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# Method for screening of solid dispersion formulations of low-solubility compounds—Miniaturization and automation of solvent casting and dissolution testing

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**Abstract**

An efficient method has been developed for screening solid dispersion formulations that are intended to enhance the dissolution of poorly soluble compounds. The method is based on miniaturization and automation of sample preparation by solvent casting, and dissolution testing, in a 96-well plate format, using less than 0.1 mg of compound per well. To illustrate the method, six polymers and eight surfactants were screened, individually and in combination, for their ability to dissolve a compound with aqueous solubility of <1 μg/ml in simulated intestinal fluid. Screening was performed at an excipient/compound ratio of 10:1, and a polymer/surfactant ratio of 3:1 for ternary formulations. Sixteen of the 48 ternary formulations dissolved the compound to a level >100 μg/ml, i.e. at least a 100-fold increase over the aqueous solubility. A number of synergies were observed wherein the performance of a ternary formulation greatly exceeded that of either of the corresponding binary formulations. Thirteen 'hits' from screening were scaled up with melt methods, and ∼2/3 of these showed comparable dissolution enhancement when tested at larger scale. Five of these were administered to rats, and the absolute oral bioavailability ranged from 10 to 23%, versus less than 1% for the unformulated compound.


*Keywords:* Solid dispersions; Solubility; High throughput; Bioavailability; Surfactants; Dissolution

## 1. Introduction

Solid dispersions have been under investigation for several decades as a means to improve the bioavailability of BCS Class 2 compounds, i.e. compounds exhibiting high permeability but low solubility and/or dissolution rate (Leuner and Dressman, 2000; Serajuddin, 1999). Solid dispersions are most commonly formed either from a drug/excipient solution (by solvent evaporation) or from a homogeneous drug/excipient melt, via melt extrusion. In the final formulation, the compound may be molecularly dispersed in the excipient matrix, or may be dispersed as fine nanocrystalline or amorphous particles which form during solvent evaporation or cooling of the melt. The matrix is typically a water-soluble polymer such as polyethylene oxide, polyvinyl pyrrolidone, or hydroxypropyl methyl cellulose. Solid dispersion formulations currently on the market include griseofulvin in poly(ethylene glycol) (Gris-PEG®, Novartis), nabilone in povidone (Cesamet®, Lilly), and itraconazole in poly(ethylene glycol) and hydroxyl propyl methyl cellulose (Sporanox®, Janssen).

While hydrophilic polymers can be very effective solid-state stabilizers, extremely insoluble drugs will tend to re-crystallize upon exposure to the gastrointestinal milieu during dissolution (Leuner and Dressman, 2000; Serajuddin, 1999). Dispersion formulations based on semi-solid surfactants, such as Vitamin E TPGS (Shin and Kim, 2003) and Gelucire 44/14 (Soliman and Khan, 2005; Yuksel et al., 2003) have the potential to facilitate dissolution and inhibit precipitation in the GI tract as a

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result of their solubilizing properties, but typically have much lower melting points and a greater degree of molecular mobility in the solid state than polymers. Therefore, formulations based on these excipients are anticipated to be less stable (Khoo et al., 2000) against recrystallization of the compound in the solid state. Semi-solids are also more difficult to process into an oral dosage form.

Formulations containing both a high-$T_g$ water-soluble polymer and a surfactant potentially combine the better long-term stability of polymeric solid dispersion formulations with the enhanced dissolution and bioavailability of liquid or semi-solid surfactant-based formulations. Ghebremeskel et al. (2006) recently reported that addition of 10% surfactant to PVP- and HPMC-based solid dispersions of a low-solubility compound led to dramatic increases in dissolution rate, with little or no adverse effect on long-term physical stability. Okonogi and Puttipipatkhachorn (2006) recently reported a similar enhancement of dissolution rate from PEG-based solid dispersions of oflaxacin when approximately 10% surfactant was included in the formulations. More generally, solid dispersions utilizing more than one excipient have the potential to allow fine-tuning of the formulation properties in order to achieve improved performance, in a manner which is not possible with single-excipient formulations. Six et al. (2004) investigated dispersions of itraconazole in a combination of two polymers (PVPVA64 and Eudragit E100) which differed in their miscibility with the drug and their dissolution rates, and found that the drug loading and dissolution rate of optimized ternary formulations were superior to those which could be achieved by binary formulations.

In all of the work reported so far on multi-excipient solid dispersions, however, only a small number of excipients and excipient combinations were studied in each case. Such focused studies of a small number of formulations allow for detailed characterization of formulation physical/chemical characteristics (e.g. studies of crystallinity and molecular interactions by DSC, XRD, and spectroscopic techniques). Given the large number of polymer and surfactant excipients available, however, the number of excipient combinations and ratios to be explored is large. Excipient selection and experimental design based on e.g. compound and polymer solubility parameters (Ghebremeskel et al., 2007) or surfactant HLB may provide a rationale for some narrowing of the scope of formulations to be explored, but still leaves many possibilities to be evaluated; and also presumes that the theoretical models used to restrict the scope of experimentation are sufficiently accurate such that 'good' formulations will not be prematurely eliminated from experimental consideration. Therefore, there is clearly value in the development of efficient strategies for experimentally evaluating large numbers of compound/polymer/surfactant combinations – or more generally, ternary and higher-order solid dispersion formulations – in order to rapidly identify systems with synergistic interactions between the components, for subsequent in-depth study using traditional techniques.

