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RESEARCH PAPER

Formulation and Performance of Danazol Nano-crystalline Suspensions and Spray Dried Powders

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ABSTRACT

Purpose This study focuses on the formulation optimization, *in vitro* and *in vivo* performance of differently sized nano-crystalline liquid suspensions and spray-dried powders of a poorly soluble BCS class II compound *i.e.* Danazol.

Methods A DoE approach was utilized to optimize stabilizer concentration and formulate danazol (BCS class II) nano-crystalline suspensions and dry powders *via* wet milling followed by spray drying. Solubility studies were performed to select best stabilizers. Particle size, PXRD, contact angle measurement and *in vitro* dissolution were utilized in characterization of the liquid and spray-dried powder formulations.

Results The liquid nano-crystalline suspensions followed particle size-dependent dissolution rates *i.e.* faster dissolution for smaller crystals. The spray-dried nano-crystal powders did not show fast dissolution profiles compared to the liquid nano-crystalline suspension. The poor dissolution of the spray-dried powder correlated to its high LogP value (*i.e.* LogP 4.53) and poor wetting (or polar surface-area). *In vivo* bioavailability studies showed superior performance of the liquid nano-crystalline suspensions compared to other milled and un-milled formulations.

Conclusion Wet-milling and spray-drying optimization for danazol nano-crystalline suspension was performed. This study indicates that drug candidates with high LogP values and low polar surface area may not be suitable for formulation as dry nano-crystals.

KEY WORDS danazol nano-crystals · DoE · *in vivo* oral bioavailability · nano-crystalline · spray-drying

ABBREVIATIONS

AUC	Area under the curve
BCS	Biopharmaceutics classification system
Cmax	Maximum concentration
DoE	Design of experiment
NS	Nanosuspensions
PSA	Polar surface area
PXRD	Powder X-ray diffraction
SD	Spray drying
Tmax	Time to reach maximum concentration

INTRODUCTION

The methodologies utilized to discover and test potential new drug candidates are largely biased to select compounds with poor aqueous solubility and/or high hydrophobicity to fulfill the requirement to appropriately bind to the target moiety [1, 2]. Formulation of these compounds is challenging and there is potential for poor bioavailability and possible failure in clinical trial [3–6]. Nano-crystalline suspensions are one of the preferred approaches to increase dissolution rate and hence oral bioavailability [7–9]. Nano-crystalline suspensions offer several advantages such as fast dissolution rates, minimal fed *versus* fasted bioavailability and enhanced bioavailability

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[7–11]. The nano-crystals have high surface area-to-volume ratio, which is a critical factor in dissolution of drug crystals (according to Noyes-Whitey equation) [12]. In addition, nano-crystals have smaller diffusion thickness (or dissolution barrier) and thus faster dissolution, as described by the Prandtl equation. They also have higher surface pressure, which leads to increased solubility as described by the Ostwald-Frendulich equation. However, this increase in solubility is marginal for pharmaceutical nano-crystalline suspensions [13]. Nano-crystalline suspensions are formulated *via* two different approaches *i.e.* top-down and bottom-up [14]. The top-down approach is based on particle size reduction *via* different patented technologies [11]. The bottom-up approach involves nano-precipitation from an appropriate drug solution.

Nano-crystalline suspensions are prone to instabilities such as Ostwald's ripening, aggregation *etc.* due to their small size and liquid formulation. Nano-crystals have high Gibb's free energy and thus can aggregate over time [15] losing their advantage of fast dissolution and high oral bioavailability. Accordingly, spray or freeze-drying can be employed. However, drying processing can also lead to formulation instability. There are very few studies that refer to drying of nano-crystalline suspensions, particularly spray drying [16–21]. Spray drying is preferred over freeze-drying due to time and cost savings.

In this present study, we have utilized milling followed by spray drying technology to produce dry nano-crystals of danazol, a poorly soluble BCS class II drug. A DoE approach was utilized to select the concentration of excipients and facilitate the formulation of a stable nano-crystalline suspension. Danazol was selected based on its poor aqueous solubility (0.6 μg/ml), hydrophobicity (logP 4.53) and lower polar surface area (PSA 46.26). It is categorized as a dissolution rate-limited drug (*i.e.* the dissolution step is the limiting factor for oral absorption). Based on the DoE study, different sizes of danazol nano-crystalline suspensions (as liquid and solid capsule) were formulated and their *in vitro* and *in vivo* performance was evaluated.

MATERIALS

Crystalline danazol (greater than 99% purity) was purchased from Jai Radhe Sales, Ahmedabad, Gujarat, India. PVP40 (polyvinyl pyrrolidone 40KDa) and trehalose were purchased from Sigma-Aldrich. HPMC E3 and Dowfax 2A1 (alkyldiphenyloxide disulfonate) were generously gifted by Dow Chemical Company (Midland, MI). The chemical structures are shown in Table I. HPLC grade solvents were purchased from Fisher Scientific. Luna C18(2) column (4.6 mm × 150 mm, 3 μm) was purchased from Phenomenex (Phenomenex, Torranance, CA).

