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Parallel screening approach to identify solubility-enhancing formulations for improved bioavailability of a poorly water-soluble compound using milligram quantities of material

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Abstract

In this article, we present a parallel experimentation approach to rapidly identify a solubility-enhancing formulation that improved the bioavailability of a poorly water-soluble compound using milligrams of material. The lead compound and a panel of excipients were dissolved in *n*-propanol and dispensed into the wells of a 96-well microtiter plate by a TECAN robot. Following solvent evaporation, the neat formulations were diluted with an aqueous buffer, and incubated for 24 h. The solubilization capacity of the excipients for the compound at 24 h ($SC_{24\,h}$), was determined by HPLC, and compared with its solubility in the corresponding neat formulations determined by a bench-scale method. The ranking order of solubilization capacity of the five tested formulations for this compound by this microscreening assay is same as the ranking order of the compound solubility in the neat formulations. Several formulations that achieved the target aqueous solubility were identified using the screening method. One of the top formulations, an aqueous solution of the compound containing 20% Tween® 80 by weight, increased the compound solubility from less than 2 μg/mL to at least 10 mg/mL. In a rat pharmacokinetic (PK) study, the Tween® 80 formulation achieved 26.6% of bioavailability, a significant improvement over 3.4% of bioavailability for the aqueous Methocel® formulation ($p < 0.01$). The results in the study suggest that this parallel screening assay can be potentially used to rapidly identify solubility-enhancing formulations for an improved bioavailability of poorly water-soluble compounds using milligram quantities of material.

Keywords: Parallel formulations screening; High-throughput formulation screening; Solubilization; Poorly water-soluble compounds; Bioavailability

1. Introduction

Poorly water-soluble compounds are usually associated with low oral bioavailability, and pose a great challenge for the development of viable dosage forms at all stages of drug development (Lipinski et al., 1997). A variety of formulation strategies have been developed to improve the solubility and bioavailability of such compounds. The formulation strategies include self-dispersing and self-emulsifying formulations, solid solutions, ionic, inclusion and lipid-based complexation, pharmaceutical salts, prodrugs, precipitation inhibitors, and particle size reduction such as micronization or nanomilling (Liu, 2000).

However, to identify an appropriate vehicle with satisfactory solubility as well as chemical and physical stability can take significant time and resources. For compounds that do not dissolve in a small set of commonly used excipients, there are a large number of possible embodiments that can be tested (e.g., combinations of excipients, ratios, and processing conditions). Therefore, current formulation screening/development for improved solubility and bioavailability is potentially time-consuming and labor-intensive, and also requires a large amount of compound. Often, the number and type of formulations that can be tested is limited by availability of compounds and the time available for testing, particularly for lead compounds that are in early development. Without rapid access to a formulation that provides adequate solubility for an initial evaluation

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in an in vivo study, compounds are often unable to advance in development (Bittner and Mountfield, 2002; Chaubal, 2004). Therefore, there is a need for developing a parallel experimentation approach to extensive screening of a wide diversity of solubility-enhancing formulations with a rapid turnaround time at the lead optimization and preclinical stages using a small quantity of material.

Parallel experimentation and high-throughput screening have been receiving increasing interest in the pharmaceutical field, including their application to selection of salts, polymorphs and cocrystals (Carlson et al., 2003; Levinson et al., 2003; Ansede and Thakker, 2004; Morissette et al., 2004), permeability profiling (Kansy et al., 1998; Avdeef, 2001; Kerns, 2001; Wohnsland and Faller, 2001), solubility profiling (Bevan and Lloyd, 2000; Avdeef, 2001; Kerns, 2001), enzymatic stability and metabolism (Di et al., 2003; Ansede and Thakker, 2004; Morissette et al., 2004), and protein binding (Fung et al., 2003a,b; Schuhmacher et al., 2004). Although parallel screening methods have been widely adopted in the pharmaceutical industry, there have been relatively few publications concerning their application to formulation screening. There has been one publication covering screening formulations of a paclitaxel injectable (Chen et al., 2003), one for screening emulsions (Bysouth et al., 2005), and several for screening transdermal permeation enhancers (Karande and Mitragotri, 2002; Karande et al., 2004) and protein formulations (Margolin, 2002; Nayar and Manning Mark, 2002).

In this article, we present a parallel formulation screening approach using miniaturized solvent-casting, combined with automation and parallel processing in a 96-well microtiter plate, which enables formulation scientists to screen a diverse set of solubility-enhancing formulations with a rapid turnaround time using milligram quantities of material. Our objective was to use the parallel screening method to identify solubility-enhancing formulations for a poorly water-soluble oncology lead compound that can support short-term proof-of-concept studies. The excipients identified by this assay were used to prepare aqueous formulations for a rat pharmacokinetic (PK) study. The bioavailability of the compound in the formulations were measured and compared.

2. Materials and methods

2.1. Lead compound

A poorly water-soluble lead compound (JNJ-10198409) was obtained from the Johnson & Johnson Pharmaceutical Research and Development compound collection. The compound has been reported to inhibit the receptor tyrosine kinase of platelet-derived growth factor for potential applications in oncology (Ho et al., 2005). The physicochemical properties of the lead compound are summarized in Table 1.

Table 1
Physicochemical properties of the lead compound

Property	Value
Molecular weight (g/mole)	325.35
Log P	2.93
pK_a	2.88
Melting point, T_m (°C)	174

Melting point was measured by DSC. Log P and pK_a were estimated computationally using ADMET Predictor by Simulations Plus, Inc., Lancaster, CA.

2.2. Formulation excipients

For this study, 38 pharmaceutical nonionic surfactants were screened. The excipients and their vendors are listed in Table 2. Phenacetin (internal standard for LC/MS/MS) was purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Liquid dispensing instrument and automation

All liquid dispensing was conducted on a TECAN GENESIS Workstation (Tecan US Inc., Research Triangle Park, NC), with a liquid handling arm and a robotic movement arm. On the TECAN workstation, a 96-well microtiter plate was positioned on the dispensing deck and all excipient solutions were placed in the troughs on the deck. The liquid handling arm has eight tips (200 or 1000 μL) to dispense the solutions from the selected positions in the troughs to 96-well locations in the microtiter plate. Scripts were written in the TECAN-specific Gemini software for the liquid handling steps such as aspiration, dispensing, and mixing. In addition, specific programs for each experiment step (e.g., dispensing individual solution for binary and ternary combinations, filtration, transferring and dilution of samples) were also written in Gemini.

