

Solidification of nanosuspensions for the production of solid oral dosage forms and inhalable dry powders

Maria Malamatari*, Satyanarayana Somavarapu, Kevin MG Taylor[#], Graham Buckton

UCL School of Pharmacy, 29-39 Brunswick Square, London, WC1N 1AX, UK

[#]Corresponding author: Kevin MG Taylor

UCL School of Pharmacy,

29-39 Brunswick Square,

London, WC1N 1AX, UK

Email: kevin.taylor@ucl.ac.uk

Tel: +44 (0) 207 753 5853

* Co-corresponding author: Maria Malamatari

Email: maria.malamatari.12@ucl.ac.uk

Solidification of nanosuspensions for the production of solid oral dosage forms and inhalable dry powders

- 1. Introduction**
- 2. Advantages of nanosuspensions**
- 3. Production of nanosuspensions**
- 4. Solidification of nanosuspensions**
- 5. Redispersibility of solidified nanosuspensions**
- 6. Conclusions**
- 7. Expert opinion**

Abbreviations

API: Active pharmaceutical ingredient

AUC: Area under the curve

BBB: Blood-brain barrier

BCS: Biopharmaceutical classification system

CGMP: Current good manufacturing practice

C_{max}: Maximum plasma concentration

COPD: Chronic obstructive pulmonary disease

D₅₀: Volume median diameter

D₉₀: Volume diameter 90% undersize

DCS: Developability classification system

DPI: Dry powder inhaler

FDA: United States Food and Drug Administration

FPF: Fine particle fraction

F_{rel}: Relative bioavailability

HPH: High-pressure homogenization

HPMC: Hydroxylpropyl methylcellulose

MIC: Minimum inhibitory concentration

MMAD: Mass median aerodynamic diameter

NCE: New chemical entity

ODT: Orally disintegrating tablet

PCL: Polycaprolactone

PVA: Polyvinyl alcohol

PVP: Polyvinylpyrrolidone

SLS: Sodium lauryl sulphate

t_{max}: Time to achieve maximum plasma concentration

TPGS: D- α -tocopheryl polyethylene glycol succinate

Abstract

Introduction: Nanosuspensions combine the advantages of nanotherapeutics (e.g. increased dissolution rate and saturation solubility) with **ease of** commercialisation. Transformation of nanosuspensions **to solid oral and inhalable dosage forms** minimises the physical instability associated with their liquid state, enhances patient compliance and enables targeted oral and pulmonary drug delivery.

Areas covered: This review outlines solidification methods for nanosuspensions. It includes spray and freeze drying as the most widely used techniques. Fluidised-bed coating, granulation and pelletisation are also discussed as they yield nanocrystalline formulations with more straightforward downstream processing to tablets or capsules. Spray-freeze drying, aerosol flow reactor and printing of nanosuspensions are **also presented as** promising alternative solidification techniques. Results regarding the solid state, *in vitro* dissolution and/or aerosolisation efficiency of the nanocrystalline formulations are given and combined with available *in vivo* data. **Focus is placed on the redispersibility of the solid nanocrystalline formulations, which is a prerequisite for their clinical application.**

Expert opinion: **A few solidified nanocrystalline products are already on the market and many more are in development.** Oral and inhalable nanoparticle formulations are expected to have great potential especially in the areas of personalised medicine and delivery of high drug doses (e.g. antibiotics) to the lungs, respectively.

Keywords: dry powder for inhalation, matrix former, nanosuspension, redispersibility, solid oral dosage forms, solidification

Article highlights

- Nanosuspensions are submicron dispersions of nanosized drug particles stabilised by surfactants, polymers or a mixture of both.
- The main advantages of nanosuspensions are increased dissolution and saturation solubility, enhanced chemical stability and rapid commercialisation.
- Transformation of nanosuspensions to the solid state ensures their long-term stability and increases patient compliance.
- Various drying techniques, with some of them well established (e.g. spray drying) while others are less applied in pharmaceutical technology (e.g. aerosol flow reactor) have been used for the transformation of nanosuspensions to solid oral dosage forms and dry powders for inhalation.
- Transformation of nanosuspensions to micron-sized nanoparticle agglomerates is a way to deliver higher doses to the lungs compared to the conventional adhesive mixtures approach using dry powder inhaler (DPI) formulations.
- Formulation and solidification process parameters influence the redispersibility (reformation of nanoparticles upon rehydration), which is a prerequisite for their superior clinical performance. Addition of matrix formers is a common strategy in order to achieve redispersible solidified nanosuspensions.

1. Introduction

Many of the new chemical entities (NCEs) arising from combinatorial screening (>40%) suffer from poor solubility in aqueous media and some of them simultaneously in organic solvents [1]. Benet [2] puts the figure of NCEs suffering from poor solubility at 90% while only 40% of marketed drugs are poorly soluble.

Whilst the vast majority of NCEs fail due to lack of efficacy **or safety**, poor solubility is frequently encountered as one of the challenges in the development of NCEs, as in the most extreme cases it results in erratic absorption and low bioavailability. In the past, the pharmaceutical industry considered these compounds as highly risky development candidates [3]. However, nowadays mainly due to their prevalence, *“industry consensus has shifted from an attitude of avoidance to one of acceptance as increasing research dedication is given to solving solubility challenges”* [4]. One strategy for addressing solubility challenges is based on nanoparticles.

Nanotechnologies are one of the most prevalent strategies not only for overcoming the problem of poor solubility and thus bioavailability, but also for targeted drug delivery. Therefore, the use of nanoparticles in drug delivery is gaining momentum and research on nanoparticulate formulations has expanded greatly in recent years. Different types of nanotherapeutics have been applied in drug delivery, such as nanocomplexes, nanoemulsions, polymeric micelles, liposomes, virosomes, polymeric nanoparticles and nanosuspensions [5].

Nanosuspensions, also called nanocrystals are submicron, colloidal dispersions of nanosized drug particles, stabilized by surfactants, polymers, or a mixture of both [6]. According to a stricter definition, a formulation should have **a volume median**

diameter (D_{50}) below 1 μm and a volume diameter 90% **undersize** (D_{90}) below 2.5 μm to be classified as a nanosuspension [7,8]. The term nanocrystals, although implying the particles are in a crystalline state, which is true for the majority of reported cases, has been also extended to describe nanosized suspensions of partially crystalline or even amorphous drugs [9].

For a nanosuspension to be stable, it must contain a third component known as stabiliser in addition to the solid particles and liquid, such as a surfactant and/ or polymer. Several theories have been proposed to explain how stabilisers function and contribute to the stability of nanosuspensions: i) the lowering of the interfacial tension, ii) the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and iii) the steric stabilisation theory [10]. Currently, the selection of their stabilisers is mainly based on trial and error. However, there is an increased interest in developing a rational approach to selecting the appropriate stabiliser based on the physicochemical properties of the drug under investigation [11,12].

Drug nanosuspensions have been suggested as a universal delivery approach for drugs that fall into classes II and IV of the Biopharmaceutics Classification system (BCS) [13,14]. However, according to the recently proposed Developability Classification System (DCS), the nanosuspensions are only beneficial as a formulation approach for class IIa drugs for which the dissolution rate is the rate-limiting step for absorption [15].

This review will focus on the conversion of liquid nanosuspensions to solid oral dosage forms and dry powders for inhalation. The reasons for the solidification of

nanosuspensions together with the various methods applied until now in order to achieve solidification, will be described in-depth. In vitro data on the dissolution and/or aerosolisation of solidified nanocrystalline formulations will be given together with available in vivo data. Finally, emphasis will be given to the factors affecting their redispersibility, which is required for their superior clinical performance.

2. Advantages of nanosuspensions

In general, the main advantages of nanosuspensions are: a) their increased dissolution rate and enhanced saturation solubility with consequent bioavailability improvement, b) their low toxicity¹ and c) the improved chemical stability.

