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*Research Article*

# Influence of Formulation and Processing Variables on Properties of Itraconazole Nanoparticles Made by Advanced Evaporative Precipitation into Aqueous Solution

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***Abstract.*** Nanoparticles, of the poorly water-soluble drug, itraconazole (ITZ), were produced by the Advanced Evaporative Precipitation into Aqueous Solution process (Advanced EPAS). This process combines emulsion templating and EPAS processing to provide improved control over the size distribution of precipitated particles. Specifically, oil-in-water emulsions containing the drug and suitable stabilizers are sprayed into a heated aqueous solution to induce precipitation of the drug in form of nanoparticles. The influence of processing parameters (temperature and volume of the heated aqueous solution; type of nozzle) and formulation aspects (stabilizer concentrations; total solid concentrations) on the size of suspended ITZ particles, as determined by laser diffraction, was investigated. Furthermore, freeze-dried ITZ nanoparticles were evaluated regarding their morphology, crystallinity, redispersibility, and dissolution behavior. Results indicate that a robust precipitation process was developed such that size distribution of dispersed nanoparticles was shown to be largely independent across the different processing and formulation parameters. Freeze-drying of colloidal dispersions resulted in micron-sized agglomerates composed of spherical, sub-300-nm particles characterized by reduced crystallinity and high ITZ potencies of up to 94% (*w*/*w*). The use of sucrose prevented particle agglomeration and resulted in powders that were readily reconstituted and reached high and sustained supersaturation levels upon dissolution in aqueous media.

**KEY WORDS:** colloidal dispersion; emulsion; evaporative precipitation into aqueous solution; itraconazole; nanoparticles; poorly water-soluble drug.

## INTRODUCTION

The fact that the number of new drug compounds characterized by low water solubility is steadily increasing represents a significant challenge to formulators necessitating advanced formulation approaches. Several products in which the drug of interest is solubilized by means of solvents, surfactants, or cyclodextrins have been developed and marketed for oral as well as intravenous administration (1). However, the occurrence of side effects ascribed to the presence of these solubilizing excipients in the formulation indicate that elimination or at least limitation of these excipients is essential for improved patient safety (2). In addition, precipitation of the drug may occur upon dilution of the formulation in the aqueous body fluids potentially negating any advantageous effects in terms of solubility (3).

Successful development and scale-up of nanoparticle engineering technologies that largely eliminate the use of potentially hazardous excipients have permitted the formulation of new drug products as well as the reformulation of already existing products with improved efficacy and safety profiles. For example, Abraxane®, a nanoparticulate paclitaxel suspension for intravenous administration co-developed by Abraxis Bioscience Inc. and AstraZeneca PLC, contains only the drug and human serum albumin as a stabilizing agent (4). Clinical trials involving patients with metastatic breast cancer demonstrated that Abraxane® resulted in significantly higher response rates as well as fewer side effects when compared with the original product Taxol® in which paclitaxel is solubilized in a mixture of polyoxyethylated castor oil (Cremophor®EL) and ethanol (5).

Generally, nanoparticles may be prepared by top–down approaches that are based on size reduction of larger particles through mechanical techniques including media milling and high-pressure homogenization (6,7). In contrast, nanoparticles may be produced by bottom–up approaches that generate particles starting from dissolved drug molecules which are precipitated (8,9). Commonly, an organic solution of the drug is added to an antisolvent momentarily causing a state of supersaturation which is followed by nucleation and growth of drug particles by condensation and coagulation (10).

Evaporative Precipitation into Aqueous Solution (EPAS) utilizes the bottom–up approach to nucleate and grow nano- or microparticles of water-insoluble drug compounds (11,12). Specifically, the drug is first dissolved in a water-immiscible,

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organic solvent with a low boiling point (*e.g.*, dichloromethane or diethyl ether) and the resulting solution sprayed through a custom-made atomizing nozzle into a heated aqueous solution representing the antisolvent. Elevated temperature of the heated aqueous solution ensures rapid evaporation of the organic solvent which in turn produces high supersaturation levels and rapid precipitation of the drug in the form of suspended particles. Stabilizers added to the heated aqueous solution adsorb onto the newly formed particle surface decreasing surface energy and preventing particle growth. At the same time, those stabilizers are generally hydrophilic and therefore improve wetting and enable rapid dissolution of EPAS-produced particles. In addition, EPAS is capable of producing particles with high drug loadings since excess, unbound stabilizer can be removed by centrifugation of the aqueous suspension. Particularly, high drug loadings of more than 90% (*w*/*w*) have been reported (13,14). Despite these advantageous characteristics of EPAS-produced particles, the EPAS process itself has certain limitations including optimization of control over particle size and size distribution and the necessity for consistent reproduction of a fine atomizing nozzle from stainless steel tubing.

The purpose of this study was to develop a more robust precipitation process that combines the advantages of EPAS with emulsion templating (*i.e.*, Advanced EPAS) to form nanoparticles of the poorly water-soluble drug itraconazole (ITZ). It was hypothesized that replacement of the organic feed solution with an oil-in-water (O/W) emulsion allows for excellent control over the particle size without requiring an atomizing nozzle since the size of precipitated particles is regulated by the droplet size of the emulsion. In fact, controlled precipitation of volatile O/W emulsions containing poorly water-soluble drugs has previously been demonstrated to be a feasible approach for the preparation of submicron drug particles which were generally found to be in a comparable size range as the emulsion droplets (15,16). Two strategies for the production of nanoparticles from O/W emulsions have been reported in the literature: (1) removal of only the organic phase under reduced pressure resulting in a colloidal dispersion or (2) direct transformation of the emulsion into a dry powder by spray-drying (15,16). The approach taken in the present study involves rapid removal of the organic phase by spraying the O/W emulsion into a heated aqueous solution, which is different from the processes described above. The present process allows for considerably shorter evaporation and consequently processing times than compared with processes by which the organic solvent is removed under reduced pressure. In addition, drug potencies of Advanced EPAS-produced particles are expected to be substantially higher compared with those particles obtained by direct conversion of the emulsion into a dry powder because unbound stabilizer may be easily removed by centrifugation of the aqueous colloidal dispersion.

In order to optimize the Advanced EPAS process, the influence of different processing and formulation parameters on characteristics of suspended as well as dried particles was investigated. ITZ was chosen as the model drug given its low water solubility of approximately 4 μg/mL at pH 1 and 1 ng/mL at neutral pH (17). Additionally, ITZ has been previously processed using EPAS processing and therefore allowed for comparison of results obtained in this study to previously reported studies (13,14).

## MATERIALS AND METHODS

### Materials

Micronized ITZ, BP was purchased from Hawkins. Inc. (Minneapolis, MN) and soy lecithin and dichloromethane (DCM) were obtained from Fisher Scientific (Fair Lawn, NJ). Sodium deoxycholate (NaDC) was purchased from Spectrum Chemical Manufacturing Corp. (Gardena, CA) and polyvinylpyrrolidone K-17 (Kollidon PF 17; PVP K-17) from BASF (Ludwigshafen, Germany). All other chemicals utilized in this study were at least of ACS grade.