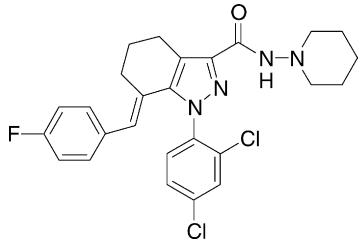
In this article, we describe a newly developed method for rapid initial screening of such formulations. The methodology is based on miniaturization and automation of formulation preparation by solvent casting, with high-throughput dissolution testing as the characterization method, and uses less than 0.1 mg of compound per sample tested. The method is illustrated by its application to a development-stage compound with aqueous solubility < 1 μg/ml. In order to evaluate the usefulness of the method, selected formulations identified through screening were prepared at larger scale by melt methods, and their *in vitro* dissolution behavior was determined. Some of these formulations were also evaluated *in vivo* (in rats) to assess the degree of bioavailability enhancement. The strengths, limitations, and most suitable applications of the method are also discussed.

## 2. Materials and methods

### 2.1. Materials

The chemical structure, molecular weight, aqueous solubility at pH 2 and 7, log *P*, melting point and Hildebrand solubility parameter of the model compound are presented in Table 1. The excipients used, and the suppliers, were: hydroxypropyl cellulose (HPC-SL, Nisso, Japan), hydroxypropyl methyl cellulose acetate phthalate (HPMCP 50, Shin-Etsu, Japan), Kollidon VA-64 (BASF, USA), Plasdone K29-32 (ISP, USA), Eudragit L100 and Eudragit RS100 (Rohm, USA), Vitamin E TPGS (Eastman Chemicals, TN, USA), Sodium lauryl suflate (SLS) [Sigma–Aldrich, USA], Pluronic F127 NF (Poloxamer 407,

Table 1
Physicochemical properties of the compound

| Compound | Structure | MW (g/mole) | Aqueous solubility (μg/ml) | Log *P* | Melting point (°C) | Solubility parameter, $\delta$ (MPa1/2) |
|---|---|---|---|---|---|---|
| JNJ-25894934 |  | 498.4 | pH 2: 1, pH 7: <1 | 5.13 | 169 | 23.67 |



The melting point was measured by DSC. Aqueous solubilities were measured by the shake-flask method. The log *P* and solubility parameter were estimated computationally using Molecular Modeling Pro, Version 6, copyright 2005 by Norgwyn Montgomery Software Inc., North Wales PA.

BASF, USA), Crodesta F-160 and Crodesta F-110 (Croda, USA), Cremophor EL (BASF, USA), Volpo 10 (Croda, USA), Tween 80 (Sigma–Aldrich, USA).

The excipients were chosen to provide a diversity of structural and physical/chemical properties, and were not selected based on any specific hypotheses regarding the likely interactions of the formulation components. Other methods of selecting the excipients may of course be used in the context of this screening method. The polymers included cellulosic (HPC-SL, HPMCP) and acrylate-based (Eudragit L100, Eudragit RS100) materials, and materials carrying anionic (Eudragit L100, HPMCP), cationic (Eudragit RS100), and neutral hydrophilic (HPC-SL, Plasdone K29-32, Kollidon VA64) functionality, as well as varying amounts and types of hydrophobic functional groups. All of the surfactants were non-ionic, except for SLS, but still represent a wide diversity of hydrophilic and hydrophobic functional groups as well as surfactant architectures, molecular weights, and melting points.

All the solvents used (acetone, ethanol, *n*-propanol) were HPLC grade and obtained from JT Baker and company. Artificial intestinal fluid (AIF) was prepared as per USP29/NF 24S1, using monobasic potassium phosphate, and was adjusted to a pH of $6.8 \pm 0.1$ using sodium hydroxide or hydrochloric acid.

### 2.2. Methods

The different stages of experimentation which make up the present study are illustrated schematically in Fig. 1, and are described in detail below.

#### 2.2.1. Screening method: solvent casting

For the screening experiments, all liquid handling operations were performed with a Tecan Genesis Freedom 200 liquid handling system (Tecan, Durham, NC, USA), using Tecan disposable tips made of electrically conducting polymer composite. Formulations were prepared, diluted, and incubated in ScienceWare 96-well deep well microtiter plates (Bel-Art Products, Pequannock, NJ, USA). Excipient and drug stock solutions were prepared in acetone: ethanol (1:1). This solvent mixture was chosen because it dissolved the widest range of polymers which were of interest. Stock solutions containing single excipients were prepared at a concentration of 2 mg/ml, while the compound stock solution was prepared at a concentration of 0.2 mg/ml. For preparing polymer/surfactant formulations, the liquid handling robot was used to dispense 225 μl of polymer stock solution, 75 μl of surfactant stock solution, and 300 μl of drug stock solution to each well, corresponding to an total excipient:compound ratio of 10:1. Formulations containing only polymer or only surfactant, in combination with the compound, were prepared using 300 μl of a single excipient stock solution and 300 μl of compound stock solution. For all formulations tested, the excipient mass per well was 0.6 mg, and the compound mass per well was 60 μg. There were three replicates prepared for each formulation tested, prepared in adjacent wells. An example of a plate map used in the screening experiments is shown in Fig. 2.

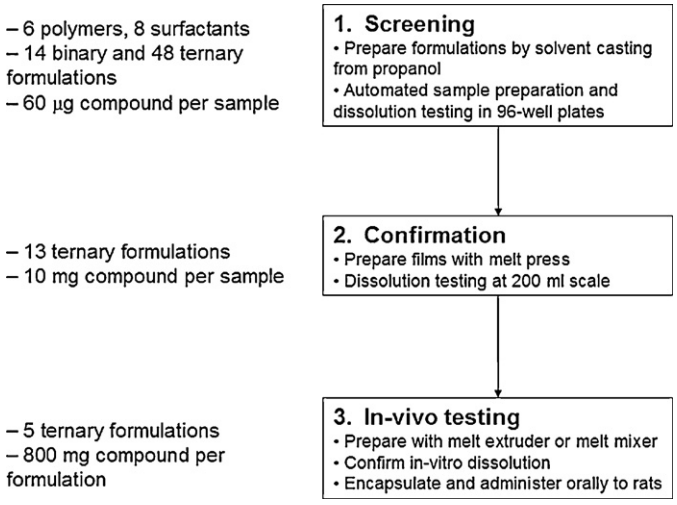



Fig. 1. Schematic illustration of the different stages of experimentation. At each subsequent stage, fewer samples are examined; the samples are larger and more compound is used per sample; and the formulation preparation and characterization methods become more relevant to traditional scale formulation development work.

