Journal of Drug Delivery Science and Technology 43 (2018) 343–352

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

The potential of synergism between ultrasonic energy and Soluplus® as a tool for solubilization and dissolution enhancement of a poorly water soluble drug. A statistically based process optimization

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ARTICLE INFO

Keywords:
Sonocrystallization
Soluplus®
Solubilization
Dissolution enhancement

ABSTRACT

The present study was a trial to improve the solubility and dissolution of a model poorly water soluble drug, nimesulide, through a combined technique of ultrasonic exposure with a surfactant treatment. A 2^3 full factorial design was applied to study the effect of varying the drug concentration, the solvent: anti-solvent ratio and the ultrasonication time on the % yield, saturated solubility and particle size of the produced sonocrystals either plain or combined with 0.025% Soluplus®. Optimized formulae were subjected to particle crystallinity and dissolution studies. Differential scanning colorimetry showed a successive lowering in the Δ H value from −25.41 J/g for the drug to −22.79 J/g for F5 and -16.7 J/g for F5+ 0.025% Soluplus® which possessed the most favorable heat of solution. X ray diffractometry showed that optimized sonocrystals acquired lower relative degree of crystallinity than untreated particles; however the addition of surfactant restored the original degree of drug crystallinity. Ultrasonic energy increased the rate of drug dissolution through a hastening in the value of $t_{1/2}$ from 236, to 151 and 88.5 min for plain drug, F5 and F5 with 0.025% Soluplus® respectively. Therefore, subjecting the drug to ultrasound energy assisted with amphiphilic surfactant proved to be a simple and promising technique to treat drug insolubility.

1. Introduction

Considerable technical challenges are met in the pharmaceutical industry when dealing with pharmacologically active drugs that possess poor aqueous solubility. Formulators have to find out efficient techniques that improve aqueous solubility of these drugs as their absorption in the gastrointestinal tract is dissolution rate limited [1,2]. Many strategies continue to arise treating the current problem [3].

Particle size reduction is a universal technique for solubility improvement of poorly soluble drugs in the pharmaceutical field [4]. The wet milling process was clearly identified and scaled up on industrial scale [5]. Although the method was widely applicable yet it was not cost effective due to specific equipments needed. Decreasing the particle size by micronization [6] or even nanonization [7,8] was reported to increase the saturated solubility and in turn, the bioavailability was improved. Various micronized formulae have emerged to increase the dissolution rate and bioavailability of poorly soluble drugs, like aprepitant [9], cilostazol [10] and nitrendipine [11] as model drugs. Formulations like solid dispersion [12], microemulsions [13], vesicular [14] and micellar systems [15,16] were extensively applied as an alternative for micronization. However, potential excipient-related toxicity might be a common drawback.

The anti-solvent re-crystallization process is a promising bottom-up method to prepare micronized drugs. It provides more convenient procedure at ambient temperature and atmospheric pressure with no requirement of expensive equipments. It is easily scalable compared to other bottom-up methods. This technique has been successfully used to prepare several drugs, such as *trans*-resveratrol [17], and atorvastatin calcium [18].

During the last decade, a new particle engineering technique based on ultrasound, have been introduced to the field of pharmaceutical technology [19]. The theory arises from the possible application of ultrasonic energy during particle formation [20]. The so-called “sonocrystallization” technique is known to influence the process of crystallization of poorly soluble drugs in different steps. First, it reduces the induction time for initiation of crystallization [21,22]. Second, it reduces the amount of anti-solvent required for crystallization [22]. Third, it tends to narrow the particle size distribution of the resulting sonocrystalized particles with a probable change in crystal geometry [19,23]. Variation in the crystal habit of drugs as a result of exposure to

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https://doi.org/10.1016/j.jddst.2017.10.023
Received 18 September 2017; Received in revised form 19 October 2017; Accepted 30 October 2017
Available online 11 November 2017
1773-2247/

Table 1
Experimental runs and measured responses of NM sonocrystals according to a full factorial design.

Formula Code	Independent variables			Measured repsonses				
	Drug conc. (%)	Solvent: antisolvent (v/v)	Sonication time (min.)	% Recovered	Saturated solubility (mg/mL)	Particle size (um)		
						d_{10}	d_{50}	d_{90}
F1-NM	Plain drug				0.225			
F2	10	1:5	5	47.99	1.108	2.96	10.29	31.08
F3	10	1:5	10	65.18	0.885	2.69	9.2	28.9
F4	10	1:10	5	37.12	1.399	2.92	9.8	34.99
F5	10	1:10	10	43.58	1.751	3.3	10.08	33.96
F6	15	1:5	5	70.81	0.706	3.53	12.66	27.73
F7	15	1:5	10	66.23	0.882	3.74	13.51	30.72
F8	15	1:10	5	52.58	1.199	3.26	11.65	28.46
F9	15	1:10	10	50.83	1.369	3.27	10.58	35.37

Table 2
Output data of the 2^3 factorial analysis of NM sonocrystals.

Responses	R^2	Adjusted R^2	Predicted R^2	Adequate precision	Significant factors
Y_1: % Recovered	0.9779	0.9631	0.9300	22.995	X_1, X_2, X_3, X_1X_3
Y_2: Saturated Solubility (mg/mL)	0.9481	0.9135	0.8360	14.048	X_1, X_2, X_3, X_2X_3
Y_3: d_{10} (%)	0.8891	0.8152	0.6496	8.964	X_1, X_1X_2
Y_4: d_{50} (%)	0.9137	0.8562	0.7273	9.616	X_1, X_2, X_1X_2
Y_5: d_{90} (%)	0.9850	0.9751	0.9527	25.019	X_1, X_2, X_3, X_1X_2, X_1X_3, X_2X_3

Table 3
Regression results of the measured responses (coded values).