2.4. Solvent evaporation instrument

A centrifugal vacuum evaporator (HT-4X, GeneVac Inc., Ipswich, UK) was used to evaporate solvents from the 96-well microtiter plates. The evaporator can hold up to eight 96-well microtiter plates for one operation, and the solvents are evaporated under high vacuum during centrifugation of plates. The evaporation parameters (evaporation time, vacuum, and temperature) for complete removal of the solvents in 96-well plates are dependent on the amount and types of both solvents and excipients in the plates, and have been determined experimentally before the formulation screening.

2.5. Parallel formulations microscreening method

A parallel formulation screening approach was used to screen a diverse set of 38 solubility-enhancing excipients in terms of their solubilization capacity, which is defined as ratio of compound solubility to excipient concentration in an aqueous medium (Yalkowsky, 1999).

A 96-well formulation screening plate map is shown in Table 3, where the first two columns in a 96-well microtiter plate could be used to profile compound aqueous solubility at different pHs. The rest of the columns are typically used for single nonionic surfactants and several binary surfactants.

Table 2
List of excipients for screening of solubility-enhancing formulations

Nonionic surfactant	Composition	Vendor
Tween 20	PEG 20 sorbitan monolaurate	Sigma–Aldrich
Tween 40	PEG 20 sorbitan monopalmitate	Sigma–Aldrich
Tween 60	PEG 20 sorbitan monostearate	Sigma–Aldrich
Tween 80	PEG 20 sorbitan monoleate	Sigma–Aldrich
Tween 21	PEG 4 sorbitan monolaurate	Sigma–Aldrich
Span 20	Sorbitan monolaurate	Sigma–Aldrich
Span Mix 2	Span 20/Tween 20 (42.0/58.0, w/w)	Sigma–Aldrich
Incrocas 30	Polyoxyl 30 caster oil	Croda
Incrocas 35	Polyoxyl 35 caster oil	Croda
Incrocas 40	Polyoxyl 40 caster oil	Croda
Acconon CA-5	PEG 5 caster oil	Abitec
Acconon CA-9	PEG 9 caster oil	Abitec
Acconon CA-15	PEG 15 caster oil	Abitec
Acconon Mix 1	Acconon CA-5/Acconon CA-15 (75/25, w/w)	Abitec
Acconon Mix 2	Acconon CA-5/Acconon CA-15 (50/50, w/w)	Abitec
Acconon CC-6	PEG 6 caprylic/capric ester	Abitec
Acconon C10	PEG 6 capric ester	Abitec
Croval A-40	PEG 20 Almond glycerides	Croda
Croval A-70	PEG 70 Almond glycerides	Croda
Croval Mix	Crovol A-40/Crovol A-70 (50/50, w/w)	Croda
Labrasol	Glyceryl caprylate/caprateand PEG-8 caprylate/caprate	Gatteffosse
Gelucire 44/14	Mono-, di- and triglycerides and di-fatty acid esters of PEG	Gatteffosse
Gelucire 50/13	Mono-, di- and triglycerides and di-fatty acid esters of PEG	Gatteffosse
Solutol HS15	Macrogol 15 Hydroxystearate	BASF
Vitamin E TPGS	d-Alpha Tocopheryl Polyethylene Glycol 1000 Succinate	Eastman
Methocel® (F4M)	Hypromellose 2906	Dow
Volpo 5	Oleth-5	Croda
Volpo 20	Polyoxyl 20 oleyl ether	Croda
Volpo Mix 2	Volpo 20/Volpo 5 (56.1/43.9, w/w)	Croda
Pluronic L44	PEG–PPG–PEG block copolymer, Mw = 2200	BASF
Pluronic F68	PEG–PPG–PEG block copolymer, Mw = 8400	BASF
Pluronic F87	PPG–PEG–PPG block copolymer, Mw = 7700	BASF
Pluronic F108	PPG–PEG–PPG block copolymer, Mw = 14,600	BASF
Pluronic F127	PPG–PEG–PPG block copolymer, Mw = 12,600	BASF
Pluronic R 17R2	PPG–PEG–PPG block copolymer, Mw = 2150	BASF
Pluronic Mix	Pluronic L44/Pluronic R17R2 (65.0/35.0, w/w)	BASF
Pluronic R 17R4	PPG–PEG–PPG block copolymer, Mw = 2650	BASF
Tetronic 304	PEG/PPG tetrafunctional block copolymer, Mw = 1650	BASF
Tetronic 701	PEG/PPG tetrafunctional block copolymer, Mw = 3600	BASF
Tetronic Mix	Tetronic 304/Tetronic 701 (61.1/38.9, w/w)	BASF

The process flow of this screening method is illustrated in Fig. 1. To dispense a small amount (i.e., 20 μg) of a compound to each well of a 96-well microtiter plate (ScienceWare-Bel-Art Products) and to avoid the difficulty of pipetting viscous, semisolid, and solid excipients, the compound and the excipients were first dissolved separately in a preferred solvent (*n*-propanol was used in this study). After complete dissolution, the compound and excipient solutions were positioned in the numbered track in the TECAN workstation. According to the formulation screening plate map in the experimental design, 40 μL of

Table 3
Formulation screening 96-well plate map

	1 2	3 4	5 6	7 8	9 10	11 12
A	pH 3	Tween 20	Incrocas 35	Croval A40	Volpo 10	Pluronic F127
B	pH 4	Tween 40	Incrocas 40	Croval A70	Volpo 20	Pluronic Mix 1
C	pH 5	Tween 60	Acconon CA-9	Croval Mix	Volpo Mix 1	Pluronic R 17R4
D	pH 6	Tween 80	Acconon CA-15	Labrasol	Volpo mix 2	Tetronic 304
E	pH 7	Tween 21	Acconon Mix 1	Gelucire 44/14	Pluronic L44	Tetronic Mix 1
F	pH 8	Span Mix 1	Acconon Mix 2	Gelucire 50/13	Pluronic F68	Hydroxypropyl-β-cyclodextrin
G	pH 9	Span Mix 2	Acconon CC-6	Solutol HS15	Pluronic F87	Methocel® (F4M)
H	pH 10	Incrocas 30	Acconon C10	Vitamin E TPGS	Pluronic F108	Bio-medium

Duplicates for each excipient in one 96-well plate.