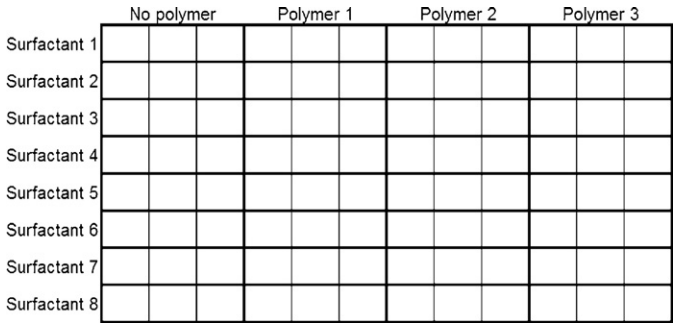



Fig. 2. Plate map used for screening of polymer/surfactant formulations and the corresponding surfactant-only formulations. All formulations were prepared in triplicate within a plate. Columns 1–3 contain only surfactant and compound, while all other wells contain polymer, surfactant, and compound. The total amount of excipient per well is 0.6 mg for all formulations, and the polymer:surfactant ratio is 3:1 for all polymer/surfactant formulations. Each well contains 60 mg of compound.

After dispensing, the plates were briefly vortexed to thoroughly mix the stock solutions, and the solvent was then evaporated using a vacuum centrifuge (Genvac® HT-4X with Genvac® CVP-100 vaccum pump, Genvac, Suffolk, UK). The rotor speed was 1300 rpm, the temperature set point for the swings was 40 °C, and the pressure set point was 5 psi. Temperature control was necessary to prevent the solvent from cooling excessively during evaporation. The majority of solvent under these conditions was removed within about 1.5 h, and the total run time was 2.5 h. At the conclusion of the evaporation run, each well in the microtiter plate contains a pellet or film of formulation at the bottom (total mass = 0.66 mg). The plates were immediately sealed with adhesive foil and held at room temperature overnight prior to dissolution testing. This was done in order to give the most unstable formulations an opportunity to begin to recrystallize. Longer storage times and accelerated storage conditions can also be used prior to dissolution testing,

to assess the longer-term stability of the formulations as part of the screening process.

*2.2.2. Screening method: dissolution testing*

The dissolution test was performed by adding 300 μl of SIF (pH 6.8) to each well, and then gently vortexing the microtitre plate on an orbital shaker for period of 1 h at room temperature. Although it is expected that dissolution/precipitation kinetics will vary significantly between formulations, requiring a high concentration of dissolved drug after 1 h is a reasonable and efficient screening criterion. The contents of each well were then transferred to a 0.2 μm PVDF membrane filter plate (Corning, New York, USA). The samples were pulled through the filter using vacuum and the filtrate was collected in collection plates (Varian, Inc., Palo Alto, CA). One hundred and fifty microliters of the filtrate was then transferred to a deep-well plate (Varian) and thoroughly mixed, by repeated pipetting, with 150 μl of *n*-propanol. Dilution of the filtrate with *n*-propanol prevents any further precipitation of the compound before analysis, and also eliminates any residual turbidity from fine aggregates or particles, which pass through the filter. This last step is essential for accurate analysis of compound concentration by UV absorption, in the presence of excipients, in order to eliminate any residual light scattering, which would otherwise interfere with the measurement.

Two hundred microliters of the diluted filtrate was then transferred to a UV measurement plate (Corning, New York, USA) for immediate analysis. The optical density (OD) was measured at 320 nm using a Spectramax 386 plate reader (Molecular Devices, Sunnyvale, CA). This wavelength was chosen after an examination of the UV spectra for all of the excipients used, which showed that none of the excipients had significant UV absorption at 320 nm, in comparison to the strong absorbance by the compound. The background OD due to absorbance by the plate and the buffer/propanol mixture was determined in a separate experiment and subtracted, and the corrected OD values were converted to compound concentration (μg/ml) using a calibration curve. Using the above procedure, we have previously demonstrated excellent correlation ($R^2 > 0.998$) between the compound concentration as determined by UV and HPLC methods (Mansky et al., 2007), for samples of filtered formulations, which were split for analysis by both methods.

*2.2.3. Melt compression: film preparation*

Thirteen formulations from the screening experiments were scaled up from 0.6 to 100 mg using a melt-press method. A blend of compound and excipients (0.5 g) was prepared by geometric mixing using a mortar and pestle. The blend was placed between two sheets of plastic release liner (3M, St. Paul, MN) made of siliconized polystyrene. A stainless steel shim with a 2 in. × 2 in. square aperture was placed between the two sheets of release liner to control the film thickness to approximately 0.25 mm. The films were compressed using a Carver® hydraulic heated press (Model: Carver M25T, Carver Inc., Wabash, IN). The compression conditions were determined in advance for each blend using a placebo excipient mixture. Generally a force of 1500 lbs and dwell time of 30 s was used, while maintaining the temperature approximately 20 ± 5 °C above the reported softening point of the polymer. The conditions were adjusted in order to produce consolidation of the powders to a homogeneous film during the compression. (This was achieved for all of the polymers except Eudragit L100, which did not flow and consolidate well, even above the softening point, resulting in somewhat fragile films with a grainy, non-uniform structure.) The film samples were stored overnight at room temperature prior to dissolution testing. To prepare the final samples for dissolution testing, a 2.5 cm diameter punch was used to obtain round films weighing between 95 and 105 mg. The measured weights were used for calculating % dissolved from the dissolution data.