	Y_1: % Precipitated		Y_2: Saturated Solubility (mg/mL)		Y_3: d_{10} (um)		Y_4: d_{50} (um)		Y_5: d_{90} (um)	
		p-value		*p-value*		*p-value*		*p-value*		*p-value*
Intercept	54.29		1.16		3.21		10.97		31.4	
X_1	5.82	$< 0.0001^*$	−0.12	0.0006^*	0.24	$< 0.0001^*$	1.13	$< 0.0001^*$	−0.83	$< 0.0001^*$
X_2	2.17	0.0040^*	0.059	0.0366^*	0.041	0.2741	−0.13	0.3721	0.84	$< 0.0001^*$
X_3	−8.26	$< 0.0001^*$	0.27	$< 0.0001^*$	−0.021	0.5633	−0.44	0.0102^*	1.79	$< 0.0001^*$
X_1X_2	−3.75	$< 0.0001^*$	0.027	0.2915	0.014	0.7069	0.074	0.6036	1.64	$< 0.0001^*$
X_1X_3	−0.14	0.8034	−0.022	0.3846	−0.16	0.0012^*	−0.54	0.0034^*	−0.45	0.0037^*
X_2X_3	−0.99	0.1144	0.071	0.0165^*	0.056	0.1467	−0.069	0.6280	0.63	0.0004^*

*Means significant.

ultrasound energy was found to improve micromeritics of crystalline drugs as well as enhancing their solubility and dissolution characteristics [24–26]. As a pharmaceutical operation, sonocrystallization seems to be an attractive tool for the improvement of biopharmaceutical properties of poorly soluble drugs specially those belonging to class BCS II [27,28].

Stabilizers are of great importance during re-crystallization, as they prevent particle aggregation in the dispersion media, by adsorption at the interface of drug particle. Moreover, they provide stabilization via two mechanisms: steric hindrance (polymeric and non-ionic stabilizers) and electrostatic repulsion (ionic stabilizers) [29]. Different examples of stabilizers have been reported in the literature [30].

Soluplus®, a graft copolymer of polyvinyl caprolactam-polyvinyl acetate polyethylene glycol is known with its excellent solubilizing properties for poorly soluble drugs. Although it is a surfactant molecule, its application as stabilizer for nanocrystals was not much explored. Yang et al., reported the successful stabilizing property of Soluplus® for the first time in the development of fenofibrate nanocrystals and reported that Soluplus® was superior to HPMC [31].

Nimesulide (NM), a BCS II potent anti inflammatory and antipyretic drug, suffers from dissolution rate limited bioavailability. Many attempts have been made to increase its aqueous solubility and dissolution from oral formulations [32,33], through the addition of excipients and/or surfactants to assist solubilization. Nowadays newer tendency is shifted towards the substitution of chemical additives used in formulation by other agents that are not hazardous to patient health [34]. In this respect, the application of ultrasonic energy is considered simple, efficient and not costly. The technique could be used extensively both at laboratory and industrial scale [35].

The present study will explore the effect of different variables in the process of sonocrystallization of NM on some of its physicochemical and micromeritics properties that can guarantee maximum drug solubilization and dissolution. Thus, as a first step, the effect of different process variables on the yield, saturated solubility and particle size of NM sonocrystals was studied using a full factorial experimental design. Dissolution rate of the optimized NM sonocrystals were also evaluated and compared to that of the raw drug. Solid state characterization of the optimized NM sonocrystals was evaluated by means of differential scanning calorimetry and X-ray diffraction. The possibility of magnifying the effect of ultrasonic energy on dissolution enhancement by the application of Soluplus® as stabilizer during the preparation of NM sonocrystals was also explored.

2. Materials

Nimesulide (NM) was kindly supplied by from Alkan Pharma, Egypt. Absolute ethanol, disodium hydrogen phosphate, potassium dihydrogen phosphate: El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Soluplus® was purchased from BASF, Germany.

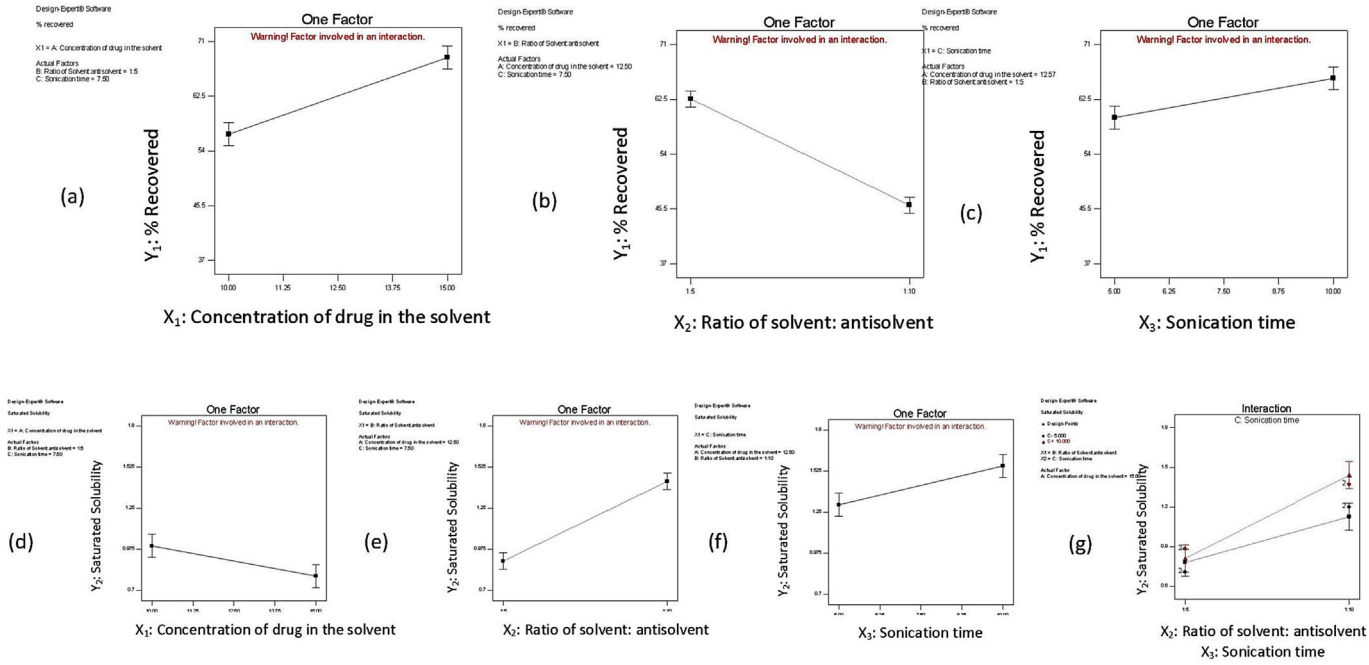


Fig. 1. Line charts showing the effects of different variables on the measured responses.
(a) Drug concentration in the solvent on the % recovered.
(b) Ratio of solvent:antisolvent on the % recovered.
(c) Sonication time on the % recovered.
(d) Drug concentration in the solvent on the % saturated solubility.
(e) Ratio of solvent:antisolvent on the % saturated solubility.
(f) Sonication time on the % saturated solubility.
(e) Ratio of solvent:antisolvent and Sonication time on the % saturated solubility.

