to yellow solution. The manufacturing target pH of the formulation was 3.3±0.1. The tonicity of the PF-00337210 formulation was adjusted by adding sodium chloride to achieve osmolality of 300±30 mOsm. Based on an intravitreal dosing volume of 50 μL and target doses of 0.3, 1.0, and 3.0 mg, the required solution concentrations were 6, 20, and 60 mg/mL, respectively. The 6- and 20-mg/mL concentrations were achieved by dilution of the 60 mg/mL using 0.9% NaCl.

Short-Term Stability and Dose Recovery Studies to Support Extemporaneously Prepared Toxicological Formulations

Prototype stability testing was conducted on 2.5- and 60-mg/mL formulations over a 48-h period, covering the amount of time for the toxicological formulations to be made extemporaneously and dosed. Formulations were made and stored upright in the intended container–closure system under 5°C and 25°C storage conditions. Formulations exhibited physical stability with no precipitation at 5°C and 25°C (Table IX). The pH and osmolarity were stable and remained within the desired ranges. Acceptable potency estimations using simulated dosing through a syringe averaged between 100% and 106%, which were within the desired 90–110% dose recovery. Formulations were chemically stable at 48 h to support toxicological studies with no detectable change in HPLC purity profile (>99.6%; Table X).

Long-Term Stability and Dose Recovery Studies to Support Clinical Drug Product Requirements

The osmolality, pH, and physical stability of the PF-00337210 prototype clinical formulation were monitored over a 6-week period (Table XI). The results show that the osmolality of 30-mg/mL PF-00337210 formulation is stable in temperature range 5–40°C for up to 6 weeks. There was some pH drift at every temperature tested. The magnitude of pH shift was temperature-dependent and was greater at higher temperature. Based on the prediction that 6 weeks of storage at 25°C approximates 12 months of storage at 5°C, the pH should remain well within the pH specification limit of 3.0 to 3.6 during refrigerated storage.

The results of purity testing and formulation assay are presented in Table XII. At the 6-week time point at 25°C, two degradants were at significant levels (0.23% and 0.11% for the two degradants, respectively). Such growth effectively eliminated the possibility of sample storage at room temperature and provided more evidence for the refrigerated storage requirement. Based on these chemical stability results and on physical stability results, a 12-month use period was assigned for the 30-mg/mL PF-00337210 formulation when stored under refrigerated conditions. The physical stability continued to be monitored since nucleation is not predictable.

Simulated Intravitreal Injection Studies

In simulated injection experiments, solution formulations reproducibly and rapidly precipitated within seconds or less to form a solid state precipitate in rabbit or dog vitreous isolates. The formation of an *in situ* precipitate was hypothesized to be a critical factor for enabling the slow vitreal clearance of the formulation over time, intended to minimize dosing frequency (preclinical species data subsequently showed *in vivo* coverage up to 3 months, data not shown). Figure 3a, b represents the percent of dose recovered as precipitate and supernatant, respectively, as a function of the overall simulated dose. Even for very small doses of less than 0.1 mg, the extent of precipitation was nearly 90%. The fact that even at low doses such a high percentage precipitates increased the confidence that the solution formulations would also precipitate *in vivo*. There appeared to be an inflection point between 0.3 and 0.4 mg, and the extent of precipitation increased with increasing dose. At a dose of 0.45 mg, >98% of the dose precipitated in both rabbit and dog vitreous. The solid form of the precipitate was determined by PXRD to be a mix of the free base hydrate form and an unknown metastable solid form. It is expected that the extent of precipitation would be concentration-dependent, increasing with increasing dose, as the competition between drug–drug collisions outweighs the propensity for the soluble drug to become involved with some part of the vitreous components. Given that the intended top therapeutic dose of 3 mg is even greater than the highest dose tested in the simulated performance studies (0.45 mg), it is estimated that the extent of precipitation for this top dose would also be >98%.