*2.2.4. Melt compression: dissolution testing*

The films were mounted on disk shaped sample holders using plastic gauze and tested for release using a modified USP type VII apparatus. The dissolution medium was 200 ml of SIF at 37 °C. One milliliter aliquots were sampled at 0, 5, 15, 30 and 60 min, centrifuged to separate solids, and the supernatant was analyzed by an Agilent HPLC system equipped with UV detector. The column used was a Thermo Hypersil BDS C18, 50 mm × 4.6 mm, 5 μm, and the mobile phase was 50 mM ammonium formate (pH 3.3):acetonitrile (40:60, v/v). The flow rate was 1.5 ml/min. The column temperature was maintained at 35 °C and the compound in the effluent was detected at 260 nm using the UV detector.

*2.2.5. Sample preparation for oral bioavailability studies*

Five formulations from the melt press/*in vitro* dissolution experiments were further scaled up for oral bioavailability testing in rats. Three of these formulations were prepared using an 8 $cm^3$ Dispersion Melt Mixer (designed and manufactured at ALZA), which is similar to the Type Six mixer manufactured by C.W. Brabender®. The batch size was 8 g, consisting of polymer, surfactant, and compound in the weight ratio 75:25:10. Processing time was 5–10 min, at the same temperature used in the melt press for each formulation. Two of the formulations were scaled up for *in vivo* studies using a HAAKE PolyLab Twin-Screw Extruder (Thermo Electron Corporation, Waltham, MA). The batch size was 8 g and the temperature was the same as that used in the melt press. The speed was maintained at 100 rpm, the samples were processed for 10 min with recirculation. After cooling to room temperature, each sample was ground briefly (<1 min) in a food processor, to convert the large/solid pieces of cooled melt to powders, and was dosed within 24 h of preparation. The ground formulations were not sieved prior to administration.

*2.2.6. In vivo pharmacokinetic studies of selected formulations*

A pharmacokinetic study was conducted in male Sprague–Dawley rats ($n = 6$ per group) with selected formulations. Rats in the weight range of 350–400 g were supplied by Charles River Laboratories (Hollister, CA). Rats were fasted overnight, water was provided ad libidum, and the animals were anesthetized with 3% isoflurane inhalant anesthesia immediately before dosing. The solid dispersion formulations were dosed at 3 mg/kg in one or two size nine gelatin capsules

(Torpac Inc., NJ, USA) using a modified 14-gauge gavage needle. Two hundred microliters of water was administered by oral gavage following the administration of the capsules. The animals recovered within 5 min after dosing and were alert throughout sampling.

Unformulated crystalline drug was dosed to one group in a manner identical to the solid dispersion formulations, as a negative control. As a positive control, an oral solution formulation consisting of 2 mg/ml compound in *N*-methylpyrrolidone:Vitamin E TPGS (1:2, v/v) was administered to one group. This had previously been identified as a high bioavailability vehicle for use in pre-clinical animal studies, but is unsuitable for clinical use due to the high NMP content. The solution formulation was given to rats via oral gavage. Both the positive and negative controls were administered at 3 mg/kg. In order to determine absolute bioavailability, an intravenous (IV) formulation of ethanol:solutol:compound (48:48:4, w/w) was prepared, diluted in saline prior to injection to provide a 2 mg/ml compound concentration, and dosed at a level of 2 mg/kg.

Blood was collected into sodium heparinized syringes at 0, 0.5, 1, 2, 4, 6, and 8 h. Plasma was transferred to cryovials and placed on dry ice followed by storage at −80 °C until analysis was performed. 100 μl of the plasma sample was transferred to a 2 ml centrifuge tube and 500 μl of JNJ-25894934 internal standard (1 ng/ml acetonitrile solution) was added. The tubes were vortexed for 4 min and centrifuged at 14,000 rpm for 5 min. 500 μl of supernatant was transferred to 96-well plate. A 10 μl aliquot was injected into the API 4000 LC/MS/MS system. The column used was a MetaChem Polaris, 3 μm C18-A 100 mm × 3.0 mm and the mobile phases consisted of 10 mM ammonium acetate in deionized water and acetonitrile. A gradient flow was used to deliver a flow rate of 0.42 ml/min. The column temperature was maintained at 22 °C. The effluent was detected using an Applied Biosystems API 4000 triple quadrupole mass spectrometer equipped with a positive Turbo ion spray source. The source temperature was set at 550 °C and the ion spray voltage was 5500 V. The system was operated in the multiple reaction-monitoring mode (MRM). The parent/product ion pairs (*m*/*z*) focused were 499.3/399.2 for JNJ-25894934 and 463.1/363.0 for internal standard. The concentration was calculated using weighted linear regression analysis of peak areas from the standard curve. The calibration range was 1–1000 ng/ml and the lowest limit of quantification were 1 ng/ml. Pharmacokinetic parameters such as $C_{max}$, $T_{max}$, $AUC_{(0–8\ h)}$, and absolute bioavailability were determined. The AUC from 0–8 h was determined using the linear trapezoidal method.

## 3. Results

### 3.1. Screening method

Fig. 3 depicts the results of the screening experiments. The results are presented as the percentage of the total compound dissolved per well after 1 h incubation. A value of 100% dissolved corresponds to a compound concentration of 200 μg/ml, i.e. approximately 200 times higher than the compound's aqueous solubility of <1 μg/ml. The color of each cell in Fig. 3 indicates whether % dissolved is <25% (orange), between 25 and 50% (yellow), or >50% (green). Numbers in a bold font and underlined (e.g. **79**) indicate formulations which were subsequently scaled up using the melt press. The number of samples prepared and tested was $n = 3$ for all polymer/surfactant/compound (ternary) formulations, and $n = 6$ for all polymer/compound and surfactant/compound (binary) formulations. Standard deviations for percent dissolved are not shown in Fig. 3, but in most cases were less than 5% (55 of 63 formulations tested), indicating a very high degree of reproducibility. Five formulations in the screening experiments had standard deviations between 15–35% dissolved. Previous studies of precipitation kinetics for surfactant-only formulations, using the same screening method, have shown that large standard deviations are observed for formulations which are in the midst of precipitating at the time of sampling and measurement (Mansky et al., 2007).