METHODS

Solubility Measurement

Equilibrium solubility was determined in different excipient solutions at 37°C under continuous shaking for 48 h. Briefly, 10 mg of the drug was added to each vial containing 10 ml of the stabilizer/excipient solution (0.2% *w/v* solution). After 48 h, 1 ml samples were withdrawn from each vial, filtered through a 0.22 μm filter and analyzed using HPLC (as described below in the method section).

Wet Milling

Danazol (1% *w/v*) was suspended in the required concentration of aqueous stabilizer solution as determined from the DoE study (described below). The prepared suspensions were stirred for 30 min for complete wetting of the drug by the stabilizer solution. The suspension (150 ml) was milled using a Netzsch media mill (Netzsch, Exton, PA) at a fixed milling intensity of 2500 rpm in the continuous mode. All the suspension formulations were continuously milled for 60 min and particle size was measured throughout the milling process. The temperature of the samples was maintained below 25°C using 2 cooling bath re-circulators (one attached to the milling and the other to the suspension re-circulation chambers).

Spray Drying

Nano- and macro-crystalline suspensions were spray dried using a Buchi B-290 spray dryer (Buchi Labortechhnik AG, Switzerland). The spray drier was equilibrated using distilled water at 110°C inlet temperature, 5 ml/min feed rate and -30 mbar aspiration rate. The outlet temperature was approximately 75°C. The inlet temperature of 110°C was selected based on (our preliminary study) minimizing nano-crystal aggregation and avoiding polymorphic phase transition as well as achieving a moisture content of less than 3% *w/w*. Once the spray dryer was equilibrated, distilled water was changed to the suspension formulation. Each formulation was spray-dried using mannitol as the excipient (drug:mannitol=1:2.5) to prevent nano-crystal aggregation. Spray-dried powders were collected from the collection chamber and immediately analyzed for particle size and crystallinity.

Design of Experiment (DoE)

Based on the solubility (Table II) and preliminary milling studies, a combination of PVP40 and Dowfax 2A1 was selected to stabilize the danazol nano-crystalline suspensions. Two critical formulation parameters: Dowfax 2A1 concentration and PVP40 concentration were tested for the preparation of

Table I Chemical Structure of the Drug and Excipients

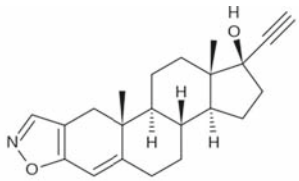
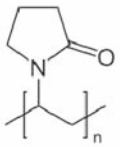
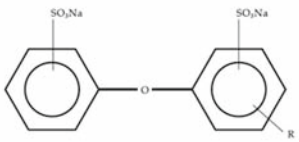
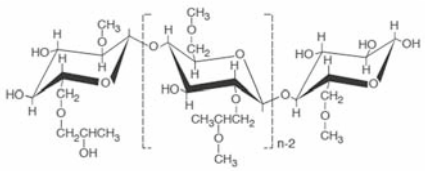
Chemical	Structure
Danazol (steroid)	
Polyvinyl pyrrolidone-40 (PVP-40KDa) (water soluble polymer)	
Dowfax 2A1 (anionic surfactant)	
Hydroxypropyl methyl cellulose E3 (Methocel E3)	

Table II Solubility of Danazol in Different Excipient Solution

Sample No	Excipient solution (0.2% *w/v*)	Solubility (ug/ml)
1	No Excipient	0.57
2	PVP 30	0.68
3	PVA	0.71
4	HPMC E5	0.68
5	HPMC E15	0.68
6	Methocel A15	0.68
7	HPMC E3	0.70
8	SLS	50.38
9	TPGS	29.49
10	Pluronic F68	4.36
11	PVP 40	0.57
12	Dowfax 2A1	4.83
13	HPC	0.66
14	Pluronic F 127	5.66

stable danazol nano-crystalline suspensions using a Netzsch media mill. A central composite design was utilized to optimize and select the right combination of stabilizer concentrations at two levels. The minimum and maximum levels (concentrations) for PVP40 were 0.15 and 0.25% *w/v*, respectively with 0.2% *w/v* as the center point and 0.05 and 0.30% *w/v* were the star or alpha points. For Dowfax 2a1, minimum and maximum levels were 0.01 and 0.02% *w/v* respectively with 0.015% *w/v* as the center point and 0.005 and 0.025% *w/v* were the star or alpha points. The critical quality attributes were particle size before and after spray drying processing. The design space is shown in Table III, a total of 12 wet millings were performed followed by spray-drying with mannitol (drug:mannitol = 1:2.5). Both the liquid suspensions and solid spray-dried powders were stored at 4°C and 25°C for storage stability testing.

