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RESEARCH PAPER

Powder-in-Bottle Formulation of SU011248. Enabling Rapid Progression into Human Clinical Trials

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ABSTRACT

SU011248 is an oral, multitargeted receptor tyrosine kinase inhibitor (anti PDGFR, VEGFR, Kit, and Flt3) for the treatment of solid tumors. The powder-in-bottle (PIB) approach was used to accelerate development and introduction into Phase I clinical trials. This approach consists of extemporaneously compounding the active pharmaceutical ingredient (API) into a solution or a suspension in the clinic prior to oral administration. The development consisted of physico-chemical assessment, constitution fluid selection, weighing and dosing validation, and stability evaluation of API, before and after constitution with the fluid. Of the oral liquids evaluated, apple juice was selected as the constitution fluid. Particle size of SU011248 had an impact on the weighing validation and the dissolution time. Particle size specifications of breadth $d_{90} < 180$ μm and length $d_{90} < 750$ μm were set to achieve pharmaceutical acceptability. Dosing validation studies showed complete recovery of SU011248 from the bottle over a dose range of 10 to 2200 mg. SU011248 is stable as the solid API. Following constitution with apple juice, the product is stable through the predicted duration of compounding and dosing at the clinical site. This approach provided a high degree of dosing flexibility during the initial phase of clinical trials. Additionally, the PIB approach reduced the time and API required for clinical development and supplies to < 2 months and < 100 gm, respectively.

Key Words: SU011248; Powder in bottle formulation; Constitution.

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INTRODUCTION

SU011248 is an oral, multitargeted receptor tyrosine kinase inhibitor, which inhibits PDGFR, KIT, Flt3, and VEGFR. This results in tumor growth delay and regression in preclinical models.[1] Preformulation studies determined SU011248 to have an ionization constant (pKa) of 8.5 and an octanol–water partition coefficient, Log P and Log D_{pH6} of 3.1 and 1.4, respectively. The solubility in certain pH 4.5–6 buffers ranged from 0.4–2.6 mg/mL. The permeability (P_{app}) of the molecule was experimentally determined using Caco-2 cells as 4.7×10^{-6} cm/sec.

The need for accelerated ''First Time in Man'' (FTIM) pharmacokinetic evaluation, predicted low dose, and acceptable pharmaceutical properties made SU011248 a good candidate for evaluation as a powder-in-bottle (PIB) formulation. The PIB approach consists of constituting the active pharmaceutical ingredient (API) extemporaneously in the clinic as a solution or a suspension. Such preparations are routinely used in the clinic, especially for pediatric, geriatric, and other patients, who have difficulty in swallowing solid dosage forms such as capsules and tablets.[2–5] The preparations are prepared extemporaneously using commercially marketed solid dosage forms and commercially available suspending solutions such as Ora-Sweet® and Ora-Plus®. However, most of these published studies[2–6] have focused on the stability of these preparations. While this concept is generally accepted in the industry, the developmental aspects or such an approach are rarely discussed in literature.[6]

This report describes the development of a PIB formulation to be constituted (as a solution or suspension) with a suitable fluid, as a FTIM formulation for SU011248. Various studies associated with the PIB development, such as constitution fluid selection, particle size evaluation, weighing and dosing analysis, and stability of preconstituted and constituted product, are described.

EXPERIMENTAL

Chemicals

SU011248 was obtained from SUGEN Chemistry Department (South San Francisco, CA). Orange and apple juices were obtained from Safeway Foods. All other chemicals and reagents were obtained from local suppliers.

Constitution Fluid Selection

Different acidic media such as low pH buffers and juices were investigated for acceptability as the reconstitution fluid. The criteria for selection were ability to solubilize SU011248, suspendability, commercial availability, and potential masking effects.

The solubility of SU011248 in various buffers such as hydrochloride and citrate, and fruit juices such as orange and apple juice was determined by adding excess compound to the constitution fluid and shaking on a mechanical shaker for 24 hours at ~21–25° C. Following shaking, the samples were filtered through 0.2 μm syringe filters (Acrodisc, Gelman Sciences, Ann Arbor, MI) and the filtrate analyzed for SU011248 content by high-performance liquid chromatography (HPLC).