Surfactant

| Polymer | None | SLS | Poloxamer 407 | Crodesta F110 | Crodesta F160 | Tween 80 | Vitamin E TPGS | Volpo 10 | Cremophor EL |
|---|---|---|---|---|---|---|---|---|---|
| None | 0 | 0 | 1 | 8 | 31 | 56 | 85 | 87 | 89 |
| Eudragit RS100 | 0 | 5 | 1 | 0 | 0 | 3 | 1 | 3 | 13 |
| Kollidon VA 64 | 0 | **48** | 21 | 31 | **70** | 32 | **52** | 64 | 18 |
| Plasdone K29/32 | 0 | 17 | 20 | 14 | **59** | 72 | **56** | 66 | **79** |
| HPC SL | 4 | 65 | 0 | 22 | **36** | 4 | **54** | 44 | 17 |
| Eudragit L100 | 17 | 4 | **59** | 40 | **56** | 50 | **59** | 62 | **70** |
| HPMCP 50 | 20 | 5 | 1 | 18 | 39 | 8 | **43** | 64 | 38 |

Fig. 3. Summary of the results of the screening experiments. The grid does not have any spatial correspondence to the plate map shown in Fig. 2. The number in each cell is the average value of % dissolved after 1 h of incubation in SIF ($n = 3$ or 6). The color of the cells indicates whether % dissolved was <25% (orange), between 25% and 50% (yellow), or >50% (green). The top row contains the results for surfactant-only formulations; the left column contains the results for polymer-only formulations; the upper left corner contains the results for unformulated compound (no excipients) which was processed by solvent casting in an otherwise identical manner to the formulations; and the remaining cells contain the results for polymer/surfactant formulations. The 13 formulations that were scaled up using the melt press method are identified by the use of a bold/underlined font (e.g. **79**) for % dissolved. Standard deviations are not shown, but were generally less than 5%, see Section 3.1.

The data are arranged so as to clearly illustrate the relationships between the percent dissolved for the ternary formulations and that for the corresponding pair of binary formulations. The data are arranged as a matrix, with each row corresponding to a polymer and each column corresponding to a surfactant. The left-most column contains data for the polymer-only formulations, and the top row contains the data for the surfactant-only formulations. The polymers and surfactants have been arranged in order of increasing % dissolved for the single-excipient (binary) formulations. The remaining cells contain data for the ternary formulations, with the polymer and surfactant corresponding to the row and column, respectively.

Samples that contained no excipients at all, i.e. which contained only 60 μg of compound, were also prepared using the solvent casting method, to determine whether solvent casting itself might lead to enhanced dissolution/solubility, e.g. by formation of amorphous precipitates during solvent casting and a stable supersaturated solution after dissolution. The compound concentration in these samples, after dilution and 1 h of incubation in SIF, was below the detection limit (0%, upper left corner in Fig. 3). This indicates that solvent casting in the absence of excipients does not result in increased compound concentration under the conditions of the experiment.

Three of the surfactants stood out for their ability to increase the dissolution of the compound, when used without a polymer–Vitamin E TPGS, Cremophor EL, and Volpo 10, which gave 85–89% dissolved at 1 h. However, given the non-equilibrium nature of the solvent casting process, the liquid or semi-solid character of these excipients, and the high compound loading level, it is doubtful that these formulations would have good long-term stability to compound recrystallization. Polymers with high $T_g$ are more likely to provide such stability, but for the best-performing compound/polymer formulations (containing only compound with HPMCP 50 or Eudragit L100), the compound was only 20 and 17% dissolved, respectively. It is interesting to note that both of these are enteric coating polymers, carrying carboxylic acid functionality on a backbone which has a partially or wholly hydrophobic character.

Of the 48 ternary formulations screened, 16 dissolved in excess of 50% of the compound after 1 h of incubation (concentration > 100 μg/ml), and 10 dissolved between 25 and 50% of the compound (concentration between 50 and 100 μg/ml). The ternary formulations giving >25% dissolved (yellow or green cells in Fig. 3) can be further divided into two categories. Some contain surfactants which are effective solubilizers when used on their own, such as Cremophor EL, Vitamin E TPGS, and Volpo 10; and the % dissolved, although high, is less than the value for the corresponding surfactant-only formulation. These formulations are on the right side of Fig. 3. In cases such as this, the performance of the ternary formulation seems to primarily reflect the performance of the surfactant—particularly for TPGS and Volpo 10, for which the choice of polymer seems to make little difference. For Tween 80 and Cremophor EL, there is a greater dependence on the choice of polymer, indicating that synergistic effects may be more important in these cases.

Other ternary formulations contain surfactants with mediocre or poor performance (i.e. <25% dissolved) when used alone, such as SLS, Poloxamer 407, and Crodesta F160, but the ternary formulations' performance greatly exceeds that of the corresponding surfactant-only and polymer-only formulations. A striking example of this is the combination of Kollidon VA64 and SLS. Both give 0% dissolved when used individually, but 48% dissolved when combined. Other formulations of this type are SLS/HPC SL, Poloxamer 407/Eudragit L100, and Crodesta F160 combined with Eudragit L100, Kollidon VA64, or Plasdone K29/32. Thus, by systematically screening polymer/surfactant combinations, it is possible to quickly identify potentially effective formulations whose performance could not have been anticipated based on the performance of the individual components. The occurrence of 'synergy' in such formulations is unambiguous. These are not only interesting scientifically, but provide additional options for further formulation development.