Particle Size Measurement

Particle size measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments). Briefly, the liquid or

Table III A Full Factorial Design (DoE) and Results for DoE Run Before and After Spray Drying (with trehalose) of Danazol Nano-Crystalline Suspensions

Batch No.	Factor 1 A:Dowfax 2A1 % *w/v*	Factor 2 B:PVP40 % *w/v*	Liquid nano-crystalline suspension (60 minutes milled)		Spray dried powder (after wet milling)	
			Z average	PDI	Z average	PDI
1	0.015	0.3	186.6	0.211	190.4	0.219
2	0.015	0.2	182	0.166	196.9	0.168
3	0.01	0.25	180.3	0.159	188	0.166
4	0.015	0.1	167.9	0.188	189.7	0.193
5	0.01	0.15	179.8	0.201	185.4	0.228
6	0.02	0.25	173.5	0.193	173.7	0.203
7	0.025	0.2	180.9	0.196	181.6	0.204
8	0.015	0.2	183.2	0.181	189.3	0.21
9	0.005	0.2	320.6	0.086	1255	0.595
10	0.015	0.2	181.8	0.232	184.1	0.227
11	0.02	0.15	179.8	0.212	190	0.2
12	0.015	0.2	171	0.192	174	0.211

spray-dried samples were suspended in a saturated and filtered (0.2 μm membrane filter) solution of danazol in 30% glycerin solution to avoid any discrepancy resulting from dissolution of the nano-particles during measurement. The viscosity of this dispersant solution was measured using a Brookfield viscometer (Model DV-III) and used to calculate the particle size of the re-dispersed and liquid nano-crystalline suspensions. All samples was analyzed in triplicate.

Powder X-Ray Diffraction (PXRD)

PXRD was utilized to determine the crystallinity of the spray-dried samples. X-ray diffraction patterns were obtained using an X-ray diffractometer (Model D5005, Bruker AXS Inc., Madison, WI) with Cu-kα radiation, a voltage of 40 kV, and a current of 40 mA. All the scans were performed with a scanning rate of 2°/min with steps of 0.02° from 5 to 40° at 2θ ranges.

HPLC Analytical Method

The quantification of danazol was conducted using a Shimadzu-HPLC system with a UV detector. The absorbance wavelength was set at 286 nm. The mobile phase was a mixture of 25 mM phosphate buffer (pH 6.1) and acetonitrile at a 35:65*v*/*v* ratio. A C18(2) Phenomenex Luna 3 μ analytical column (4.6 mm×150 mm) was used with a flow rate of 1 ml/min and the column temperature was maintained at 40°C using a column heater.

Storage Stability Testing

All the spray-dried powder formulations were stored at two different temperatures *i.e.* 4 and 25°C for 2 months. Samples were withdrawn regularly and analyzed for particle size and crystallinity.

In Vitro Dissolution Testing

USP apparatus II (AT7 smart, Sotax AG Switzerland) was utilized for the *in vitro* dissolution experiments. In the case of spray-dried powders, the samples were filled into hard gelatin capsules (size 9e, Torpac) and the basket (instead of paddle) was utilized. All the dissolution experiments were conducted at 37°C in 900 ml (sink conditions) of 0.1 N HCl (pH 1.2) with 100 rpm basket speed or 50 rpm paddle speed for capsules and liquid formulations, respectively. At each time point, 2 ml samples were withdrawn from the dissolution chamber and replaced with fresh 0.1 N HCl. The samples were filtered using 0.1 μm filters to avoid any erroneous results from un-dissolved nano-particulates. All samples were analyzed using the HPLC method as described above.

Contact Angle Measurement

The spray-dried nano-crystalline powders (approximately 150 mg) were compressed using a Carver press at a pressure of 5 t for 2 min, after which the pellet was carefully removed from the die. The pellets were vacuum dried overnight at room temperature to remove any moisture adsorbed during processing. The dried pellets were utilized to measure contact angle with distilled water. The contact angle was measured using a contact angle goniometer. A drop of 2.5 μl of distilled water was selected for measurement. All measurements were performed in triplicate and the mean values and standard deviations were reported.

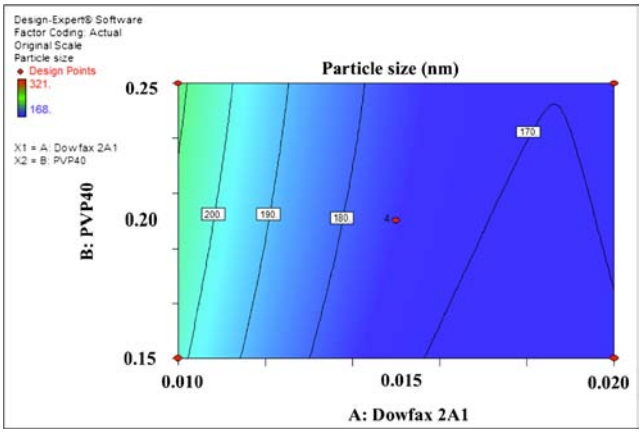


Fig. 1 Contour plot showing the effect of Dowfax 2A1 and PVP40 concentrations (% w/v) on particle size reduction.