The fact that this solution formulation precipitates to form a depot is key to its performance and is the likely reason that *in vivo* coverage remained for at least 3 months post

Table VIII. Final Formulation Composition for High-Strength (60 mg/mL) PF-00337210 Solution Formulation

Component	Concentration (mg/mL)	Function
PF-00337210 (freebase)	60.0	Active
Sodium chloride, parenteral grade	3.80	Tonicity agent
Citric acid, monohydrate, parenteral grade	2.10	Buffer agent
1.0 N Hydrochloric acid solution, parenteral grade	238.3[a]	pH adjustment
Sterile water for injection	707.3	Solvent
Total	1,012[b]	

[a] Approximate value; pH adjustment dictates final amount. The density of 1.0 N HCl is 1.014 g/mL

[b] The density of the 60 mg/mL formulation is 1.012 g/mL

Table IX. Physical Stability Attributes of Low- and High-Strength PF-00337210 Solution Formulations Intended for Toxicological Studies

		Initial (t=0)		Final (t=48 h)		
Storage temperature	PF-00337210 formulation strength (mg/mL)	Initial pH[a] (t=0)	Osmolarity[a] (mOsm) (t=0)	Final pH[a] (t=48 h)	Osmolarity[a] (mOsm) (t=48 h)	Visual observation[a]
5°C	2.5	4.00±0.00	294±0	4.00±0.01	294±1	Clear, colorless solution
	60	3.34±0.00	305±1	3.23±0.00	305±1	Clear, yellow solution
25°C	2.5	4.00±0.00	294±0	3.98±0.01	294±0	Clear, colorless solution
	60	3.34±0.00	305±1	3.25±0.01	304±1	Clear, yellow solution

Data displayed as average ± standard deviation
[a] n=2

Table X. Chemical Stability of Extemporaneously Prepared Low- and High-Strength PF-00337210 Solution Formulations Intended for Toxicological Studies

		Initial (t=0)		Final (t=48 h)	
Storage temperature	PF-00337210 formulation strength (mg/mL)	Purity[a] (HPLC peak area (t=0)	% dose recovery from syringe[a] (t=0)	Purity[a] (HPLC peak area) (t=48 h)	% dose recovery from syringe[a] (t=48 h)
5°C	2.5	99.62±0.01	106±3	99.63±0.01	105±4
	60	99.61±0.00	106±1	99.63±0.01	100±3
25°C	2.5	99.62±0.01	106±3	99.66±0.02	101±1
	60	99.61±0.00	106±1	99.60±0.01	100±2

All data are displayed as average ± standard deviation
[a] n=3

Table XI. Physical Stability Attributes for Longer-Term Storage of 30 mg/mL PF-00337210 Solution Formulations Intended for Clinical Studies

	Initial (t=0)		t=3 weeks		T=6 weeks	
Storage temperature	pH	Osmolarity (mOsm)	pH	Osmolarity (mOsm)	pH	Osmolarity (mOsm)
5°C	3.35	297	3.32	298	3.29	298
25°C	3.35	297	3.30	298	3.23	298
40°C	3.35	297	3.21	301	3.00	302

Table XII. Chemical Stability Attributes for Longer-Term Storage of 30 mg/mL PF-00337210 Solution Formulations Intended For Clinical Studies

	Initial (t=0)		t=3 weeks		T=6 weeks	
Storage temperature	Purity (HPLC peak area)	% dose recovery from syringe	Purity (HPLC peak area)	% dose recovery from syringe	Purity (HPLC peak area)	% dose recovery from syringe
5°C	99.7	98.4	99.68	100.3	99.67	98.6
25°C	99.7	98.4	99.63	102.3	99.34	97.4
40°C	99.7	98.4	98.54	97.0	97.30	99.6

