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A novel particle engineering technology: spray-freezing into liquid

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Abstract

Spray-freezing into liquid (SFL) is a novel particle engineering technology where a feed solution containing an active pharmaceutical ingredient (API) and pharmaceutical excipient(s) is atomized beneath the surface of a cryogenic liquid, such as liquid nitrogen. Intense atomization results from the impingement that occurs between the liquid feed and the cryogenic liquid. The atomized feed droplets instantly solidify within the liquid nitrogen continuous phase to form a suspension. The frozen microparticles are then collected and lyophilized to obtain the dry SFL micronized powder. The novel SFL process has been used in this study to enhance the dissolution rates of two poorly water soluble APIs, carbamazepine and danazol. The SFL process has also been used to produce stable peptide particles of insulin.

Keywords: Spray-freezing into liquid; Dissolution and stability enhancement; Danazol; Carbamazepine; Insulin; Micronization; Particle engineering

1. Introduction

Spray-freezing into liquid (SFL) is a novel particle engineering technology where a feed solution containing an active pharmaceutical ingredient (API) and pharmaceutical excipient(s) is atomized beneath the surface of a cryogenic liquid, such as liquid nitrogen. The impingement between the feed solution and cryogenic liquid results in intense atomization of the feed into microdroplets, which freeze instantly in the cryogen. Thus, the frozen microparticles are suspended within the cryogenic continuous phase. The suspended frozen phase can be separated from the continuous phase by sieve separation or by allowing the cryogen to evaporate. Once the frozen microparticles are collected, the solvent(s) can be removed by lyophilization. The dry SFL micronized powder contains the API molecularly embedded within a pharmaceutical excipient matrix.

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The objective of this study is to utilize SFL technology to produce micronized powders containing: (1) poorly water soluble APIs with high dissolution rates, and (2) a peptide with high stability against degradation and dimerization. Advantages of these engineered microparticulate powders relative to unprocessed powders will be demonstrated. To enhance the dissolution rate of a poorly water soluble API, it may be dispersed within a matrix of an excipient such as a surfactant, polyethylene glycol, polyvinylpyrrolidone (PVP), or cyclodextrin derivatives (Badawy et al., 1996; Brown et al., 1998; Liversidge and Cundy, 1995; Moneghini et al., 2001; Zerrouk et al., 2001; El-Zein et al., 1998). The stability of a labile API, such as a protein or peptide may be enhanced by dispersing it within an excipient matrix composed of a lyoprotectant sugar, surfactant, or polymer (Hora et al., 1999; Hagen et al., 1996; Wang, 2000; Roser, 1991). It will be shown that dissolution rates and stabilities may be enhanced in matrix formulations produced by SFL.

2. Materials and methods

Carbamazepine (CBZ), micronized danazol, insulin, amyl acetate, hydrolyzed poly(vinyl alcohol) (PVA, MW 22 000), poloxamer 407, PVP K-15, tyloxapol, lactose, trehalose, sodium lauryl sulfate (SLS), tris(hydroxymethyl)aminomethane, 1 N hydrochloric acid (HCl), sodium (tetra) ethylenediaminetetraacetic acid (EDTA), ammonium sulfate, triethylamine, and L-arginine were purchased from Spectrum Chemicals (Gardena, CA). Tetrahydrofuran (THF) was purchased from Mallinckrodt (Paris, KY). Acetonitrile and glacial acetic acid were purchased from EM Sciences (Gibbstown, NJ). Liquid nitrogen was obtained from Boc Gases (Murray Hill, NJ).

2.1. Preparation of SFL micronized powders

The poorly water-soluble SFL formulations containing CBZ and danazol were prepared by dissolving each API in THF. The pharmaceutical excipients were dissolved in purified water. The organic and aqueous solutions were mixed to form one-phase cosolvent SFL feed solution formulations, which are listed in Table 1. The insulin SFL feed solution formulation was prepared by dissolving the insulin alone or in combination with lyoprotectants in purified water at the concentrations listed in Table 1. Both the CBZ and insulin feed solution formulations were processed using the laboratory-scale ($\leqslant 50$ ml) SFL apparatus (schematic shown in Fig. 1a). The large volume danazol feed solution formulation was processed using the pilot-scale ($\geqslant 50$ ml) SFL apparatus (schematic shown in Fig. 1b). The CBZ

