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# Development of a precipitation-resistant solution formulation to increase in vivo exposure of a poorly water-soluble compound

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ABSTRACT

In vitro and in vivo investigations were conducted to develop a suitable formulation for early toxicology and clinical studies of ((R)-7-(3,4-dichlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidin-6-yl)((S)-2-(4-fluorophenyl)pyrrolidin-1-yl)methanone (Compound A), a nonionizable and poorly water-soluble compound that selectively inhibits the ultrarapid potassium current (IKur) and is intended for the treatment of arrhythmia. Various nonaqueous solution formulations were evaluated, in vitro, for ability to prevent or delay precipitation of Compound A from solution following dilution with water. The plasma exposures of precipitation-resistant solutions, non precipitation-resistant solutions, and aqueous suspensions were then compared in rats, dogs, and/or humans. The data indicated that a solubilized, precipitation-resistant formulation achieved the highest plasma concentrations in all species and also improved dose proportionality, particularly in rats. Development of such formulations may be highly valuable for achieving in vivo blood levels often required for successful toxicological and early clinical evaluation of poorly soluble compounds.



## 1. Introduction

One of the significant challenges in the formulation of Biopharmaceutical Classification System (BCS) Class II (low aqueous solubility, high permeability) compounds (Amidon et al., 1995; Yu et al., 2002) to support toxicology and/or early clinical studies is the achievement of sufficiently high plasma concentrations following oral dosing. Traditional solid dosage forms and aqueous suspension formulations may be unsuitable for high-dose studies of poorly water-soluble compounds, as they commonly exhibit solubility- or dissolution-limited absorption and plateau in exposure with increasing dose (Chen et al., 2006; Hörter and Dressman, 2001; Li et al., 2002; Neervannan, 2006; Paulson and Maziasz, 2004; Takano et al., 2008). Non dose-proportional absorption can be especially problematic in early toxicology studies, which generally require high plasma concentrations in order to establish comfortable safety multiples to support clinical studies.

For compounds that are ionizable, it may be possible to improve exposures by formation of a salt (Engel et al., 2000; Gould, 1986; Gwak et al., 2005; Nielsen et al., 2005; Serajuddin, 2007; Wadke et al., 1989). For nonionizable compounds, however, many alternative approaches, including particle size reduction (Chaumeil, 1998; Cooper, 2010; El-Shabouri, 2002; Kumar et al., 2007; Liversidge and Cundy, 1995; Sigfridsson et al., 2009; Wu et al., 2004), chemical complexation with cyclodextrins or other agents (Carrier et al., 2007; Higuchi and Ikeda, 1974; Jambhekar et al., 2004; Järvinen et al., 1995; Lim and Go, 2000; Orienti et al., 2009; Rajewski and Stella, 1996; Wong and Yuen, 2001), formulation as a solid dispersion (Jachowicz et al., 2000; Leuna and Dressman, 2000; Nazzal et al., 2002; Sethia and Squillante, 2003; Sheen et al., 1991, 1995; Shin and Kim, 2003; Sinha et al., 2009) or amorphous system (Ambike et al., 2004, 2005; Hancock and Zografi, 1997; Karimian et al., 1998; Kennedy et al., 2008; Shimpi et al., 2005; Yu, 2001), or delivery using a lipid/surfactant combination to form an emulsion or microemulsion (Dollo et al., 2003; Gao et al., 2003, 2004; Gershanik and Benita, 2000; Gursoy and Benita, 2004; Kang et al., 2004; Kawakami et al., 2002; Pouton, 2000; Tang et al., 2007;

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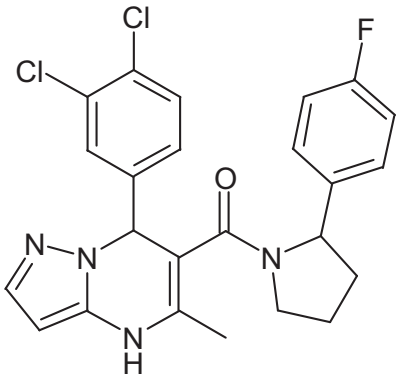


**Fig. 1.** Chemical structure of Compound A: ((R)-7-(3,4-dichlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidin-6-yl)((S)-2-(4-fluorophenyl)pyrrolidin-1-yl)methanone.