### Methods

#### *Particle Formation Using Advanced EPAS*

For all experiments, O/W emulsions (20% DCM, *v*/*v*) were used. Specifically, ITZ and lecithin were dissolved in DCM while a water-soluble stabilizer (NaDC or PVP K-17) was dissolved in water. Both phases were combined and O/W emulsions produced by sonication employing a Branson Sonifier 450, (Branson, Danbury, CT) at 10% duty cycle and output control of 8. Emulsions were then sprayed into purified water maintained at 80°C to induce precipitation of particles. During initial experiments this was achieved by injecting emulsions into the heated aqueous solution using a glass syringe with a 23-G needle. In later experiments, emulsions were sprayed *via* an HPLC-pump (LabAlliance, Series III, State College, PA) through 0.005 in. (127 μm) inner diameter polyetheretherketone (PEEK) tubing. If dry particles were sought, aqueous colloidal dispersions were centrifuged (Avanti J 25, Beckman, Fullerton, CA) at 20,000 rpm (48,384×*g*) for 30 min to concentrate particles. The supernatant was then decanted to increase ITZ potency in the precipitate by removing unbound stabilizer. Finally, particles were rapidly frozen by submerging in liquid nitrogen and then lyophilized employing a bench top tray lyophilizer (The VirTis Company, Inc., Gardiner, NY). A schematic of the Advanced EPAS process is depicted in Fig. 1.

#### *Particle Size Analysis*

Particle size distributions of O/W emulsions and aqueous colloidal dispersions were measured by laser diffraction using a Malvern Mastersizer 2000 equipped with a Hydro MU sample dispersion unit (Malvern Instruments, Ltd. Worcestershire, UK). Aliquots of samples were diluted with approximately 500 mL of purified water to obtain a laser obscuration of 10% to 15%. Volume distributions were calculated with a refractive index of 1.610 for ITZ and reported by listing the Dv 10, Dv 50 and Dv 90, which correspond to the diameters at which the cumulative sample volume is under 10%, 50% and 90%, respectively. Span indices were calculated according to Eq. 1 and used to describe the polydispersity.

$$\text{Span} = \frac{(\text{Dv90} - \text{Dv10})}{\text{Dv50}} \quad (1)$$

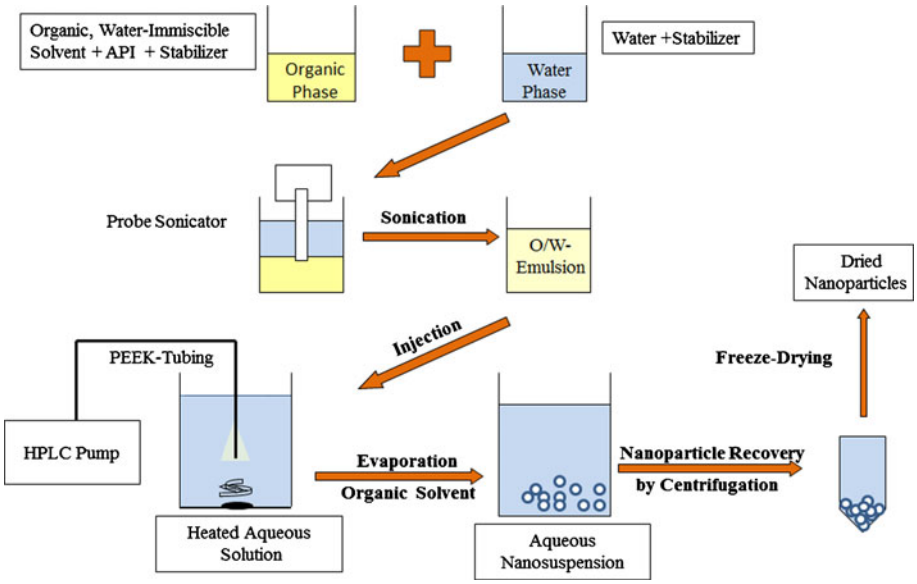


**Fig. 1.** Schematic of the "Advanced EPAS" process

*Optical Microscopy*

Emulsion morphology was characterized by optical microscopy using an Axioskop2 plus microscope (Carl Zeiss Microimaging Inc., Thornwood, NY). Images were captured and electronically analyzed by Axio Vision 4.0 (Carl Zeiss Microimaging Inc., Thornwood, NY).

*Drug Potency*

The ITZ potency (in percent, *w*/*w*) was determined in triplicate by dissolving about 5 mg of dry powder in 25 mL of acetonitrile:water (50:50, *v*/*v*). Aliquots were then analyzed for ITZ content using a Shimadzu VP-AT series LC10 HPLC system with a photodiode array detector (Model 996) extracting at a wavelength of 263 nm. The mobile phase consisted of 70:30:0.05 acetonitrile:water:diethanolamine and ITZ was eluted from an Inertsil 5 μm ODS-2 column (4.6 mm i.d.× 150 mm; GL Sciences Inc.) at approximately 5.4 min. Drug potency (%, *w*/*w*) is represented by Eq. 2:

$$\text{Drug potency}(\%) = \left(\frac{\text{Weight of drug in nanoparticles}}{\text{Total weight of nanoparticles}}\right) * 100 \quad (2)$$

*Scanning Electron Microscopy*

Samples were mounted onto an aluminum stage and sputter-coated with silver for 30 s. Particle morphology was evaluated employing a LEO-1530 scanning electron microscope (SEM) operated at an accelerating voltage of 10 kV.

*Differential Scanning Calorimetry*

Differential scanning calorimetry (DSC) was conducted using modulated temperature DSC, Model 2920 (TA Instruments, New Castle, DE). Samples were weighed to 5–10 mg in aluminum crimped pans and heated at a ramp rate of 10°C/min using a modulation temperature amplitude of 1°C and a modulation period of 60 s. Dry nitrogen was used as the purge gas through the DSC cell at a flow rate of 40 mL/min. All data were analyzed using TA Universal Analysis 2000 software (TA Instruments, New Castle, DE) and are presented in terms of heat flow. The percent of crystallinity of samples was estimated according to the method previously described by Matteucci *et al.* (18).

*Stabilizer Adsorption*

To quantify the amount of stabilizers adsorbed onto ITZ particles, mass balance experiments were conducted. Emulsions were sprayed into 250 mL water maintained at 80°C using an HPLC pump and PEEK tubing at a flow rate of 7 mL/min for 5 min. Aqueous suspensions were then centrifuged as described previously. Supernatant and precipitate were weighed before and after drying at 40°C to gravimetrically determine the amount of stabilizer adsorbed. Specifically, the amount of stabilizer ($W_{\text{Stab, ads}}$) adsorbed on ITZ particles is given by Eq. 3 (19):

$$W_{\text{Stab, ads}} = W_{\text{Ppt, dry}} - W_{\text{Drug, Ppt}} - \left(W_{\text{Ppt, wet}} - W_{\text{Ppt, dry}}\right) * \left(\frac{W_{\text{Sup, dry}}}{W_{\text{Sup, wet}} - W_{\text{Sup, dry}}}\right) \quad (3)$$

where $W_{\text{Ppt, wet}}$ and $W_{\text{Ppt, dry}}$ are the weight of the precipitate before and after drying, respectively. Likewise, $W_{\text{Sup, wet}}$ and $W_{\text{Sup, dry}}$ are defined as the weight of the supernatant before and after drying. $W_{\text{Drug, Ppt}}$ is the amount of ITZ in the precipitate as measured by HPLC.

*Redispersion of Freeze-Dried Particles*

Freeze-dried particles were redispersed in deionized water by sonication for 30 s. Particle size distributions were determined as described above.