The reduced particle size and high surface area per unit mass of the nanoparticles lead to a more rapid dissolution as described by **the** Nernst and Brunner equation [16,17]. Regarding the saturation solubility, which for drug particles in the micrometer range and above is a constant depending on temperature and dissolution medium, in the case of sub-micron particles it depends on their size and is reported as "apparent" saturation solubility [18]. The enhanced "apparent" saturation solubility of nanosuspensions has been attributed to the increased curvature of nanoparticles resulting in increased dissolution pressure and hence drug solubility as described by Ostwald-Freundlich [19]. Due to the increased dissolution rate and enhanced saturation solubility, nanosuspensions result in improved bioavailability [14,20].

¹ **Recently, there is an increasing concern regarding nanotoxicology mainly due to the ability of nanoparticles to enter cells. The toxicity of nanoparticles is related to their cellular uptake and intracellular fate. According to the nanotoxicological classification system proposed by Müller et al. [21], nanocrystals are in the low/non-risk group. Their typical size (100 nm < d < 1000 nm) does not allow cell endocytosis and their biodegradable nature is associated with low danger of biopersistence. However, studies should be conducted on the interaction of nanocrystals with cells and their cellular uptake has to be considered when developing nanocrystalline-based products [21].**

More specifically, regarding oral drug delivery, nanosuspensions have been used as a way to address the issue of low bioavailability with reduced food-effect compared to micronised drug [21,22]. Nanosuspensions **also increase** adhesiveness to the gastrointestinal mucosa leading to prolonged gastrointestinal residence time and thus enhanced uptake via the gastrointestinal tract [23]. Mucoadhesive nanosuspensions of the antiprotozoal drug buparvaquone containing chitosan were proposed as a drug delivery system against intestinal *Cryptosporidium* infections due to their increased gastrointestinal residence time and thus prolonged action [24].

The high drug loading of nanosuspensions compared to other formulation approaches (e.g. cyclodextrin formulations) is beneficial as it is associated with reduced toxicity due to the very **small amounts** of stabilisers/excipients used [25]. Formulating a drug as a nanosuspension has also been proposed as a method of improving chemical stability with respect to solution formulations. For example, quercetin nanosuspensions exhibited no significant change in drug content or color over 1 month. In contrast, for the solution, a 28.3% reduction in drug content and discoloration was observed over the same period [26].

Formulating drugs as nanosuspensions may change their pharmacokinetic profile and extend their therapeutic use, as in the case of melarsoprol, an organoarsenic compound. Melarsoprol as a nanosuspension was unable to cross the blood-brain barrier (BBB), but rather accumulated preferentially in the Mononuclear Phagocyte System, which is a family of cells comprising bone marrow progenitors. The high concentration of the drug in the bone marrow resulted in reduced arsenic-related encephalopathies, due to the inability of the formulation to cross the BBB, and opens

the way for the use of melarsoprol against refractory leukemias and other cancer diseases [27].

Apart from their superior clinical performance, nanosuspensions have attracted the interest of drug formulators as **they can** extend the life cycle of an active pharmaceutical ingredient (API) after patent expiration, as occurred for a fenofibrate nanosuspension launched by Abbott Laboratories [14]. Nanosuspensions are also suitable formulations for preclinical animal studies mainly due to their quantitative and easy oral administration [28]. **Moreover, the techniques for the production of nanosuspensions (e.g. wet-bead milling) are easy to scale-up and can provide the amount required (10 ml up to a few liters) for toxicological and pharmacokinetic studies in animals, and for clinical trial material under current good manufacturing practices (CGMP) [25,29]. All these characteristics have resulted in the rapid commercialisation of nanosuspensions.**

The application of nanosuspensions in pulmonary drug delivery has been limited until recently. However, many of the advantages mentioned above can also be extended to this route of administration. Nanosuspensions have been proposed as a formulation approach to increase the dissolution rate and thus absorption of poorly water-soluble inhaled corticosteroids such as fluticasone propionate and budesonide [30]. Britland *et al.* [31] compared the bioavailability, emission characteristics and efficacy of a budesonide **nanoparticulate** formulation with those of a proprietary **micronized suspension of the drug** after delivery as a nebulized aerosol to a human airway epithelial culture cell line. For an equivalent dose, the budesonide nanosuspension achieved improved uptake, retention and efficacy in the culture cells.

Furthermore, there are two key advantages in using nanosuspensions instead of solution formulations for pulmonary delivery of drugs with poor aqueous solubility. Preparation of solutions requires the use of organic co-solvents while the need for co-solvents is limited or even non-existent for the production of nanosuspensions. As a result, the danger of potential co-solvent related toxicity is reduced [32]. In nanosuspensions, the drug concentration is not limited by the solubility in the vehicle as is the case for solutions, allowing the delivery of higher doses in the lungs [32]. Exploiting this advantage, Rundfeldt *et al.* [33] prepared itraconazole nanosuspensions which achieved high and long lasting lung tissue concentrations well above the minimum inhibitory concentration (MIC) against *Aspergillus* species, when administered using standard mesh nebulizer technology. The high concentration achieved indicates the potential of itraconazole nanosuspensions as a treatment for allergic bronchopulmonary aspergillosis in patients with cystic fibrosis, administered only once daily.

Finally, nanosuspensions can be used as formulations facilitating pulmonary drug discovery, as they are easy to dose with a syringe-type delivery device in preclinical animal studies [30]. **The advantages of nanosuspensions for both oral and pulmonary drug delivery are summarised in Table 1.**

3. Production of nanosuspensions

Methods for the production of nanosuspensions can be categorized as top-down and bottom-up methods, depending on the starting material. In top-down methods, such as wet-bead milling, high-pressure homogenization (HPH) and microfluidisation, the starting material comprises larger solid particles than the resulting nanoparticles and

mechanical processes are the fundamental mechanism causing particle size reduction. In bottom-up methods, particles are formed from the molecular level. Such methods are further subdivided into solvent evaporation (e.g. spray drying, electrospraying, cryogenic solvent evaporation, etc.) and antisolvent methods (e.g. liquid antisolvent, supercritical antisolvent, etc.) [34,35].

The main advantage of top-down over bottom-up methods is the production of nanosuspensions with high drug loading. Moreover, they do not involve harsh organic solvents since the solvent in which drug is dispersed, but not dissolved, is water for the majority of poorly water-soluble drugs, making the top-down methods eco-friendly. This permits the formulation of many **poorly soluble** APIs, characterized as “brick dust”, suffering from poor solubility in a wide range of solvents. In general, because of the more streamlined process-flow and the solvent-free feature of top-down methods, the majority of marketed and developmental nanosuspension-based pharmaceutical formulations have been produced by top-down methods (Table 2).

In this review, the production methods of nanosuspensions will not be discussed in detail since several relevant reviews have been published [13,25,36]. Rather, focus will be given on the transformation of nanosuspensions to the solid form, which will in this text be referred to as "solid nanosuspensions".

4. Solidification of nanosuspensions

Liquid nanosuspensions are associated with physical instability issues including sedimentation, creaming, crystal growth (also known as Ostwald ripening), aggregation and solid state transformation. A detailed review of the physical and

chemical instability issues of drug nanoparticles has been provided by Wu *et al.* [37]. These instability phenomena make it difficult to ensure that the particle size of the nanosuspensions will remain the same upon storage. Moreover, nanosuspensions, due to their liquid media, are prone to microbial growth upon storage whilst addition of antimicrobial preservatives has been reported to cause destabilisation of the nanosuspensions in some cases [38].

On the other hand, solid dosage forms exhibit enhanced physical and chemical stability. In addition, there is a general preference of patients for solid oral forms over liquid forms, enhancing compliance [22,39]. Transformation of nanosuspensions to more convenient dosage forms (e.g. tablets, capsules, pellets, granules etc.) is required in order to expand the application of nanocrystalline-based formulations into late development and commercial stages [40].