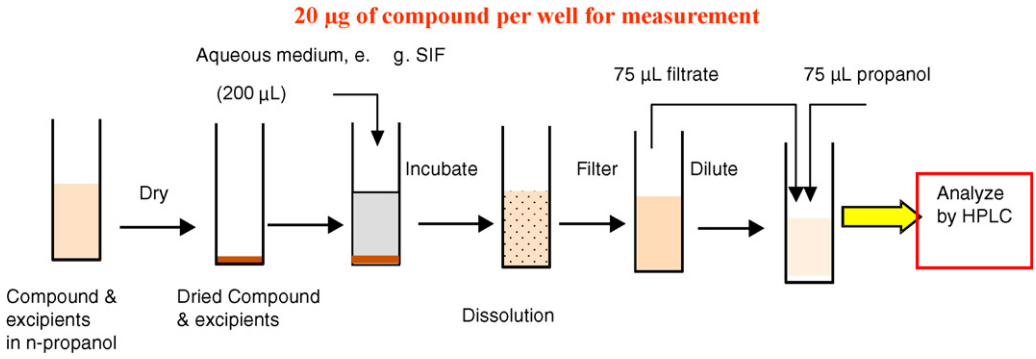

Fig. 1. Schematic diagram illustrating formulations screening process workflow.

compound solution (0.5 mg/mL) and 200 μL of the corresponding excipient solutions (2 mg/mL) were automatically dispensed into the desired locations in a 96-well microtiter plate by the TECAN robot following specific Gemini programs. The solutions were well mixed by two aspirations. The 96-well microtiter plate with the solutions was then placed in a GeneVac evaporator for solvent evaporation, which usually took about 2 h for evaporation of 200 μL of *n*-propanol in each well. After removal of the solvent in the plates, 200 μL of simulated intestinal fluid (SIF) (pH 7.4) was added to each well of a 96-well microtiter plate, and the plate was vortexed at high speed for about 5–10 s. Following incubation at room temperature for a desirable period of time, the plate was placed on a vacuum set and the solution was filtered automatically through a 0.2-μm polyvinylidene fluoride (PVDF) filter plate (pION, Inc., Woburn, MA) under vacuum to remove compound particles. After discarding the first 40-μL volume, the filtrate was diluted with *n*-propanol and the compound concentration in the filtrate was determined using an HPLC method with a lower limit of quantification of 0.1 μg/mL.

As shown in the process flow of this screening method, the compound/excipients were diluted with an aqueous medium and then incubated for a set time. During the incubation, compound could precipitate out of the formulations with time. Therefore, to determine equilibrium solubilization capacity of the excipients for the tested compounds, an adequate period of time is needed to reach equilibrium solubility for measurement. However, practically we usually use a 24-h incubation time following addition of an aqueous medium in the formulation microscreening assay, since compound precipitation from the solution often occurs predominantly in the first 24 h.

In this study, a 24-h incubation time following addition of an aqueous medium was used. Solubilization capacity of the excipients for the compound reported in the study, $SC_{24\,h}$, was defined by the ratio of measured apparent solubility of compound in the aqueous medium containing the excipients to the excipient concentration following a 24-h incubation upon addition of an aqueous medium. The compound/excipient ratio was kept at the weight ratio of 1:20. A maximum of solubilization capacity (50 mg/g, compound/excipient), or 100 μg/mL compound concentration, was reached if the entire compound dissolved in an aqueous medium without precipitation within a 24-h incubation time. For our screening purpose in the study, we used 100 μg/mL as a bench mark for screening the excipients because it is generally accepted that solubility is not likely to limit bioavailability at levels above 65 μg/mL (Lipinski et al., 1997).

2.6. Determination of the compound solubility in neat formulations at bench scale

About 100 mg of the lead compound was added to approximately 1 g of an excipient. A ratio of 1/10 (compound/excipients) was used to assure excess amount of compound solids present during the measurement. The mixture was sonicated at 40 °C for 1 h. After a 24-h incubation at 37 °C, the sample was shaken at room temperature for another 2 h. Finally, the sample was centrifuged for 40 min at 16,000 rpm. The supernatants were sampled and compound equilibrium solubility in the neat formulation was determined by an HPLC method.

2.7. HPLC assay

An HP1100 HPLC with an auto-sampler module for a 96-well microtiter plate (Agilent, Palo Alto, CA) was used to analyze the compound concentration. The final data from HPLC analysis were imported directly into a database for tracking and data mining. In the HPLC assay, a Phenomenex C18 column (150 mm × 4.6 mm, 3 μm) (Phenomenex USA, Torrance, CA) was used. The mobile phase consisted of 45% (v/v) acetonitrile with 0.1% (v/v) trifluoroacetic acid (TFA) and 55% (v/v) water with 0.1% (v/v) TFA. The flow rate was controlled at 0.8 mL/min with 20 μL of injection volume. The compound was eluted at 30 °C and quantitated at a wavelength of 235 nm. The retention time of the compound was 3.3 min during a total 5.5-min run time per sample. None of the excipients used in the study interfere with the assay.

2.8. Measurement of compound permeability

Passive transcellular permeability of the lead compound was estimated using Parallel Artificial Membrane Permeability Assay (PAMPA) by a Double-Sink Protocol (pION, Inc.). Sandwich configuration of donor/artificial membrane/acceptor in PAMPA mimics gastrointestinal absorption for the measurement of passive transcellular diffusion of compounds through the artificial membrane. In the study, the donor chambers were filled with 200 μL of diluted compound with the proprietary Sys-

tem Solution with constant buffer capacity in the pH range 3–10 (pION Inc., Cat# P/N 110158), while the acceptor chambers were filled with 200 μL of the proprietary acceptor sink buffer (ASB-7.4, pION Inc.). The acceptor wells and donor wells were in close contact but separated by a 0.45 μm filter (0.3 cm^2 cross-sectional area, 125 mm in thickness), which was filled with 4 μL of lipid solution (GIT-O, pION, Inc.). Following a 4-h incubation at ambient condition without agitation, the compound concentrations in the donor and acceptor solutions were assayed by an ultraviolet (UV) 96-well plate reader (Molecular Devices Crop., Sunnyvale, CA). The buffer in donor wells and the compound concentration in donor wells at time zero were treated as blank and reference. All liquid dispensed into a 96-well microtiter plate was handled by a TECAN robot. PAMPA flux, an indication of compound permeability, was calculated as ratio of amount of compound in acceptor chambers to amount of compound in donor chambers using the PAMPA software (pION, Inc).