Suspendability was determined following selection of a suitable particle size for manufacture. Particles of the selected size of SU011248 (~100 mg) were added to the media (5 mL) and time-to-settle determined qualitatively. Commercial acceptability was based on market availability and potential masking effects such as color and taste were evaluated qualitatively.

Particle Size Evaluation

Sizing

A weighed sample of SU011248 was taken and sieved on a sonic sifter for 30 minutes. Following sieving, samples were collected on sieves US Standard Size 20, 30, 40, 60, 80, 100, 200, and 325. Representative samples of the sieved SU011248 from the different sieves were observed under a microscope (Nikon, Photostat light microscope, Millburn, NJ) and particle size determined.

Weighing and Suspendability

The effect of particle size on weighing the low dose PIB was evaluated by weighing 10 mg of the sieved SU011248 samples on an analytical balance into containers equivalent to the one for the clinical trials. The samples were weighed and difficulty in obtaining the target weights within a 10% deviation was determined qualitatively. The effect of particle size on suspendability was determined by suspending the sieved fractions of SU011248 (~100 mg) in media (5 mL) followed by an estimation of ''time required for settling.''

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Dissolution Profiles

Dissolution profile of the various sieved fractions of SU011248 was determined by adding approximately 100 mg SU011248 from the various sieved fractions to 800 mL apple juice. A Distek dissolution system (Distek Inc., North Brunswick, NJ) equipped with a Hewlett Packard autosampler and UV-Visible spectrophotometer (Hewlett Packard, Wilmington, DE) was used. The paddles were run at 50 rpm and the temperature maintained at 25° C. Samples were withdrawn using the autosampler at various time points and analyzed at 445 nm using a standard curve. The dissolution profiles were plotted and effect of particle size on the dissolution noted.

Weighing and Dosing Validation

For the weighing validation, the specified quantity of the SU011248 active was weighed into Type I glass containers (100 mL capacity, equivalent to the ones to be used in the clinical trials) at the low (10 mg) and the mid (35 mg) dose levels and appropriately labeled. The contents of each container were dissolved completely in the HPLC diluent to obtain approximately 0.2 mg/mL API concentration. The samples were then assayed using the HPLC method.

The dosing validation studies were conducted by simulating the dosing process and assaying any residual SU011248 in the PIB container. The specified quantity of SU011248 was weighed into Type I glass vials (100 mL capacity) at three doses, low (10 mg), mid (35 mg), and high (2200 mg) and appropriately labeled. SU011248 in the PIB container was constituted with 75 mL apple juice added as 50- and 25-mL aliquots, with a minute swirling after each addition. The constituted fluid was discarded. This was followed by two 50-mL water rinses with a one-minute swirl between rinses of the PIB container. These rinses were discarded. The residual API content of the PIB container was collected in a 50-mL volumetric flask, with two 20-mL washings using the HPLC diluent. The volume was made up to mark with the same diluent and contents assayed using a HPLC method. The contents of the volumetric flask represent the leftover dose in the PIB container post constitution and administration to the patient.

Stability Studies

Preconstitution Stability

A specified quantity of SU011248 was weighed into Type I glass vials (100 mL capacity) at a single dose (50 mg). These samples were closed with rubber caps, sealed with an aluminum seal, and placed at various conditions (40° C/75% RH, 60° C, 60° C/75% RH, and 80° C). At specified time points (0 and 30 days), stability samples were dissolved completely in the diluent and analyzed for SU011248 content by HPLC.

Postconstitution Stability

The containers were prepared as indicated in the dosing validation studies. SU011248 in the PIB container was constituted with 75 mL apple juice added