Table 1
SFL formulations containing either a poorly water soluble API or a protein utilized in the current study

Ingredient	% (w/w)	Amount in formulation (g)
SFL micronized CBZ formulation		
Carbamazepine	0.22	0.20
THF	33.11	29.80
SLS	0.22	0.20
Purified water	66.44	59.60
SFL micronized danazol formulation		
Danazol	0.22	2.50
THF	33.11	372.50
PVA (MW 22 000)	0.11	1.25
Poloxamer 407	0.11	1.25
PVP	0.11	1.25
Purified water	66.33	746.25
SFL micronized insulin (A)		
Insulin	0.25	0.10
Purified water	99.75	39.40
SFL micronized insulin (B)		
Insulin	0.25	0.10
Tyloxapol	0.05	0.02
Purified water	99.70	39.38
SFL micronized insulin (C)		
Insulin	0.25	0.10
Tyloxapol	0.05	0.02
Lactose	1.25	0.49
Purified water	98.45	38.89
SFL micronized insulin (D)		
Insulin	0.25	0.10
Tyloxapol	0.05	0.02
Trehalose	1.25	0.49
Purified water	98.45	38.89

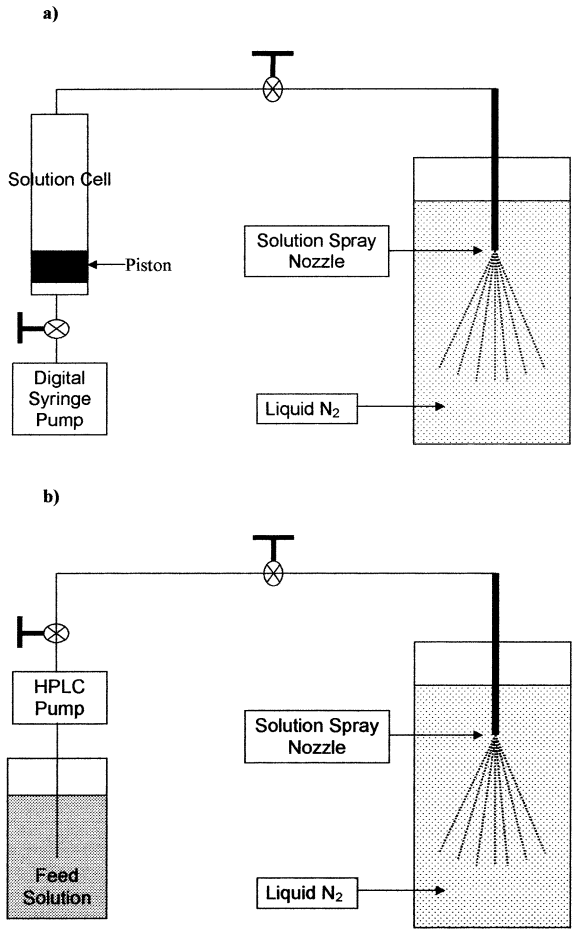

Fig. 1. Schematic representation of the (a) laboratory-scale and (b) pilot-scale SFL process utilizing liquid nitrogen as the cryogenic medium.

and insulin SFL feed solution formulations were atomized beneath the liquid nitrogen surface at 5000 psi constant pressure through a 63.5 μm I.D. polyether-ether-ether-ketone (PEEK) nozzle measuring 10 cm in length. Constant pressure was supplied by an ISCO Model 100DX syringe pump (ISCO, Inc, Lincoln, NE). The pilot-scale danazol SFL feed solution formulation was atomized beneath the liquid nitrogen surface at 5000 psi constant pressure through a 127 m I.D. PEEK nozzle measuring 15 cm in length using a Jasco Model PU-1586 HPLC pump (Jasco, Inc, Easton, MD). Each of the frozen powders were then collected on a 150-mesh sieve and lyophilized in a VirTis Advantage Benchtop Tray Lyophilizer (The VirTis Company, Inc, Gardiner, NY).

A co-ground physical mixture and a slowly frozen control, which was lyophilized after the feed solution formulation was solidified in a −40 °C freezer, were used as the controls for comparison with the SFL micronized formulations.