2.9. Measurement of compound melting point

A differential scanning calorimeter (Hyper-DSC, Perkin-Elmer, Boston, MA) was calibrated using indium. Samples (2–3 mg) were heated from 25 to 210 °C at 10 °C/min in aluminum pans under nitrogen atmosphere. The melting points were calculated using the Pyris software (Perkin-Elmer).

2.10. Pharmacokinetic study

Tween® 80 and Methocel®, which showed different solubilization capacity for the tested compound in the microscreening assay, were selected in the PK study. The formulations were prepared as follows. Compound was first added to Tween® 80. After a 30-min sonication of the mixture at 45 °C, deionized water was added to the mixture to prepare 10 mg/mL of compound in 20% (by weight) Tween® 80 for duodenal dosing. Methocel® formulation was prepared by adding the compound into 0.5% (by weight) Methocel® aqueous solution to target 10 mg/mL of compound.

For intravenous (i.v.) dosing, Solutol® HS15 (10% by weight) was used to achieve 2 mg/mL of compound concentration in an aqueous medium. Also no compound precipitation was observed when this formulation was diluted 250 times in water. The i.v. formulation was prepared as follows. The compound was first added to Solutol® HS15. After a 30-min sonication of the mixture at 45 °C, deionized water was added to the mixture to prepare 2 mg/mL of compound in 10% (by weight) Solutol® HS15 for i.v. dosing.

Male and/or female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200–450 g were used to evaluate the formulations. For duodenal dosing studies, each of the catheterized rats had the duodenal catheter for dosing and the carotid catheters for blood sampling. The surgeries were done several days prior to shipping to ALZA. Rats were used within 2–3 days after arrival. All animals were fasted overnight before dose administration and fed 4 h post-dose. The duodenal catheters were flushed with 0.1 mL deionized water prior to the dosing, and the carotide catheters were flushed with heparinized saline solution at regular intervals and after each sampling. All rats were fitted with Covance Infusion Harness™ for rats (Model CIH95, Instech Laboratories, Plymouth Meeting, PA) and housed in the standard tubs during the study. The test animals were administered at the doses of 10 and 2 mg/kg rat body weight, respectively, for the duodenal and i.v. dosing. Blood samples were collected at the timepoints of 0 h (predosing), 0.25 h, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h. After collection, the blood samples were centrifuged and the plasma from the centrifugation were stored at −80 °C until bioanalysis. The PK study was conducted in accordance with the principles outlined in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, and had received approval by the Institutional Animal Care and Use Committee (IACUC) at the local authorities.

2.11. Bioanalysis of plasma samples

The rat plasma samples (100 μL) were treated with 300 μL of acetonitrile containing 100 ng/mL of an internal standard (phenacetin). The mixture was vortexed for 10 min, and then centrifuged at 4500 rpm for 10 min. The supernatant (10 μL) was injected into a Hypersil BetaBasic C18 column (5 cm × 2.1 mm, 5 μm) (Thermo Electron, Waltham, MA), and eluted at 0.3 mL/min by a gradient system consisting of mobile phase A, 0.1% (v/v) formic acid in water; and mobile phase B, 0.1% (v/v) formic acid in acetonitrile. The gradient system was programmed by increasing the proportion of mobile phase B from 35 to 75% (v/v) linearly within 2.1 min, then decreasing the proportion of mobile phase B back to 35% at 2.2 min. The retention times for the compound and the internal standard were 1.1 and 0.9 min, respectively, with a total run time of 3.2 min.

The compound was detected by an API 3000 mass spectrometer with a Turbo Ionspray interface (Applied Biosystems, Foster City, CA). Electrospray ionization was performed in the positive ion mode heated nebulizer with a temperature of 300 °C. Liquid chromatography–tandem mass spectroscopy (LC/MS/MS) chromatograms were acquired in multiple reaction mode (MRM). The MRM transitions for the lead compound and the internal standard were 326.3 to >190.6, and 181.1 to >110.3, respectively. A calibration curve was prepared at a concentration range of 1.0–1000 ng/mL.

2.12. Pharmacokinetic data analysis

The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were determined directly from the individual plasma concentration of parent compound versus time profiles. The area under the plasma concentration versus time (AUC_t) was estimated by trapezoidal integration to the last sampling point (t). The AUC from time t to infinity ($AUC_{t-\infty}$) was determined by the ratio of the compound concentration of the plasma sample at the last sampling point to the apparent elimination rate constant. The apparent elimination rate constant was estimated from i.v. dosing by linear regression of log plasma concentration at the terminal phase versus time. The AUC from time zero to infinity ($AUC_{0-\infty}$) is the sum of AUC_t and $AUC_{t-\infty}$. The abso-

Table 4
Aqueous solubility of the lead compound vs. pH

pH	Solubility (μg/mL) ($n=4$)
3.0	5.2 ± 1.0
4.0	1.0 ± 0.0
5.0	0.8 ± 0.2
6.0	1.8 ± 0.2
7.0	1.4 ± 0.6
8.0	1.8 ± 0.4
9.0	1.5 ± 0.4

lute bioavailability (BA) for PK studies was calculated using $AUC_{0-\infty}$ by the following equation:

$$BA\% = 100 \times \frac{AUC_{duodenal}}{AUC_{i.v.}} \times \frac{D_{i.v.}}{D_{duodenal}}$$

where $AUC_{duodenal}$ and $AUC_{i.v.}$ are the area under the parent compound concentration versus time curves for the duodenal dosing and i.v. dosing, respectively; and $D_{i.v.}$ and $D_{duodenal}$ are the doses for i.v. and duodenal dosing, respectively.