3. Methods

3.1. Preparation on NM sonocrystals

NM sonocrystals were prepared by anti-solvent re-crystallization technique. In this process, ethanol and distilled water were chosen as solvent and anti-solvent of NM, respectively. A certain amount of raw NM was dissolved in absolute ethanol and the solution was then exposed to ultrasonic treatment using a probe sonication apparatus (Chrom Tech) with an ultrasonic processor, Model: UP-500, SN: UH005 0102, Input power: 220V (England) for 10 min to make sure that all drug particles were completely dissolved. Ten milliliters of NM–ethanol solution were injected gradually into a certain volume of the anti-solvent. The mixed solutions were sonicated with a probe of 1/8 inch diameter for different time intervals. The sonication worked for 9 s and then solutions were kept under rest for 1 s. NM sonocrystals were then recovered by filtration (through whatman filter paper n° one) and dried in an oven at 40° C for 24 h.

3.2. Evaluation of the percentage yield recovered

The percentage yield recovered will be measured indirectly through measuring the un-crystallized drug during filtration of NM sonocrystals. An aliquot of the filtrate was properly diluted and measured spectrophotometrically at 393 nm to determine the percentage of NM recovered, using the following equation:

$$\%\ \text{NM yield recovered} = \frac{\text{Total NM} - \text{uncrystallized NM}}{\text{Total NM}} \times 100$$

3.3. Characterization of the prepared sonocrystals

3.3.1. Determination of the saturated solubility of NM sonocrystals

An excess amount of NM sonocrystals or NM raw powder was added to 5 mL phosphate buffer saline pH = 7.4, and shaken at 37 ± 0.5 °C for 72 h in a thermostatically controlled water bath (Clifton, Nickel-Electro LTD, England). The samples were then filtered through a 0.45 μm membrane filter, suitably diluted and the concentration in the solution was determined spectrophotometrically at λ_{max} 393 nm.

3.3.2. Particle size analysis

The particle size of the prepared batches of sonocrystals was detected by a Mastersizer 2000 laser diffractometer (Malvern Worcs, UK). The size distributions were calculated by the cumulative volume diameter and expressed as d_{10}, d_{50}, and d_{90} mean diameter (d_{10}, d_{50} and d_{90} indicated that 10%, 50% and 90% of the particles were below that corresponding size in um, respectively).

3.3.3. Optical microscopy

A thin layer of NM raw drug and the selected NM sonocrystals were examined using an ordinary light microscope (Leica Imaging Systems, Cambridge, UK). Photomicrographs were taken using a digital camera (Victor, Yokohama, Japan).

3.3.4. Effect of Soluplus® on the characteristics of optimized NM sonocrystals

Another batch of optimized sonocrystals was prepared by the same procedure mentioned in the preparation of the optimized NM sonocrystals, except that 0.025% w/w Soluplus® was dissolved in the anti-solvent before addition of NM-ethanolic solution.