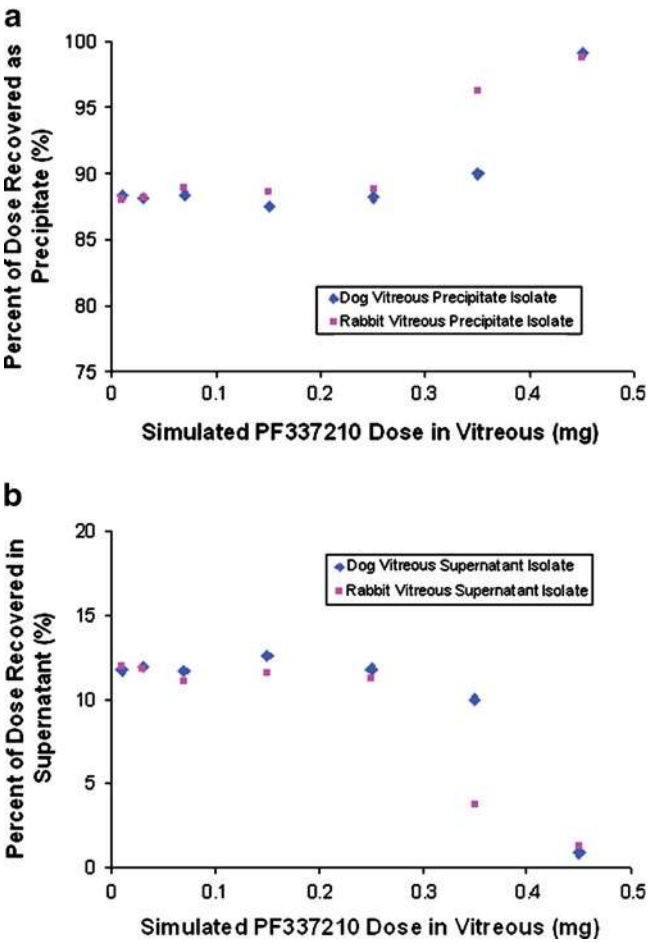

Fig. 3. *In situ* vitreous precipitation of PF-00337210 solution formulations into extracted rabbit and dog vitreous. **a** Percent of dose recovered as solid precipitate as a function of PF-00337210 dose. **b** Percent of dose recovered in the supernatant as a function of PF-00337210 dose

injection. The fact that intravitreal half-life is highly dependent on drug form in the vitreous is demonstrated by Durairaj and coworkers (27). In that work, diclofenac administered as a solution had a half-life of 2.85 h whereas when administered as a suspension, the resulting solid depot had a greatly extended half-life of 581 h.

We believe that the PF-00337210 solution formulation represents the first time that a nonpolymeric *in situ*-forming depot formulation has been developed for intravitreal delivery, with the drug as the precipitating agent. There have been precedented formulations designed to precipitate upon injection. However, those in which the drug is the precipitating agent were for nonocular drug delivery routes. For example, Pohl and coworkers (28) described an invention for a solution formulation of insulin that precipitates upon subcutaneous or intramuscular injection for sustained release. Formulations containing polymers that precipitate or gel, such as ELIGARD® or ATRIDOX®, are also precedented (29–33). In one particular system designed for intravitreal delivery, Mitra and coworkers designed a sustained release system consisting of drug-loaded poly (DL–lactide–co–glycolide) (PLGA) microspheres dispersed in thermogelling PLGA–PEG–PLGA polymer gel (34). Those systems rely on polymers rather than the drug as the precipitating or gelling agent.

CONCLUSIONS

Multiple considerations of a complex delivery route and unique drug properties were balanced to develop an ophthalmic drug candidate solution formulation for intravitreal injection. This formulation maximized drug properties such that the formulation would precipitate upon injection into the vitreous for sustained delivery. The precipitation was critical for the slow clearance of the formulation out of the vitreous over time in order to minimize dose frequency down to once every 3 months or less. Isotonic PF-00337210 solution formulations comprised of PF-00337210 free base in a citrate-buffered vehicle containing NaCl were developed to support both regulatory toxicological and clinical studies. The highest strength was 60 mg/mL, supporting a dose of 3.0 mg upon injection of 0.05 mL into the vitreous.

ACKNOWLEDGMENTS

The supporting contributions of the following colleagues are gratefully acknowledged: Dr. Danhua Chen, Brian Samas, Chris Seadeek, Dr. Jaymin Shah, Chang Shu, and Dr. Anand Sistla.

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