*Dissolution Testing at pH 1*

Dissolution testing in 0.1 N hydrochloric acid was performed using the paddle apparatus at 50 rpm and 37°C (Vankel 7000 Dissolution Tester, Vankel Technology Group, Cary, NC). An equivalent of 80 μg/mL ITZ (equal to 20 times the equilibrium solubility of crystalline ITZ in the dissolution media) was added to the dissolution vessels. Aliquots of dissolution media were sampled at predetermined time points and filtered through 0.2 μm GHP filters. In all cases, the filtrate was completely clear upon visual inspection. Filtered aliquots were then diluted in a 1:1 ratio with acetonitrile and analyzed for ITZ content employing HPLC as previously described.

*Dissolution Testing at pH 7.4*

Dissolution testing was performed using the paddle apparatus at 100 rpm (Vankel 7000 Dissolution Tester, Vankel Technology Group, Cary, NC). Specifically, formulations were tested in phosphate-buffered saline containing 10% (*v*/*v*) fetal bovine serum held at 37°C. An equivalent of 20 μg/mL ITZ (equal to 1,333 times the equilibrium solubility of crystalline ITZ in the dissolution media) was added to the dissolution vessels. Aliquots of dissolution media were sampled and filtered through 0.2 μm GHP filters. In all cases, the filtrate was completely clear upon visual inspection. Filtered aliquots were then diluted in a 1:1 ratio with acetonitrile, vortexed for 3 min and centrifuged (Microfuge 18 Centrifuge, Beckman, Fullerton, CA) at 3,000 rpm for 15 min. The supernatant was assayed by HPLC.

## RESULTS AND DISCUSSION

### Influence of Emulsion Template on Size Distribution of Suspended Particles

The original EPAS technology has been successfully employed to enhance dissolution rates of poorly water-soluble drugs by forming submicron to micron-sized drug particles. For instance, ITZ particles characterized by high wettability and surface areas only stabilized with small amount of surfactants and/or polymers were prepared by EPAS in previous studies (13,14). Specifically, Chen *et al.* produced ITZ particles stabilized with a combination of Poloxamer 407, and varying surfactants/polymers such as Poloxamer 188 and PVP K-15 that displayed significantly enhanced dissolution rates than compared with the bulk drug (13). Particle sizes (Dv 50) of these EPAS-produced ITZ particles ranged from 8.7 to 18.7 μm, but rather high surface areas suggest that particles were agglomerates of smaller primary particles. In order to produce micron- or submicron-sized particles by EPAS processing, the organic feed solution has to be broken up into very fine droplets. Key factors affecting the size of droplets obtained by pressure atomization of a fluid stream are the fluid characteristics (*i.e.*, surface tension, viscosity, and density) and the nozzle geometry (*i.e.*, diameter of the orifice) (20). In terms of nozzle geometry, a smaller diameter of the orifice generally results in reduced average droplet sizes. Based on this, the EPAS process utilizes a custom-made nozzle with an extremely thin orifice (~5 μm) that yields high pressure drops of at least 3,000 psi resulting in intense atomization of the organic feed solution.

In this study, ITZ nanoparticles were produced by utilization of an emulsion template/solvent evaporation process, Advanced EPAS, which does not require a special atomizing nozzle. O/W emulsions containing ITZ and lecithin in the organic phase and NaDC in the aqueous phase were produced by sonication and subsequently injected into heated water (80°C) by means of a syringe with a 23-G needle (inner diameter, 300 μm). The corresponding particle size distribution of particles in suspension is shown in Fig. 2a. Even though injection by means of a syringe/needle did not cause the liquid to break up into droplets but rather resulted into a continuous fluid stream, nanoparticles with a Dv 50 value of 290 nm were obtained.

In addition to an ITZ emulsion, an ITZ solution in DCM containing lecithin was injected into a heated aqueous solution containing NaDC by means of a syringe/needle. Concentrations of ITZ and stabilizers were matched to the concentrations applied for the emulsion formulation to allow for comparison. Particles created from the ITZ solution were significantly larger (Dv 50, 13.4 μm) than the ones obtained from the ITZ emulsion (Fig. 2b). The results indicate that utilization of an emulsion template enables preparation of ITZ-loaded nanoparticles without requiring intense atomization of the feed stock by a custom-made nozzle. It is assumed that homogenization of the emulsion by sonication results in submicron-sized droplets that are converted into solid nanoparticles upon evaporation of the organic solvent during Advanced EPAS processing.

To further investigate this hypothesis and to gain an insight into the relationship of the size of emulsion droplets and the size of Advanced EPAS-produced particles, emulsions were sonicated for different time periods (0.5, 1, and 5 min) in order to vary their droplet sizes. Increasing the sonication time from 0.5 to 1 min resulted in a decrease in the Dv 50 value of emulsion droplets from around 1 μm to 430 nm (Fig. 3). After

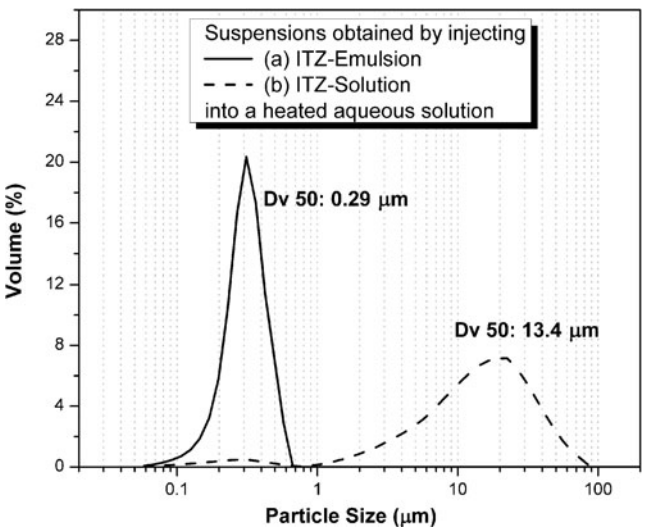


**Fig. 2.** Particle size distributions of suspensions obtained by injecting **a** an ITZ emulsion (ITZ/lecithin/NaDC=1:1:0.1; 20% DCM, *v*/*v*) into water and **b** a solution of ITZ and lecithin in DCM into heated water containing NaDC (ITZ/lecithin/NaDC=1:1:0.1). Temperature of aqueous solution (water or NaDC solution) was maintained at 80°C. Injection was performed using a glass syringe with a 23-G needle

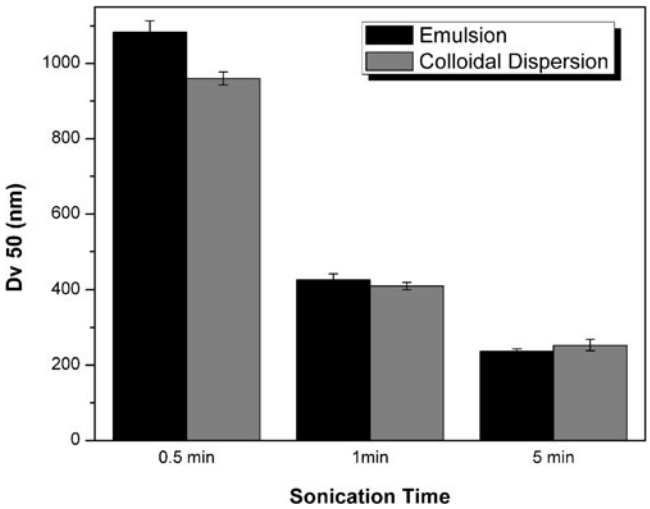


**Fig. 3.** Dv 50 values of emulsion droplets (*black columns*) and ITZ particles (*grey columns*) as a function of sonication time. Particles were obtained by injecting emulsion (ITZ/lecithin/NaDC=1:1:0.1; 20% DCM, *v/v*) into water maintained at 80°C using a glass syringe with a 23-G needle

5 min of sonication droplet size distributions were substantially monodisperse with a Dv 50 value of 250 nm. The three emulsions were then injected into a heated aqueous solution and the size distribution of precipitated particles determined. Overall, Dv 50 values of ITZ particles correlate well with Dv 50 values obtained for ITZ emulsion droplets (Fig. 3). The results suggest that the particle size is directly controlled by the emulsion droplet size and can therefore be controlled by adjusting the latter.