Dry powder inhalers (DPIs) constitute one of the main types of inhalation devices for drug delivery to the lungs, and their market is estimated to reach over USD 31.5 billion in 2018 [41]. The inherent stability of the dry powders, the ability to administer high doses, the absence of propellants and the easier use compared to pressurised metered-dose inhalers (pMDIs) as they are breath-actuated can explain the increasing popularity of DPIs (in patients ≥ 5 years old and with adequate inspiratory flow), especially in Europe and North America [42].

For pulmonary delivery the particles should have an aerodynamic diameter between 1-5 μm so as to deposit in the lungs [43]. Therefore, delivery of individual nanoparticles to the lungs (with the exception of particles below 50 nm) appears to be problematic, as due to their submicron size, the probability of exhalation before

deposition is increased [44,45]. Moreover, nanoparticles tend to strongly aggregate upon aerosolisation under the normal airflow rates in passive DPIs and their cohesive nature makes their handling extremely difficult [46]. In order to overcome these limitations, the controlled agglomeration of nanoparticles to micron-sized clusters has been recently proposed as “*an approach to harmonise the advantages of nanoparticles with the aerodynamics of small microparticles so as to achieve an improved bioavailability² and aerosolisation behaviour of the drug*” [47]. It is clear then, that solidifying nanosuspensions to respirable nanoparticle agglomerates of 1 - 5 μm , is a prerequisite for administering nanoparticles effectively to the lungs using DPIs.

From the above, it becomes clear that, in general, the reasons for solidification of nanosuspensions lies in the disadvantages of the liquid state and the advantages associated with solid dosage forms. The usual problem of solidifying the nanosuspensions is the difficulty of readily redispersing "solid nanosuspensions" to yield nanosuspensions upon hydration with aqueous media in vitro and in the biological fluids of the gastrointestinal tract or lungs.

4.1. Methods of solidification

Spray drying, spray-freeze drying, freeze drying, deposition as coatings, incorporation in granules and pellets, aerosol flow reactor and printing methodologies which have

² Regarding pulmonary drug delivery, drug absorption and local bioavailability depends upon the fraction of deposited drug that dissolves in the lungs. Mucociliary clearance and absorption are two competing mechanisms and this can lead to bioavailability reduction for drug particles with low dissolution velocity [48]. Nanoparticle-based formulations have been found to promote more rapid absorption following inhalation of poorly water-soluble drug that have dissolution-limited drug absorption (e.g. fluticasone propionate, beclomethasone dipropionate, budesonide, itraconazole, fentanyl)[49].

been applied for the solidification of nanosuspensions will be discussed in-depth in this review.

Illustration of the various solidification techniques for the production of solid oral nanocrystalline dosage forms is given in Fig.1. Fig.2 summarises the solidification approaches for the preparation of inhalable micron-sized nanoparticle agglomerates with suitable aerodynamic properties for delivery to the lungs using DPIs.

4.1.1 Spray drying

Spray drying is a one-step process whereby a liquid feed is converted to a dried particulate form. The main principles behind spray drying are the atomization of the liquid feed into fine droplets by a nozzle and the evaporation of the solvent by means of a hot drying gas [50]. The characteristics of the produced particles (e.g. particle size distribution, shape, residual moisture content and density) can be controlled by adjusting the formulation and process parameters (e.g. feed rate, inlet temperature) during spray drying. This makes spray drying a fundamental particle engineering technique for the development of both oral and inhalable formulations. Moreover, it is a popular process, from an industrial perspective, as it is quicker and more cost-effective compared to freeze drying. Some examples of spray drying applications in developing “solid nanosuspensions” for oral and pulmonary delivery will follow.

Zuo *et al.* [51] prepared nanosuspensions of fenofibrate by wet-bead milling and then applied spray drying for their solidification with simultaneous incorporation of mannitol as a matrix former. They elucidated the effect of formulation and process parameters on the redispersibility of the produced powders and found that: a) a low

drug to mannitol ratio and b) an inlet temperature lower than the melting point of fenofibrate should be used in order to minimize irreversible aggregation. Finally, the spray-dried nanocrystalline formulation with the optimal redispersibility characteristics was compared with **a commercial nanocrystalline formulation licensed by Pharma ULC in Canada**. No significant differences were observed between the in vitro and in vivo performance of the two formulations (relative bioavailability, F_{rel} : 89.6%).

Freag *et al.* [52] prepared nanosuspensions of diosmin (350-550 nm) by acid-base neutralisation (bottom-up technique), which were spray dried in the presence or absence of mannitol. The intestinal permeation of the formulations was studied using the ex vivo technique of the non-everted sac. It was found that formulating diosmin as a nanosuspension remarkably improved the permeation of the drug while for the solidified nanosuspensions the permeability was dependent on the incorporation of mannitol. Nanosuspensions spray dried without mannitol exhibited a decreased permeation compared to the liquid nanosuspensions, which was attributed to incomplete dispersion within the sac. However, no decrease in permeation was observed for nanosuspensions solidified by spray drying together with mannitol, due to their enhanced redispersibility.

Spray drying has a long tradition in pulmonary drug delivery and until now the majority of reported studies on the development of inhalable particles by this methodology have focused on spray drying of drug solutions. But, as spray drying is a rapid solidification procedure, the obtained particles (at least from solution feed) are usually amorphous with the characteristic example being that of salbutamol sulfate (albuterol sulfate) after spray drying from aqueous solution (**Fig.3a**) [53].

Amorphy is regarded as a disadvantage for respirable particles as it is associated with the danger of recrystallisation upon storage, which may influence adversely the stability, dissolution, absorption and aerosolisation efficiency of the product [54]. Recently, a few studies on solidified nanosuspensions for the development of pulmonary formulations by applying spray drying have been reported. The observed important formulation factors and characteristics of the resulting respirable "solid nanosuspensions" are summarized below.

Pilcer *et al.* [55] prepared carrier-free respirable agglomerated nanoparticle formulations of tobramycin with enhanced dispersion properties by combining high-pressure homogenization with spray drying. This can be considered as an attractive formulation approach for treating lung infections as a high dose can be delivered to the site of infection reducing the side effects related to systemic antibiotic administration.

Yamasaki *et al.* [56] prepared nanosuspensions of cyclosporine A by anti-solvent precipitation (bottom-up technique) using a multi-inlet vortex mixer. The nanosuspensions were spray dried after the addition of different amounts of mannitol (**Fig.3b**), resulting in respirable nanoparticle agglomerates with enhanced fine particle fraction (FPF > 50%). The drug in the nanoparticle agglomerates was found to be in the amorphous state and this was attributed to the method of nanosuspension production rather than to the spray-drying step. The authors also investigated the effect of cyclosporine A to mannitol mass ratio on the dissolution rate and on the aerosolisation performance of the solidified nanosuspensions and concluded that

mannitol, as a hydrophilic excipient, improved the dissolution rate of cyclosporine A, while not influencing the aerosolisation behaviour.

Pomázi *et al.* [57] developed respirable nanoparticle agglomerates of meloxicam embedded in mannitol and other adjuvants (polymers and L-leucine), by applying high-pressure homogenisation followed by spray drying. The presence of the amino acid L-leucine was found to enhance the aerosolisation of the nanoparticle agglomerates due to decreased particle-to-particle adhesion [58]. The nanoparticle agglomerates of meloxicam were crystalline and exhibited enhanced in vitro aerosolisation (FPF > 53%, mass median aerodynamic diameter, MMAD < 3.52 μm) and dissolution performance. Similar results have been reported for nanoparticle agglomerates of itraconazole as a formulation approach for the treatment of pulmonary aspergillosis [59].