3. Results and discussion

3.1. Physiochemical properties of the lead compound

The molecular weight, Log P, pK_a and melting points are summarized in Table 1. The aqueous solubility of the lead compound at different pHs is shown in Table 4. The aqueous solubility of the lead compound was in the range of 1–5.2 μg/mL between pH 3 and 9. The PAMPA flux, an indication of compound permeability, ranged from 18.6 to 25.2% (Table 5), but was statistically insignificantly different in the tested pH range ($p > 0.05$). About 20% of PAMPA flux of this compound in the physiological pH indicates a reasonably good permeability of compound (Kansy et al., 1998). The compound has a melting point at 174 °C as measured by DSC, and a Hansen solubility parameter of 23.39 $(MPa)^{1/2}$ as estimated using Molecular Modeling Pro software (ChemSW, Fairfield, CA). The compound is thus typical of a Biopharmaceutics Classification System (BCS) Class II compound. One of the factors that lead to the poor oral bioavailability of this compound is its low aqueous solubility. Therefore, solubility-enhancing formulations identified by a formulation screening approach would improve oral bioavailability of this compound.

Table 5
PAMPA flux of the lead compound vs. pH

pH	PAMPA flux % ($n=3$)
4.0	21.5 ± 1.4
4.5	22.4 ± 3.8
5.0	22.7 ± 1.1
6.0	23.2 ± 3.1
7.0	25.2 ± 3.3
7.5	20.1 ± 0.5
8.0	18.6 ± 8.7
9.0	25.0 ± 3.0
10.0	23.6 ± 2.6

3.2. Parallel formulations screening results

The $SC_{24\,h}$ values of 38 nonionic excipients for this compound are illustrated in Fig. 2. Among the excipients, seven commonly used surfactants (Tween®, Incrocas®, Croval®, Gelucire®, Solutol® HS15, Vitamin E TPGS®, and Volpo®) significantly enhanced the solubilization of the compound in an aqueous medium, with their solubilization capacities close to the targeted 50 mg/g (compound/excipient). We also found that this enhanced solubilization of the compound in the excipients was not due to a pH change. As shown in the process flow of this microscreening method (Fig. 1), 200 μL of SIF (pH 7.4) was added to each well of a 96-well microtiter plate containing the excipients after removal of the solvent in the plates. Re-measurement of the pH of SIF in the presence of the excipients showed the same pH for all aqueous excipient solution in each well (7.4). This could be explained by the buffer capacity of SIF and the use of nonionic excipients in the study.

The enhancement of compound solubilization in an aqueous medium by those surfactants can be attributed to micelle formation. The final concentration of excipients after addition of an aqueous medium is 2 mg/mL (0.2%), far above the critical micelle concentrations of these surfactants (typically <0.02%). Each type of surfactant tested had a different solubilization capacity due to its different structure/property and its interaction with the compound. However, the solubilization capacity following a 24-h incubation time upon addition of an aqueous medium, $SC_{24\,h}$, within the same family of surfactants seemed to increase with their hydrophile–lipophile balance (HLB) value, or the polyethylene oxide (PEO) chain length for a given hydrophobic block (Table 6). This result is in agreement with observations reported previously (Ismail et al., 1970; Mulley and Winfield, 1970; Collett and Koo, 1975; Saettone et al., 1988; Hamza and Kata, 1989). The compound tested in the study has low aqueous solubility (Table 4), but also has several polar groups that account for 12% of total surface area (estimated by the ADMET Predictor software). Nonpolar compounds are solubilized primarily within the hydrocarbon core region of the micelles of the surfactants, and highly polar compounds are anchored in the micelle surface region. In comparison, hydrophobic compounds with polar groups are likely solubilized in both the core and the palisade layer, and also in the mantle of the micelles (Saettone et al., 1988; Kondo et al., 1993). Therefore a longer PEO chain (high HLB value) for a given hydrophobic block leads to an increase in the solubilization of compounds of this type in the hydrophilic (PEO) outer mantles of the micelles.

Pluronic® block copolymers have been used widely in the formulations for poorly water-soluble compounds. However, in the microscreening assay, all Pluronic® surfactants tested (Pluronic® L44, F68, F87, F108, F127 and R17R4) did not show a solubilization enhancement for the compound (Fig. 2). For example, the solubility of the compound in Pluronic® L44 was 4.72 mg/g (compound/excipient), but $SC_{24\,h}$ of this excipient was found to be extremely low (0.76 mg/g). The same phenomenon was also observed for other poorly water-soluble compounds when those Pluronics® were tested in the parallel formulation screening assay (data not published). The low sol-