2.2. X-ray powder diffraction

A Philips 1710 X-ray diffractometer with a copper target and nickel filter (Philips Electronic Instruments, Inc, Mahwah, NJ) and Jade 5 XRD pattern-processing software (Materials Data, Inc, Irvine, CA) were used to obtain the XRD patterns of the samples. The XRD patterns of the leveled powders were measured from 5 to 50 2θ° using a step size of 0.05 2θ° and a dwell time of 1 s at each step.

2.3. Scanning electron microscopy

A Hitachi S-4500 field emission scanning electron microscope was used to obtain SEM micrographs of the powder samples, which had been gold-palladium sputter coated prior to analysis.

2.4. Dissolution testing

Dissolution testing was performed using a U.S.P. Type II VanKel VK6010 Dissolution Testing Station (Cary, NC). Powder containing approximately 5 mg of each API was placed into each of six dissolution vessels. Five-milliliter aliquots were taken at 2, 5, 10, 20, 30 and 60 min. Paddle speed and bath temperature were set at 50 rpm and 37.0 ± 0.2 °C, respectively. Dissolution media for CBZ consisted of 900 ml purified water in each vessel. Dissolution media for danazol consisted of 900 ml of a SLS (0.75% w/v)/Tris(hydroxymethyl)aminomethane (1.21% w/v) solution adjusted to pH 9.0 with 1 N HCl.

2.5. HPLC analyses

2.5.1. Reverse-phase HPLC quantitation of CBZ

Mobile phase consisting of 50% purified water,

35% methanol and 15% (v/v) acetonitrile was used to quantitate CBZ at a flow rate of 1.5 ml/min and 288 nm UV wavelength. CBZ was eluted from an Inertsil 5 μm ODS-2 (15.0×4.6 mm^2) column (Metachem Technologies, Inc, Torrance, CA) at 5 min.

2.5.2. Reverse-phase HPLC quantitation of danazol

Mobile phase consisting of 70% acetonitrile and 30% (v/v) purified water was used to quantitate danazol at a flow rate of 1.0 ml/min and a wavelength of 288 nm. Danazol was eluted from an Inertsil 5 μm ODS-2 (15.0×4.6 mm^2) column (Metachem Technologies, Inc, Torrance, CA) at 5 min.

2.5.3. Reverse-phase quantitation of insulin and A-21 desamido insulin degradant

Mobile phases consisting of (A) 60% aqueous buffer containing 0.22 M ammonium sulfate with 0.5% triethanolamine (adjusted to pH 2.3 with 1 N HCl) and 40% acetonitrile and (B) 73% aqueous buffer and 27% acetonitrile were used in a gradient method to quantitate insulin and A-21 desamido insulin at a flow rate of 1.0 ml/min and 214 nm UV wavelength. Mobile phase B was run at 100% initially, then ramped to 25.64% Mobile Phase A and 74.36% Mobile Phase B by 20 min. Insulin and A-21 desamido insulin were eluted from a Macrosphere RP 300 C18 5 μm (250×4.6 mm^2) column (Alltech Associates, Inc, Deerfield, IL) at 9 and 10 min, respectively.

2.5.4. Size-exclusion chromatography quantitation of insulin dimer and monomer

Mobile phase consisting of 64.9% purified water with 0.1% L-arginine, 15% glacial acetic acid and 20% (v/v) acetonitrile was used to quantitate the insulin dimer and monomer at a flow rate of 0.5 ml/min and 280 nm wavelength. The insulin dimer and monomer were eluted from a Waters Protein-Pak 125 10 μm column (7.8×300 mm^2, Milford, MA) at 16 and 18 min, respectively.

3. Results and discussion

3.1. SFL micronized CBZ powder produced from laboratory-scale feed solution

CBZ exists as a highly crystalline powder, as indicated from the crystalline peaks detected between 10° and 20° in the bulk CBZ XRD pattern