### 3.2. Formulations scaled up by melt compression

Thirteen 'hits' identified from the screening study were further studied at larger scale by measuring the dissolution of melt-compressed films. The criteria applied in selecting the formulations for scale up were not strictly defined, but generally comprised two requirements: (1) the surfactant is a solid or semi-solid, which should favor the stability of the formulation and (2) the % dissolved at 1 h in screening exceeds 50%. Eleven of the scaled up formulations met the first criterion, ten met the second criterion, and eight met both criteria.[5]

Percent drug dissolved at 1 h for the melt-pressed samples is presented in Table 2, along with the corresponding screening data (with standard deviations) for comparison. Due to limited compound availability, melt-compressed samples were prepared and tested as either duplicates or single samples. For samples which were tested in duplicate, the two independent measurements of percent dissolved at a particular time point agreed to better than 10% in all cases. Although there is not a rank order correlation between the results from screening and the melt press, the screening method successfully identified multiple formulations which, when prepared by the melt press method, increased the compound concentration from 1 μg/ml to the range 50–150 μg/ml. Of 13 melt-press formulations tested, 9 dissolved >25% of the compound after 1 h of incubation; and 4 dissolved between 50 and 75%.

### 3.3. In vivo pharmacokinetic studies

Five of the 13 melt-pressed formulations were further scaled up using a melt mixer or melt extruder (see Section 2). These formulations were dissolution tested to confirm their effectiveness at dissolving JNJ-25894934 prior to proceeding to *in vivo* studies, and the % dissolved at 1 h is listed in Table 2 alongside the screening and melt press results. Due to limited compound availability, melt-compressed samples were prepared and tested as either duplicates or single samples. For samples which were tested in duplicate, the two independent measurements of percent dissolved at a particular time point agreed to better than 10% in all cases. These formulations were then orally administered to rats to determine whether the *in vitro* enhancement of dissolution would translate into improved bioavailability *in vivo*. Crystalline compound in a capsule and a solution formulation (Vitamin E TPGS/NMP) were also administered, as negative and positive controls, respectively. The plasma serum concentration curves are presented in Fig. 4, and the PK parameters determined from analysis of this data are presented in

[5] Formulations consisting of polymer plus liquid surfactant may still be suitable for use as vehicles for pre-clinical in vivo studies, but should be excluded from screening in future work if the sole objective is identification of potential clinical or commercial formulations.

Table 2
Summary of *in vitro* dissolution data from screening and melt-press samples

| Formulation | % dissolved, 1 h | | | Absolute BA, % ($n = 6$) |
|---|---|---|---|---|
| | Screening ($n = 3$) | Melt press ($n = 1$ or 2) | Melt mixer/extruder ($n = 1$ or 2) | |
| JNJ-25894934 (crystals in capsule) | | | | 0.5 (0.2) |
| IV | | | | 100 (1) |
| Oral solution, VitETPGS:NMP | | | | 27 (6) |
| HPMCP:VitETPGS | 43 (1.7) | 69 | 87 | 23 (7) |
| HPC-SL:VitETPGS | 54 (0.2) | 34 | 61 | 10 (4) |
| PVPVA:SLS | 48 (1.5) | 70 | 40 | 14 (2) |
| PVPVA:VitETPGS[a] | 52 (7.2) | 62 | 99 | 12 (4) |
| PVPVA:Crodesta F-160 | 70 (0.9) | 70 | 87 | 16 (12) |
| Eudragit L100:CRODESTA F160 | 56 (0.8) | 7 | | |
| Eudragit L100:CREMPHOR EL | 70 (2.0) | 19 | | |
| Eudragit L100:POLOXAMER 407 | 59 (3.0) | 42 | | |
| Eudragit L100:VitETPGS | 59 (4.9) | 18 | | |
| HPC-SL:CRODESTA F160 | 36 (2.0) | 40 | | |
| PVP:CRODESTA F160 | 59 (1.0) | 34 | | |
| PVP:VitETPGS | 56 (0.4) | 16 | | |
| PVP:CREMOPHOR EL | 79 (8.7) | 45 | | |

The polymer:surfactant ratio was 3:1 (by weight) for all polymer/surfactant formulations. The compound content in the solid dispersion formulations was 10 wt%, except for PVPVA/TPGS (7 wt%). The absolute BA is included in the last column for those formulations that were scaled up and administered orally to rats. Numbers in parentheses are standard deviations. Standard deviations are not reported for *in vitro* dissolution of melt processed samples as only one or two samples were prepared and tested for each formulation. When tested, replicates agreed to better than 10% in all cases.

[a] 7.7% drug loading.

Table 3. The absolute bioavailability data are also presented graphically in Fig. 5, and are included in Table 2 for direct comparison with the *in vitro* dissolution test results from solvent-cast (screening) and melt pressed films. The absolute BA for the crystalline compound powder and the oral solution formulations was 0 and 27%, respectively, while the absolute BA for the solid dispersion formulations ranged from 10 to 23%.

There was no statistically significant difference between the bioavailability of the various solid dispersion formulations tested, with the exception of the HPMCP/TPGS formulation versus HPC/TPGS, PVPVA/SLS and PVPVA/TPGS ($p < 0.05$). For all other pair-wise comparisons of bioavailability, $p > 0.05$. Thus, there were only limited differences in bioavailability despite differences of as much as a factor of 2 in % dissolved *in vitro* at 1 h. We note that obtaining *in vitro*/*in vivo* correlation for solid dispersion formulations of Class 2 compounds is a challenging task (Verreck et al., 2004), irrespective of any issues specific to the high throughput screening methodology presented here.

## 4. Discussion

In order to be useful, a high throughput (HT) technique must at least be able to (1) prepare and test many more systems (molecules, materials, or formulations) per unit time than would be possible with traditional techniques, e.g. by a factor of 10 or more; (2) identify with acceptable accuracy those systems which are worthy of further study using more resource-intensive traditional techniques (i.e. a screening method must have a reasonably low rate of false positives and false negatives); and (3) use a minimal quantity of the drug substance compared to traditional methods. If these criteria are met, then the HT technique may be used as a 'front end' to traditional experimentation. More predictive and resource-intensive traditional techniques can then be applied to the study of systems which have been pre-selected through high-throughput screening, and which are therefore both more diverse and more likely to succeed than would otherwise be the case.