Wu et al., 2009; Yin et al., 2009), could be used. For compounds that cannot be complexed or solubilized in lipid/oil-based systems, polar cosolvents such as PEG 400, propylene glycol, ethanol, etc., may be considered, particularly in cases where toxicology investigations require very high exposures. Use of these types of cosolvent systems may necessitate formulation of the drug at high concentrations to reduce dose volume and, thereby, minimize any potential safety or tolerability concerns associated with the cosolvents. While fairly high concentrations of drug are often achievable using cosolvent systems, immediate precipitation of the compound from solution can occur upon contact with the aqueous environment of the gastrointestinal tract. Such an event can then result in significantly limited or variable oral absorption and unpredictable exposures. For this reason, development of a suitable cosolvent formulation, designed to overcome the potential for in situ precipitation of hydrophobic compounds at high concentration, may be very useful for preclinical and/or early clinical investigations. Similar issues, related to dilution-induced precipitation, have been encountered in the development of cosolvent-based systems for the intravenous administration of water-insoluble compounds (Li and Zhao, 2007; Straubinger, 1995; Yalkowski and Valvani, 1977). These formulations are typically diluted, from concentrate, with an aqueous medium such as normal saline or 5% dextrose prior to dosing. They must, therefore, be resistant to precipitation of the compound during and after this process. Surfactants such as polysorbate or Cremophor® have been used to reduce the risk of dilution-induced precipitation in several intravenous products (Li and Zhao, 2007; Orienti et al., 2009; Straubinger, 1995). In addition to increasing solubility, there have been a significant number of studies suggesting that cosolvents and surfactants may also enhance oral absorption through mechanisms such as inhibition of p-glycoprotein or other efflux carrier systems (Aungst, 1993; Grube and Langguth, 2007; Hugger et al., 2002; Johnson et al., 2002; Nornoo et al., 2009; Rege et al., 2002). The current work, however, will focus on the role of these excipients in solubilization and inhibition of precipitation of insoluble compounds during and following dilution in an aqueous environment.

((R)-7-(3,4-dichlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidin-6-yl)((S)-2-(4-fluorophenyl)pyrrolidin-1-yl)methanone, or Compound A (Fig. 1), is a selective inhibitor of the ultrarapid potassium current (IKur) and is intended for the treatment of arrhythmia. Compound A is a nonionizable compound with very low aqueous solubility (~4 μg/mL) and relatively low solubility in oils (<5 mg/mL). Its measured Caco2 permeability is 99 nm/s, and its Log D value (octanol/aqueous buffer) is 4.0 at pH 2.5 and 4.1 at pH 7.4. The melting point of Compound A is 196–203 °C. The solubility of Compound A in pharmaceutically acceptable nonaqueous cosolvents such as PEG 400, propylene glycol, and 95% ethanol (alcohol, USP) is ~20–60 mg/mL, but solutions of the compound in these cosolvents tend to precipitate immediately, and with uncontrolled particle size, upon dilution with aqueous media. Preliminary pharmacokinetic studies conducted in rats, dogs, and monkeys showed substantial interspecies variability, with relatively high exposures from aqueous suspension in rats, poor exposures in dogs, and variable exposures in monkeys. The challenging physicochemical properties and high variability in the in vivo absorption profile of Compound A increased the risk that a traditional solid dosage form or a simple cosolvent solution could fail to provide the required maximum plasma concentrations to support nonrodent toxicology and Phase I clinical studies. The current in vitro and in vivo screening studies were conducted to develop a solubilized, precipitation-resistant formulation providing reliable plasma exposures of Compound A.

## 2. Materials and methods

### 2.1. Materials

Compound A, with purity >98%, was produced by Bristol-Myers Squibb Co., New Brunswick, NJ. All other chemicals were reagent or analytical grade and were used as received.

### 2.2. Preparation of formulation prototypes for in vitro and/or in vivo screening

Solubilized and precipitation-resistant formulations of Compound A were designed to improve the solubility of the drug and/or maintain the drug in solution upon contact with the aqueous environment of the gastrointestinal tract. Multiple solubilized formulation prototypes, both precipitation-resistant and non-precipitation-resistant, were prepared for evaluation. Non-precipitation-resistant formulations, containing mixtures of PEG 200, PEG 400, polysorbate 80, ethanol, N-methyl pyrrolidone, Cremophor EL®, and/or propylene glycol, were prepared by combining the ingredients in proportions listed in Tables 1–3. Concentrations of Compound A in these solutions ranged from approximately 2 to 45 mg/mL. Precipitation-resistant solution formulations consisted of 0.8–22.5 mg/mL Compound A in a vehicle containing varying combinations of PEG 400, ethanol, polysorbate 80, and d-α tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS, Eastman Chemicals). Actual vehicle compositions evaluated are listed in Tables 1–5. The vehicles were prepared by dissolving the vitamin E TPGS in ethanol, followed by addition of PEG 400 and polysorbate 80. A semisolid dispersion formulation composed of 50% PEG 400/50% vitamin E TPGS was also evaluated. This formulation was prepared by dissolving Compound A at 50 mg/g in molten vitamin E TPGS at 50 °C, followed by blending with PEG 400. The mixture was then filled into gelatin capsules and allowed to cool to room temperature, during which time the contents of the capsule became partially solidified. An additional precipitation-resistant vehicle was prepared by dissolving polyethylene glycol-15-hydroxystearate (Solutol® HS 15, BASF) in PEG 400.