From the above it can be summarized that spray drying of nanosuspensions has great potential for the production of solid formulations with predefined solid state and enhanced dissolution and aerosolisation properties that can be either administered orally or via a DPI. Addition of matrix formers and careful selection of process parameters is essential for the production of solid nanosuspensions with enhanced redispersibility, a prerequisite for superior clinical performance. Therefore, spray drying of nanosuspensions can be used as a platform for the oral and pulmonary delivery of poorly water-soluble drugs.

4.1.2. Spray-freeze drying

Spray-freeze drying is an alternative technique to spray drying, which has been used for the preparation of thermolabile inhalable particles, especially proteins [60]. In the spray-freeze drying process, the liquid feed is atomised by a nozzle and the atomised droplets are snap frozen in liquid N₂. The iced droplets are then lyophilized in order to remove the frozen solvent (water) via sublimation and obtain a powdery product. In comparison to spray drying, the spray-freeze drying is a low-temperature solidification process that produces porous particles [61].

Niwa and Danjo [62] applied spray-freeze drying for the preparation of phenytoin "solid nanosuspensions" with enhanced redispersibility. The nanosuspensions were prepared by wet-bead milling, with polyvinylpyrrolidone (PVP) and sodium lauryl sulphate (SLS) as dispersing agents, and were further solidified by spray-freeze drying and conventional spray drying. The spray-freeze dried particles comprised porous network structures in which drug nanocrystals were embedded (**Fig.4a, b**). Both their redispersibility and dissolution were improved compared to solid nanosuspensions produced by conventional spray drying. The superior properties of spray-freeze dried particles were attributed to the better penetration of water into the pores of the particles resulting in rapid reconstitution of nanosuspensions. Consequently, spray-freeze drying of drug nanosuspensions was suggested as a novel dissolution-enhancing technique for poorly water-soluble drugs.

Few studies have been published regarding the spray-freeze drying of drug nanosuspensions to produce particles suitable for inhalation. Cheow *et al.* [63] prepared inhalable composite particles by spray-freeze drying suspensions of

levofloxacin-loaded polycaprolactone (PCL) nanoparticles, in order to avoid problems associated with the low melting point of PCL and the limitation of elevated temperature use, which is inevitable in spray drying. Prior to the spray-freeze drying, adjuvants such as mannitol or polyvinyl alcohol (PVA) were added to the nanosuspensions to act as cryoprotectants and also to ensure redispersibility of the particles produced. Porous inhalable particles with suitable flowability and redispersibility were produced when high adjuvant concentrations were used.

From the above it appears that spray-freeze drying is an alternative method for the solidification of thermolabile drug nanosuspensions, where the use of spray drying is not appropriate. Moreover, regarding pulmonary drug delivery, spray-freeze drying allows the preparation of particles with large geometric diameters but still having suitable aerodynamic properties due to their high porosity/low particle density [63]. In this way the poor flowability problems usually associated with inhalable particles can be overcome.

4.1.3. Freeze drying

Freeze drying or lyophilisation is a classical process of removing water from high value products (e.g. antibiotics, enzymes, vaccines, etc.) without excessive damage, enhancing stability on storage and reconstitutable by adding water, or other suitable aqueous diluent, prior to use [64]. In freeze drying, the liquid solution or suspension is firstly frozen under atmospheric pressure and subsequently heated under vacuum to remove the ice crystals by sublimation. At the end of the process, a highly porous cake with low moisture is obtained. Freeze drying together with spray drying are the most commonly used techniques for the solidification of nanosuspensions due to their

easy application and scale up possibility and therefore industrial acceptability. Recent applications of freeze drying for the solidification of nanosuspensions to oral and pulmonary formulations have been the following.

Fu *et al.* [65] prepared nanosuspensions of nimodipine by high-pressure homogenisation, which were then freeze dried so as to be transformed to the solid state. Freeze drying **had** no effect on the redispersibility of the nanocrystalline formulations compared to a commercial solid dispersion of nimodipine **marketed by Bayer Healthcare Pharmaceuticals**. However, when the *in vitro* and *in vivo* profiles were compared, while the freeze-dried nanocrystals exhibited lower dissolution than **the solid dispersion** under non-sink conditions, their bioavailability was 2.6-fold higher, providing a way to reduce the high administered dose of nimodipine associated with its extensive first pass metabolism. Moreover, according to the authors, focus should be placed on the permeability mechanisms of nanocrystals and especially on the role of enterocytes and M cells on their oral absorption, which may explain the unfavourable *in vitro in vivo* correlation.

Lai *et al.* [66] prepared orally disintegrating tablets (ODTs) of piroxicam, using freeze-dried nanosuspensions as a way to enhance the drug's dissolution rate and also minimise the unwanted effects associated with its oral administration (e.g. gastric irritation). The piroxicam nanosuspensions were prepared by high-pressure homogenisation and stabilised with **Poloxamer 188**. The type of excipient added to the nanosuspension prior to the freeze-drying step was found to influence the dissolution rate of the nanocrystalline formulations. Crosscarmellose and gelatine resulted in higher dissolution rate than a commercial piroxicam ODT product

marketed by Pfizer, while xanthan gum reduced dissolution rate which was attributed to the increased viscosity of the hydrated gum upon dissolution.

For the formation of respirable nanoparticle agglomerates, El-Gendy *et al.* [47] employed freeze drying for the transformation of the nanosuspensions obtained by a controlled flocculation process (protocol under the name NanoClustersTM, owned by Savara Pharmaceuticals). Budesonide nanoparticle agglomerates prepared by this protocol, exhibited enhanced dissolution rate compared to the raw drug and had MMAD $2.1 \pm 1.8 \mu\text{m}$ (mean \pm SD) on aerosolisation, appropriate for deep lung deposition of the particles. The NanoClustersTM protocol has been applied for the preparation of nanoparticle agglomerates of various drugs, such as ciprofloxacin, paclitaxel, diatrizoic acid, nifedipine and fluticasone propionate (Fig. 4c) [47,67,68].

Although spray drying and freeze drying are the most widely used techniques for the solidification of nanosuspensions, the powders produced by these methods often suffer from poor flowability and high hygroscopicity. These limitations make their downstream processing into final dosage forms such as tablets and capsules challenging [69]. Therefore, other methodologies have been applied to transform nanosuspensions into solid oral dosage forms, e.g. deposition as coatings and incorporation in granulations and pellets.

4.1.4. Deposition as coatings

Layering of nanosuspensions onto the surface of multiparticulates (e.g. granules, pellets, sugar beads, non-pareils, etc.) using a fluidised-bed coater has been used as an alternative method for the solidification of nanosuspensions [69-71]. In this method,

sufficient amounts of excipients, such as d- α -tocopheryl polyethylene glycol succinate (TPGS) and hydroxypropyl methylcellulose (HPMC) are used, both to stabilise the nanosuspensions, and to act as coating agents, required to ensure nanoparticles adhere onto the multiparticulates [69].

Möschwitzer and Müller [71] produced mucoadhesive nanosuspensions of hydrocortisone acetate by **high pressure homogenisation (HPH)**, which were stabilised by **Poloxamer 407** and chitosan chloride. The nanosuspensions were layered onto the surface of sugar spheres using a fluidized-bed coater equipped with a Wurster insert and bottom sprayer. Using the same equipment, the layered cores were further coated with an enteric polymer **containing methacrylic acid as functional group**, to achieve a controlled-release profile. The authors highlighted the triple role of chitosan chloride in the formulations: stabiliser of the nanosuspension, binder in the layering process and mucoadhesive excipient. Formulations of layered nanosuspensions exhibited enhanced dissolution rates compared to those layered with a drug microsuspension.

4.1.5. Incorporation in granules and pellets

Figuerola and Bose [72] studied the incorporation of nanosuspensions in fluidized-bed granulates. Nanosuspensions of naproxen and a proprietary Novartis compound were prepared by wet-bead milling and were further sprayed onto a mannitol-based substrate using a fluidized-bed granulator. Spray mode was identified as a critical process parameter, with top spraying yielding granules with enhanced redispersibility and faster release rates than those produced by bottom spraying. In addition, *in vivo* studies regarding the proprietary Novartis compound in fasted beagle dogs revealed

no significant differences between the pharmacokinetic profiles of the parent nanosuspension and the granulated nanocrystalline formulation.