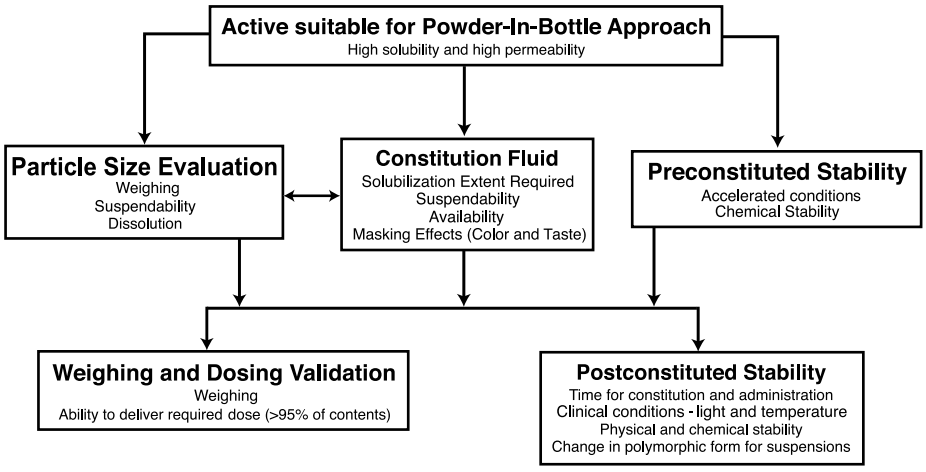

Figure 1. Developmental aspects of a powder-in-bottle approach for first-time-in-man study.

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Table 1. Solubility of SU011248 in various solvents.

Excipients	Solubility of SU011248 (mg/mL)	pH at equilibrium
Water	<0.1	9.2[a]
20 mM HCl–KCl, pH 2.0	2.6[a]	4.6[a]
10 mM citrate buffer pH 3.5	3.7±0.02[b]	4.1
5 mM HCL–KCl with 1% Tween 20	4.8[a]	ND[d]
Orange juice	4 to 7[c]	3.8–4.2[c]
Apple juice	10–15[c]	4.4[c]

[a]Results reported are mean of duplicate determinations.
[b]Results reported are mean ± standard deviation of n=3.
[c]Results reported as a range.
[d]Not determined.

as 50- and 25-mL aliquots, with a minute swirling after each addition. The constituted solution was then placed on stability under ambient temperature (~22–25° C) and light conditions (fluorescent light), samples collected at set time points, and analyzed by HPLC.

High Pressure Liquid Chromatographic Method

An Agilent HPLC (HP1100 or equivalent) equipped with a diode array detector was used. The column was a Waters XTerra™ PR-18, 5 μm, 4.6 × 150 mm at 1 mL/min. The mobile phase consisted of Solvent A and acetonitrile. Solvent A consisted of a mixture of 0.05 M potassium phosphate and 0.1% triethyl amine, pH adjusted to 5. The elution method was a linear gradient from 90% to 37% Solvent A over 36 minutes and then back to 90% Solvent A over 10 minutes. The column was then held at 90% Solvent A for 10 minutes. The injection volume was 50 μL. The column was maintained at 45° C and detection wavelength was 268 nm.

The HPLC diluent for the standards and the samples was a 90:10 mixture of Solvent A and acetonitrile.

RESULTS AND DISCUSSION

The developmental aspects of the PIB approach are shown in Fig. 1. The chart outlines the steps required and taken to enable early FTIM studies using this approach.

Constitution Fluid Selection

The PIB formulation is constituted with an appropriate fluid to form a solution or a suspension prior to administration. Since SU011248 can be expected to show high solubility at low pH and the preference to administer the PIB as a solution, various buffers such as hydrochloride and citrate and fruit juices such as orange and apple juice were considered appropriate for constitution and evaluated. The solubility results are listed in Table 1. The solubility of SU011248 is much higher (2.6–4.8 mg/mL) in the low pH buffers as compared to that in water (<0.1 mg/mL). High solubility (4–15 mg/mL) was also observed in commercially available juices such as orange and apple juices. This is expected, since orange juice and apple juice contain citric and malic acids, respectively, and the pH of these juices is typically between 3 to 4.

Table 2. Evaluation of various fluids for constitution of the SU011248 PIB.

Constitution fluids evaluated	Solubilization	Suspendability	Commercial availability	Potential taste masking
Water	–	–	++	–
HCl–KCl buffer[a]	++	+	–	Unknown
Citrate buffer[b]	+	+	+	+
Orange juice	+	++	++	++[c]
Apple juice	++	++	++	++[c]
Ora-Sweet®	ND	++[d]	++	++[e]
Ora-Plus®	ND	++[d]	++	++[e]

[a]20 mM HCl–KCl buffer at pH 2.0.
[b]300 mM citrate buffer at pH 4.5.
[c]Citrates or juices containing sugars may attenuate bitterness (if any) of the API.
[d]High suspendability expected due to presence of suspension agents.
[e]Contains sugars and flavoring agents.