Bioanalytical Method

Quantification of danazol was conducted using a Shimadzu-HPLC system attached to a UV detector. The mobile phase was a mixture of 25 mM phosphate buffer (pH 6.1) and acetonitrile at a 35:65 *v*/*v* ratio. A C18(2) Phenomenex Luna 3 μ analytical column (4.6 mm × 150 mm) was used with the flow rate of 1 ml/min and the column temperature was maintained at 40°C using a column heater. Griseofulvin was chosen as the internal standard. The absorbance wavelength was set at 286 nm and 292 nm for danazol and griseofulvin, respectively. Danazol extraction was performed *via* a protein precipitation method with methanol. Briefly, the internal standard (approximately 10 ng griseofulvin) was added to the plasma samples and vortexed. Next 500 μl of methanol was added and the samples were vortexed for 15 min. The precipitated samples were centrifuged at 14,000 rpm for 15 min. The supernatants were decanted to clean centrifuge tubes. The samples were evaporated to dryness using a stream of nitrogen at 45°C. The dried samples were re-suspended in 50 μl methanol and 25 μl of the centrifuged samples were used for HPLC analysis.

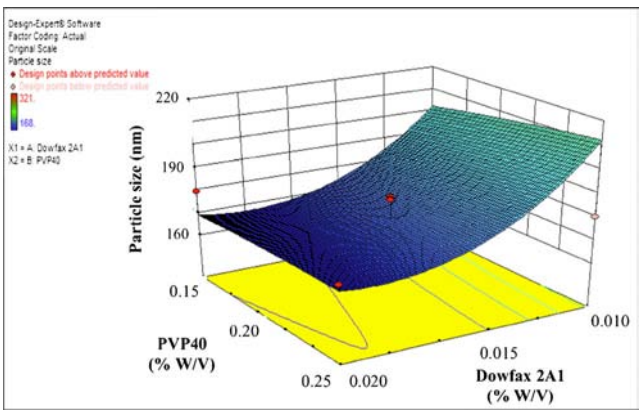


Fig. 2 3D surface plot showing the effect of Dowfax 2A1 and PVP40 concentrations (% w/v) on particle size reduction.

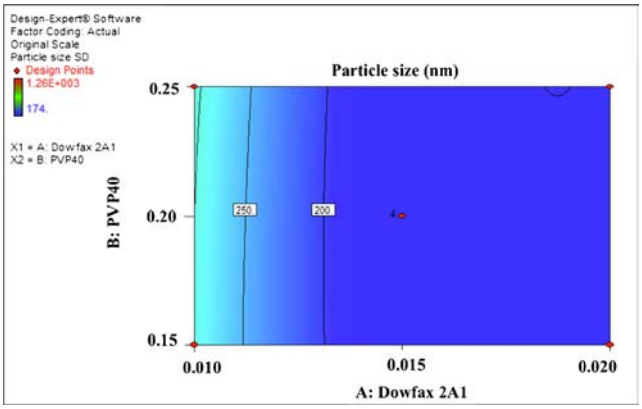


Fig. 3 Contour plot showing the effect Dowfax 2A1 and PVP40 concentration (% w/v) on nano-crystal aggregation following spray-drying processing.

In Vivo Oral Bioavailability

Three danazol formulations with different milling times or particle sizes (180 nm, 740 nm and 8 μm) were dosed at 30 mg per kg of rat body weight. All animal studies were approved by the University of Connecticut IACUC committee. The animals were starved overnight (with free access to water) before the study. The liquid nanocrystalline formulations were administered *via* oral gavage using a dosing needle. At 20 min, 40 min, 1, 1.5, 2, 2.5, 3, 4, 6, 12 and 26 h following oral administration, 175 μL blood samples were collected from a jugular catheter and placed into pre-heparinized centrifuge tubes. The blood samples were centrifuged and the drug extraction procedure was performed as described in the bioanalytical method. The *in vivo* data analysis was performed using Kinetica software 4.4 (Thermo Scientific), using the trapezoidal rule (0–24 h).

RESULTS

Selection of Stabilizer/s

The drug solubility was obtained in different excipient solutions (as shown in Table II). The equilibrium solubility of

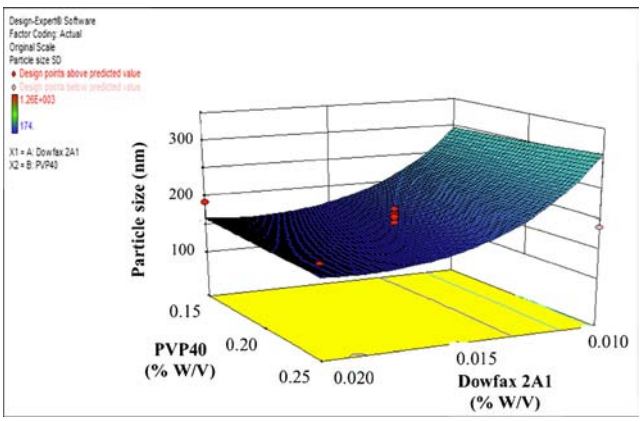


Fig. 4 3D surface plot showing the effect Dowfax 2A1 and PVP40 concentration (% w/v) on nano-crystal aggregation following spray-drying processing.