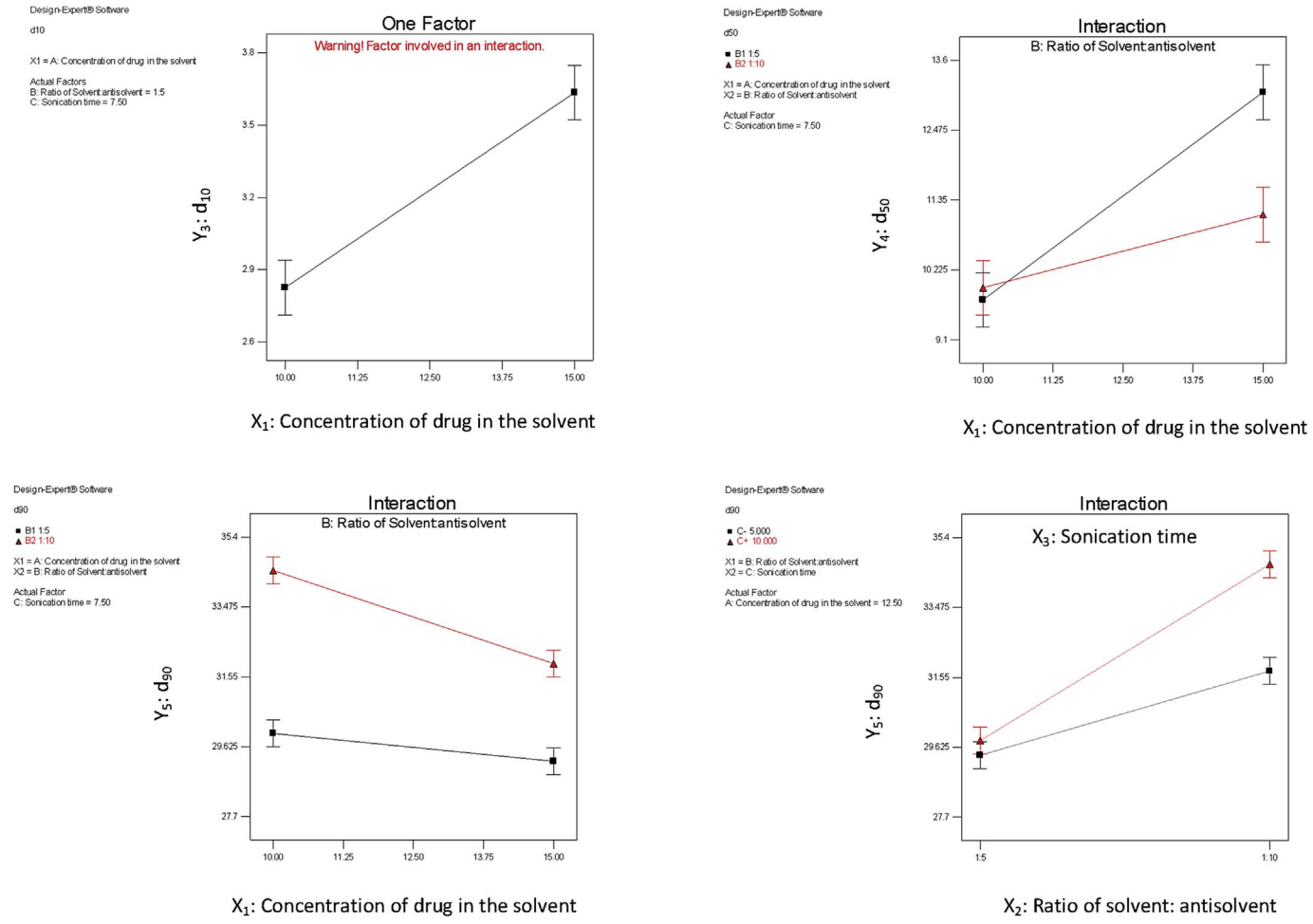


Fig. 2. Line charts showing the effects of different variables on the particle size of the prepared sonocrystals.

3.4. Evaluation of particle crystallinity of the optimized NM sonocrystals

3.4.1. Differential scanning calorimetry (DSC)

Powder crystallinity was assessed by DSC (TA Instruments Q1000, USA). Thermal analysis was carried out using DSC (TA instruments, model DSC 204) for NM pure powder and the optimized sonocrystals. Analysis was performed for 2.0 mg samples at a temperature heating rate of 10°C/min and a temperature range of 30–250 °C using nitrogen purge.

3.4.2. X-ray diffraction (XRD)

XRD of NM pure powder and the optimized sonocrystals were performed by X-ray diffractometer (D5000, Siemens, Germany). The XRD was performed at room temperature using Cu Ka1 radiation generated at 100 mA and 50 kV during the range from 5°to 60°.

The relative degree of crystallinity (RDC) was calculated according to the following equation:

$$RDC = I_{sam}/I_{drug}$$

where I_{sam} is the peak height of the sample under investigation and I_{drug} is the peak height of the drug at the same angle [56].

3.4.3. In-vitro dissolution study

The dissolution of raw drug, and optimized sonocrystals was performed using USP dissolution tester (Pharma Test Dissolution Tester, Germany), Apparatus II (paddle method). The solution temperature was set at 37 ± 0.5 °C and paddle speed was maintained at 100 rpm. Accurately weighed samples (20 mg) were dipped into 600 mL phosphate buffer (pH = 7.4) as dissolution medium. Samples (3 mL) were withdrawn at different time intervals, replaced with equal volumes of fresh medium, filtered using 0.22 μm filters and measured spectrophotometrically for NM at λ_{max} 393 nm after appropriate dilution. All experiments were done in triplicate (n = 3).

3.4.4. Kinetic analysis of dissolution data

In order to explore and compare the mechanism and rate of dissolution of optimized sonocrystals with or without carrier and that of the plain drug particles, the data obtained from dissolution experiments were treated statistically according to linear regression analysis (using Microsoft office Excel 2007 software). The data were fitted to zero order, first order and Higuchi diffusion model. Kinetic treatment of the data was then performed for the order of the best fit.

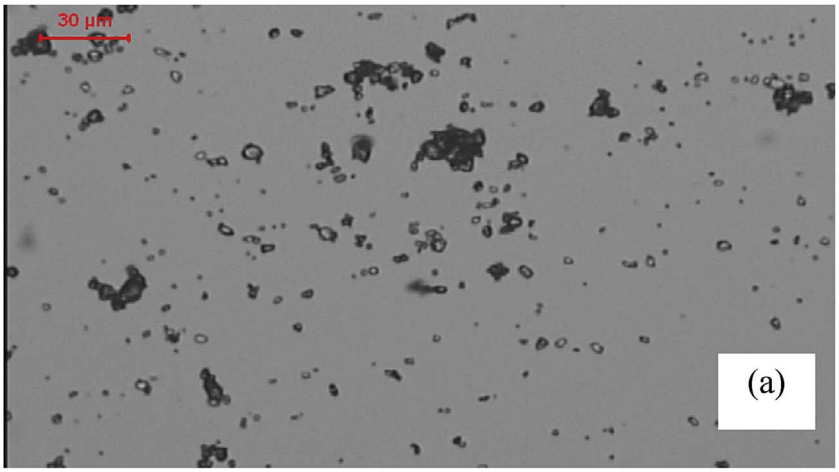
Equation for zero order: $C = C_\circ - K_\circ t$

Equation for first order: $\log C = \log C_\circ - \frac{Kt}{2.303}$

Simplified equation for Higuchi diffusion model: $Q = K \times t^{1/2}$

3.4.5. Determination of dissolution efficiency

The extent of dissolution is an important parameter that measures the amount of drug dissolved after a specified time. Dissolution efficiency was used to calculate the total amount of drug available in solution in dissolution experiments for optimized products as well as for plain drug crystals according to the following formula [36]:



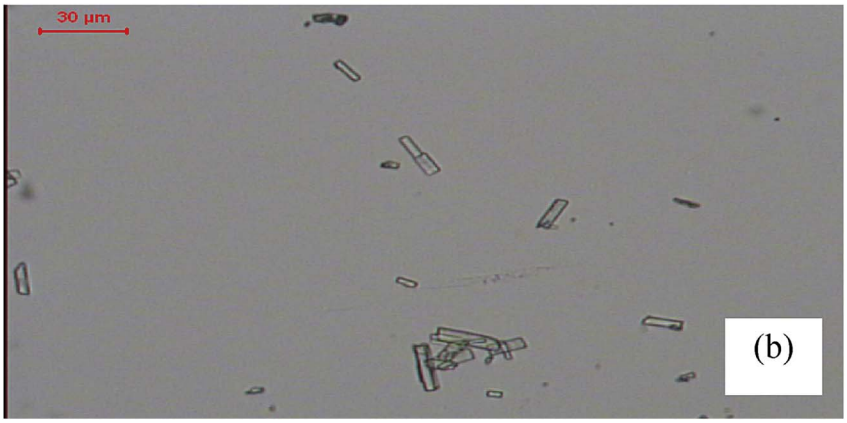


Fig. 3. Photo-images of (a) NM plain powder, and (b) NM sonocrystals F5.