Vergote *et al.* [73] prepared spray-dried nanosuspensions of ketoprofen with and without sodium lauryl sulphate (SLS) and applied melt pelletisation to incorporate them in a controlled-release formulation. The pellets incorporated the spray-dried nanosuspension of ketoprofen, drum-dried corn starch and microcrystalline wax. It was reported that incorporation of SLS during the preparation of the nanosuspension not only acted as a stabiliser but also helped to achieve complete drug release from the pellet matrix. The formulations containing nanocrystalline ketoprofen exhibited faster *in vitro* release than the wax-based pellets of microcrystalline drug and the commercial long-acting product. However, no significant differences in the area under the curve (AUC) values of the above formulations were observed, after oral administration to dogs [74]. The pellets containing nanocrystalline ketoprofen exhibited an *in vitro* burst release achieving a higher maximum plasma concentration (C_{\max}) and shorter time to achieve maximum concentration (t_{\max}) than the commercial formulation. Tablets prepared by compressing the ketoprofen pellet formulations with placebo wax-based pellets containing disintegrant had an *in vivo* profile similar to the commercial product.

It appears that fluidised bed coating or granulation as well as pelletisation can be attractive options for the incorporation of solid nanocrystalline formulations in multiparticulates. As a result, the benefits of nanoparticles and multiparticulates can be combined and the handling difficulties of the solid nanosuspensions could be resolved.

4.1.6. Use of the aerosol flow reactor

The aerosol flow reactor, patented by Teicos Pharma, has been used for the solidification of nanosuspensions. It is a one-step continuous process involving the atomisation of a liquid feed into a carrier gas (usually N₂) for drying. More specifically, the liquid feed is atomized by an ultrasonic air-jet nebulizer and the droplets produced, suspended in the carrier gas, are directed into a heated tubular laminar flow reactor with constant temperature where evaporation takes place [76]. Varying the temperature of the reactor tube was found to have an effect on the particle morphology. Specifically, increasing the reactor temperatures up to 160 °C increased the particle size due to formation of hollow nanoparticles. Above 160 °C, the trend was reversed and the morphology of the particle changed to smaller spherical particles with smooth surfaces, **which was attributed to the collapse of the hollow structure of the nanoparticles** [75].

Laaksonen *et al.* [76] prepared indomethacin nanosuspensions by wet-bead milling stabilised by **Poloxamer 188**. The nanosuspensions were diluted with an aqueous solution of mannitol and leucine and then solidified using the aerosol flow reactor. In this way, fast-dissolving composite microparticles of indomethacin nanocrystals embedded in a mannitol matrix and a leucine-rich coating layer were produced.

Using the aerosol flow reactor technology, Raula *et al.* [77] prepared inhalable microparticles for the concomitant pulmonary administration of budesonide and salbutamol sulfate. **Combination therapy has been found to be advantageous for the treatment of respiratory diseases by reducing the need for multiple therapies**

and as a result it holds potential to improve patient adherence to medication and therapeutic outcomes. Especially for chronic obstructive pulmonary disease (COPD), combination therapy enables delivery of more than one drug to the same area of the lungs where they can exhibit synergistic action [78]. The method for the preparation of the inhalable microparticles was as stated above, with the difference that the budesonide nanosuspension was diluted with an aqueous solution of salbutamol sulfate, mannitol and leucine. The obtained microparticles exhibited improved dissolution properties, with budesonide nanocrystals dissolving in 20 min and at the same rate as salbutamol sulfate, ensuring that the drugs will have a synergistic effect at the site of deposition/action. The microparticles also had enhanced aerosolisation efficiency with FPF: $\approx 50\%$, due to the leucine coating, which reduced the adhesion between particles.

4.1.7. Printing

Printing technologies have been used as a deposition method of dissolved or dispersed APIs for the production of solid oral dosage forms and inhalable formulations [79,80]. The main advantages of printing technologies are the preparation of personalised medicines, precise dosing that is especially important for drugs with a narrow therapeutic index and the ability for administering a combination of drugs by printing multiple drug layers using barrier coatings [81]. Printing technologies applied up until now in pharmaceutical formulations involve inkjet printing, flexographic printing and 3D printing.

Pardeike *et al.* [81] prepared nanosuspensions of folic acid by high-pressure homogenisation and applied piezoelectric inkjet printing onto edible substrates (e.g.

rice sheet) as the solidification technique. They stated that in this way the advantages of nanosuspensions as formulations for poorly water-soluble drugs were combined with the potential of preparing personalised medicines by printing.

Flexographic printing has been characterised as a modern version of letterpress printing with the ability to print in almost any type of substrate. Its use for the printing of pharmaceutical formulations was first reported by Genina *et al.* [82]. Recently, Palo *et al.* [83] used flexographic printing for the conversion of nanosuspensions into solid dosage forms. Nanosuspensions of indomethacin and itraconazole, produced by wet-bead milling and stabilised by **Poloxamer 407**, were printed on edible substrates (**e.g. rice paper**). The authors reported that homogeneous distribution of nanocrystals on the substrates was achieved, without any evident aggregation. The dissolution rate of the nanocrystals incorporated in the printed formulations was found to be slightly lower than the liquid nanosuspension, but still much higher than the raw drug.

All in all, printing of nanosuspensions is a rapid technique with great potential as it can be used as a screening tool and can also produce personalised dosage forms.

5. Redispersibility of solidified nanosuspensions

After the description of different methods that have been used for the solidification of nanosuspensions, it is important to highlight the need for production of redispersible solid nanosuspensions [8].

During the solidification processes (e.g. spray drying, freeze drying, etc.) the nanosuspensions are exposed to thermal or freezing stresses that may lead to phase

and composition changes of the formulations [84]. Moreover, as solidification progresses the solvent volume is reduced and this may lead to a decrease in the solubility of the stabiliser that may precipitate, destabilising the colloidal system [85,86]. Nanocrystals also start approaching each other as the volume of the liquid media is reduced and attractive forces (e.g. capillary and van der Waal's) are developed among adjacent nanocrystals [87]. All these effects may cause irreversible aggregation of the nanocrystals and loss of the advantages of nanoformulations [88].

A few studies have elucidated the factors involved in the irreversible aggregation of solid nanosuspensions. Van Eerdenbrugh *et al.* [86] studied the effect of spray drying and freeze drying of nanosuspensions on the dissolution properties of drugs with different physicochemical properties. They suggested that the tendency for irreversible aggregation of nanocrystals during solidification is mainly drug-dependent. Solid nanosuspensions of drugs with high surface hydrophobicity (e.g. itraconazole) exhibited decreased dissolution compared to the liquid nanosuspension. The same trend **occurred** between log P of the drugs and dissolution of the solid nanosuspensions. The authors concluded that during solidification, drugs with higher surface hydrophobicity and/or log P resulted in aggregates that were harder to disintegrate than drugs with higher hydrophilicity.

Yue *et al.* [8] studied the effect of various stabilisers on the redispersibility of solid suspensions of baicalin. Compared to other stabilisers, tocopherol polyethylene glycol succinate (TPGS) was more effective in protecting baicalin nanosuspensions from detrimental effects during solidification. This was explained by the high affinity of TPGS for the drug crystals and the strong surface adsorption. As a result, the polymer

chain of TPGS has the ability to counter effectively the solidification-related stresses faced by the nanocrystals. The inferior performance of other stabilisers (e.g. HPMC, methylcellulose, polyvinylpyrrolidone K30, Tween 80, etc.) was attributed to polymer chain entanglement during solidification. In this study, the solidification methods used included spray drying, freeze drying and vacuum drying. By varying the process parameters, it was found that drying the nanosuspensions at higher temperatures negatively impacted their redispersibility. Regarding freeze drying, increasing the freezing rate was found to enhance the redispersibility of the formulations [8]. Contradictory results have been reported in other studies with respect to whether a fast or slow freezing rate prevents irreversible aggregation of nanocrystals [85]. However, it is evident that in order to achieve successful solidification, process parameters should be selected carefully and working within well-defined processing windows is necessary [89].