Table 3
Summary of the PK parameters derived from the plasma concentration data in Fig. 5

| Formulation | Absolute BA (%) | $C_{max}$ (ng/ml) | $T_{max}$ (h) | AUC (ng h/ml), 0–8 h |
|---|---|---|---|---|
| JNJ-X crystals in capsule | 0.5 (0.2) | 2.8 (1) | 4.0 (1.6) | 14 (1) |
| IV | 100 (1) | 2990 (349) | <1(1.6) | 1789 (10) |
| Oral solution | 27 (6) | 138 (26) | 3.2 (2.6) | 832 (193) |
| HPMCP/VitETPGS | 23 (7) | 227 (105) | 2.0 (1.1) | 620 (197) |
| PVPVA/Crodesta F-160 | 16 (12) | 144 (368) | 2.3 (2.8) | 432 (333) |
| PVPVA/SLS | 14 (2) | 84 (116) | 4.0 (1.3) | 369 (61) |
| PVPVA/VitETPGS | 12 (4) | 103 (77) | 2.0 (1.6) | 308 (107) |
| HPC-SL/VitETPGS | 10 (4) | 58 (59) | 4.3 (0.8) | 278 (158) |

Numbers in parentheses are standard deviations ($n = 6$ animals per group).

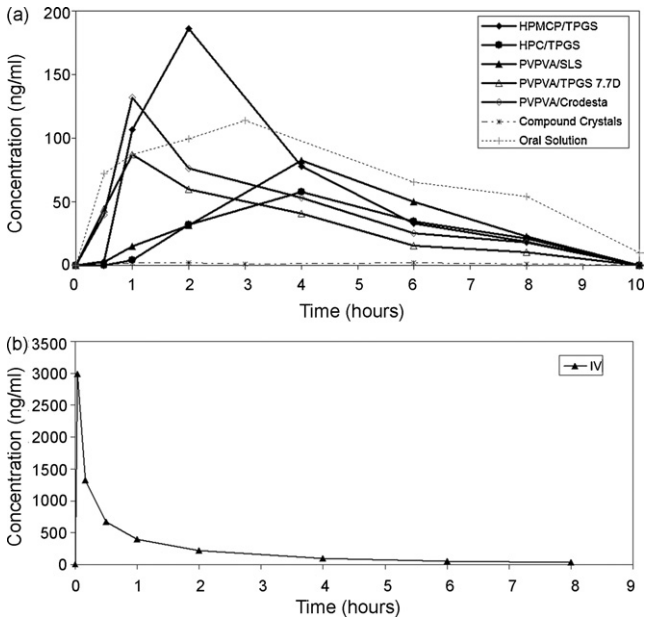



Fig. 4. Plasma concentration versus time for the formulations that were administered orally (top) and intravenously (bottom). Standard deviations are not shown here, but standard deviations of parameters derived from these curves ($C_{max}$, $T_{max}$, AUC, BA) are included in Table 3.

To our knowledge, the present work is the first published use of a high-throughput method for screening of polymer-based solid dispersion formulations of pharmaceutical compounds with low solubility. Other published work on high-throughput formulation of low-solubility compounds has focused on salt and polymorph screening (Desrosiers et al., 2002; Morissette et al., 2004) and on liquid formulations (Chen et al., 2003). The methodology described here involves two major simplifications to permit a high throughput workflow: use of solvent casting for sample preparation, and use of dissolution testing as the experimental method for selection of hits. We here consider the advantages and limitations of this approach, and highlight directions for further improvement of the screening method.

The key advantage of solvent casting in this context is that it allows rapid study of many formulations using small amounts of compound and easily automated sample preparation procedures. In contrast, preparation of a formulation by melt extrusion requires specialized equipment, is time consuming compared to solvent casting, and requires much larger amounts of material.

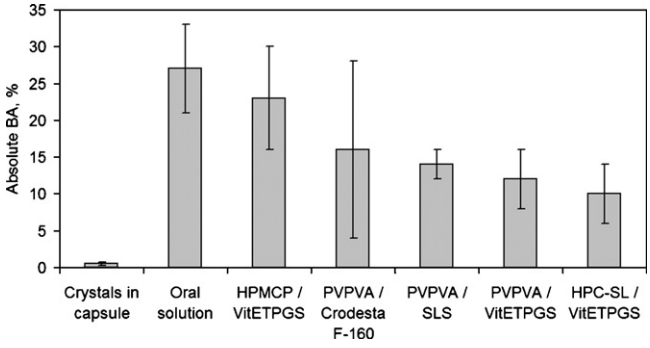



Fig. 5. Oral bioavailability of different formulations in rat ($n = 6$).

Melt extrusion is currently the method of choice for manufacturing of solid dispersions, however, and one cannot expect a formulation prepared by solvent casting to have identical characteristics to the same one prepared by melt extrusion. In melt extrusion, mixing and dispersion of the formulation components are induced by high temperature and shear; whereas in solvent casting, the initial uniform dissolution of the formulation components in a common solvent plays a key role in achieving dispersion.