Table 4
Dissolution efficiencies and kinetic analysis of the dissolution results.

Formula Code	DP_{360}[a]	DE360[b]	Linear Regression Analysis of Data						Kinetic treatment of data			
			Slope			R^2			K	t½(min)	Y intercept	
			Zero	First	Diffusion	Zero	First	Diffusion			value	significance
NM	59.68	35.67	0.1574	−0.0011	3.6756	0.9701	0.9963	0.9977	3.6756	272.8	−10.709	8.5[c]
F6	62.1	40.03	0.1587	−0.0011	3.7154	0.9583	0.9900	0.9901	3.7154	235.6	−7.0327	3.6[c]
F5	76.51	54.23	0.1664	−0.0015	3.8883	0.9702	0.9982	0.9991	3.8883	150.9	2.2383	2.2[d]
F5/0.025% Soluplus	95.65	72.14	0.19	−0.0034	4.6131	0.8710	0.9970	0.9686	0.0078	88.5	1.9015	20.29[d]

[a] Amount dissolved at 360min.
[b] Dissolution efficiency at 360min expressed as percent.
[c] Lag time in min.
[d] Flush dissolution in mg%.

$$DE\% = \int_0^t \frac{y \cdot dt}{y100 \cdot t} \times 100\%$$

4. Results and discussion

4.1. Analysis of the factorial design

Factorial designs are commonly used to analyze the influence of different variables on the properties of a drug delivery system. Table 1 shows the experimental runs and the measured responses of the prepared NM sonocrystals. The predicted R^2 values were in reasonable agreement with the adjusted R^2 in all responses (approximately 0.2 differences between them) (Table 2). Adequate precision measured the signal-to-noise ratio to ensure that the model can be used to navigate the design space. A ratio > 4 (the desirable value) was observed in all responses. Polynomial equations were generated to establish the relationship between the factors and the responses. A positive sign before a factor in a polynomial equation indicates a synergistic effect, whereas a negative sign represents an antagonistic effect. Table 3 represents the regression results of the measured responses (coded values) for NM sonocrystals. The values of the coefficients X_1–X_3 relate to the effects of these variables on the corresponding response. Coefficients with more than one factor term (X_1X_2, X_1X_3, X_2X_3) represent the interaction terms. The significance of each coefficient was determined on the basis of *p*-values as listed in Table 3.

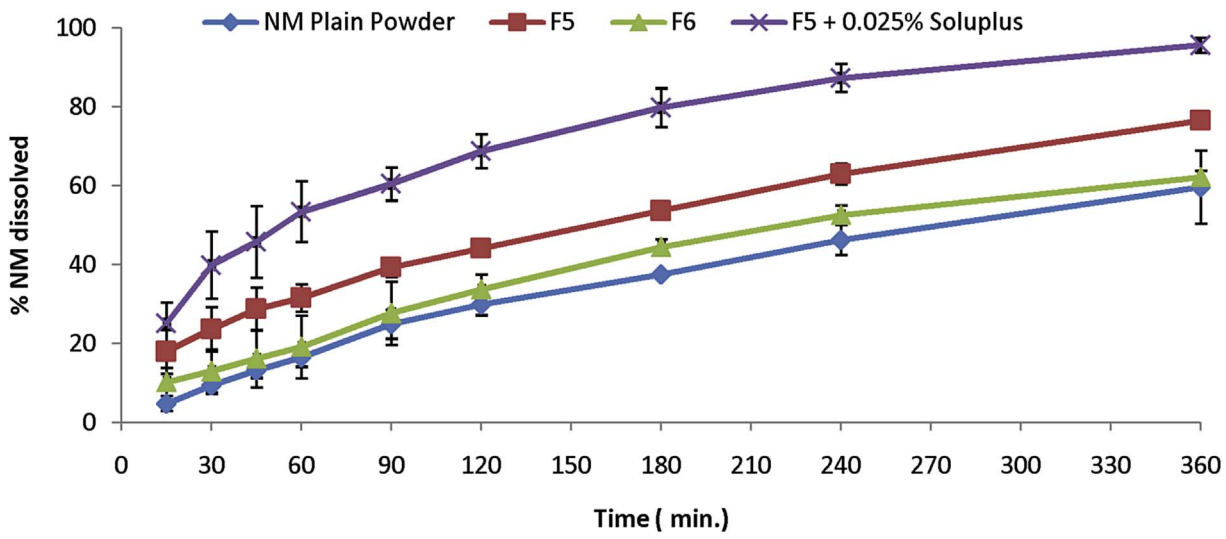


Fig. 4. Dissolution profiles of plain NM, F5, F6, and F5/0.025% Soluplus.

4.2. Effect of varying the concentration (% w/v) of NM

ANOVA results showed that the percentage concentration of drug in the solvent had a significant effect on the % NM recovered ($p < 0.0001$). Fig. 1 shows the line charts which illustrate the effect of different variables on the %NM recovered. It is clear that increasing the drug concentration in the solvent from 10% to 15% resulted in significantly higher yield % of NM recovered from the solution (Fig. 1). Under identical condition of preparation, the higher the concentration of the parent solution, the higher will be the state of supersaturation where the solution retains the ability to held excess solute under the influence of ultrasonic energy. When conditions are restored (reorientation of drug molecules in solution after cessation of sonication), a high % of drug crystals are delivered from solution. The circulation of ultrasonic waves throughout the crystallization container might caused a more effective microscopic mixing of solution with anti-solvent [37]. The cavitation effects were also thought to influence greatly the nucleation rate and hence a higher yield of drug was obtained [38,39].