For comparison, aqueous suspensions, with average particle sizes ranging from ~60 μm to <1 μm and concentrations of ~20–25 mg/mL, were also evaluated in rats and/or dogs. An “as is” suspension with no particle size reduction was prepared by suspending Compound A, as received (average particle size ~60 μm, determined by microscopy), in a vehicle composed of 0.5% methylcellulose, with 0.1% Cremophor EL® included as a wetting agent (final concentration 20 mg/mL Compound A). Two different methods were used for the preparation of aqueous suspensions with

**Table 1**
In vitro screening of the resistance of various formulations of compound A to dilution-induced precipitation.

| Vehicle | Conc. of Compound A (mg/mL) | Approximate precipitation time (min) | | | | |
|---|---|---|---|---|---|---|
| | | Dilution factor | | | | |
| | | 2× | 5× | 10× | 25× | 100× |
| PEG 400 | 20 | ND[a] | ND | Instant | ND | ND |
| 33.3% PEG 400/33.3% ethanol/33.3% water | 15 | Instant | ND | Instant | ND | ND |
| 5% NMP/5% Cremophor EL®/90% PEG 400 | 10 | ND | ND | Instant | ND | ND |
| 50% ethanol/50% PG | 45 | ND | ND | Instant | ND | ND |
| 9% polysorbate 80/91% PEG 400 | 30 | ND | ND | Instant | ND | ND |
| 15% polysorbate 80/85% PEG 400 | 20 | ND | ND | 60 | ND | 90 |
| 23% Solutol® HS15/77% PEG 400 | 20 | ND | ND | 60 | ND | ND |
| 50% ethanol/50% Cremophor EL® | 45 | ND | ND | 5 | ND | 5 |
| | 22.5 | ND | ND | 30 | ND | 30 |
| 35% ethanol/65% Cremophor EL® | 40 | ND | ND | 20 | ND | 60 |
| | 30 | ND | ND | 30 | ND | 90 |
| 20% vitamin E TPGS/8% polysorbate | 55 | ND | 15 | 15 | 15 | ND |
| 80/7% ethanol/65% PEG 400 | 6.25 | >300 | ND | >300 | >300 | >300 |
| 20% vitamin E TPGS/8% polysorbate 80/10% ethanol/62% PEG 400 | 15 | >300 | >300 | >300 | ND | >300 |

PEG, polyethylene glycol; NMP, N-methyl polyvinylpyrrolidone; TPGS, d-alpha tocopheryl polyethylene glycol 1000 succinate.
[a] Value not determined.

particle size below 1 μm. In the first method, Compound A, with average initial particle size ~60 μm, was suspended in 4 mL of a solution of polysorbate 80 in water (concentration of Compound A and polysorbate 80 in this initial suspension were approximately twice that for the final formulation). The mixture was then passed, manually, at least 10 times through an EmulsiFlex-B3™ microfluidizer (Avestin, Inc.) to reduce the particle size of Compound A to less than 1 μm. A solution of methylcellulose, with concentration also approximately twice that of the final suspension, was added to the particle size-reduced suspension of Compound A, in a ratio of 1:1, and mixed well to produce the final suspension formulation with a drug concentration of approximately 25 mg/mL. Suspensions prepared by this technique were used for in vivo evaluation in rats. In the second method, Compound A was slurried with 2.6% hydroxypropyl cellulose (HPC)-SL and was milled in an Elan NanoMill-01® for 45 min at a speed of ~600 rpm. The resulting suspension was diluted to 25 mg/mL with vehicle and filtered through a 5-μm filter. Measurement of potency by HPLC confirmed that no drug was lost upon filtration. Suspensions prepared by this technique were used for in vivo evaluation in dogs. The particle size distribution of each suspension was monitored over several days for any significant growth using a Horiba LA-910 Laser Light Scattering Particle Sizer.

### 2.3. Evaluation of precipitation resistance

Resistance of solutions to dilution-induced precipitation of Compound A was evaluated by rapid, bolus addition of water to solutions containing various concentrations of Compound A. Similar precipitation resistance studies, using simulated gastric and intestinal fluids, have been conducted by others (Dai et al., 2007, 2008). However, for the present studies, water was selected as the diluent rather than simulated gastric or intestinal fluid, because Compound A is nonionizable, and its solubility is unaffected by the pH of the medium. In addition, water was considered as worst case for inducing precipitation, as gastrointestinal fluids may contain surfactants or other components that could potentially enhance solubility of Compound A. The dilution factors ranged from 2- to 100-fold. Following dilution, the samples were agitated manually and observed visually for appearance of any precipitate. Solutions showing no immediate precipitate formation were considered to be "precipitation-resistant." It should be noted that the level of environmental particulates was not controlled for any of the formulations evaluated. Given that such particulates can serve as nucleation sites and accelerate precipitation in supersaturated solutions, the study design further enables worst case evaluation of the ability of various formulations to resist dilution-induced precipitation. A summary of solvent combinations screened can be found in Table 1.

The results of initial screening by visual inspection were confirmed using a more quantitative dilution experiment in which 2 mL of a 10 mg/mL solution of Compound A in 20% Solutol® HS15/80% PEG 400 was added into 900 mL of water at 37 °C in a USP dissolution apparatus type II (rotating paddle), with paddle speed 50 rpm. Following introduction of the Compound A/Solutol® HS15/PEG 400 solution, samples were periodically withdrawn from the vessel, filtered through a 0.45 μm syringe filter to remove any precipitated compound, and analyzed for drug concentration by HPLC.

The effect of dilution of Compound A, formulated in a vehicle composed of 20% vitamin E TPGS, 8.5% polysorbate 80, 10% ethanol, 61.5% PEG 400, was also evaluated at drug concentrations of 15 mg/mL, 30 mg/mL and 60 mg/mL. The USP rotating paddle method with paddle speed 50 rpm was used for these studies. The dilution medium used was 480 mL of water at 37 °C. A volume of 20 mL of formulation was added to the medium, and the concentration of Compound A was measured by a fiber optic UV probe detector (Model DPT-DISS, LeapTec) at 315 nm.