This finding is in accordance with previous results by Sjöström *et al.* who prepared nanoparticles of the sparingly water-soluble drug cholesteryl acetate by precipitation of an O/W emulsion under reduced pressure (16). Removal of the organic phase containing the drug at concentrations close to saturation resulted in particle sizes that closely matched droplet sizes of the emulsion supporting the assumption that one particle is formed from each organic droplet. In the process employed by Sjöström *et al.* the organic, water-immiscible solvent is removed from the emulsion at temperatures significantly below its boiling point requiring reduced pressure and long evaporation times of several hours. In contrast, elevated temperatures of the aqueous receiving solution used in Advanced EPAS processing provide immediate evaporation of the organic solvent thereby eliminating any downstream separation methods. In addition, rapid evaporation of the organic solvent produces high supersaturation and consequently rapid nucleation thus offering the potential to produce very small particles.

## Influence of Formulation Variables on Size Distribution of Suspended Particles

### *Concentration of Emulsion Stabilizers*

It was found that ITZ nanoparticles could be produced by precipitation of emulsions stabilized with lecithin and NaDC. Both substances naturally occur in the human body with lecithin presenting an essential component of cell membranes and NaDC being a bile salt (21). Accordingly, they are regarded as highly biocompatible and exhibit good safety profiles.

To optimize the emulsion formulation, the concentration of ITZ was kept constant (1%, *w/v*) and the concentration of lecithin and NaDC was varied. Sizes of particles precipitated from different emulsion formulations are presented in Table I. Results indicate that concentrations as low as 0.2% (*w/v*) for lecithin and 0.1% (*w/v*) for NaDC are sufficient to create stable emulsions and consequently ITZ nanoparticles.

The combined use of lecithin and bile salts has been previously shown to significantly enhance emulsion stability (22,23). Additive adsorption of both stabilizers leads to a mixed lipid emulsion droplet interface which results in higher stability than emulsion systems stabilized by either surfactant alone.

At a concentration of 1% ITZ and 1% lecithin, an increase in the concentration of NaDC from 0.05% to 0.1% resulted in a narrower particle size distribution as indicated by a decrease in the span index (Table I). A further increase in the concentration of NaDC did not provide additional benefits in terms of polydispersity These results are in accordance with previous studies which demonstrated that an increase in the concentration of anionic bile salt results in increased zeta potentials until a maximum value is reached (22,23). The increase in zeta potential indicates significant adsorption of the bile salt at the emulsion interface thereby providing enhanced stability through electrostatic repulsion. Furthermore, it has been proposed that in addition to electrostatic repulsion, steric hindrance through residual liquid crystalline phospholipid at the emulsion interface enhances stability of mixed lecithin/bile salt systems (22).

Generally, the formation of a stable emulsion is of utmost importance for successful preparation of nanoparticles as the particle size is directly controlled by the emulsion droplet size. Overall, monodispersed ITZ nanoparticle distributions were prepared from emulsions over a wide range of stabilizer concentrations with ITZ potencies of up to 76.9% (*w/w*) (Table I), indicating robustness of the formulation and potential application for other poorly soluble drug substances.

### *Solids Concentration of the Emulsion*

The effect of the solids concentration of the emulsion on the particle size distribution of Advanced EPAS-produced particles was investigated. Specifically, the total solids concentration of the emulsion was varied between 0.525% and 4.2% (*w/v*) while the ratio of ITZ to stabilizers was kept constant. A solids concentration of 4.2% (*w/v*) was determined to be the upper limit for this specific formulation based on the solubility of ITZ and lecithin in DCM. The size distributions of ITZ particles obtained by injecting emulsions with varying solids concentration into heated water are shown in Fig. 4c. ITZ nanoparticles could be obtained at all solids concentrations. However, at the lowest solids concentration of 0.525% (*w/v*) a significant portion of micron-sized particles was detected. A microscopic picture of the corresponding emulsion (Fig. 4a) shows a broad droplet size distribution with micron- and submicron-sized droplets indicating that the low stabilizer concentrations of 0.25% (*w/v*) lecithin and 0.025% (*w/v*) NaDC as employed for the lowest solid concentration of 0.525% (*w/v*) were not able to sufficiently reduce the interfacial tension between DCM and water and/or to prevent coalescence of the emulsion droplets. In contrast, the

**Table I.** Particle Sizes, Span Indices, and Theoretical ITZ Potencies (in percent, *w/w*) of ITZ Particles as a Function of Emulsion Stabilizer Concentrations

| Concentration (%, *w/v*) | | | | | | | |
|---|---|---|---|---|---|---|---|
| ITZ | Lecithin | NaDC | Theoretical ITZ potency (%) | Dv 10 (μm) | Dv 50 (μm) | Dv 90 (μm) | Span |
| 1.0 | 0.2 | 0.1 | 76.9 | 0.17 | 0.28 | 0.41 | 0.88 |
| 1.0 | 1.0 | 0.05 | 48.8 | 0.19 | 0.30 | 0.46 | 0.91 |
| | | 0.1 | 47.6 | 0.18 | 0.29 | 0.42 | 0.85 |
| | | 0.5 | 40.0 | 0.14 | 0.28 | 0.46 | 1.13 |
| | | 1.0 | 33.3 | 0.15 | 0.28 | 0.43 | 0.98 |
| 1.0 | 2.0 | 0.1 | 32.3 | 0.16 | 0.28 | 0.43 | 0.92 |

ITZ particles obtained by injecting O/W emulsion (20% DCM, *v/v*) into heated water (80°C) *via* a glass syringe with 23-G needle

emulsion with the highest solids concentration of 4.2% (*w/v*) (Fig. 4b) displays fairly monodispersed droplets in the nanometer size range.

Overall, precipitation of particles from emulsions with solids concentrations of 1.05%, 2.1%, and 4.2% (*w/v*) resulted in similar size distributions with Dv 50 values of 260, 290, and 310 nm, respectively. The slight increase in the Dv 50 value with increasing solids concentration of the emulsion may be attributed to the increasing solids content of the final suspension favoring particle–particle collisions and thus particle growth (24). From a manufacturing viewpoint, the 4.2% (*w/v*) formulation is the most desirable since it combines satisfactory particle size with high manufacturing efficiency.

## Influence of Processing Variables on Size Distribution of Suspended Particles

### *Temperature and Volume of Heated Aqueous Solution*

The effect of temperature of the aqueous receiving solution on the particle size of EPAS-produced particles has been investigated in a previous study (11). It was demonstrated that the type of stabilizer used in the heated aqueous receiving solution is more important than its actual temperature as an increase in temperature either resulted in an increase (Tween 80) or decrease (PVP 40,000) in the mean particle size. However, this previous study did not evaluate the influence of processing temperature on particle size in combination with an emulsion but with an organic drug solution.