As shown in Fig. 1, increasing the drug concentration in the solvent resulted in significantly lower NM saturated solubility ($p < 0.0001$). This was a practical issue since higher drug concentration offered a higher state of supersaturation which implicates that higher weights of particles could not be held in solution rather they tended to crystallize out. Ultrasonic waves assisted this action by accelerating the diffusional path inside the vessel as a result of more intimate contact between solvent and anti-solvent. A faster induction for nucleation thus occurred and opposed the enhancement in the saturated solubility of the drug [39,40].

Fig. 2 shows the line charts which demonstrate the effect of different variables on the mean particle size (MPS) of NM sonocrystals. Increasing the concentration of drug in the solvent had a profound effect on the mean particle size (MPS) of NM sonocrystals ($p < 0.0001$ for both d_{10}, and d_{50}). MPS increased with increased drug concentration in the solvent. This appeared to depend on crystallization speed, the latter increased in more concentrated solution causing a tendency for agglomeration with the net result of increasing the diameter of the recovered crystals [41].

4.3. Effect of the sonication time

Increasing the sonication time from 5 to 10 min resulted in significantly higher percent of drug recovery from the solution ($p = 0.0039$) (Fig. 1) due to higher opportunity for efficient mass transfer and nucleation [26]. Significantly higher NM saturated solubility ($p = 0.0366$) upon longer sonication was also observed and might be explained on the basis of the shock waves initiated by cavitation during the sonication process (Fig. 1). These waves were thought to be responsible for inter-particulate collision between particles in the vessel causing an intense localized heating which in turn promoted an enhancement in the equilibrium solubility of the drug [20,26].

4.4. Effect of the solvent: anti-solvent ratio

The solvent: anti-solvent ratio also significantly affected the % NM recovered from the solution and its saturated solubility titre (Fig. 1). Changing the ratio from 1:5 to 1:10 resulted in significantly lower % NM recovered from the solution ($p < 0.0001$) as well as higher value of saturated solubility ($p < 0.0001$). Ruecroft et al. revealed that in case of sonocrystallization less volume of anti-solvent is usually needed to induce crystallization than in case of conventional crystallization [22,42]. The solvent-anti-solvent used (ethanol, water) are miscible in all proportion, so a higher volume of anti-solvent might have a stronger interaction with the solvent molecules causing a dilution effect to the formed nuclei. Thereby, causing a decrease in the % of crystallized yield along with an increased tendency of drug particles to remain in solution resulting in a higher NM saturated solubility ($p < 0.0001$) [43].

The solvent: anti-solvent ratio also significantly affected NM MPS (Fig. 2). Changing the ratio from 1:5 to 1:10 resulted in significantly lower NM MPS d_{50} ($p = 0.0102$). The suggested dilution effect of the formed nuclei at higher volume of anti-solvent might protect the formed crystals from further aggregation. Furthermore, slight inter-particulate collision induced by shock waves [23] could cause crystal breakage, the so-called mechanical attrition of the crystallized drug molecules under the effect of ultrasound field, giving rise to smaller mean particle diameter for the end product [20,37].

Since a successful formulation on industrial scale necessitates the presence of an acceptable solubility for the drug included along with the availability of high yields for production batches, F6 and F5 were chosen as optimized formulae on the basis of highest yield and greatest value of saturated solubility respectively. The sonocrystals formulation F5 was selected for further optimization using 0.025% Soluplus® as a surfactant in the solvent phase.

4.5. Dissolution study

Since the dissolution of poorly soluble drugs in the gastrointestinal tract is a prerequisite for their oral absorption, a study of the factors influencing dissolution enhancement of these drugs is an important

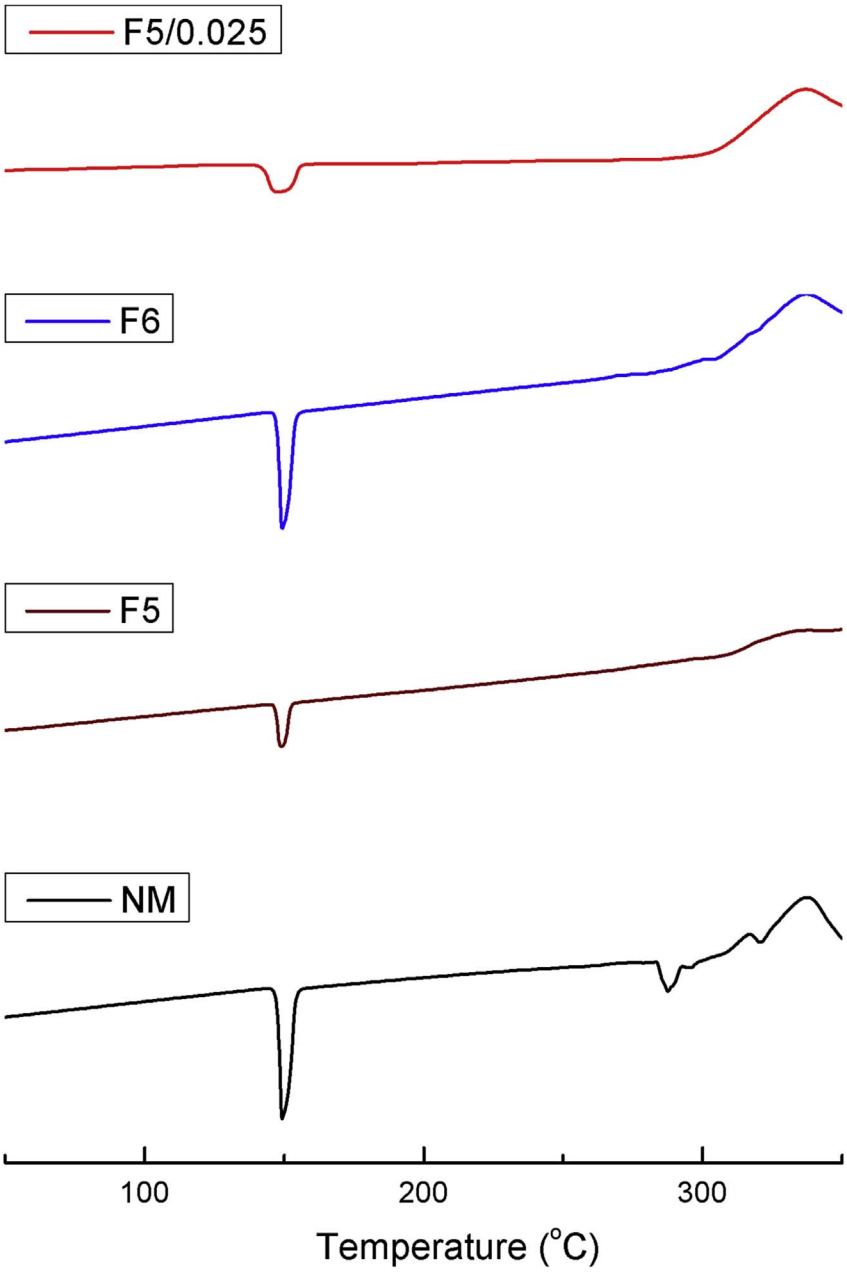


Fig. 5. DSC of plain NM, F5, and F5/0.025% Soluplus.