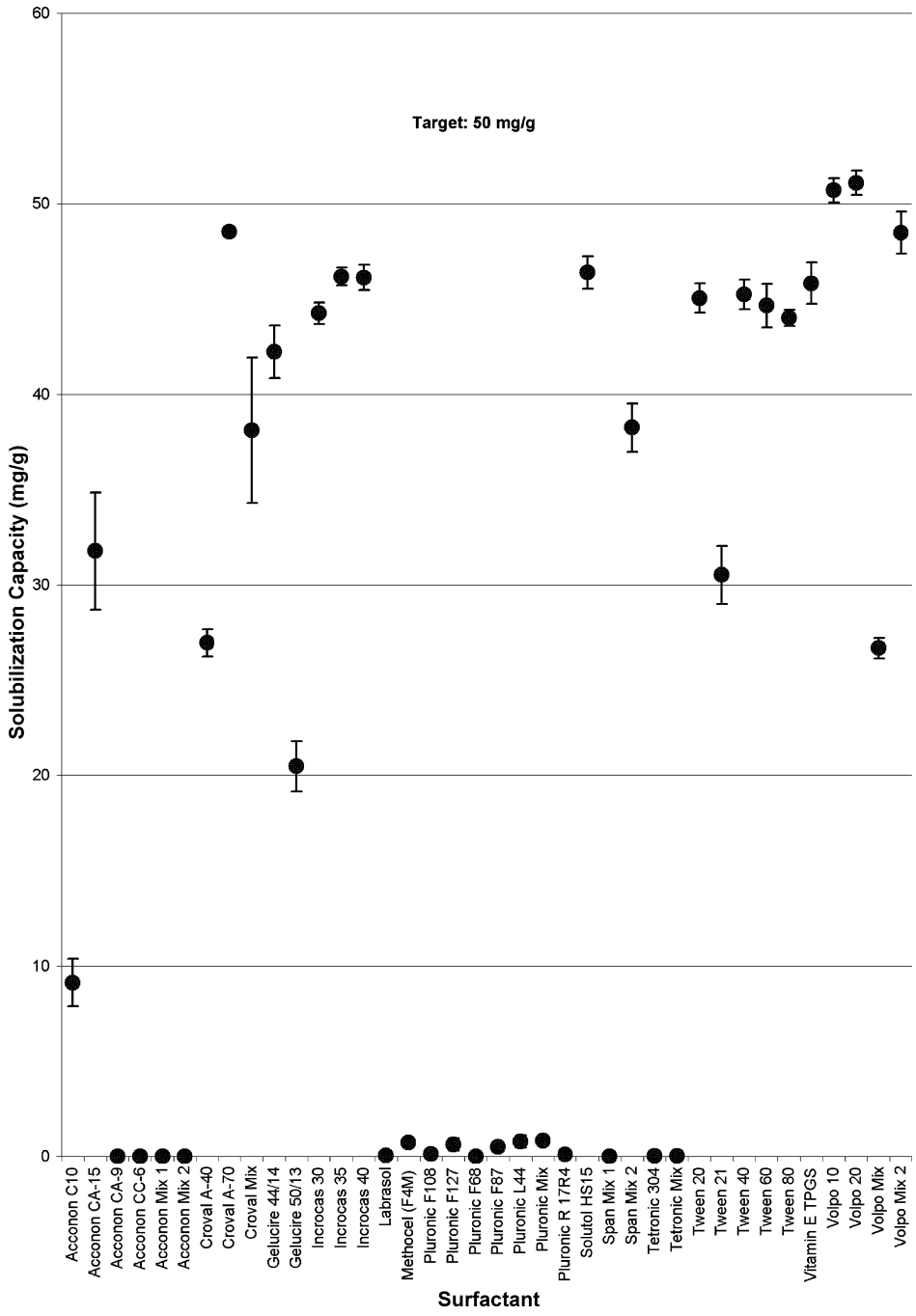

Fig. 2. Results of formulation microscreening for the lead compound; plot of the solubilization capacity following 24-h incubation upon addition of an aqueous medium, $SC_{24\,h}$, for the 38 excipients tested; loading = 20 μg compound and 400 μg excipient in 200 μL SIF per well; $n = 4$.

ubilization of compound is attributed to recrystallization of the compounds in Pluronics after removal of solvents in the assay. The Pluronics® used in the study are hydrophilic and have very limited ability to solubilize crystallized compounds (Hammad and Muller, 1999; Oh et al., 2004).

It is also interesting to note that Labrasol®, a commonly used solubilizing agent in pharmaceuticals, did not enhance solubilization of the compound in an aqueous medium ($SC_{24\,h} < 0.05$ mg/g) (Fig. 2) although solubility of the compound in Labrasol® was high (91.8 mg/g). Other water-miscible organic solvents such as polyethylene glycol (PEG), ethanol, and 1-methyl-2-pyrrolidinone (NMP), are often used to enhance compound solubility in neat formulations for poorly water-soluble compounds. The compounds solubilized in these formulations, however, tend to precipitate upon dilution with water since the compound solubility in an aqueous medium is exponentially related to the concentration of these solvents (Yalkowsky, 1999). Like other water-miscible organic solvents, Labrasol® is a good solubilizing agent for poorly water-soluble compounds (Strickley, 2004). However, after diluting the compound in Labrasol® with SIF, the compound precipitates, which could limit in vivo bioavailability.

Table 6
$SC_{24\,h}$ of the excipients for the lead compound vs. their HLB values

Excipient	Composition	HLB	$SC_{24\,h}$ (mg/g) (compound/excipient) ($n=4$)
Tween® 21	PEG 4 sorbitan monolaurate (100 wt.%)	13.3	30.1 ± 1.5
Tween® 20	PEG 20 sorbitan monolaurate (100 wt.%)	16.7	45.1 ± 0.8
Span® Mix 1	Span® 20/Tween® 20 (82.72/17.28, w/w)	10	0.5 ± 0.0
Span® Mix 2	Span® 20/Tween® 20 (42.0/58.0, w/w)	13.3	38.2 ± 1.3
Croval® A40	PEG 40 Almond glycerides (100 wt.%)	10.0	27.0 ± 0.7
Croval® Mix	Croval® A-40/Croval® A-70 (50/50, w/w)	12.5	38.1 ± 3.9
Croval® A70	PEG 70 Almond glycerides (100 wt.%)	15.0	48.6 ± 0.1
Volpo® Mix 1	Volpo® 20/Volpo® 5 (17.3/82.7, w/w)	10.0	26.7 ± 0.6
Volpo® Mix 2	Volpo® 20/Volpo® 5 (56.1/43.9, w/w)	12.4	48.5 ± 1.1
Volpo® 20	Polyoxl 20 oleyl ether	15.4	51.1 ± 0.7

Fig. 3 is a theoretical plot illustrating the apparent solubility versus the time following addition of an aqueous medium in aqueous media for five different formulation types in the parallel screening assay. The plot is illustrative of the possible solubility behavior of five different formulation types and was constructed using hypothetical numbers.

In a Type I formulation the compound is completely dissolved in an excipient after removal of *n*-propanol, and no precipitation occurs after diluting with aqueous media. In a Type II formulation, the compound is recrystallized after removal of *n*-propanol, but the recrystallized compound can be solubilized into the aqueous media by the excipients. In a Type III formulation, the compound is dissolved in an excipient after removal of *n*-propanol. The compound tends to precipitate following a addition of an aqueous medium with aqueous media, but precipitation is inhibited or reduced within a desirable time period by the excipients. These surfactants with high solubilization capacity belong to either Formulation Types I, II, or III. Formulation Type IV is similar to III, but excipients cannot inhibit or reduce the compound precipitation upon addition of an aqueous medium in aqueous media. Labrasol® and other water-miscible organic solvents such as PEG, ethanol, propylene glycol, and NMP belong to Type IV. For a Type V formulation (the worst scenario), the compound is recrystallized after removal of *n*-propanol, and the recrystallized compound is hardly resolubilized by the excipients. The Pluronics® are in this category.