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Table 3. Size analysis of sieved fractions.

Sieve fraction		Size analysis by microscopy 90% below	
Sieve #	Pore size (μm)	Length (μm)	Breadth (μm)
60–40	250–425	1000	400
80–60	180–250	750	250
100–80	150–180	750	180
200–100	75–150	750	150

The attributes used to evaluate the constitution fluids and the results obtained are given in Table 2. Fruit juices such as apple and orange juice met the desirable criteria for a constitution fluid such as suspendability and commercial availability. The juices provided color masking (complement with yellow-orange color of SU011248) and have the potential to provide a degree of taste masking due to the presence of sugars in the media. In addition, juices have a viscosity closer to water compared to commercially available suspending vehicles such as Ora-Sweet and Ora-Plus, which can affect dissolution due to their higher viscosity.[6]

Apple juice was selected as the constitution fluid and allows the dosing of up to 750 mg SU011248 (using 75 mL apple juice) as a solution. Although orange juice was acceptable and has not been indicated in potential inhibition of enzymes as seen with grapefruit juices,[7,8] the possibility of such interactions precluded its use.

Particle Size Evaluation

Studies were designed to identify the desirable range of particle size based on two factors: the ability to accurately weigh required doses and to identify a maximum particle size that does not affect the dissolution profile of SU011248 in the chosen constitution fluid.

Size analyses of the sieved fractions were carried out and are given in Table 3. SU011248 used for formulation development had a wide particle size distribution and sometimes, hard agglomerates, depending on the API lot. However, the sieved material gave a narrow size range where the breadth of the particles corresponded to the pore size of sieve through which the material was passed. Since SU011248 was observed to show a needle-shaped particle, the length was also monitored and observed to be constant at about 750 μm.

Weighing studies showed that fractions collected over sieves 20 and 30 (particle size>600 μm) were difficult to weigh accurately at the 10-mg level. This is attributed to the large sizes of the agglomerated SU011248, which prevented minor adjustments of weights. Suspension studies indicated that efficient suspension with long-time-to-settle was obtained with fractions passed through sieve # 80 and below corresponding to a particle size of<180 μm.

The dissolution profiles of SU011248 in apple juice are shown in Fig. 2. The dissolution of SU011248 was observed to reach completion after 50 to 60

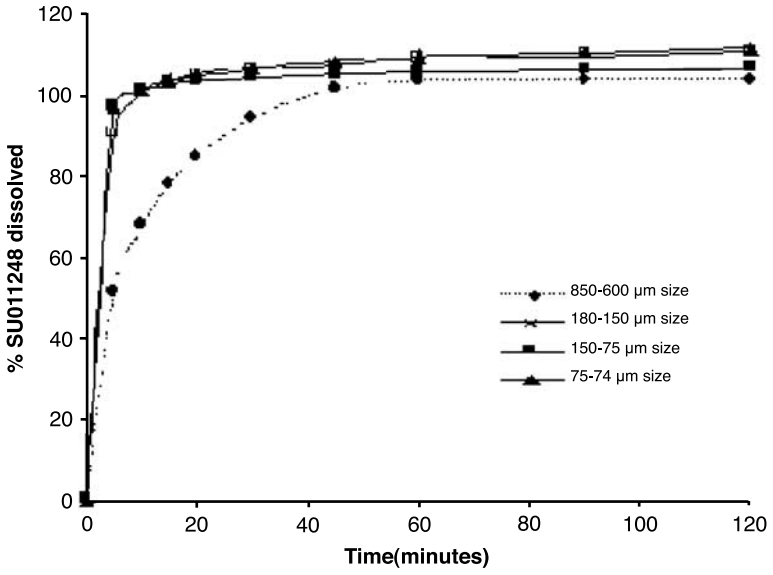

Figure 2. Effect of particle size on dissolution of SU011248 in apple juice.

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Table 4. Weighing and dosing validation of the SU011248 PIB.