While the relatively limited number of formulations screened in these initial studies could be analyzed using a manual approach, high-throughput methods for evaluating precipitation resistance using a 96-well plate format with spectrophotometric or HPLC analysis have been developed and can be particularly useful for quantitatively screening a wide range of formulation compositions (Dai et al., 2007, 2008; Chandran et al., 2011).

### 2.4. Pharmacokinetic studies

The in vivo exposures of selected precipitation-resistant formulations were compared to aqueous suspension in rats, dogs, and humans. Preclinical formulations and dose levels evaluated in rats and dogs are listed in Tables 2 and 3. For rat PK studies, each formulation was administered by oral gavage to fasted adult Sprague Dawley rats ($n$ = 2–18), and blood samples were withdrawn at pre-determined intervals for analysis of drug concentration. For dog PK studies, formulations were dosed either by capsule or oral

**Table 2**
Comparison of in vivo exposures of Compound A obtained using various formulation approaches in rats.

| Dosage form type | Vehicle[a] | Concentration Compound A (mg/mL) | Dose volume (mL) | Approximate dose (mg/kg) | Mean AUC[b] (μM h) | Mean $C_{max}$ (μM) |
|---|---|---|---|---|---|---|
| Non precipitation-resistant solution | 33.3% PEG 200/33.3% ethanol/33.3% water | 2 | 1.5 | 10 | 6.4 ± 1.5[c] | 3.3 ± 1.1 |
| | PEG 400 | 20 | 0.03 | 2 | 2.6[d,e] | 0.3 |
| | | 20 | 0.3 | 20 | 23.3[d,e] | 2.4 |
| | | 20 | 3 | 200 | 57[d,e] | 4.9 |
| | 95% PEG 400/5% polysorbate 80 | 4 | 1.5 | 20 | ND[f] | 4.0 ± 0.2 |
| | 90% PEG 400/10% polysorbate 80 | 4 | 1.5 | 20 | ND | 4.4 ± 0.2 |
| | 95% PEG 400/5% Cremophor EL® | 4 | 1.5 | 20 | ND | 4.0 ± 0.2 |
| Precipitation-resistant solution | 80% PEG 400/20% Solutol® HS15 | 30 | 3 | 300 | 34.1 ± 3.4 | 5.3 ± 0.8 |
| | 61.2% PEG 400/20.4% vitamin E TPGS/10.2% polysorbate 80/8.2% ethanol | 0.8 | 4.5 | 12 | 11.4 ± 0.9[e,g] | 0.8 ± 0.2 |
| | | 4 | 4.5 | 60 | 36.3 ± 6.9[e,g] | 3.5 ± 1.0 |
| | | 20 | 4.5 | 300 | 170.8 ± 20.6[e,g] | 17.0 ± 8.1 |
| Aqueous suspension (EmulsiFlex™) (particle size: $D_{84}$ < 1 μm) | 0.1% (w/v) methylcellulose/0.1% (w/v) polysorbate 80 | 26 | 3.1 | 270 | 65 ± 13[c] | 10 ± 2.1 |

[a] Vehicle proportions expressed as weight/weight unless otherwise indicated.
[b] $n = 3$, unless otherwise indicated. AUC values are reported as $AUC_{0-\infty}$ except in cases where $AUC_{t-\infty}$ is greater than 20% of $AUC_{0-\infty}$, where $t$ represents the time of the last measured plasma concentration. In the latter case, AUC values are reported as $AUC_{0-t}$.
[c] AUC reported as $AUC_{0-10\,h}$ for these selected formulations.
[d] $n = 2$ for these selected formulations.
[e] AUC reported as $AUC_{0-24\,h}$ for these selected formulations.
[f] ND: Value not determined.
[g] $n = 18$ for these selected formulations.

**Table 3**
Comparison of in vivo exposures of Compound A obtained using various formulation approaches in dogs.

| Dosage form type | Vehicle[a] | Concentration Compound A (mg/mL) | Dose volume (mL) | Approximate dose (mg/kg) | Mean AUC[b] (μM h) | Mean $C_{max}$ (μM) |
|---|---|---|---|---|---|---|
| Non-precipitation-resistant solution | 33.3% PEG 200/33.3% ethanol/33.3% water | 10 | 3.5 | 3.5 | 3.6 ± 0.9 | 1.0 ± 0.3 |
| | PEG 400 | 3.5 | 7 | 2.5 | 2.5 ± 1.5[c] | 0.6 ± 0.4 |
| | | 30 | 16.5 | 50 | 26 ± 8.0[d] | 4.2 ± 1.9 |
| Precipitation-resistant solution | 80% PEG 400/20% Solutol® HS15 | 10 | 5 | 5 | 1.2 ± 0.1 | 0.7 ± 0.1 |
| | 65% PEG 400/20% vitamin E TPGS/7.5% polysorbate 80/7.5% ethanol | 6.25 | 5.5 | 3.5 | 1.9 ± 0.4[c] | 1.1 ± 0.8 |
| Precipitation-resistant semisolid dispersion | 50% PEG 400/50% vitamin E TPGS | 2.5 | 20 | 5 | 1.6 ± 0.9[e] | 0.8 ± 0.3 |
| | | 25 | 20 | 50 | 25 ± 13[e] | 9.1 ± 3.2 |
| Aqueous suspension (NanoMill®) (particle size: $D_{50}$ < 276 nm) | 2.6% (w/v) hydroxypropylcellulose SL | 25 | 20 | 5 | 1.7 ± 0.3 | 0.9 ± 0.3 |
| Aqueous suspension (unprocessed) (particle size: 10–100 μm) | 0.5% (w/v) methylcellulose/0.1% (w/v) Cremophor EL® | 20 | 20 | 40 | 6.8 ± 2.4[c] | 1.1 ± 0.7 |