Table IV Particle Size of the All the DoE Formulations Stored at 4 and 25°C for 60 days

			4°C				25°C			
	Initial		7 days		60 days		7 days		60 days	
Batch No.	Z-Average	PDI	Z-Average	PDI	Z-Average	PDI	Z-Average	PDI	Z-Average	PDI
1	190.4	0.219	194	0.208	211.1	0.193	195.5	0.216	214.3	0.239
2	196.9	0.168	193.3	0.171	209.9	0.159	204.3	0.187	215.4	0.188
3	188	0.166	188.6	0.2	203.4	0.193	188.8	0.184	206.5	0.194
4	189.7	0.193	180.8	0.213	207.6	0.187	191.9	0.194	206.7	0.21
5	185.4	0.228	193.5	0.242	205.9	0.272	194.4	0.21	204.2	0.253
6	173.7	0.203	181.6	0.196	193.3	0.205	179.3	0.223	194.1	0.259
7	181.6	0.204	187	0.208	202	0.188	191	0.193	207.7	0.19
8	189.3	0.21	199.3	0.199	211	0.198	200.6	0.233	216.9	0.227
9	1255	0.595	1401	0.508	1258	0.577	1848	0.571	1724	0.625
10	184.1	0.227	186.1	0.199	195.7	0.216	182.4	0.23	202.1	0.225
11	190	0.2	183.4	0.281	204.7	0.25	191.6	0.235	210.8	0.269
12	174	0.211	183	0.203	193.5	0.228	177.1	0.196	189.7	0.207

danazol significantly increased in the SLS and TPGS solutions and therefore these surfactants were not utilized for nano-crystalline suspension preparation due to the probability that they would affect stability *via* Ostwald's ripening. None of the polymeric stabilizers investigated were suitable for the preparation of small size nano-crystals within 60 minutes of media milling. Based on this observation, combinations of polymeric and small molecule surfactants were selected and wet milling was performed. Pluronic F68, Pluronic F127, and Dowfax 2A1 were tested in combination with different polymeric stabilizers. Either HPMC E3 or PVP40 together with Dowfax 2A1 were the best stabilizer combinations as they produced small nano-crystals (data not shown). Based on these observations, a combination of PVP40 and Dowfax 2A1 was selected for the DoE optimization and formulation study. The design ranges were 0.10–0.20% *w/v* for Dowfax 2A1 and 0.15–0.25% *w/v* for PVP40.

Wet Milling

Wet milling was performed for all suspension formulations as reported in Table III. 150 ml of the required stabilizer solution (a combination of PVP40 and Dowfax 2A1) was prepared with 1% *w/v* of drug. The particle size of the milled liquid nano-suspension is shown in Table III. The particle size of the milled liquid nano-suspensions decreased with increase in Dowfax 2A1 concentration (Figs. 1 and 2). The PVP40 polymeric stabilizer had little effect on particle size reduction.

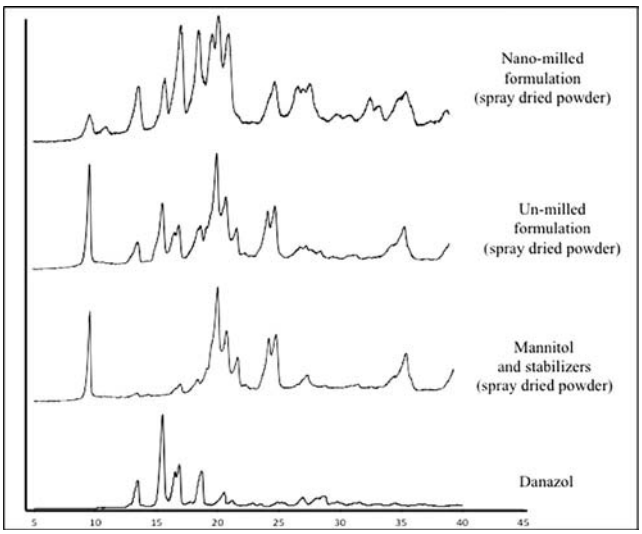


Fig. 5 PXRD of the wet milled and un-milled formulations. (Note: Danazol (raw) and mannitol + stabilizer spray dried powder for comparison).