ITZ emulsions were injected into the aqueous solution maintained at 70°C, 80°C, or 90°C. Particle size distributions, as summarized in Table II, were comparable with Dv 50 values of 290 nm for 70°C and 80°C and 310 nm for 90°C. The process of DCM removal can be generally broken down into the extraction of DCM into the aqueous phase followed by its evaporation at the air–liquid interface. The extent and speed of DCM extraction from the emulsion droplets into the aqueous phase depends primarily on the solubility of DCM in water. The solubility of DCM in water is known to decrease with increasing temperature which will lead to slower extraction of DCM into the aqueous phase at higher temperatures (25). Evaporation of DCM at the air–water interface however, will be more rapid at higher temperatures maintaining a steep concentration gradient between the two liquids (26).

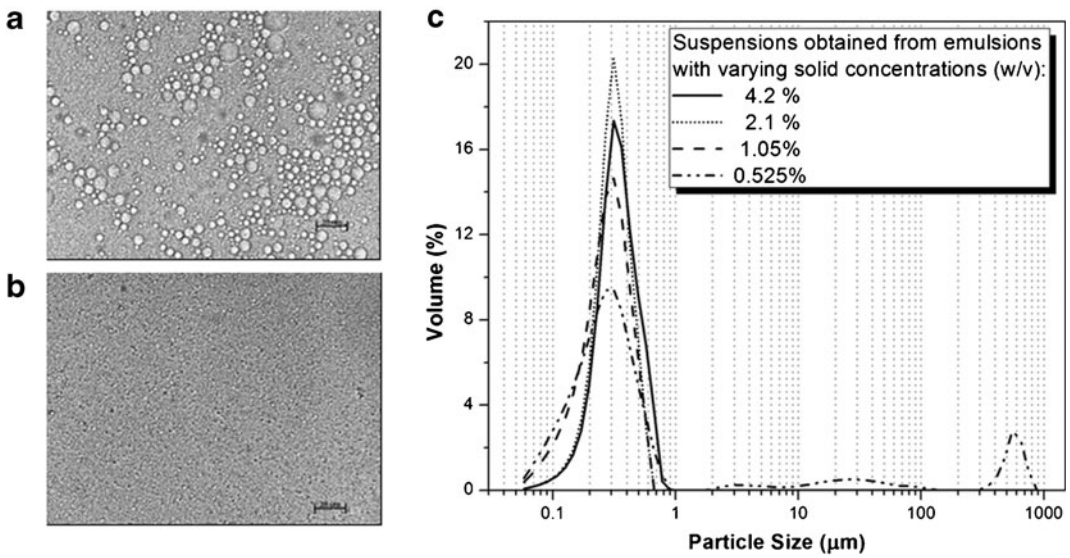


**Fig. 4.** Morphology of O/W emulsions (ITZ/lecithin/NaDC=1:1:0.1; 20% DCM, *v/v*) produced with varying solids concentrations at **a** 0.525% (*w/v*) and **b** 4.2% (*w/v*). The *scale bar* corresponds to 20 μm. **c** Particle size distributions of colloidal dispersions obtained by injection of emulsions with varying solids concentrations into a heated aqueous solution maintained at 80°C using a glass syringe with a 23-G needle

**Table II.** Particle Sizes and Span Indices of ITZ Particles as a Function of Temperature and Volume of Aqueous Heated Solution

| Temperature aqueous solution (°C) | Volume ratio aqueous solution to emulsion | Dv 10 (μm) | Dv 50 (μm) | Dv 90 (μm) | Span |
|---|---|---|---|---|---|
| 70 | 20:1 | 0.18 | 0.29 | 0.42 | 0.85 |
| 80 | 3:1 | 0.12 | 0.28 | 0.74 | 2.22 |
| | 5:1 | 0.20 | 0.29 | 0.40 | 0.70 |
| | 10:1 | 0.18 | 0.28 | 0.41 | 0.82 |
| | 20:1 | 0.18 | 0.29 | 0.42 | 0.85 |
| 90 | 20:1 | 0.22 | 0.31 | 0.47 | 0.80 |

ITZ particles obtained by injecting O/W emulsion (ITZ/lecithin/NaDC=1:1:0.1; 20% DCM, *v/v*) into heated aqueous solutions of varying volume (volume of emulsion kept constant) and temperature *via* a syringe and 23-G needle
*ITZ* itraconazole, *NaDC* sodium deoxycholate, *DCM* dichloromethane

Since the processing temperatures investigated are all well above the boiling point of DCM (39.8°C) evaporation of DCM is assumed to be very rapid at all temperatures making the small differences in solubility at the different temperatures tested negligible. For further experiments, a temperature of 80°C was chosen. Still, based on the data lower temperatures may be employed for example in the case of drugs that exhibit a low melting point or are not as thermally stable at higher temperatures.

High processing temperatures also ensure almost complete removal of DCM from aqueous dispersions formed. A previous study conducted by our research group demonstrated that residual DCM concentrations in EPAS-produced suspensions processed between 75°C and 85°C were extremely low (0.0021–0.0041 mg/mL) (11). According to the International Conference on Harmonization-Guidance for Industry, DCM is considered a class II solvent with a residual solvents limit of 600 ppm in a finished product and a permitted daily exposure of 6 mg (27).

Furthermore, the influence of the volume of the aqueous heated solution on the size distribution of precipitated particles was investigated. Specifically, the volume of the emulsions was kept constant while the volume of the heated aqueous solution was varied. Comparable particle size distributions were produced from volume ratios of aqueous receiving solution to emulsion between 20:1 and 5:1 (Table II). In terms of manufacturing efficiency, a small volume ratio is desirable since less water has to be removed from the colloidal dispersion in order to obtain the dried particles. The data demonstrate however, that a decrease in the volume ratio below 5:1 results in a significantly increased Dv 90 value and span index (Table II). As the volume ratio is reduced, the rate of mass transfer from emulsion droplets into the aqueous phase will be reduced since less water is available for extraction of DCM. This in turn reduces the degree of supersaturation favoring the generation of larger, less uniform particles. In addition, a decrease in the volume ratio leads to the formation of a more concentrated suspension which in turn increases the collision frequency and growth of particles.

### *Type of Nozzle*

In contrast to EPAS processing, Advanced EPAS processing does not require a customized atomizing nozzle for the production of submicron or micron-sized particles. In fact, injection of an ITZ emulsion *via* a syringe/needle produced nanoparticles even though no atomization of the feed stock occurred as the inner diameter of the needle (300 μm) employed was 60 times larger than that of the atomizing nozzle (5 μm) commonly reported for EPAS-processing.

However manual injection of the emulsion into the aqueous receiving solution is not a sufficiently controlled and reproducible way of creating particles. For this reason, an HPLC pump, which provides a reproducible flow rate, was investigated in combination with two different types of tubing. Specifically, ITZ emulsions were sprayed into the aqueous receiving solution by means of either stainless steel or PEEK tubing. Owing to the difference in the inner diameter of stainless steel tubing (762 μm) and PEEK tubing (127 μm) both provided a very different pressure drop of 30 and 450 psi, respectively (Table III). This is still well below the pressure drop that is produced by the atomizing EPAS nozzle which usually lies between 3,000 and 4,500 psi (13,19). Particle sizes obtained from both types of tubing employed are almost identical, once again indicating that the particle size distribution is governed by the emulsion droplet size distribution and not by the degree of atomization provided by the nozzle employed (Table III).