parameter to evaluate. Fig. 3 demonstrated a comparison between the dissolution profile of plain NM and the optimized sonocrystals of the drug. The extent of plain drug dissolved was ~17 mg% after 60 min. The values increase to ~20 and 32 mg% for F6 and F5 sonocrystals respectively [44–48]. This could be interpreted by the change in crystal morphology from the ill defined for plain drug particles to the rod shape of sonocrystals with larger surface area exposed to the solvent action of the aqueous medium [49]. The difference in extent between F5, F6 was a function of the smaller particle size and consequently slightly higher surface area of the former (Table 1). The addition of Soluplus to F5 showed an additional enhancement in NM dissolution to 53.5 mg% after 60 min. The hydrophilicity of Soluplus® has contributed to increase the wetting of drug particles when placed in contact with the dissolution medium. This phenomenon enabled more dissipation of the stagnant layer and a maximum increase in the amount of drug dissoluted [50].

4.6. Dissolution efficiency (DE %)

The term dissolution efficiency has been used by many workers [51,52] to describe and compare the extent of drug dissolution from different formulae to that of the plain drug. Table 4 demonstrated the impact of different variables on the increase in DE%. F6, with lower solvent: anti-solvent ratio, lower sonication time and higher drug concentration than F5, produced only 12.2% increase versus 52% for the latter. Addition of Soluplus® to F5 enabled a jump of 102.2% in DE%. This result supports the new assumption that hydrophilic surfactants can greatly modify the in vitro performance of sonocrystalized drug products.

4.7. Kinetic treatment of dissolution data

Table 4 shows that a diffusion controlled dissolution prevailed for plain drug particles as well as for sonocrystals of F6 and F5. The addition of Soluplus® changed the mechanism of NM dissolution. The

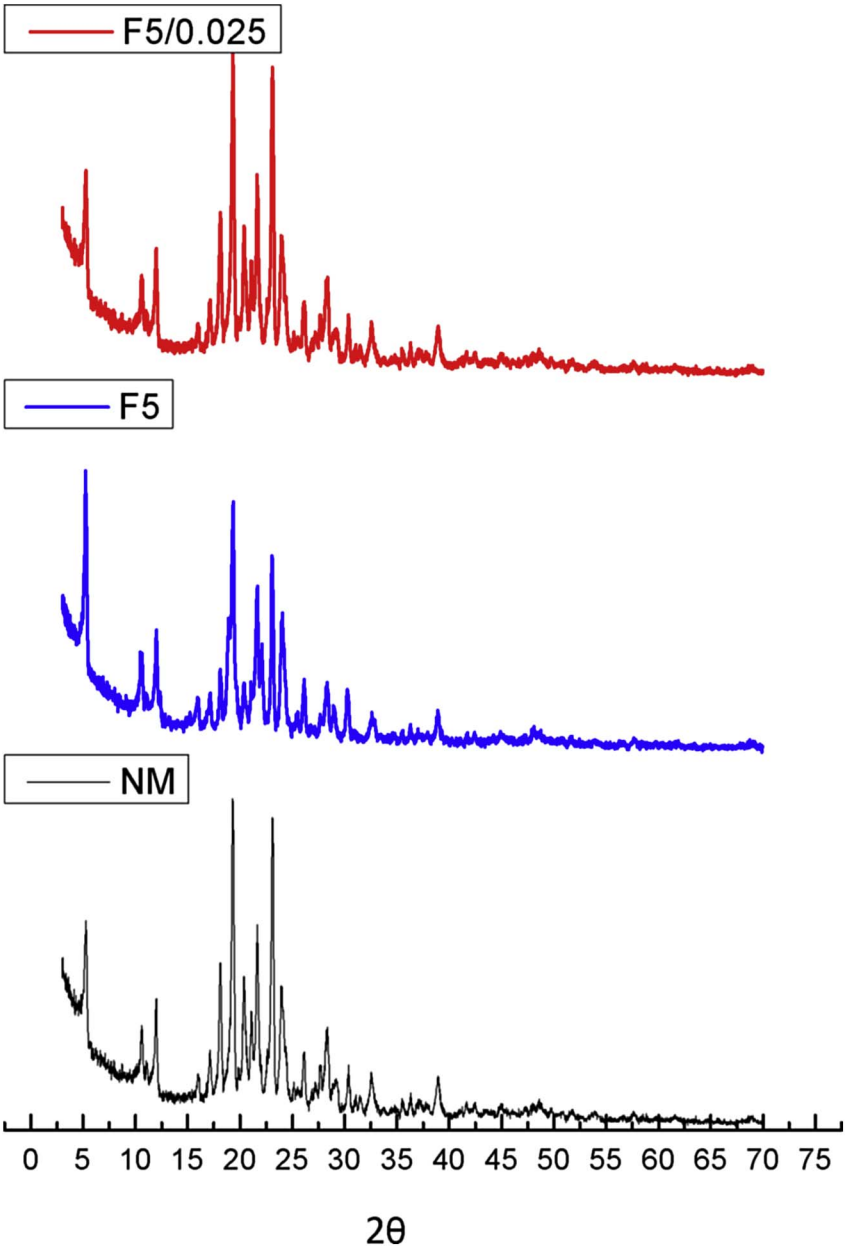


Fig. 6. XRD patterns of plain NM, F5, F6, and F5/0.025% Soluplus.