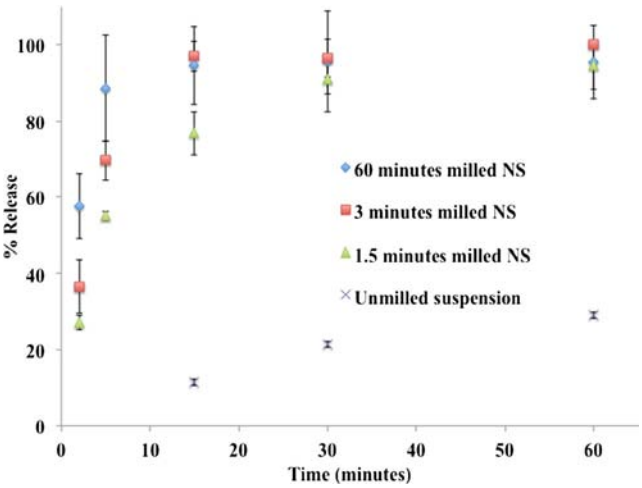


Fig. 6 *In vitro* drug release of different sized (milled *versus* un-milled) danazol liquid suspension formulations (please note: NS represents nanosuspension).

Table V Particle Size of Differently Milling Minutes Formulation and After Spray Drying Processing

Formulation	Particle size (Liquid formulation)	Particle size (Spray dried powder)
60 min milled sample	170.8	180
3 min milled sample	550.5	580
1.5 min milled sample	710	740
Un-milled sample	8 μm	8 μm

Spray Drying

All wet milled samples were spray dried as described in the methods section using mannitol as an auxiliary excipient. Minimal aggregation was observed following spray drying with the exception of batch number 12 (Table III). This formulation had a low concentration of Dowfax 2A1 (*i.e.* 0.005% *w/v*), a negatively charged surfactant. It is speculated that this concentration was insufficient to adequately cover the nano-crystal surface resulting in irreversible aggregation. Contour and 3D surface plots of the nano-crystalline spray dried powders are shown in Figs. 3 and 4, respectively. No polymorphic changes were observed for any of the DoE formulations as determined by PXRD (data not shown). Based on wet milling and spray drying results, a combination of 0.2% *w/v* PVP40 and 0.02% *w/v* Dowfax 2A1 was selected for the danazol nano-crystalline formulation.

Stability

All the DoE samples were stored at 4°C and at 25°C for 60 days as described in the methods section. All formulations were stable at both 4°C and 25°C for at least 60 days (Tables IV). No polymorphic changes were observed (data not shown).

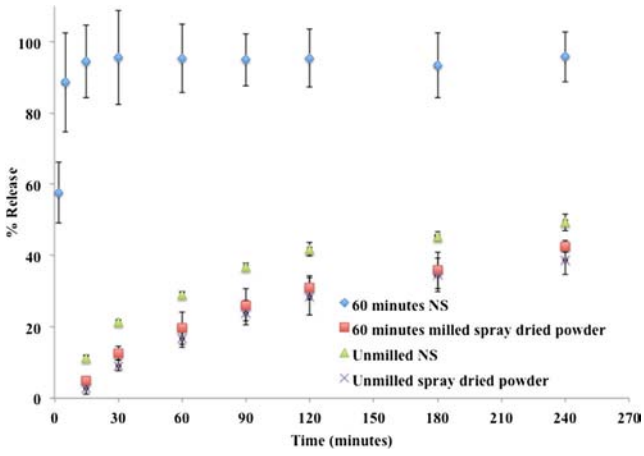


Fig. 7 *In vitro* drug release of different sized (milled *versus* un-milled) crystalline spray dried formulations as liquid and solid (capsule) dosage forms (please note: NS and SD represents nanosuspension and spray dried powder, respectively).

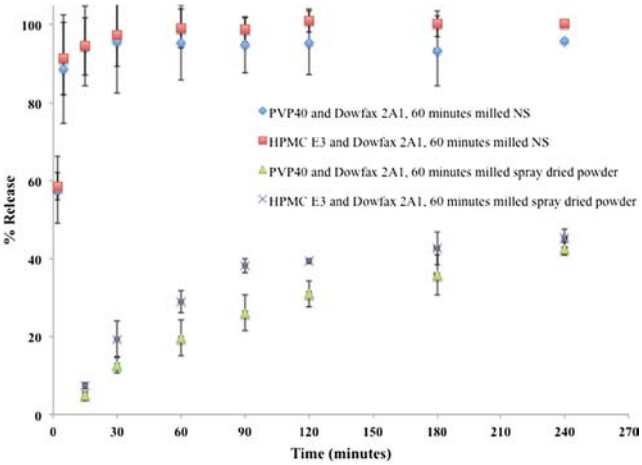


Fig. 8 *In vitro* drug release of milled liquid and spray dried powder (as capsules) containing different stabilizers (please note: NS and SD represents nano-suspension and spray dried powder, respectively).