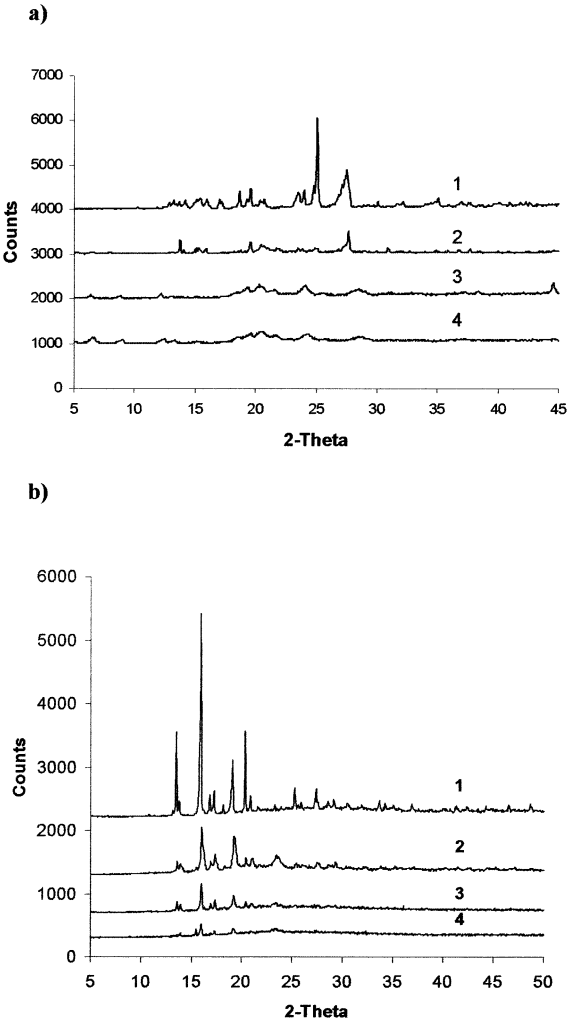

Fig. 2. XRD patterns of (a) bulk CBZ (1), the physical mixture containing CBZ with pharmaceutical excipients (2), the slowly frozen control (3) and the SFL micronized carbamazepine powder (4) and (b) bulk danazol (1), the physical mixture containing danazol with pharmaceutical excipients (2), the slowly frozen control (3) and the SFL micronized danazol powder (4).