[a] Vehicle proportions expressed as weight/weight unless otherwise indicated.
[b] $n = 3$, unless otherwise indicated. AUC values are reported as $AUC_{0-\infty}$ except in cases where $AUC_{t-\infty}$ is greater than 20% of $AUC_{0-\infty}$, where $t$ represents the time of the last measured plasma concentration. In the latter case, AUC values are reported as $AUC_{0-\infty}$.
[c] AUC reported as $AUC_{0-32\,h}$ for these selected formulations.
[d] AUC reported as $AUC_{0-24\,h}$ for this selected formulation.
[e] $n = 6$ for these selected formulations.

gavage to fasted adult beagle dogs ($n$ = 3–6), and blood samples were withdrawn at pre-determined intervals for analysis.

Human PK studies were conducted in fasted, healthy volunteers ($n$ = 5–6). Compositions of the oral dosing solutions and suspensions are listed in Tables 4 and 5, respectively. The formulations were prepared by constituting the clinical drug in bottle product with two different vehicles, at a designated compounding pharmacy, using instructions and components supplied by Bristol-Myers Squibb. The precipitation-resistant solution formulation was dosed using an oral syringe, followed by 240 mL of water, while the aqueous suspension formulation was administered by instructing the subject to drink the entire contents of the vial, followed by a complete vial washout to obtain the full dose. In case of oral solution, the total dose volume administered to each subject was 20 mL. For dose levels in which active dose volume was less than 20 mL, each subject received a separate volume of placebo solution to adjust the total dose volume to 20 mL. Similar to rats and dogs, blood samples were withdrawn at pre-determined intervals for analysis. Human PK studies comparing solution to suspension were conducted at a dose of 100 mg, using crossover design, with a 7-day washout period between formulations.

All plasma concentration data were treated using non-compartmental methods to generate plasma concentration profiles as a function of time, as well as $C_{max}$ and AUC values for each formulation.

### 2.5. Sample analysis

#### 2.5.1. In vitro

Drug concentrations of in vitro samples were analyzed using reversed phase HPLC. The chromatographic system for assay consisted of a Waters Alliance 2690 HPLC Separations Module, equipped with a controlled-temperature autosampler, maintained at 5 °C, a Waters 486 tunable UV absorbance detector, operated at a wavelength of 215 nm, and a Millennium$^{32}$ Chromatography Manager.

Chromatographic analysis was performed under linear gradient conditions, at ambient temperature, using a YMC ODS-AQ column (150 × 4.6 mm, 3 μm), with a flow rate of 1 mL/min and an injection volume of 50 μL. The mobile phases consisted of water and acetonitrile, each containing 0.1% trifluoroacetic acid.

#### 2.5.2. In vivo

Plasma drug concentrations were analyzed using an LC/MS/MS method. The chromatographic system for assay consisted of a Shimadzu LC 10 AD/VP pump, a Perkin-Elmer PE 200 LC autosampler, and a Micromass Quattro LC mass spectrometer.

Chromatographic analysis was performed under isocratic conditions, at ambient temperature, using a Phenomenex Luna C18(2) column (50 × 2 mm, 5 μm), with a flow rate of 0.3 mL/min and an injection volume of 10 μL. The mobile phase consisted of 15% 10 mM ammonium acetate in water and 85% 10 mM ammonium acetate in a mixture of 90% acetonitrile/10% water. The mass spectrometric parameters used are summarized in Table 6.

## 3. Results and discussion

### 3.1. In vitro precipitation-resistance screening

Results of in vitro precipitation-resistance screening for Compound A in various cosolvent combinations are presented in Table 1 and in Figs. 2 and 3. Formulations containing the surfactants, vitamin E TPGS, polysorbate 80, and Solutol® HS15, showed the highest level of precipitation-resistance. Based on the in vitro data presented in Table 1, a combination of vitamin E TPGS with polysorbate 80 appeared to be most effective in inhibiting dilution-induced precipitation of Compound A compared to formulations containing Solutol® HS15, Cremophor EL®, or polysorbate 80, alone. The potential of vitamin E TPGS to show a synergistic effect in preventing dilution-induced precipitation, when combined with other surfactants, has been observed by others in the literature (Dai et al., 2008).