In Vitro Dissolution Testing

Three wet milled optimized formulations were spray-dried using mannitol as the stabilizer. The PXRD of the milled *versus* un-milled spray dried powder is shown in Fig. 5. No polymorphic changes were observed after milling or spray drying of the danazol nanosuspensions. These optimized danazol crystalline formulations were utilized for *in vitro* dissolution. All the dissolution experiments were conducted as described in the methods section. The effect of milling time/particle size on danazol dissolution is shown in Fig. 6. Nano-crystalline formulations (milled for 60 min) showed faster dissolution rates compared to the 3 min milled, 1.5 min milled and un-milled formulations. In the case of the 60 min milled nano-crystalline formulation, drug dissolution was complete in approximately 5 min compared to 10 min for the 3 min milled and 30 min for 1.5 min milled nano-crystalline formulations. Approximately, 20% dissolution was observed for the un-milled danazol formulation at 60 min. The dissolution rate appeared to be particle size dependent. Interestingly, in case of the spray-dried optimized nano-crystalline powder formulations (60 min milled and then spray dried), although the particle size did not change following drying (Table V) the dissolution profile was similar to that of the un-milled spray dried powder formulation (Fig. 7). In addition, both liquid suspension formulations (*i.e.* 60 min milled and un-milled nanosuspension formulations) were faster than the spray dried powder formulations. To further investigate these findings, a

Table VI Contact Angle Values of Un-Milled and Milled Danazol Powder Formulations

Sample	Contact angle (θ)
Un-milled powder	35.7
Nano-crystalline spray dried formulation (60 min milled)	34.4

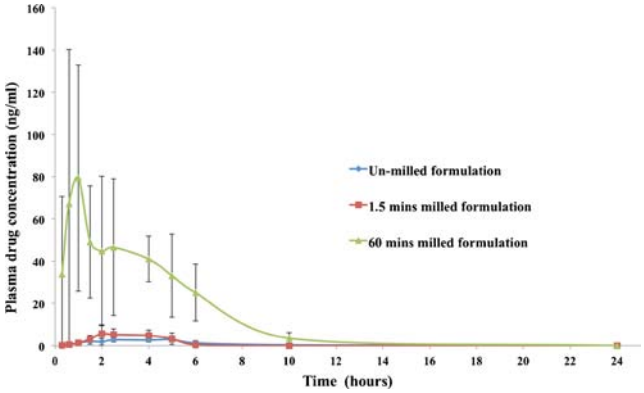


Fig. 9 *In vivo* oral absorption profiles of danazol formulations as liquid dosage forms.

different stabilizer combination (HPMC E3 and Dowfax 2A1 at concentrations of 0.2 and 0.02% *w/v*, respectively) was utilized and nano-crystalline formulation was prepared. However, similar results of poor dissolution were obtained for the spray-dried powders utilizing either stabilizer combination (Figs. 7 and 8).

Contact Angle Measurement

The contact angles of the samples were measured as described in the methods section. As shown in Table VI, the contact angles of the un-milled formulation *versus* the nano-crystalline spray dried powder formulation were similar.

In Vivo Performance

As shown in Fig. 9, the nano-milled liquid formulation (Cmax=80.16 ng/ml) has a superior absorption profile compared to the 1.5 min milled (Cmax=5.97 ng/ml) and un-milled formulations (Cmax=3.88 ng/ml). The bioavailabilities of the nano-milled and 1.5 min milled formulations are more than 13 and 1.5 times that of the un-milled formulation, respectively (Table VII).

DISCUSSION

Nano-crystalline suspensions are prone to undergo Oswald ripening, aggregation *etc*. due to their small size and thus high Gibb's free energy. One of the foremost criteria for stabilizer selection for nanosuspensions is that drug solubility should not significantly increase in the stabilizer solution. Increase in drug solubility can further promote nano-crystalline instability. Danazol equilibrium solubility increased in almost all the excipient solutions except a few where the solubility increase was marginal, as shown in Table II. Danazol solubility was significantly higher in the case of SLS and of TPGS, which could be attributed to their high HLB values (*i.e.* the HLB of SLS is 40 and the HLB of TPGS is 13) compared to the other excipients. Surfactants with high HLB values will act as solubilizers instead of wetting agents. Pluronics should also be avoided with danazol nano-crystalline formulations, as they have significant sticking tendency to the spray drier glass walls, which affects powder yield (unpublished lab data). Dowfax 2A1 had a significant effect on particle size reduction during wet milling, as shown in Figs. 1 and 2. Particle size reduction with increase in Dowfax 2A1 concentration may be due to increase in wetting of the danazol crystals. Wet milling followed by spray drying was performed based on the results of the DoE study to achieve different sized danazol nano-crystals (Table V). The *in vitro* dissolution profiles of these nano-crystalline suspensions followed the particle size *i.e.* faster dissolution rate with smaller danazol crystals.