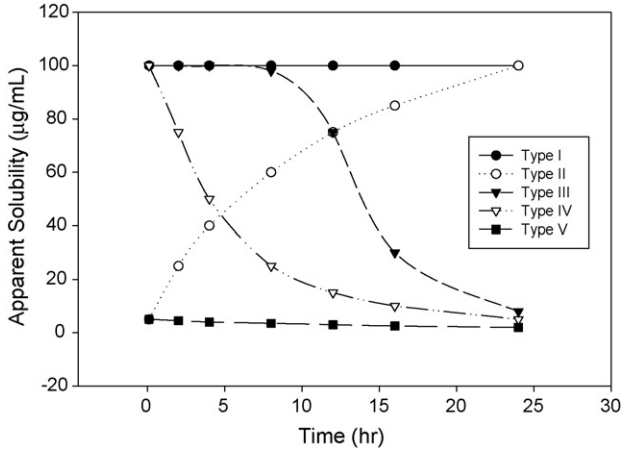

Fig. 3. Schematic diagram showing five types of formulations in the screening assay.

High-throughput formulation screening methods reported in the literature primarily use solid compound, and therefore require more material (e.g., 1–10 mg per well), powder dispensing equipment, and viscous/heated liquid dispensing capability. In this microscreening assay, a miniaturized solvent-casting method with automation enables formulation scientists to rapidly prepare and screen hundreds of formulations in parallel, using only tens of microgram quantities of compound per formulation tested. Use of solvent-casting in the method also avoids the use of powder dispensing equipment and the difficulty of dispensing/heating viscous, semisolid, and solid excipients. With automation and parallel processing in 96-well microtiter plates, a proper experimental design can finish about 96 formulations with duplicate measurement within 2 days per person using HPLC analysis, compared with 10–30 formulations per week by a bench-scale method. The approach also allows screening of a variety of single excipients and their mixtures for possible synergistic effects. In addition, it allows scientists to rank order a diverse set of solubility-enhancing formulations such as microemulsion, complexation, and solid solution (data not published), which would otherwise be difficult to perform manually.

This formulation microscreening approach is suitable for application to the compounds that can be dissolved in the volatile solvents with the excipients, and stable in an aqueous medium. Use of solvent-casting in the microscreening assay simplifies the screening procedure and equipment and makes it possible to dispense small quantities of compounds accurately. After removal of solvent, however, this approach may create a different crystalline form in the formulations, or form a non-equilibrium solid solution of compound/excipients and therefore the compounds may be supersaturated. This microscreening assay is most suitable for use in discovery and early preclinical development, where limitation associated with supersaturation, changes in crystal form and potential instability are less relevant.

3.3. Solubilization capacity ($SC_{24\,h}$) by the microscreening assay versus the solubility of the compound in the neat formulations by a bench-scale method

Fig. 4 shows the $SC_{24\,h}$ of five surfactants, Pluronic® L44, Acconon® CA-15, Tween® 21, Tween® 80 and Cremophor

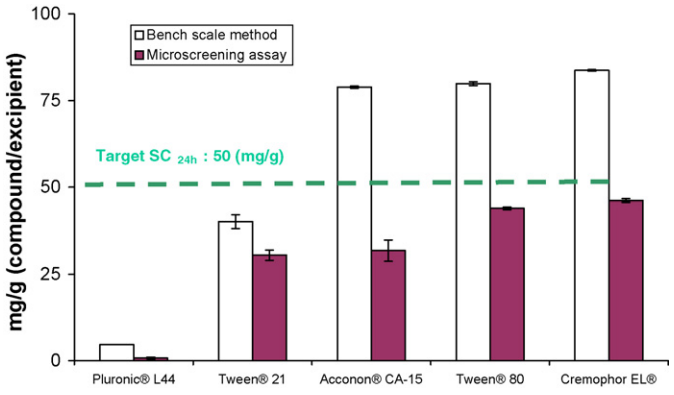

Fig. 4. $SC_{24\,h}$ measured by the microscreening assay vs. the solubility of the lead compound in the neat formulations by a bench-scale method ($n = 4$).

EL®, versus the solubility of the lead compound in the neat formulations measured by a bench-scale method. Those five surfactants represent different types of excipients with a HLB range of 10–16. It would be technically difficult to measure the compound solubility in all excipients used in the microscreening assay. Some excipients such as Pluronics® (F68, F87, F108, and F127), Solutol® HS15 and Vitamin E TPGS® are solid or semisolid at room temperature. Also some excipients become very viscous or gel when the compound concentration in the excipients is high.

As shown in Fig. 4, the values of $SC_{24\,h}$ for those surfactants measured by microscreening approach did not match with the solubility results in surfactants by bench-scale method. The $SC_{24\,h}$ values of Tween® 80 and Cremophor EL® were close to the maximum targeted 50 mg/g (compound/excipient). The $SC_{24\,h}$ values of other three tested excipients, Pluronic® L44, Acconon® CA-15, and Tween® 21, were well below the compound solubility in the neat formulations.

The bench-scale method measures the equilibrium solubility of the compound in neat formulation, while the microscreening approach in the study measures the kinetic solubility of the compound after the formulation disperses in aqueous media. Consequently, the final results in microscreening approach are determined by not only the drug initial solubility in neat formulations, but also the extent of drug precipitation in an aqueous medium.

As an example of this phenomenon observed in this study, the compound's solubility in a neat formulation containing Acconon® CA-15 was higher than its solubility in Tween® 21, but the $SC_{24\,h}$ was comparable. This could be due to the fact that Acconon® CA-15 is more hydrophilic (HLB ~ 16) than Tween 21 (HLB ~ 10). Hydrophilic surfactants such as Acconon® CA-15, tend to diffuse into the aqueous media after following dilution, leading to drug precipitation (Pouton, 2000; Gao et al., 2004). The risk of precipitation is greater when the formulation contains a more hydrophilic surfactant and a higher proportion of such surfactants (Pouton, 2000). Consequently, more compounds precipitate out in Acconon® CA-15 than in Tween® 21 after the formulations disperse in an aqueous medium, leading to similar $SC_{24\,h}$ values (Fig. 4).