hydrophilic carrier might dissolve first surrounding drug crystals with a boundary layer from which a first order release kinetics prevailed. Although F6 sonocrystals possessed the highest yield among formulated sonocrystals yet its dissolution rate (K) (Table 4) was very similar (3.72 mg $min^{-1/2}$) to that of the plain drug (3.68 mg $min^{-1/2}$), only a slight improvement accounting for a lower value of lag time (3.6min.) in F6 sonocrystals, compared to that of NM (8.5min.). This result proved the necessity to optimize different variables controlling the sonocrystallization technique. Although F5 sonocrystals exhibited only a slight improvement in K value (3.89 mg $min^{-1/2}$), yet a considerable hastening in $t^{½}$ value occurred (~151min) relative to that of F6 sonocrystals (~273 min) and plain NM (~236 min) due to a deviation in the dissolution pattern from a system with a lag time (for NM and F6 sonocrystals) to another (F5 sonocrystals) with a 2.2 mg% flush dissolution at zero time. The addition of 0.025% Soluplus® to F5 sonocrystals gave rise to the best enhancement in dissolution kinetics, where the $t_{1/2}$ attained (88.5 min) which is the lowest value among studied formulae. The highest magnitude of flush dissolution accounting for 20.29 mg% might be explained by the strong ability of the amphiphilic polymer to make micellar solubilization [53]. During initial preparation of the drug solution in the presence of Soluplus®, the hydrophobic inner portion of the polymer (polyvinyl caprolactame) formed an inner core entrapping drug molecules in it, while the hydrophilic outer portion (polyethylene glycol unit) was easily hydrated with aqueous medium enabling the whole micellar system including the drug to dissolve in water [54]. Upon application of ultrasound to the above mixed solution, the duel effect of ultrasonication and micellar state might cause drug molecules to recrystallize in a nano crystal form with high dissolution extent and rate [50]. In order to confirm the aforementioned assumption, particle size analysis using Malvern Zetasizer (DLS, Zetasizer Nano ZS, Malvern instruments, Malvern, UK) was performed, and the particle size of the formed sonocrystals was found to be 628 nm.

4.8. Optical microscopy

An image of NM raw drug (Fig. 4a) and the selected NM sonocrystals (Fig. 4b) were taken using an ordinary light microscope equipped with a digital camera. NM raw drug appeared as an ill-defined crystals,

whereas the prepared sonocrystals appeared as sharp needle like crystals of defined shape and sharp edges.

4.9. Differential scanning calorimetry (DSC)

Figure 5 shows the DSC thermograms of NM raw powder, Soluplus®, F5, and F6, and F5/0.025% Soluplus® sonocrystals. Raw NM showed a main sharp endothermic peak at 149.30 °C with an enthalpy of fusion (ΔH) −24.6 J/g., other less sharp peak appeared at 287.59 °C. Soluplus® had a main weak endothermic peak at 76.4 °C which represented the transition of the polymer from the glassy to the rubbery state with an enthalpy of −65.43 J/g. F6 was characterized by nearly the same main peak of the drug at 149.35 °C with a very similar value for ΔH (−25.41 J/g), however the weak peak characteristic of NM at 287.59 °C had disappeared. This slight deviation indicated that the crystallinity of NM has not been changed greatly with the preparation of sonocrystals F6, only minor changes might happen in the crystal shape (Fig. 5) and/or particle size. This result was in agreement with the kinetic data of dissolution and confirmed the necessity for full optimization of all influencing variables controlling the sonocrystallization process.

In contrast, the sonocrystals of F5 showed a deviation from NM, with a main endothermic peak at 148.74 °C and a lower ΔH value of −22.79 J/g. The slight change in melting temperature corresponds to a development of crystals with a different morphology and habit (Fig. 5). The decrease in the enthalpy of fusion was interpreted by a less energy retained in the crystals. Formula F5 with 0.025% Soluplus® demonstrated the higher magnitude of shift in endothermic peak (147.74 °C). As previously reported by Meriani et al., 2004 [32], the reduction in melting temperature is probably due to crystal size reduction to the nanoscale. The lower value for ΔH (−16.7J/g) than that of unprocessed NM crystals indicated that the sonocrystallization process along with the presence of an amphiphilic carrier with the drug seemed to produce sonocrystals in the nanoscale with the most favorable heat of solution to dissolve when come in contact with dissolution medium [55].

4.10. X-ray diffraction study: (XRD)

Figure 6 shows the XRD patterns of NM raw powder, F5, and F5/0.025% Soluplus® sonocrystals. Unprocessed NM exhibited a strong crystalline nature as demonstrated by the appearance of intense peaks at 12.03°, 19.29°, and 21.6° 2θ in the x ray diffractogram (Fig. 6). Crystallinity was maintained in optimum formulae since both F5 and F5/0.025% Soluplus® showed the same characteristic peaks at the same diffraction angle as the drug. In order to compare the degree of crystallinity among formulae, the relative degree of crystallinity (RDC) was calculated according to the following equation:

$$RDC = I_{sam}/I_{drug}$$

where I_{sam} is the peak height of the sample under investigation and I_{drug} is the peak height of the drug at the same angle [56].

Pure drug peak at 19.29° 2θ was used for calculating the RDC. The calculated RDC values were 0.733 and 1.0 for F5 and F5/0.025% Soluplus® respectively. Therefore, sonocrystallization technique under controlled conditions has succeeded to disrupt the strong crystallinity of the drug through the formation of crystal topography with lower crystal lattice energy [46]. The addition of Soluplus® during sonocrystallization seemed to overshadow the impact of ultrasound on recrystallized NM since the drug in solution might be entrapped inside a micelle of the carrier, so the obtained crystals maintained the same physical characteristics as the untreated NM.

5. Conclusion

By proper selection of the optimum experimental conditions, sonocrystallization was found to be a successful tool for solubilization and dissolution enhancement of NM. The addition of Soluplus® with the drug solution seemed to execute a magnification to the effect of ultrasonic energy which was reflected in a better *in-vitro* performance of the drug. Therefore sonocrystallization assisted by amphiphilic carriers could be considered as a simple and efficient solution for treating poorly soluble drugs in the pharmaceutical industry.

Declaration of interest

The authors report no conflict of interest.

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