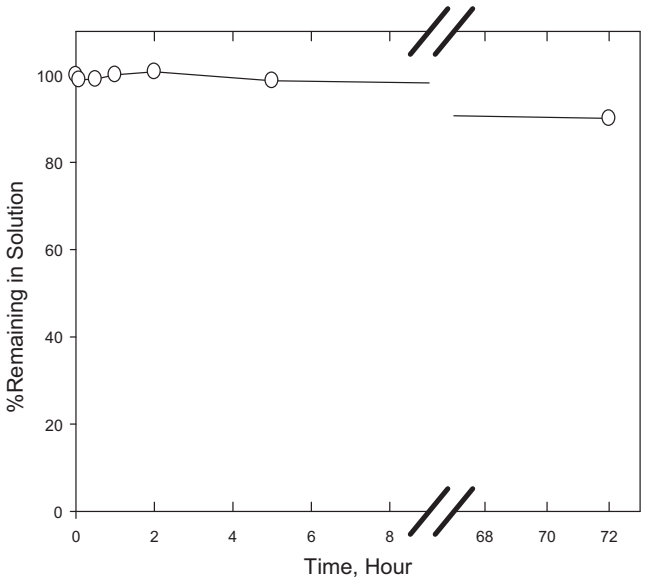


**Fig. 2.** Percent Compound A remaining in solution after dilution when formulated as a precipitation-resistant solution of 10 mg/mL in ~80% PEG 400/20% Solutol HS15® (USP Paddle Method, 50 RPM in 900 mL water at 37 °C).

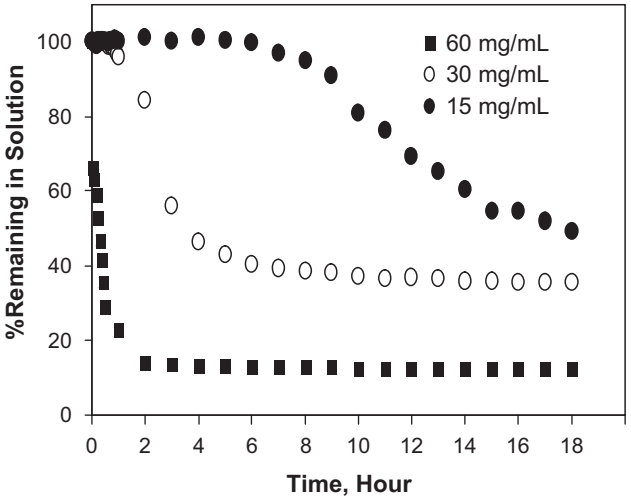


**Fig. 3.** Effect of drug concentration on precipitation resistance of Compound A, formulated in 20 mL of a vehicle containing 20% vitamin E TPGS, 10% alcohol USP, 8.5% polysorbate 80 and 61.5% PEG 400 (USP Paddle Method, 50 RPM in 480 mL water at 37 °C).

Fig. 2 shows the percent Compound A remaining in solution after dilution when formulated as a precipitation-resistant solution, at a concentration of 10 mg/mL, in a vehicle composed of 20% Solutol® HS15/80% PEG 400. The data indicate that the concentration of Compound A in solution remained relatively unchanged for up to 72 h following introduction of 2 mL of the solution concentrate into a dissolution vessel containing 900 mL of water at 37 °C, stirred at 50 rpm.

The effect of the initial formulation concentration of Compound A on the resistance to dilution-induced precipitation is presented in Fig. 3. As would be expected, the precipitation-resistance of

**Table 4**
Dose-dependence of Compound A exposure from precipitation-resistant solution[a] in humans.

| Concentration Compound A[b] (mg/mL) | Dose (mg) | Mean AUC[c] (μM h) | Mean $C_{max}$ (μM) |
|---|---|---|---|
| 5 | 1 ($n$ = 6) | Not determinable | Not determinable |
| 5 | 3 ($n$ = 5) | 0.007 ± 0.009 | 0.002 ± 0.002 |
| 5 | 10 ($n$ = 6) | 0.024 ± 0.010 | 0.009 ± 0.003 |
| 5 | 30 ($n$ = 6) | 0.070 ± 0.019 | 0.026 ± 0.006 |
| 5 | 100 ($n$ = 5) | 0.51 ± 0.30 | 0.16 ± 0.10 |
| 20 | 300 ($n$ = 5) | 3.77 ± 0.02 | 1.11 ± 0.83 |

[a] Vehicle: 61.2% PEG 400/20.4% vitamin E TPGS/10.2% polysorbate 80/8.2% ethanol (proportions expressed as weight/weight).
[b] Total dose volume for precipitation-resistant formulation brought to 20 mL across all dose levels using blank vehicle (administered separately from active solution).
[c] AUC values are reported as $AUC_{0-\infty}$.

**Table 5**
Comparison of in vivo exposures of Compound A, obtained using two formulation approaches in humans (dose = 100 mg, $n$ = 6).

| Dosage form type | Vehicle[a] | Concentration Compound A (mg/mL) | Mean AUC[b] (μM h) | Mean $C_{max}$ (μM) |
|---|---|---|---|---|
| Precipitation-resistant solution | 61.2% PEG 400/20.4% vitamin E TPGS/10.2% polysorbate 80/8.2% ethanol | 5 | 0.30 ± 0.14 | 0.11 ± 0.03 |
| Aqueous suspension (unprocessed; particle size: 10–100 μm) | 0.05% (w/v) polysorbate 80 | 10 | 1.09 ± 0.96[c] | 0.03 ± 0.01 |