Weighing validation	
Dose (mg/vial)	Percent LC
10	102.8
35	99.7
Dosing validation	
Dose (mg/vial)	Percent LC remaining
10	0.11
35	0.04
2200	0.10

Results reported are mean of duplicate determinations.
LC: label claim.
Target for weighing validation: 100±5% of label claim.
Target for dosing validation: <5% of label claim remaining in the vial following dosing.

minutes, irrespective of particle size. However, there is a difference in the dissolution profiles between the fractions above and below the 180 μm pore size. While >90% was dissolved rapidly within 2–5 minutes for fractions collected below 180 μm particle size, the dissolution in the 850–600 μm fraction was slower with approximately 80% dissolved after 20 minutes and complete dissolution only after 60 minutes. This difference in dissolution rates can be attributed to the lower surface area available for dissolution of the larger particles compared to the surface area of the smaller particles.

Since particle size impacted weighing, suspension, and dissolution of the API, specifications were set for a desirable particle size. Consequently, for the clinical manufacture the API was passed through US sieve # 80 (180 μm pore size), which provided the recommended particle size of 90% below 180 μm breadth and 750 μm length.

Weighing and Dosing Validation

The weighing validation was carried out at doses ≤35 mg since the % error can be higher at low doses. The recovery of SU011248 from the 10- and 35-mg vials was determined as 103% and 100% (Table 4), respectively, indicating that the compound could be weighed accurately at these doses.

The dosing validation studies mimicked the dosing in the clinic. The recovery studies were carried out at three doses corresponding to 10, 35, and 2200 mg (low, mid, and high doses). Residual SU011248 in the PIB container was determined following either solubilization (doses below 35 mg) or suspension (doses at 2200 mg) in 75 mL of apple juice followed by two rinses with 50 mL water. The results are summarized in Table 4. The residual API in the vial at all dose levels was observed to be <0.2%, indicating a complete dose administration. Typically, complete dose administration in the clinic requires that >95% of the indicated dose has been administered and <5% remains in the container.

Stability

The preconstituted stability studies in clear glass vials indicated that the API was stable after 30 days at 40° C/75%RH, 60° C, 60° C/75%RH, and 80° C. The results are given in Table 5. The results clearly indicate that the compound is stable for at least 30 days following storage at regular and accelerated conditions. There is no increase in the impurity levels at temperatures ≤60° C and only a slight increase at 80° C.

The postconstituted stability studies of the product were carried out at 50-mg dose levels following constitution with 75 mL apple juice. The stability of the product was studied over 2 hours, since the product is to be administered within 1 hour of constitution. Considering a clinical setting, the study was carried out at ambient conditions of light (fluorescent light) and temperature (~22–25° C) conditions. The results are listed

Table 5. SU011248 stability profile before constitution.

	40° C/75% RH		60° C		60° C/75% RH		80° C	
Time (days)	%	% total imp	%	% total imp	%	% total imp	%	% total imp
0	99.5	0.6	99.5	0.6	99.5	0.6	99.5	0.6
30	99.2	0.5	102.0	0.7	101.2	1.2	100.5	1.9

%, % recovery; % total imp, % total impurities.
Results reported are mean of duplicate determinations.

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Table 6. SU011248 stability profile post-constitution with apple juice.

Time (hr)	Potency (% label claim)	% compared to t_0	% total imp[a]
0	96.7	100	1.6
1.5	96.3	98.6	2.0
2.0	98.1	101.2	2.0

Results reported are mean of duplicate determinations.
[a]Major impurity is the E isomer (Average value: 1.6%).

in Table 6. The recovery of SU011248 was 96–98% of the target concentration and is within the required specification of 95–105% of label claim. The constituted solutions were observed to be stable after 2 hours with recoveries of >98% compared to T_0, indicating that the product can be administered to the patient. The only major impurity in the product was observed to be the E isomer of SU011248 (~1.6%), which was formed due to the exposure of the analytical solutions to light. There was also no discernible difference in the color of the solution/suspension.

CONCLUSIONS

A PIB formulation for SU011248 was developed and transferred to the clinic for accelerated FTIM studies. The clinical development, including provision of supplies, was carried out using about 30 to 100 grams of the active compound, which is significantly lower than that required for development and supplies of conventional dosage forms such as capsules and tablets. In addition, the development and preparation of the clinical formulation was achieved in <2 months, resulting in an accelerated FTIM study. This acceleration is in comparison to capsule form development, which typically requires 3–6 months for development and production of clinical supplies. The PIB approach enabled an early readout on the pharmacokinetic profile of the compound in humans and rapid progression into Phase I clinical trials.

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