The fact that colloidal dispersions of ITZ can be produced without the use of a customized atomizing nozzle presents a significant advantage of the Advanced EPAS process over the first reported EPAS process. The requirement for consistent reproduction of a fine atomizing nozzle as needed for EPAS as well as its use for the production of drug

**Table III.** Particle Sizes and Span Indices of ITZ Particles as a Function of Nozzle Type

| | Type of tubing | |
|---|---|---|
| | PEEK | Stainless steel |
| Inner diameter (μm) | 127 | 762 |
| Pressure drop (psi) | 450 | 30 |
| Dv 10 (nm) | 150 | 150 |
| Dv 50 (nm) | 280 | 290 |
| Dv 90 (nm) | 480 | 510 |
| Span | 1.17 | 1.22 |

ITZ particles obtained by spraying O/W emulsion (ITZ/lecithin/NaDC=1:1:0.1; 20% DCM, *v/v*) into a heated aqueous solution maintained at 80°C using an HPLC-pump connected to either polyetheretherketone (PEEK) or stainless steel tubing
*ITZ* itraconazole, *NaDC* sodium deoxycholate, *DCM* dichloromethane

**Table IV.** Characterization of Dried ITZ Particles in Terms of ITZ Potency (in percent, *w*/*w*) and Stabilizer Adsorption (in percent)

| Formulation | Initial drug-to-stabilizer ratio | Theoretical ITZ potency (%, *w*/*w*) | Actual ITZ potency (%, *w*/*w*) | $\frac{W_{Stab,ads}}{W_{Drug,ppt}}$ (%) |
|---|---|---|---|---|
| ITZ/lecithin/PVP K-17 (1:0.25:1) | 1:1.25 | 44.44 | 94.40 | 3.98 |
| ITZ/lecithin/NaDC (1:1:0.1) | 1:1.1 | 47.62 | 83.83 | 16.49 |

ITZ particles obtained by injecting O/W emulsions (20% DCM, *v*/*v*) into heated water (80°C) *via* an HPLC pump connected to PEEK tubing at a flow rate of 7 mL/min
*ITZ* itraconazole, *NaDC* sodium deoxycholate, *DCM* dichloromethane

particles is still at the research stage and scale-up to commercial batch sizes represents a challenge. In contrast, emulsion technology is established in the pharmaceutical industry allowing for potential scale-up of the Advanced EPAS process.

## Characterization of Freeze-Dried ITZ Particles

Aqueous colloidal dispersions are commonly at risk of physical and chemical instabilities including agglomeration, crystal growth and drug degradation (28). Conversion into the solid form, *e.g.*, through freeze-drying, evades instabilities encountered in the liquid state and additionally allows incorporation of nanoparticles into solid dosage forms (29).

Besides the emulsion stabilized with lecithin and NaDC, which was discussed previously, a second emulsion stabilized with lecithin and polyvinylpyrrolidone (PVP K-17) was investigated. Firstly, the use of PVP which was added to the aqueous phase of the emulsion resulted in stable ITZ nanoemulsions suitable for Advanced EPAS processing. Secondly, it was hypothesized that the polymeric stabilizer PVP would be effective in stabilizing precipitated particles in the amorphous state. This assumption was based on several studies reporting that PVP successfully inhibited crystallization of poorly soluble drugs including indomethacin, ketoconazole, and felodipine (30–32).

### *Stabilizer Adsorption and Drug Potency*

In order to quantify the amount of stabilizer adsorbed onto ITZ particles after Advanced EPAS processing, stabilizer adsorption was measured based on mass balance between the supernatant and precipitate, a method previously described by Chen *et al.* (19). As particles precipitate, stabilizers present in the aqueous phase of the emulsion (NaDC or PVP K-17) will adsorb onto the newly formed particle surface. For the amphiphilic NaDC significantly greater adsorption values (16.5%, *w*/*w*) were obtained than for the homopolymer PVP (4%, *w*/*w*) as shown in Table IV. This is expected since the hydrophobic steroid backbone of the NaDC molecule facilitates strong adsorption at the solid–liquid interface while the hydrophilic domain (hydroxyl groups) is orientated into the aqueous medium (33). In contrast, homopolymers such as PVP generally show little surface activity at the O/W interface because the homopolymer segments are highly water-soluble therefore showing little affinity to the interface (34). Yet, adsorption at the solid–liquid interface does occur once the minimum energy of adsorption per polymer segment, which compensates for the loss in configurational entropy, is overcome.

As suspensions obtained from Advanced EPAS processing were centrifuged and the supernatant removed, unbound stabilizer was effectively removed yielding freeze-dried nanoparticles with high ITZ potencies of greater than 90% for PVP-stabilized and more than 80% for NaDC-stabilized particles (Table IV). Chemical stability of dried particles was further confirmed by HPLC analysis where no additional peaks that would indicate the formation of degradation products were seen in chromatograms.

### *Crystallinity*

Furthermore, DSC was used to assess the crystalline/amorphous nature of dried ITZ particles. DSC thermograms of crystalline ITZ and the physical mixtures of Advanced EPAS compositions show only one endothermic peak at around 170°C corresponding to the melting of the crystalline drug. In contrast, DSC thermograms of ITZ particles (Fig. 5) show a glass transition, followed by an exothermic peak corresponding to recrystallization of amorphous ITZ and a sharp melting endotherm of crystalline ITZ at 165°C. The percent crystallinity of dried particles was estimated as the

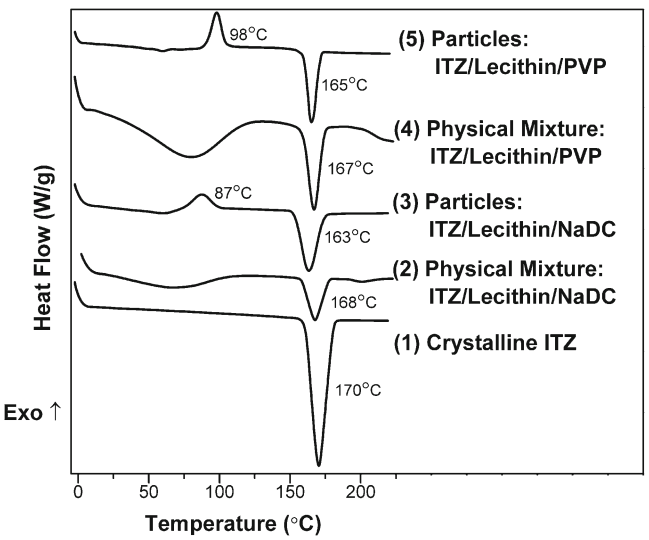


**Fig. 5.** DSC thermograms of *1* bulk, crystalline ITZ, *2* physical mixture of ITZ/lecithin/NaDC=1:1:0.1, *3* Advanced EPAS-produced particles obtained from processing O/W emulsion containing ITZ/lecithin/NaDC=1:1:0.1, *4* physical mixture of ITZ/lecithin/PVP K-17=1:0.25:1, and *5* Advanced EPAS-produced particles obtained from processing O/W emulsion containing ITZ/lecithin/PVP K-17=1:0.25:1