[a] Vehicle proportions expressed as weight/weight unless otherwise indicated.
[b] AUC values are reported as $AUC_{0-\infty}$ except in cases where $AUC_{t-\infty}$ is greater than 20% of $AUC_{0-\infty}$, where $t$ represents the time of the last measured plasma concentration. In the latter case, AUC values are reported as $AUC_{0-t}$.
[c] AUC reported as $AUC_{0-72\,h}$

**Table 6**
Mass spectrometric conditions used in the analysis of plasma samples for Compound A.

| Parameter | Value |
|---|---|
| Ion polarity | Positive |
| Source type | Electrospray |
| Drying gas | Nitrogen, UHP 838 L/h |
| Capillary voltage (V) | 1000 |
| Cone (V) | 33 |
| Extractor (V) | 3.0 |
| RF lens (V) | 0 |
| Source block temperature (°C) | 120 |
| Desolvation Temperature (°C) | 300 |
| Multiplier (V) | 650 |
| LM resolution 1 | 12.5 |
| HM resolution 1 | 12.5 |
| Ienergy 1 (V) | 1.6 |
| Entrance (V) | 20 |
| Collision (V) | 17 |
| Exit (V) | 22 |
| LM resolution 2 | 12.5 |
| HM resolution w | 12.5 |
| Ienergy 2 (V) | 1.5 |

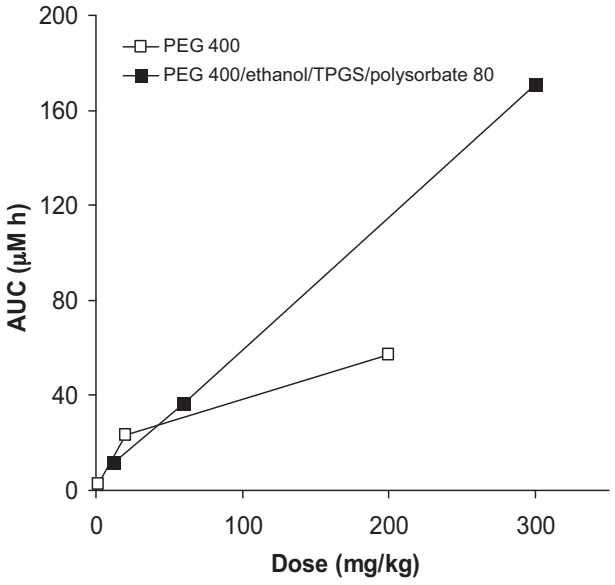


**Fig. 4.** Comparison of dose proportionality of Compound A exposures from precipitation-resistant and non precipitation-resistant solution formulations in rats. Formulations consisted of 20 mg/mL Compound A in PEG 400 or 61.2% PEG 400; 20.4% vitamin E TPGS; 8.2% alcohol, USP; 10.2% polysorbate 80 (additional details listed in Table 2).

Compound A in a given formulation was dependent upon initial concentrations of Compound A. A solution of 60 mg/mL Compound A in a vehicle composed of 20% vitamin E TPGS, 10% alcohol USP, 8.5% polysorbate 80, and 61.5% PEG 400 exhibited immediate dilution-induced precipitation, while a solution of 15 mg/mL Compound A in the same formulation was resistant to precipitation for 6–8 h following addition to water at 37 °C. At 30 mg/mL, dilution-induced precipitation of Compound A was also observed, but at a slower rate than for 60 mg/mL (resistance to precipitation was maintained for approximately 30 min to 1 h following addition of the 30 mg/mL formulation to water). Given that precipitation of compounds from supersaturated solutions is dependent upon the rate of particle nucleation, more highly concentrated solutions would be expected to nucleate, and therefore, precipitate, more rapidly than less concentrated solutions.

### 3.2. In vivo exposure evaluations

Results of preclinical in vivo screening of various formulations investigated for Compound A are summarized in Tables 2 and 3. For lower dose levels (~10 mg/kg) in rats, a precipitation-resistant solution, consisting of PEG 400/polysorbate 80/vitamin E TPGS/ethanol, showed somewhat greater plasma exposure but a lower $C_{max}$ value than the non precipitation-resistant system, composed of PEG 200/ethanol/water (Table 2). At higher doses (~200–300 mg/kg), the precipitation-resistant system provided significant advantage over both non precipitation-resistant solutions and particle size-reduced aqueous suspensions in terms of

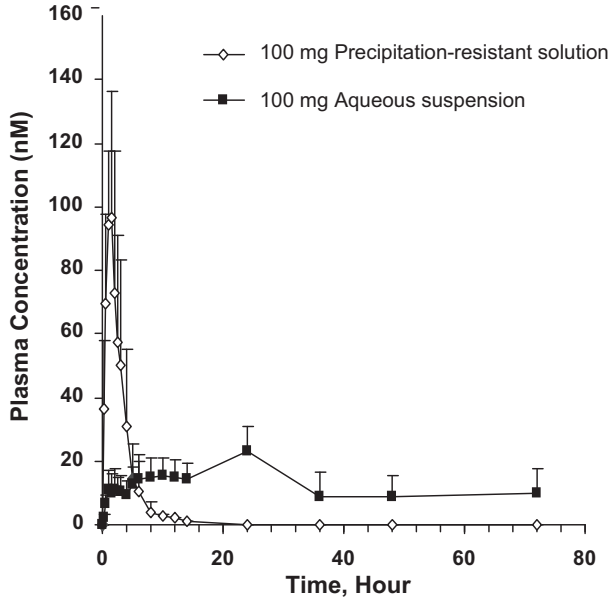


**Fig. 5.** Comparison of plasma exposures of Compound A from precipitation-resistant nonaqueous solution and aqueous suspension in humans. Formulations consisted of 5 mg/mL Compound A in 61.5% PEG 400; 20% vitamin E TPGS; 10% alcohol, USP; 8.5% Polysorbate 80 (solution) or 0.05% Polysorbate 80, NF, in Sterile Water for Injection (suspension).