The most common way to enhance the redispersibility of solid nanocrystals is the incorporation of bulking agents/matrix formers as a part of the formulation before the solidification step. The matrix former should immediately dissolve upon redispersion releasing the nanocrystals into the dispersion medium (Fig.4). Water-soluble sugar-based compounds such as mannitol, sucrose and lactose have been the most commonly used matrix formers. However, the list of excipients investigated as matrix formers is continuously expanding, with microcrystalline cellulose and even clay reported to enhance the redispersibility of solid nanosuspensions [89,90]. A detailed study on the effect of sugars as bulking agents in order to prevent nanocrystal aggregation during spray and freeze drying has been provided by Kumar *et al.* [91].

6. Conclusions

Nanosuspensions are a useful formulation approach for an ever-increasing number of poorly water-soluble drugs in the industrial pipeline. Transformation of nanosuspensions from the liquid form to the solid state can help to ensure their long-term stability and potentially increase patient compliance. A range of drying techniques, some well-established, others with only few years of application in the area of pharmaceutical technology, have been used for transformation of nanosuspensions to solid oral dosage forms and dry powders for inhalation. The “solid nanosuspensions” are recognised as advanced formulations compared to traditional systems. The number of nanocrystalline-based products already commercially available, the increasing number of scientific publications and patents in this area from several research groups, indicate that both the pharmaceutical industry and academia have embraced this flexible formulation approach, which is estimated to advance even more over the coming years [92,93].

7. Expert opinion

Nanosuspensions combine the advantages of increased dissolution rate and saturation solubility, low toxicity and enhanced chemical stability. In this way, nanocrystals are one of the most industrially feasible aspects of nanotechnology, with some nanocrystalline-based products already on the market and others in development. Transformation of nanosuspensions from the liquid to the solid state can help to ensure their long-term stability, increase patient compliance and also open the way for the pulmonary administration of nanoparticles using DPIs.

Regarding the oral administration of “solid nanosuspensions”, spray and freeze drying are the most commonly used techniques; the powders produced being blended with other excipients and either filled into capsules or compacted to tablets. Fluidised-bed coating or granulation as well as pelletisation are alternative solidification techniques having the advantage of a more straightforward downstream processing to a final product (multiparticulates in capsules or tablets). Printing of nanosuspensions is a rapid technique with great potential as it can be used as a screening tool but can also produce personalised dosage forms.

Conversion of nanosuspensions to micron-sized nanoparticle assemblies with suitable aerodynamic properties can be used as a platform for the delivery of poorly water-soluble drugs to the lungs using DPIs. This platform, after suitable modifications, can also benefit the aerosolisation of water-soluble drugs [94,95]. Spray drying and the aerosol flow reactor have been successfully applied for the production of inhalable nanoparticle agglomerates with enhanced dissolution and aerosolisation efficiency. Spray-freeze drying of nanosuspensions produced larger lower density nanoparticle agglomerates, which were still suitable for pulmonary drug delivery and also exhibited better flowability. As spray drying is a well-established technique in particle engineering for pulmonary drug delivery and also popular from an industrial perspective, it is likely to be the technique of choice for the scaling up and commercialisation of the concept of respirable nanoparticle agglomerates.

One of the current limitations is the empirical nature of the stabiliser selection for the production of nanosuspensions. At present there is no systematic approach to guide the choice of stabiliser with respect to the active ingredient. Focus should be given to approaches such as molecular modelling and scanning probe microscopy that can

provide valuable insight into the way the stabiliser adsorbs to the surface of the crystal particles [96,97]. In this way, these two techniques may be able to prioritise and narrow down the number of stabilisers that need to be screened. Moreover, potential within the areas of nanosuspension preparation and solidification can be accelerated with the implementation of the concept of quality by design [98]. In this way, the knowledge of the interplay between formulation, process and quality attributes will facilitate rational design of nanocrystalline-based formulations.

Regarding pulmonary drug delivery, one of the distinct advantages of inhalable nanoparticle agglomerates is their enhanced aerosolisation performance even without the addition of carrier particles (e.g. lactose). In this way, high drug payloads can be administered to the lungs, which are difficult with the traditional approach of adhesive mixtures of carrier particles and micronised drug. **This approach can revolutionise the administration of antibiotics to patients with chronic respiratory conditions (e.g. cystic fibrosis), as it enables local delivery of higher drug concentration at the lung tissue while concentrations elsewhere are kept at a minimum. In this way, maximum therapeutic effect can be achieved by administering lower inhaled doses compared to those required for systemic administration, and as a result side effects can be minimised.**

Finally, future studies should generate more *in vivo* data, especially concerning the pulmonary drug delivery of nanoparticle agglomerates, so as to ensure that the beneficial *in vitro* properties of this formulation approach are translated in superior clinical performance and pave the way for new therapies worldwide.

Bibliography

1. Lipinski C. Poor aqueous solubility - An industry wide problem in drug discovery. *Am Pharm Rev* 2002;5: 82–85
 2. Benet LZ. Predicting DMPK of NMEs: **What do we need in terms of science and tools?** New England Drug Metabolism Discussion Group: Gerald Miwa Retirement Symposium, 2007
 3. Kipp JE. The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. *Int J Pharm* 2004;284:109–22
 4. Brough C, Williams RO. Amorphous solid dispersions and nano-crystal technologies for poorly water-soluble drug delivery. *Int J Pharm* 2013;453:157–66
- * Comparative analysis between nanocrystalline delivery forms and amorphous solid dispersion. The thermodynamic and kinetic theories relative to these technologies are discussed along with commercially available products.**
5. Hafner A, Lovrić J, Lakoš, GP, Pepić I. Nanotherapeutics in the EU: an overview on current state and future directions. *Int J Nanomedicine* 2014;9:1005–23
 6. Chingunpituk J. Nanosuspension technology for drug delivery. *Walailak J Sci Tech* 2007;4:139–53
 7. Wong J, Brugger A, Khare A, et al. Suspensions for intravenous (IV) injection: a review of development, preclinical and clinical aspects. *Adv Drug Deliv Rev* 2008;60:939–54
 8. Yue PF, Li Y, Wan J, et al. Study on formability of solid nanosuspensions during nanodispersion and solidification: I. Novel role of stabilizer/drug property. *Int J Pharm* 2013;454:269-77
- * Article on the role of physicochemical drug and stabiliser characteristics on the successful formation of solid nanosuspensions.**
9. Lindfors L, Skantze P, Skantze, U, et al. Amorphous drug nanosuspensions. 3. Particle dissolution and crystal growth. *Langmuir* 2007;23:9866–74
 10. Nuttan MTH, Reddy IK. General principles of suspensions. In: Kurshreshtha AK, Singh ON, Michael Wall G. eds. *Pharmaceutical Suspensions*. New York: Springer, 2010, 39-66
 11. Ghosh I, Schenck D, Bose S, Ruegger C. Optimization of formulation and process parameters for the production of nanosuspension by wet media milling technique: effect of Vitamin E TPGS and nanocrystal particle size on oral absorption. *Eur J Pharm Sci* 2012;47:718–28