Interestingly, in the case of the spray-dried nano-crystalline powder formulations (60 min milled and then spray dried), the particle size remained approximately the same (Table V) but the dissolution profile did not show any advantage compared to the un-milled spray dried powder formulation (Fig. 7). This was not considered to be due to the stabilizers (*i.e.* Dowfax 2A1 and PVP40) as similar results were obtained with another spray-dried nano-crystalline powder formulation with a different combination of stabilizers (*i.e.* HPMC E3 with Dowfax 2A1) (Fig. 8). These results suggest that the spray-dried danazol nano-crystalline powders suffer from poor wetting (not aggregation, as no particle size increase was observed after spray drying) leading to delayed-release. The contact angle values of the un-milled *versus* milled spray-dried powder formulations support this conclusion (Table VI). (Please note: The contact angle measurement was performed on spray-dried milled or un-milled powders containing water-soluble mannitol, which resulted in smaller contact angle values compared to the neat drug powder). The molecular properties of danazol, *i.e.* high LogP of 4.53, high contact angle of 90°θ (neat

Table VII Pharmacokinetics Parameters After Per-oral Administration of the Liquid Crystalline Suspension

Formulation	Cmax (ng/ml)	Tmax (h)	AUC (0–24) (ng/ml h)	Fold increase compared to un-milled formulation
60 min milled suspension (180 nm)	80.16	1	316.92 ± 196.54	13
1.5 min milled suspension (710 nm)	5.97	2	36.69 ± 13.31	1.5
Un-milled suspension (8 μm)	3.88	3	24.07 ± 8.86	1

Table VIII List of Few Marketed Formulations Based on Nano-Crystalline Suspension Technology (1- Literature Reported Values)

Product	Drug compound	Indication	LogP[1]	Polar surface area[1]	Dosage
Rapamune®	Sirolimus	Immuno-suppresant	4.85	195.43	Oral tablet and suspension
Emend®	Aprepitant	Anti-emetic	4.5	75.19	Oral capsule
TriCor®	Fenofibrate	Hypo-cholestermic	5.3	52.6	Oral tablet
Megace ES®	Megestrol acetate	Appetite stimulant	3.48	60.44	Oral suspension

danazol powder) and poor polar surface area of 46.26, suggest that it is very hydrophobic with poor wetting properties. However, the liquid danazol nano-crystalline suspension formulation behaved as expected *i.e.* the dissolution rate increased with increase in milling minutes (Fig. 6). It has been previously reported that re-dispersion of dried nano-crystal powders correlated to the hydrophobicity of the drug (*i.e.* drugs with high contact angles and/or poor wetting due to hydrophobicity resulted in poor dissolution) (16). In addition, a few outliers were observed when comparing percent drug dissolution (within 15 min) and hydrophobicity (*i.e.* LogP) of the freeze-dried nano-crystalline powders. However, these authors did not report correlation of drug dissolution and bioavailability to the polar surface area or wettability (*i.e.* by contact angle measurement). In another study, nanosuspensions of different drugs were formulated and their *in vivo* performance was correlated to drug molecular properties [22]. It was concluded from that study that LogP, melting point and polar surface area were the critical factors that influenced *in vivo* drug performance or oral bioavailability. However, the influence of dried powders *versus* liquid nano-suspensions on *in vitro* and *in vivo* oral performance was not investigated. In addition, all the marketed formulations based on nano-technology that are dried powders (such as tablets or capsules) have a polar surface area of more than 50 (Table VIII). All these results suggest polar surface area (or wettability *via* contact angle measurement) along with LogP needs to be carefully evaluated, if the required final product is a nano-crystalline solid dosage form such as capsules or tablets.

In the present work, it was observed that both LogP and wettability (or polar surface area) are important characteristics for *in vitro* and *in vivo* performance of the dried nano-crystalline powder. The *in vivo* oral bioavailability of the liquid danazol suspensions increased significantly with decrease in the particle size as expected.

CONCLUSIONS

This study shows the importance of the molecular properties of poorly soluble drug candidates (BCS class II/IV) for drying of nano-crystalline suspension formulation. Drug candidates with poor wetting properties (due to high LogP and high contact angle) formulated as nano-crystalline suspensions may not be appropriate for drying processing. Much of the published work in the area of drying of nano-crystalline suspensions is focused on freeze-drying technology. The present study shows that the more cost effective spray drying technology may be utilized to achieve non-aggregating nano-crystalline powders. A DoE approach was successfully utilized to produce nano-crystalline suspensions *via* wet milling followed by spray drying. The *in vivo* bioavailability of danazol nano-crystalline suspensions showed superior performance compared to micro-sized and un-milled nano-crystalline suspension formulations. The *in vivo* oral absorption data for the danazol nano-crystalline suspensions follows the *in vitro* dissolution results, indicating that *in vitro* dissolution may be used as a predictor of particle size and hence bioavailability. The bioavailability of the nano-crystalline danazol formulation was 13 times that of the un-milled formulation.

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