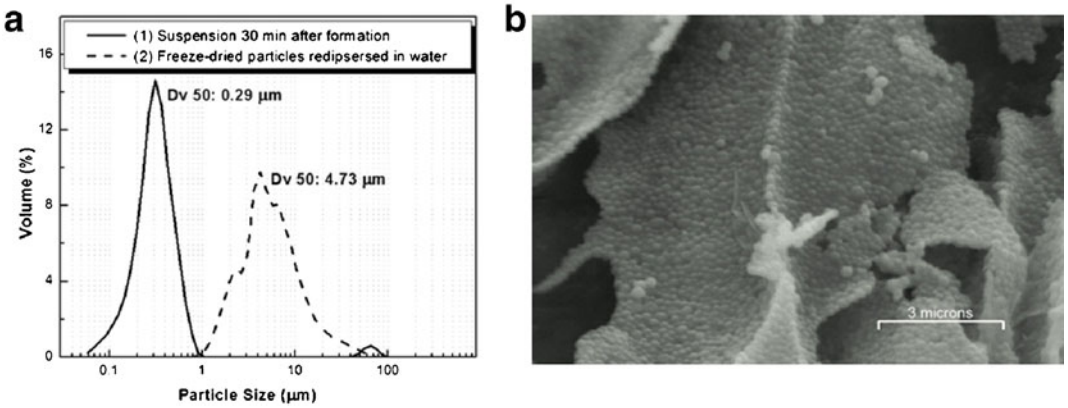


**Fig. 6.** **a** Particle size distributions of *1* colloidal dispersion obtained by injection of emulsion (ITZ/lecithin/PVP K-17=1:0.25:1) into a heated aqueous solution maintained at 80°C and *2* freeze-dried Advanced EPAS-produced particles (ITZ/lecithin/PVP K-17=1:0.25:1) redispersed in water by sonication for 30 s. **b** SEM image of freeze-dried Advanced EPAS-produced particles (ITZ/lecithin/PVP-K 17=1:0.25:1). The *scale bar* corresponds to 3 µm

area under the crystallization and melting peaks as previously described by Matteucci *et al.* (18). Freeze drying of ITZ colloidal dispersions resulted in powders with 47% and 26% crystallinity for particles stabilized with lecithin/NaDC and lecithin/PVP, respectively.

Rapid addition of a drug dissolved in an organic solvent to an aqueous stabilizer solution will most likely result in suspensions containing particles of amorphous morphology (35). However, without the addition of appropriate stabilizers, crystallization occurs over time as particle surfaces are exposed to the surrounding aqueous medium (36). Specifically, the amorphous character of the precipitated particles significantly increases solubility in the aqueous media relative to the crystalline state resulting in a supersaturated solution which is prone to nucleation and crystal growth (37). A number of excipients, especially hydrophilic polymers such as PVP and cellulose derivatives, *i.e.*, hypromellose, have been reported in the literature to inhibit nucleation or crystal growth (38–40). Even though only a small amount of PVP adsorbed onto the particle surface compared with NaDC (Table IV), its stabilizing effect against recrystallization seems to be much more significant. Lindfors *et al.* have reported that PVP concentrations as low as 0.01% (*w*/*w*) significantly reduced the crystal growth rate of bicalutamide in aqueous supersaturated solutions (38). It was demonstrated that PVP adsorbs to already formed crystals thereby preventing further accumulation of the drug at the growing crystal surface. In addition, PVP may cause stabilization against crystal growth by increasing the local viscosity thus hindering diffusion of drug molecules to join a new crystal lattice (41).

*Redispersibility*

To ensure that nanoparticles maintain their beneficial characteristics when converted into solid dosage forms it is critical that they easily reconstitute back to their original size. Redispersion of freeze-dried ITZ particles in water resulted in particle size distributions with considerably increased Dv 50 values. Specifically, the Dv 50 increased from 290 nm for particles in the original suspension to 4.73 µm for particles stabilized with lecithin and PVP K-17 (Fig. 6a) and 5.65 µm for particles stabilized with lecithin and NaDC (size distribution not shown) indicating that agglomeration of nanoparticles occurred. During freezing of colloidal dispersions phase separation into ice and a cryo-concentrated phase composed of nanoparticles occurs. This in turn may induce agglomeration or even irreversible fusion of nanoparticles (29). A SEM image of freeze-dried particles (Fig. 6b) revealed micron-sized agglomerates of distinct spherical nanoparticles indicating that agglomeration, but no fusion of particles occurred.

The addition of sucrose to ITZ particles concentrated by centrifugation of the aqueous colloidal dispersion resulted in fluffy powders which were easily and rapidly reconstituted in water. Increasing the sucrose concentration improved the level of stabilization against agglomeration as seen in particle size distributions (Table V) and SEM images (Fig. 7). The lowest percentage of 0.1% (*w*/*v* of emulsion) sucrose was not effective in preventing agglomerate formation since particles redispersed in water were mainly micron-sized (Dv 50, 3.87 µm) (Table V). At a sucrose concentration of 1% particles were

**Table V.** Particle Sizes, Span Indices, and Actual ITZ Potencies (in percent, *w*/*w*) of ITZ Particles as a Function of Sucrose Concentration

| Sucrose (% (*w*/*v*) emulsion) | Dv 10 (µm) | Dv 50 (µm) | Dv 90 (µm) | Span | ITZ potency (%, *w*/*w*) |
|---|---|---|---|---|---|
| 0.1 | 1.71 | 3.87 | 8.79 | 1.83 | 83.05 |
| 1 | 0.10 | 0.31 | 2.63 | 8.24 | 43.91 |
| 2 | 0.10 | 0.24 | 0.54 | 1.80 | 24.43 |
| 4 | 0.11 | 0.27 | 0.54 | 1.58 | 14.45 |

Particle sizes obtained after redispersion of freeze-dried powders in water by sonication for 30 s. ITZ particles obtained by injecting O/W emulsion (ITZ/lecithin/PVP K-17=1:0.25:1; 20% DCM, *v*/*v*) into heated aqueous solution (80°C) *via* an HPLC pump connected to PEEK tubing at a flow rate of 7 mL/min
*ITZ* itraconazole, *DCM* dichloromethane

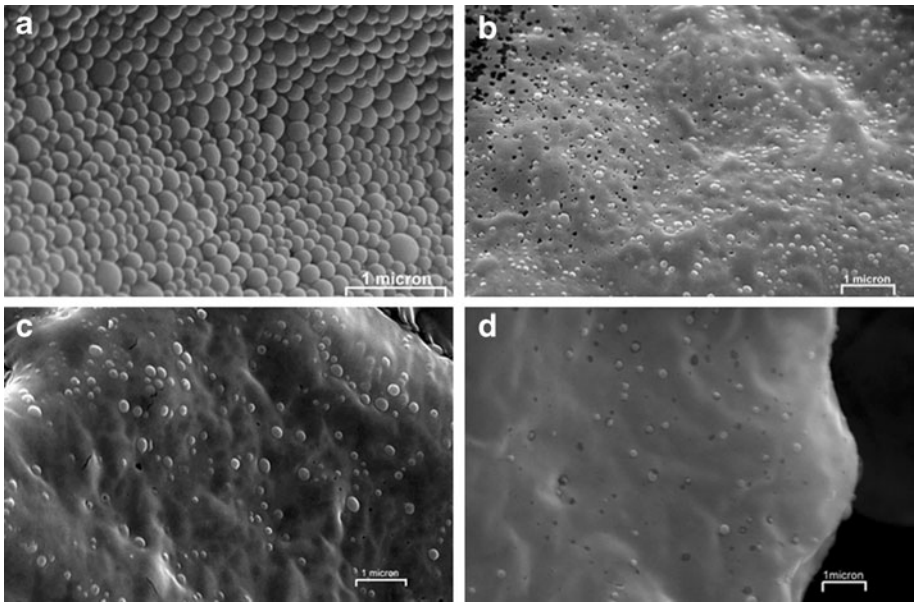


**Fig. 7.** SEM images of Advanced EPAS-produced particles (ITZ/lecithin/PVP K-17=1:0.25:1) formulated with varying percentages of sucrose (in percent (*w*/*v*) of emulsion): **a** 0.1% sucrose, **b** 1% sucrose, **c** 2% sucrose, and **d** 4% sucrose. The *scale bar* corresponds to 1 μm

mostly in the nano-size range (Dv 50, 0.31 μm), however micron-sized particles were present as well (Dv 90, 2.63 μm). As the sucrose concentration was further increased to 2% and 4%, narrower particle size distributions with nano-sized particles were obtained (Table V). The protective effect of sucrose can mainly be attributed to its ability of forming a glassy matrix around particles thereby preventing the particles from agglomeration during freeze-drying (42).