Nevertheless, the study results indicated that the ranking order of $SC_{24\,h}$ of the five tested formulations by this screening assay is the same as the ranking order of the solubility results from bench-scale method. This indicates that for these formulation drug precipitation is not significant enough to change the ranking order of formulations.

It should be also pointed out that the primary goal of this paper was to develop the screening assay to identify the vehicles that may improve the bioavailability of the compounds for initial preclinical studies using small quantities of material. Therefore our intention using this approach was to identify a Type 1 formulation (Fig. 3), in which the compound was completely solubilized in the neat formulation after solvent removal and did not precipitate when the formulations dispersed into an aqueous medium. Without drug precipitation, we expected the Type I lead formulations identified by the screening assay would achieve an acceptable bioavailability for initial assessment of pharmacology and toxicity studies.

3.4. Pharmacokinetic study

In order to demonstrate an improved bioavailability of a solubility-enhancing formulation identified by the parallel screening assay, Tween® 80 and Methocel®, which showed the solubilization capacity of 44 and 0.73 mg/g (compound/excipient), respectively, in the parallel screening results, were selected. A three-arm rat PK study was conducted by duodenal dosing of the Tween® 80/water (20/80, wt.% formulation), duodenal dosing of 0.5% aqueous Methocel® formulation and i.v. dosing of a lead compound solution in Solutol® HS15 (10% by weight). The compound concentration profiles for these three arms are shown in Fig. 5 and the rat PK studies results are summarized in Table 7.

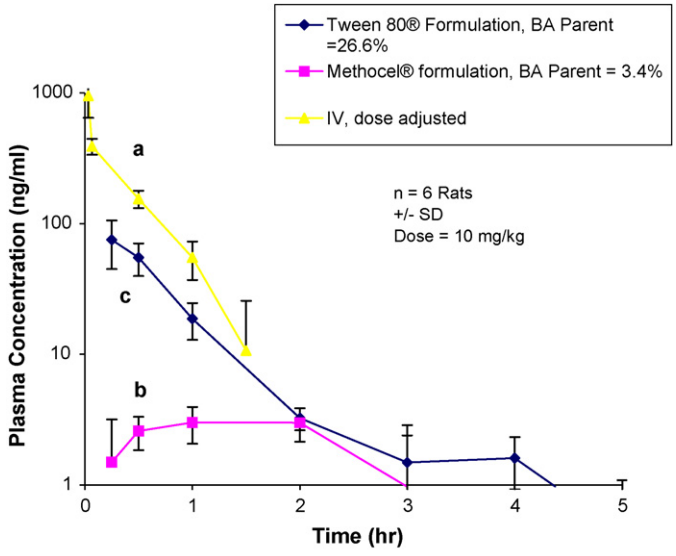

Fig. 5. Parent plasma concentrations vs. time following i.v. dosing (a), and intraduodenal administration of a Methocel® formulation (b) and a Tween® 80 formulation (c); $n = 6$ rats; dose = 10 mg/kg.

Table 7
Summary of rat pharmacokinetic study of the lead compound following i.v. dosing and duodenal dosing of a Tween® 80 formulation vs. a Methocel® formulation

	i.v. Bolus[a] ($n=6$)	Duodenal dosing Methocel® formulation[b] ($n=6$)	Duodenal dosing Tween® 80 formulation[c] ($n=6$)
Average AUC (ng h/mL)	228.1 ± 39.3	7.8 ± 2.4	60.7 ± 18.1
C_{max} (ng/mL)	950.1 ± 305.3	3.8 ± 0.7	76.0 ± 29.0
T_{max} (h)	0.03 ± 0.00	1.3 ± 1.0	0.29 ± 0.10
Relative BA%	100	3.4 ± 1.0	26.6 ± 8.0

Dose: 2 mg/kg for i.v., 10 mg/kg for the formulations.
[a] Corrected for dose 10 mg/kg.
[b] 10 mg/mL in 0.5% aqueous Methocel®.
[c] 10 mg/mL in Tween® 80/deionized water (20/80, wt.%).

The Tween® 80 formulation achieved 26.6% of bioavailability, a significant improvement over the bioavailability (3.4%) for the Methocel® formulation ($p<0.01$). The compound has low aqueous solubility (1–5 μg/mL) in a range of pH 3 and 9 (Table 4), and is subject to metabolism by uridine diphosphate glucocuronosyltransferase (UGT) in the gastrointestinal tract (Hanninen, 1985) and livers upon absorption (Yan et al., 2006). As shown in the parallel screening results (Fig. 2), Tween® 80 significantly enhanced the solubilization of the compound in an aqueous medium, with a solubilization capacities close to the targeted 50 mg/g (compound/excipient). Following dosing of the Tween® 80 formulation, the compound was thus absorbed quickly due to the improved solubilization of the compound. This rapid absorption rate minimizes the enzymatic reaction. The more rapid the rate of absorption, the more likely the enzyme in both gastrointestinal tract and hepatic systems becomes saturated, leading to a greater plasma concentrations of parent drugs (Levy and Matsuzawa, 1967). In contrast, Methocel® showed a very low solubilization capacity for the tested compound in the parallel screening assay. This low aqueous solubility of the compound in the Methocel® formulation may lead to the poor bioavailability of this compound observed in the PK study. The PK results clearly demonstrate that the solubility-enhancing formulation identified by the formulation screening assay increased the bioavailability for the poorly water-soluble compound.

4. Conclusions

We developed a formulation microscreening approach using miniaturized solvent-casting, combined with automation and parallel processing in 96-well microtiter plates. This method was used to screen 38 solubility-enhancing excipients in terms of their solubilization capacity for preclinical studies, using about 2 mg of material. As identified by the parallel formulations screening approach, a preclinical vehicle comprising Tween® 80 significantly enhanced bioavailability of the lead compound in rats over a 0.5% Methocel® formulation.

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