Although processing is important, however, the inherent physical–chemical interactions of the formulation components are equally critical to formulation performance, and are likely to manifest themselves whether the formulation is prepared by a melt or solvent method. If the compound is highly immiscible with the excipients, or if the excipients have poor ability to solubilize or stabilize the compound in an aqueous medium, then using melt extrusion to prepare the formulation is unlikely to overcome these issues. Similarly, if the compound and excipients interact favorably with regard to miscibility and solubilization, then solvent casting can often be used to produce good solid dispersions, as many workers have shown. For example, Verreck et al. (2003) used solvent casting to prepare Itraconazole/HPMC solid dispersions at five different drug/excipient ratios, and used dissolution testing to select the ratio which was subsequently successfully scaled up by melt extrusion. Numerous other publications report the study of fundamental compound/excipient interactions and miscibility using solvent casting as a method of solid dispersion sample preparation (Kalaiselvan et al., 2006; Sarisuta et al., 1999; Sethia and Squillante, 2004; Wang et al., 2005).

The screening methodology presented here uses only dissolution behavior as a measure of formulation performance, and when used by itself, does not provide any information on the physical state of the solid formulations (crystallinity and phase behavior). For the purposes of initial screening, however, dissolution behavior is not only the most easily measured formulation characteristic, but is also arguably the most relevant to the ultimate purpose of improving dissolution and bioavailability (Leuner and Dressman, 2000). A formulation which is a true molecular dispersion is not useful if the drug precipitates rapidly upon dissolution of the formulation. A partially crystalline formulation with rapid dissolution and good solubilizing or precipitation-inhibiting characteristics may be preferable. Full characterization of the formulation physical state and stability is obviously of great importance, but can be performed on selected formulations which show promising dissolution behavior in screening.

In the present work, we have tested the predictive value of the screening method by selecting 13 hits from screening, preparing them by melt methods at the 0.1–1 g scale, and testing the dissolution *in vitro*. The screening method was quite successful in identifying formulations which, when prepared by melt methods, greatly enhanced compound dissolution in comparison to unformulated compound (e.g. by a factor of 50 or more). Although the correlation of solvent and melt results was not quantitative among the "hit" formulations which were scaled up, we consider the high success rate in transitioning from sol-

vent casting to melt preparation to be a strong indication of the utility of the screening technique. We have further tested the predictive value by selecting five formulations for preparation at larger scale by melt extrusion or melt mixing, and determining the oral bioavailability in rats. The bioavailability of all formulations tested exceeded that of the unformulated compound by at least a factor of 20, although there was not a rank order correlation between *in vitro* and *in vivo* performance. Therefore, the methodology appears to hold great promise for rapid initial screening of polymer/surfactant solid dispersion formulations.

Several issues will need to be studied in order to further develop this screening method. First, the solvent used for casting of the solid dispersion formulations can be expected to have a potentially important impact on the physical state of the resulting solid formulations and their dissolution behavior. A variety of solvents should be studied in combination with a single large set of formulations, to determine how much of an impact the choice of solvent may have. One may even contemplate routinely using several different solvents in screening, i.e. preparing additional replicate formulations with different casting solvents, in order to minimize the chances of missing a promising formulation due to solvent effects. With suitable liquid handling/automation, low compound use per well, and the use of a UV/vis plate reader for analysis, the additional number of experiments could easily be accommodated.

Another issue which should be addressed systematically is formulation physical stability in the solid state, which is typically one of the most significant challenges for solid dispersions. Once initial screening has been done, selected formulations can be re-prepared in the screening format and subjected to accelerated stability testing, e.g. by exposing microtiter plates containing the formulations to elevated temperature and humidity conditions, before repeating dissolution testing. Alternatively, stability of 'hit' formulations may be addressed in a more conventional way using samples subsequently prepared by melt extrusion.

In the present study, we have not made any effort to determine the frequency of 'false negatives' produced by the screening method. A false negative in this context is a formulation which performs well when produced by a manufacturing process such as melt extrusion or spray drying, but does not show up as a "hit" in screening. This issue should be systematically studied by selecting multiple formulations which performed poorly in screening, and scaling them up by melt extrusion to determine whether a significant number of good formulations were missed by the screening technique. An alternative approach to searching for false negatives is to select formulations which are already known to be successful when prepared by melt extrusion or spray drying, and prepare and test them using the screening methodology.

Finally, there is considerable room for modifying *in vitro* testing conditions for improved *in vivo* correlation. However, this issue is not specific to the screening methodology described in this paper, and is a general one for formulation of low-solubility compounds. The goal of improved IVIVC is being actively pursued by many researchers, using approaches such as the development and use of bio-relevant dissolution media (Dressman and Reppas, 2000; Nicolaides et al., 1999, 2001), and the combination of dissolution/precipitation and permeability testing in a single experiment (Corti et al., 2006; Wexler et al., 2005). The issues of improved IVIVC on the one hand, and miniaturization and automation on the other hand, are independent to a significant extent, and it would be valuable to ultimately combine the best practices of both approaches in a single assay format.

## 5. Conclusions

We have developed an efficient method for screening solid dispersion formulations of Class 2 compounds by solvent casting and dissolution testing. The method uses miniaturization, parallel processing, and automation to increase the efficiency, throughput, and scope of the initial screening stages of formulation development, and appears to be particularly useful for screening of ternary and higher-order systems (compound plus two or more excipients). The potential value of the method was evaluated by systematically studying all binary and ternary formulations of a development-stage compound with six polymers and eight surfactants. Some of the 'hits' identified by the screening method appeared to derive their improved performance primarily from the high solubilizing power of the surfactant, while others clearly involved strong synergistic interactions between the polymer, surfactant, and compound. Selected formulations were scaled up using melt methods, and evaluated through *in vitro* dissolution testing and *in vivo* determination of bioavailability in rats. The screening method was quite successful in identifying formulations which significantly enhanced dissolution and bioavailability. Bioavailability in rats ranged from 10 to 23% for the solid dispersion formulations identified by the screening method, versus 0.5% for unformulated (crystalline) compound.

Although the methodology clearly requires further evaluation and development, the data presented here therefore suggest that this screening method is potentially quite useful as a 'front end' for traditional formulation development—i.e. a method for rapidly surveying large numbers of excipients and their combinations and selecting promising formulations for more intensive study with traditional techniques.

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