both AUC and $C_{max}$ values. The formulation containing Solutol® HS15 provided lower exposures than the formulation containing vitamin E TPGS. However, the concentration of Compound A in the Solutol®-containing formulation evaluated in rats was higher than that used both in the in vitro testing and in the TPGS-containing formulation. Since the rate of precipitation from solution is dependent upon solution concentration prior to dilution, it is possible that the Solutol®-containing formulation was not completely resistant to precipitation at 30 mg/mL. It is also interesting to note that the particle size-reduced suspension produced greater exposures and $C_{max}$ levels than did the solution in PEG 400. Precipitation of large or clumped particles of Compound A from PEG 400 in the GI tract after dosing could be a possible explanation for this observation.

The dose proportionality of exposures of Compound A from precipitation-resistant and non-precipitation-resistant solution formulations in rats is presented in Fig. 4. The data show that nonlinear exposures are observed with increasing dose of Compound A from PEG 400, a non-precipitation-resistant system, while good dose proportionality was achieved using the precipitation-resistant solution formulation in rats.

Dogs showed negligible differences in maximum plasma concentrations and overall exposures of Compound A for precipitation-resistant solutions, non precipitation-resistant solutions, and particle size-reduced aqueous suspensions at low doses (~5 mg/kg) (Table 3). The AUC values for the solution formulations were also similar at high dose (40–50 mg/kg) but were greater than suspension. The greatest maximum plasma concentrations, however, were achieved using the precipitation-resistant system containing vitamin E TPGS. Other surfactant-containing formulations were not evaluated for performance at high dose in dogs. In general, absorption from all formulations was lower in dogs, and large increases in $C_{max}$ and AUC of Compound A were more challenging to achieve in dogs compared to rats. These results suggest that solubility and dissolution rate play a significant role in the absorption of Compound A in rats, but may contribute less significantly in dogs. Of the various formulation approaches screened, precipitation-resistant solutions containing vitamin E TPGS provided the greatest $C_{max}$ and AUC values at high doses needed for toxicology studies.

Results of pharmacokinetic evaluations comparing nonaqueous, precipitation-resistant solution and aqueous suspension formulations of Compound A after a single oral dose in humans are presented in Tables 4 and 5 and in Fig. 5. Exposures increased approximately linearly with dose levels from 3 to 10 mg but increased more than dose proportionally between dose levels of 10 and 300 mg. In contrast, plasma concentrations from aqueous suspension, dosed at 100 mg, were much lower (approximately one quarter that of solution at the same dose level). In addition, surprisingly prolonged drug exposures that were sustained for at least 72 h following dosing were observed, suggesting absorption-controlled pharmacokinetics from the suspension formulation in humans (suspension formulations did not show similar sustained exposures in animal studies). Dissolution rate-limited absorption could have contributed to this type of pharmacokinetic profile, but additional studies would be needed to better understand critical factors responsible for the pharmacokinetic profile of Compound A from aqueous suspension.

In addition, a wide range of dose volumes and surfactant types and concentrations were used in the PK studies of solution formulations of Compound A in rats and dogs. As surfactant concentration in the gastrointestinal tract could also potentially influence in vivo performance, it may be informative to further evaluate the impact of these variables for specific surfactant types on the absorption of Compound A in future investigations.

## 4. Conclusions

A nonaqueous, precipitation-resistant formulation approach was shown to increase plasma concentration of Compound A significantly, compared to aqueous suspension or non-precipitation-resistant formulations, in rats, dogs, and humans. While particle size reduction also increased absorption, particularly in rats, greatest overall $C_{max}$ values were achieved with precipitation-resistant solubilized systems containing vitamin E TPGS. Inhibition of nucleation and crystal growth of Compound A due to increased viscosity of the vehicle components may be a possible mechanism contributing to the resistance of these formulations to dilution-induced precipitation. In addition, several of the excipients evaluated are similar to those used in self-emulsifying drug delivery systems (SEDDS) (Gao et al., 2003; Dai et al., 2007). Therefore, solubilization of Compound A through micelle formation could also have contributed to precipitation resistance. Solubilized formulations lacking surfactants showed erratic behavior, possibly due to rapid precipitation of Compound A, with uncontrolled particle size, upon contact with the aqueous environment of the gastrointestinal tract following dosing. Results of the current investigations show that precipitation-resistant formulations may be highly valuable for obtaining in vivo exposures needed for successful toxicological and early clinical pharmacokinetic and tolerability evaluation of poorly water-soluble compounds, particularly in cases where salt formation is difficult or impossible.

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