12. Van Eerdenbrugh B, Vermant J, Martens JA, et al. A screening study of surface stabilization during the production of drug nanocrystals. *J Pharm Sci* 2009;98: 2091–103
13. Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. *Eur J Pharm Biopharm* 2006; 62:3–16
- ** Review dedicated to the physicochemical background of size reduction process, influence of process parameters, clinical batch production and scaling up of the production of nanocrystals using high pressure homogenisation.**
14. Shegokar R, Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. *Int J Pharm* 2010;399:129–39
- * Review on industrially available production processes, regulatory aspects and nanotoxicology of nanocrystals of first and second generation.**
15. Butler JM, Dressman JB. The Developability Classification System: application of biopharmaceutics concepts to formulation development. *J Pharm Sci* 2010;99: 4940–54
16. Nernst W. Theorie der reaktionsgeschwindigkeit in heterogenen systemen. *Z Phys Chem* 1904;47:52
17. Brunner E. Reaktionsgeschwindigkeit in heterogenen systemen. *Z Phys Chem* 1904;43:56-102
18. Buckton G, Beezer AE. The relationship between particle size and solubility. *Int J Pharm* 1992;82:7–10
19. Mauludin R, Müller RH, Keck CM. Kinetic solubility and dissolution velocity of rutin nanocrystals. *Eur J Pharm Sci* 2009;36, 502–10
20. Kesisoglou F, Panmai S, Wu Y. Nanosizing oral formulation development and biopharmaceutical evaluation. *Adv Drug Deliv Rev* 2007;59:631–44
21. Muller RH, Gohla S, Keck CM. State of the art of nanocrystals- special features, production, nanotoxicology aspects and intracellular delivery. *Eur J Pharm Biopharm* 2011;78:1-9
22. Rabinow BE. Nanosuspensions in drug delivery. *Nat Rev Drug Discov* 2004;3: 785–96
23. Jacobs C, Kayser O, Müller RH. Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone. *Int J Pharm* 2001;214:3–7
24. Kayser O. A new approach for targeting to *Cryptosporidium parvum* using mucoadhesive nanosuspensions: research and applications. *Int J Pharm* 2001;214:83–85

25. Verma S, Burgess D. Solid Nanosuspensions: The Emerging Technology and Pharmaceutical Applications as Nanomedicine. In: Kurshreshtha AK, Singh ON, Michael Wall G. eds. Pharmaceutical Suspensions. New York: Springer, 2010, 285-318

**** Book chapter dealing with the physicochemical principles, benefits and application of nanosuspensions as an emerging technology area for nanotherapeutics.**

26. Gao L, Liu G, Wang X, et al. Preparation of a chemically stable quercetin formulation using nanosuspension technology. *Int J Pharm* 2011;404:231–7

27. Ben Zirar S, Astier A, Muchow M, Gibaud S. Comparison of nanosuspensions and hydroxyl-beta-cyclodextrin complex of melarsoprol pharmacokinetics and tissue distribution in mice. *Eur J Pharm Biopharm* 2008;70:649-56

28. Niwa T, Miura S, Danjo, K. Universal wet-milling technique to prepare oral nanosuspension focused on discovery and preclinical animal studies - Development of particle design method. *Int J Pharm* 2011;405:218–27

29. Moschwitz J. Special aspects of nanomedicines: viewpoint from the industry. European Medicines Agency 1st International Workshop on Nanomedicine, 2010

30. Yang JZ, Young AL, Chiang P-C, et al. Fluticasone and budesonide nanosuspensions for pulmonary delivery: preparation, characterization and pharmacokinetic studies. *J Pharm Sci* 2008;97:4869-78

31. Britland S, Finter W, Crystyn H et al. Droplet aerodynamics, cellular uptake and efficacy of a nebulizable corticosteroid nanosuspension are superior to a micronized dosage form. *Biotechnol Prog* 2012;28:1152-59

32. Chiang P-C, Alsup JW, Lai Y, et al. Evaluation of aerosol delivery of nanosuspension for pre-clinical pulmonary drug delivery. *Nanoscale Res Lett* 2009;4:254-61

33. Rundfeldt C, Steckel H, Scherliess H, et al. Inhaled highly concentrated itraconazole nanosuspension for the treatment of bronchopulmonary aspergillosis. *Eur J Pharm Biopharm* 2013;83:44-53

34. Peltonen L, Valo H, Kolakovic R, et al. Electro spraying, spray drying and related techniques for production and formulation of drug nanoparticles. *Expert Opin Drug Deliv* 2010;6:705–19

35. Zhang J, Wu L, Chan HK, Watanabe W. Formation, characterization, and fate of inhaled drug nanoparticles. *Adv Drug Deliv Rev* 2011;63:441–55

**** Review on pulmonary drug delivery of nanoparticle therapeutics. Particle formation methods, their *in vitro* and *in vivo* performance together with their clearance mechanisms are reviewed.**

36. Sinha B, Müller, RH, Möschwitzer JP. Systematic investigation of the cavi-precipitation process for the production of ibuprofen nanocrystals. *Int J Pharm* 2013; 458:315–23
37. Wu L, Zhang J, Watanabe W. Physical and chemical stability of drug nanoparticles. *Adv Drug Deliv Rev* 2011;63:456–69
38. Kobierski S, Ofori-Kwakye K, Müller RH, Keck CM. Resveratrol nanosuspensions: Interaction of preservatives with nanocrystal production. *Pharmazie* 2011;66:942–47
39. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. *J Pharm Pharmacol* 2004;56:827–40
40. Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. *Int J Pharm* 2008;364:64–75
41. Pulmonary Drug Delivery Systems: Technologies and Global markets. BCC Research, 2014. Available at: www.bccresearch.com/market-research/healthcare/pulmonary-drug-delivery-systems-hlc094b.html [Last accessed 02 October 2015]
42. Geller DE. Comparing clinical features of the nebulizer, metered-dose inhaler and dry powder inhaler. *Respir Care* 2005;50:1313-22
43. Heyder J, Gebhart J, Rudolf G, et al. Deposition of particles in the human respiratory tract in the size range 0.005–15 μm . *J Aerosol Sci* 1986;17:811–25
44. Byron P. Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. *J Pharm Sci* 1986;75:433–38
45. Rogueda P, Traini D. The nanoscale in pulmonary drug **delivery**. Part 1: deposition, fate, toxicology and effect. *Expert Opin Drug Deliv* 2007;4:595–606
46. Watts AB, Williams III RB. Nanoparticles for Pulmonary Delivery. In: Smyth H, Hickey A. eds. *Controlled Pulmonary Drug Delivery*. New York: Springer, 2011, 335-64
47. El-Gendy N, Gorman EM, Munson EJ, Berkland C. Budesonide nanoparticle agglomerates as dry powder aerosols with rapid dissolution. *J Pharm Sci* 2009;98:2731–46
48. Forbes B, Richer NH, Buttini F. Dissolution: A Critical Performance Characteristic of Inhaled Products?. In: Nokhodchi A, Martin GP. eds. *Pulmonary Drug Delivery: Advances and Challenges*. West Sussex:Wiley, 2015, 223-40
49. Tolman JA, Williams III RO. Advances in the pulmonary delivery of poorly water-soluble drugs: influence of solubilization on pharmacokinetic properties. *Drug Dev Ind Pharm* 2010;36:1-30

50. Masters K. Spray drying handbook. (5th ed). Essex: Longman Scientific and Technical, 1991.

51. Zuo B, Sun Y, Li H, et al. Preparation and in vitro/in vivo evaluation of fenofibrate nanocrystals. *Int J Pharm* 2013;455:267–75

52. Freag MS, Elnaggar YSR, Abdallah OY. Development of novel polymer-stabilized diosmin nanosuspensions: in vitro appraisal and ex vivo permeation. *Int J Pharm* 2013;454:462–71

53. Chawla A, Taylor KMG, Newton JM, Johnson MCR. Production of spray dried salbutamol sulphate for use in dry powder aerosol formulation. *Int J Pharm* 1994;108:233–40

54. Chow AHL, Tong HHY, Chattopadhyay P, Shekunov BY. Particle engineering for pulmonary drug delivery. *Pharm Res* 2007;24:411–37