In fact, SEM pictures (Fig. 7) revealed that ITZ particles are embedded into a sucrose matrix which separates particles from each other. At a sucrose concentration of 1%, ITZ particles (Fig. 7b) are still packed relatively tightly and therefore locally able to agglomerate. With increasing sucrose concentrations (Fig. 7c, d) particles are however spread out to a greater extent so that agglomeration is successfully prevented.

Even though particles formulated with 4% sucrose gave the best result in terms of particle size, only the formulations with 1% and 2% sucrose were chosen for further evaluation since they combine acceptable particle size distributions with reasonable ITZ potencies (Table V).

### *Supersaturated Dissolution Testing*

Dissolution testing under supersaturated conditions was conducted at acidic (pH 1) and neutral pH (pH 7.4) with ITZ nanoparticles formulated with 1% and 2% (*w*/*v* of emulsion) sucrose. The saturation solubility of crystalline ITZ at pH 1 is approximately 4 μg/mL as indicated by the dashed line Fig. 8a. Dissolution of ITZ nanoparticles at pH 1 yielded high and sustained plateau levels exhibiting supersaturation of up to ten

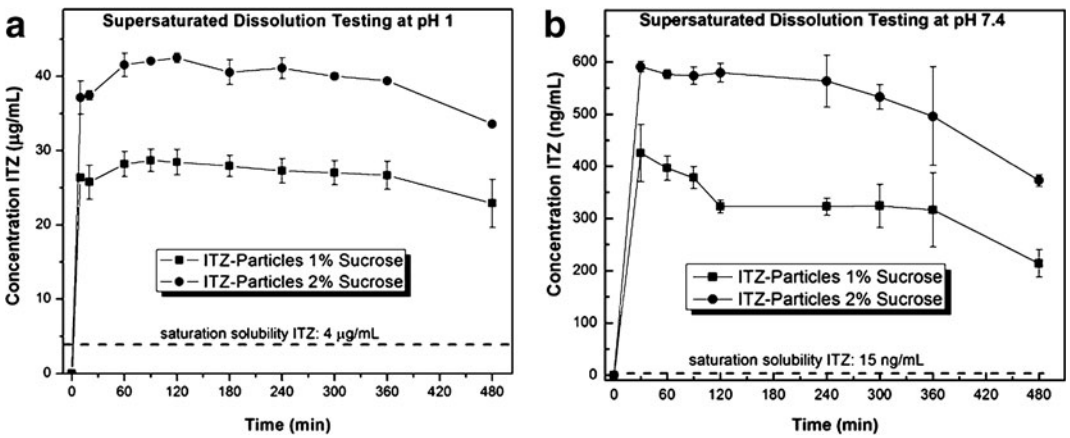


**Fig. 8.** Dissolution profiles of freeze-dried ITZ particles (ITZ/lecithin/PVP K-17=1:0.25:1) formulated with 1% and 2% sucrose in **a** 0.1 N hydrochloric acid at supersaturation conditions (20 times equilibrium solubility of crystalline ITZ) using 100 mL vessels and small paddle apparatus at 50 rpm and 37°C and **b** phosphate-buffered saline (pH 7.4) containing 10% (*v*/*v*) fetal bovine serum at supersaturation conditions (1,333 times equilibrium solubility of crystalline ITZ) using 100 mL vessels and small paddle apparatus at 100 rpm and 37°C

times for nanoparticles formulated with 2% sucrose and six times for particles formulated with 1% sucrose (Fig. 8a). Similar results were obtained when particles where tested at pH 7.4 where the saturation solubility of crystalline ITZ was determined to be 15 ng/mL. Dissolution of ITZ particles resulted in maximum supersaturation levels of 40 and 27 times for nanoparticles formulated with 2% and 1% sucrose, respectively. Once the Advanced EPAS powders come in contact with the aqueous dissolution media, the hydrophilic sucrose matrix will immediately wet and dissolve and then release the primary particles of ITZ.

The partially amorphous nature of ITZ particles as well as their nanometer size will facilitate rapid dissolution rates as well as high levels of supersaturation.

A significantly greater area under the supersaturation curve was obtained for the 2% sucrose formulation (18,796 μg*min/mL at pH 1; 245,288 ng*min/mL at pH 7.4) in comparison to the 1% sucrose formulation (12,741 μg*min/mL at pH 1; 150,037 ng*min/mL at pH 7.4). Looking at the particle size distributions of both formulations, ITZ particles formulated with 2% sucrose have a very narrow size distribution with 90% of particles being smaller than 540 nm. ITZ particles formulated with 1% sucrose however, contain a considerable portion of particles that are in the micron-size range directly translating into a slower dissolution rate. The slower dissolution rate in turn increases the time that is available for crystallization of undissolved particles in the presence of the dissolution media thereby decreasing the maximum attainable supersaturation level (18). Previous studies have shown that generating and sustaining high ITZ supersaturation levels is beneficial for increasing bioavailability in rats after oral gavage (43,44).

## CONCLUSIONS

ITZ nanoparticles have been prepared employing Advanced EPAS, a novel process combining emulsion templating and EPAS processing. The use of an emulsion as feed stock for EPAS processing was found to offer superior control over the size of precipitated particles than compared with an organic solution as utilized in the EPAS process. The particle size distribution of Advanced EPAS-produced particles was shown to be independent across several processing parameters, indicating robustness of the process and potential to be adapted for other poorly soluble compounds. In addition, Advanced EPAS overcomes the current limitation of the conventional EPAS process in terms of scale-up since the requirement for consistent reproduction of an atomizing stainless steel nozzle was eliminated.

Advanced EPAS produced spherical sub-300-nm particles with decreased crystallinity which could be stabilized with only small amounts of stabilizers yielding drug potencies of more than 90% (*w*/*w*). The use of sucrose as cryoprotectant enabled easy reconstitution in aqueous media where particles reached high and sustained ITZ supersaturation levels. Improving the solubility/dissolution of drugs like ITZ, that are characterized by low solubility and high intestinal permeability, is of significant interest for enhancing their bioavailability and thus therapeutic effectiveness. Advanced EPAS-produced particles have immediate applications for oral administration where they may be incorporated into various dosage forms including tablets, capsules or pellets. In addition, they can be valuable for the delivery of poorly soluble drugs through other routes of administration that require more stringent control over the particle size such as the parenteral and pulmonary routes.

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