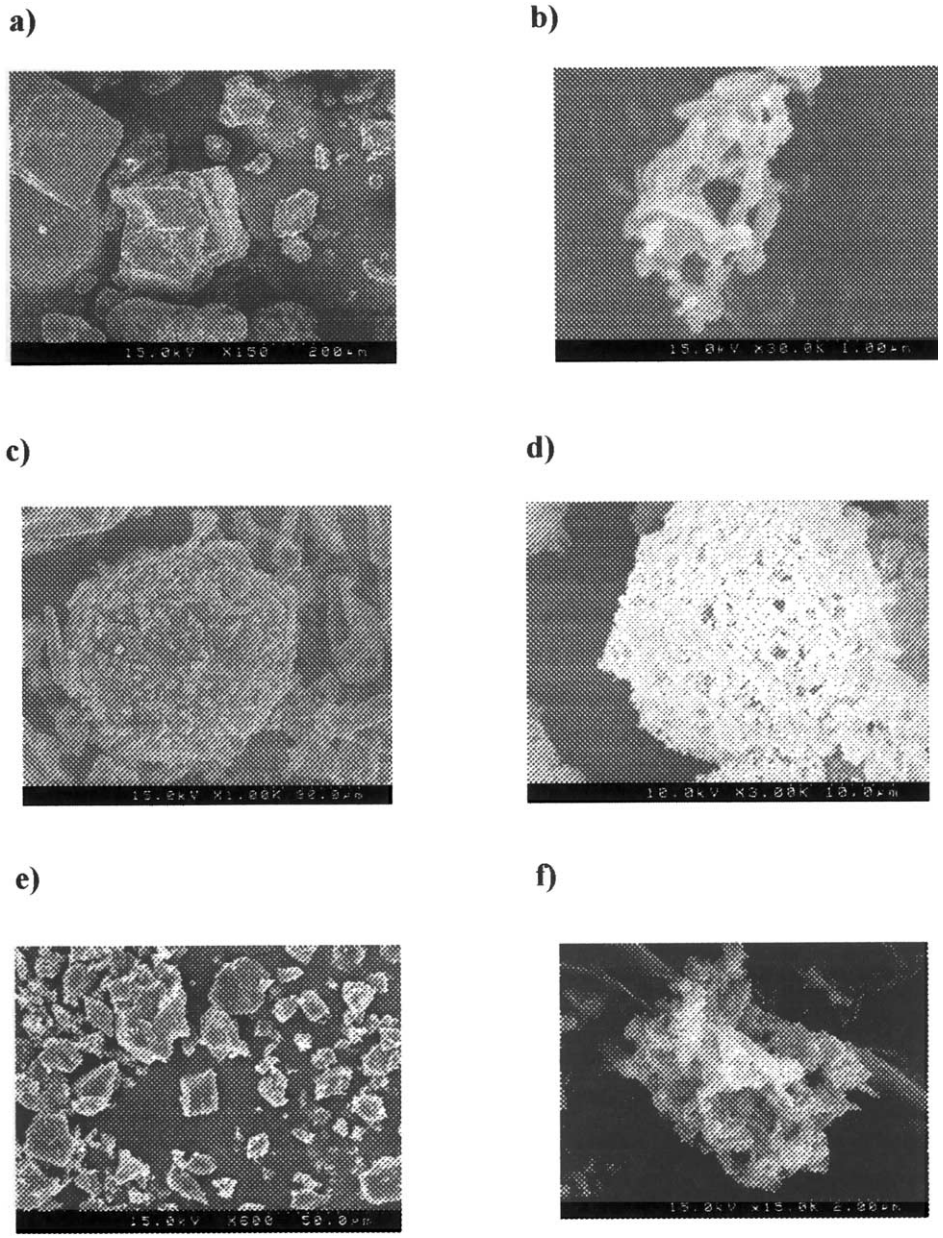

Fig. 3. SEM micrographs of (a) the physical mixture containing CBZ and excipients, (b) the SFL micronized CBZ powder, (c) the physical mixture containing danazol and excipients, (d) the SFL micronized danazol powder, (e) bulk insulin and (f) the SFL micronized insulin powder.

shown in Fig. 2a. The most intense peaks at 25° and 28° are characteristic of bulk CBZ. In the XRD pattern of the physical mixture, the characteristic peak at 28° remains, which indicates that crystalline CBZ is present. No characteristic CBZ peaks are present in the XRD patterns of either the slowly frozen control or the SFL micronized powder, indicating that the CBZ is completely amorphous within these two formulations.

In Fig. 3a and b, the physical mixture and SFL micronized CBZ powder SEM micrographs are shown. In Fig. 3a, bulky CBZ rhomboid particles can be distinguished from smaller irregular SLS particles. This distinction cannot be made in the SFL powder (Fig. 3b). The porous micron-sized particles consist of CBZ imbedded within the SLS matrix.

The amount of CBZ dissolved from the SFL micronized powder is significantly greater than any of the control formulations. Within 10 min, 100% of the danazol from the SFL formulation dissolved. Eighty percent of the CBZ from the slowly frozen control dissolved within the first 10 min, and 90% dissolved in 60 min. The physical mixture control has a dissolution profile similar to that of bulk CBZ because the bulk SLS in the formulation must first dissolve in the dissolution media to allow the lipophilic CBZ to wet and dissolve. In the bulk state, SLS dissolution in water is slow, thus retarding CBZ dissolution; however, with the SFL processed powder, the porous micronized particles wet and dissolve immediately.

3.2. SFL micronized danazol powder produced from pilot-scale feed solution

Micronized danazol is highly crystalline, as can be seen from the intense crystalline peaks detected between 10° and 25° in the bulk danazol XRD pattern shown in Fig. 2b. The physical mixture and slowly frozen control formulation produced similar XRD patterns, in which the crystalline danazol peaks were reduced in intensity, but the SFL micronized danazol powder had the highest amorphous API fraction of the formulations investigated in Fig. 2b.

In Fig. 3c and d, the physical mixture and SFL micronized danazol powder SEM micrographs are shown. In Fig. 3c, the bulk danazol adheres to the surface of the poloxamer particles within the physical mixture, but distinctions can be made between individual ingredients. This distinction cannot be made in the SFL powder (Fig. 3d). The porous microparticles consist of danazol imbedded within the micronized excipient matrix.

The amount of danazol dissolved from the SFL micronized powder is significantly greater than any of the control formulations after 20 min dissolution (Fig. 4.) Within 2 min, 95% of the danazol from the SFL formulation dissolved, and by 10 min 100% danazol was in solution. The physical mixture control has a slower dissolution profile than that of the bulk unprocessed danazol because the excipients used in this formulation swell when introduced to aqueous media in the bulk form, thus trapping the danazol within the swollen bulk excipient network. However, the SFL micronized powder formulation wets and dissolves immediately without swelling upon introduction to the dissolution media.

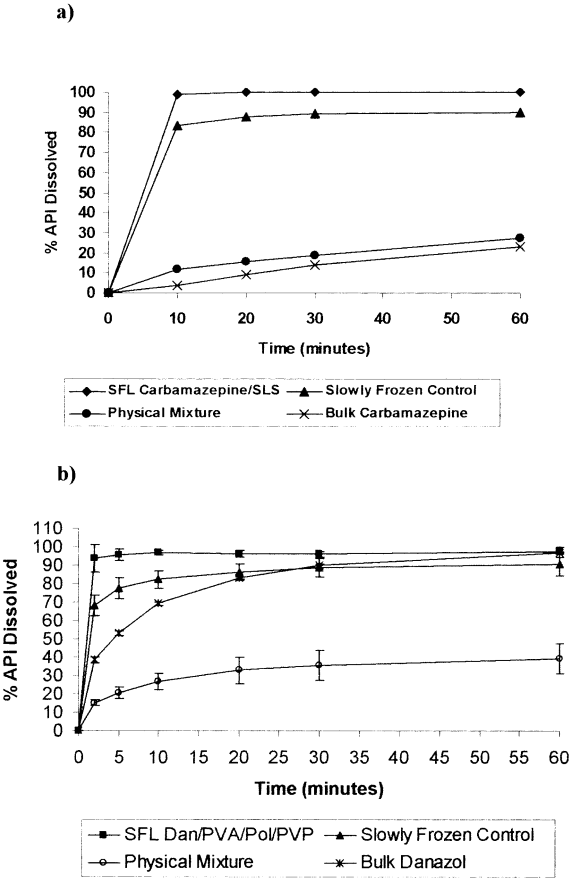

Fig. 4. Dissolution profiles of the (a) CBZ and (b) danazol formulations investigated.

Table 2
Comparison of the degradation and dimerization of unprocessed bulk insulin compared to various SFL micronized insulin formulations

Sample	A-21 desamido insulin peak area	Insulin peak area	Percent degradation
Bulk insulin	225 694	7 232 668	3.03
SFL insulin	168 677	5 380 040	3.04
SFL insulin/tyloxapol	174 295	5 524 328	3.06
SFL insulin/tyloxapol/lactose	212 090	5 488 444	3.72
SFL insulin/tyloxapol/trehalose	168 022	5 344 029	3.05

Sample	Dimer peak area	Insulin peak area	Percent of dimer in samples
Bulk insulin	2233	886 346	0.25
SFL insulin (A)	2535	703 137	0.36
SFL insulin/tyloxapol (B)	1953	769 149	0.25
SFL insulin/tyloxapol/lactose (C)	980	507 767	0.19
SFL insulin/tyloxapol/trehalose (D)	1261	683 937	0.18

3.3. SFL micronized insulin powder produced from laboratory-scale feed solution

In Fig. 3e, the SEM micrograph of bulk insulin shows that the primary particle size is 10–20 μm. The particle size of the SFL micronized insulin powder is approximately 3 μm, as shown in Fig. 3f.

The short-term stability of various SFL micronized insulin powders were measured and compared to that of unprocessed bulk insulin, as described in Table 2. Whereas previous studies have shown that significant protein degradation occurs upon freezing, the SFL processed powders have A-21 desamido insulin levels similar to those of unprocessed bulk insulin (Wang, 2000). When formulated without a lyoprotectant sugar or surfactant, the SFL micronized insulin formulation contained a similar level of A-21 desamido insulin (3.04%), compared to the unprocessed bulk insulin (3.03%). The covalent insulin dimer concentration in the SFL micronized insulin formulated without a lyoprotectant is only slightly higher than that of the unprocessed bulk insulin.

If formulated alone or in combination with stability enhancing pharmaceutical excipients, the SFL micronized insulin powders are as stable as the unprocessed bulk insulin, but have a smaller primary particle size, which could be desired for targeted drug delivery.

4. Conclusions

The SFL particle engineering technology may be utilized to micronize a wide range of APIs, for the purpose of enhancing the stability of potentially labile compounds and the dissolution rate of poorly water-soluble compounds.

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