**** Review on particle technologies for the production of respirable particles (e.g. spray drying and spray freeze drying). Emphasis is placed on particulate properties and their influence on pulmonary drug delivery.**

55. Pilcer G, Vanderbist F, Amighi K, Spray-dried carrier-free dry powder tobramycin formulations with improved dispersion properties. *J Pharm Sci* 2009;98:1463–75

*** Article on the preparation of inhalable tobramycin nanoparticle agglomerates as a way to administer high doses of antibiotics to the lungs.**

56. Yamasaki K, Kwok PCL, Fukushige K, et al.. Enhanced dissolution of inhalable cyclosporine nano-matrix particles with mannitol as matrix former. *Int J Pharm* 2011; 420:34–42

*** Detailed examination of the aerosol and dissolution performance of spray dried cyclosporine nanosuspensions.**

57. Pomázi A, Buttini F, Ambrus R, et al. Effect of polymers for aerolization properties of mannitol-based microcomposites containing meloxicam. *Eur Polym J* 2013;49:2518–27

58. Sou T, Orlando L, McIntosh MP, et al. Investigating the interactions of amino acid components on a mannitol-based spray-dried powder formulation for pulmonary delivery: A design of experiment approach. *Int J Pharm* 2011;421:220–9

59. Duret C, Wauthoz N, Sebti T, et al. New inhalation-optimized itraconazole nanoparticle-based dry powders for the treatment of invasive pulmonary aspergillosis. *Int J Nanomedicine* 2012;7:5475–89

60. Maa YF, Nguyen PA, Sweeney T, et al. Protein inhalation powders: Spray drying vs spray freeze drying. *Pharm Res* 1999;16:249–54

61. Mumenthaler M, Leuenberger H. Atmospheric spray-freeze drying: a suitable alternative in freeze-drying technology. *Int J Pharm* 1991;72:97–110
62. Niwa T, Danjo K. Design of self-dispersible dry nanosuspension through wet milling and spray freeze-drying for poorly water-soluble drugs. *Eur J Pharm Sci* 2013;50:272–81
63. Cheow WS, Ng MLL, Kho K, Hadinoto K. Spray-freeze-drying production of thermally sensitive polymeric nanoparticle aggregates for inhaled drug delivery: effect of freeze-drying adjuvants. *Int J Pharm* 2011;404:289–300
64. Tang X, Pikal MJ. Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharm Res* 2004;21:191-200
65. Fu Q, Sun J, Zhang D, et al. Nimodipine nanocrystals for oral bioavailability improvement: preparation, characterization and pharmacokinetic studies. *Colloids Surf B Biointerfaces* 2013;109:161–6
66. Lai F, Pini E, Corrias F, et al. Formulation strategy and evaluation of nanocrystal piroxicam orally disintegrating tablets manufacturing by freeze-drying. *Int J Pharm* 2014;467:27–33
67. El-Gendy N, Pornputtipitak W, Berkland C. Nanoparticle agglomerates of fluticasone propionate in combination with albuterol sulfate as dry powder aerosols. *Eur J Pharm Sci* 2011;44:522–33
68. Plumley C, Gorman EM, El-Gendy N, et al. Nifedipine nanoparticle agglomeration as a dry powder aerosol formulation strategy. *Int J Pharm* 2009;369:136–43
69. Kayaert P, Anné M, Van den Mooter G. Bead layering as a process to stabilize nanosuspensions: influence of drug hydrophobicity on nanocrystal reagglomeration following in-vitro release from sugar beads. *J Pharm Pharmacol* 2011;63:1446–53
70. He W, Lu Y, Qi J, et al. Formulating food protein-stabilized indomethacin nanosuspensions into pellets by fluid-bed coating technology: physical characterization, redispersibility, and dissolution. *Int J Nanomedicine* 2013;8:3119–28
71. Möschwitzer J, Müller RH. Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. *Eur J Pharm Biopharm* 2006;62:282–7
72. Figueroa CE, Bose S. Spray granulation: importance of process parameters on in vitro and in vivo behavior of dried nanosuspensions. *Eur J Pharm Biopharm* 2013;85:1046–55
73. Vergote G, Vervaet C, Van Driessche I, et al. An oral controlled release matrix pellet formulation containing nanocrystalline ketoprofen. *Int J Pharm* 2001;219:81–7

74. Vergote G, Vervaet C, Van Driessche I, et al. In vivo evaluation of matrix pellets containing nanocrystalline ketoprofen. *Int J Pharm* 2002;240:79–84

75. Eerikäinen H, Watanabe W, Kauppinen EI, Ahonen PP. Aerosol flow reactor method for synthesis of drug nanoparticles. *Eur J Pharm Biopharm* 2003;55:357–60

76. Laaksonen T, Liu P, Rahikkala A, et al. Intact nanoparticulate indomethacin in fast-dissolving carrier particles by combined wet milling and aerosol flow reactor methods. *Pharm Res* 2011;28:2403–11

77. Raula J, Rahikkala A, Halkola T, et al. Coated particle assemblies for the concomitant pulmonary administration of budesonide and salbutamol sulphate. *Int J Pharm* 2013;441:248–54

*** Article on the use of aerosol flow reactor for the preparation of inhalable nanoparticle agglomerates.**

78. Arun JJ, Lodha R, Kabra SK. Bronchodilatory effect of inhaled budesonide/formoterol and budesonide/salbutamol in acute asthma: a double-blind, randomized controlled trial. *BMC Pediatr* 2012;12:21

79. Boehm RD, Miller PR, Daniels J, et al. Inkjet printing for pharmaceutical applications. *Mater today* 2014;17:247-52

80. Mueannoom W, Srinongphan A, Taylor KMG, et al. Thermal ink-jet spray freeze-drying for preparation of excipient-free salbutamol sulfate for inhalation. *Eur J Pharm Biopharm* 2012;80:149-55

81. Pardeike J, Strohmeier DM, Schrödl N, et al. Nanosuspensions as advanced printing ink for accurate dosing of poorly soluble drugs in personalized medicines. *Int J Pharm* 2011;420:93–100

82. Genina N, Fors D, Vakili H, et al. Tailoring controlled-release oral dosage forms by combining inkjet and flexographic printing techniques. *Eur J Pharm Sci* 2012;47:615–23

*** Article on the flexographic printing of nanosuspensions.**

83. Palo M, Kolakovic R, Laaksonen T, et al. Fabrication of drug-loaded edible carrier substrates from nanosuspensions by flexographic printing. *Int J Pharm* 2015; published online 16 January 2015, doi:10.1016/j.ijpharm.2015.01.027

84. Chaubal MV, Popescu C. Conversion of nanosuspensions into dry powders by spray drying: a case study. *Pharm Res* 2008;25:2302-8

**** One of the first papers investigating the impact of formulation and process parameters on the redispersibility of spray dried nanosuspensions.**

85. Beirowski J, Inghelbrecht S, Arien A, Gieseler H. Freeze-drying of nanosuspensions, 1: freezing rate versus formulation design as critical factors to preserve the original particle size distribution. *J Pharm Sci* 2011;100:1958–68
86. Van Eerdenbrugh B, Froyen L, Van Humbeeck J, et al. Drying of crystalline drug nanosuspensions-the importance of surface hydrophobicity on dissolution behavior upon redispersion. *Eur J Pharm Sci* 2008;35:127–35
87. Zhang X, Guan J, Ni R, et al. Preparation and solidification of redispersible nanosuspensions. *J Pharm Sci* 2014;103:2166–76
88. Chung NO, Lee MK, Lee J. Mechanism of freeze-drying drug nanosuspensions. *Int J Pharm* 2012;437:42–50
89. Dong Y, Ng WK, Hu J, et al. Clay as a matrix former for spray drying of drug nanosuspensions. *Int J Pharm* 2014;465:83–9
90. Van Eerdenbrugh B, Froyen L, Van Humbeeck J, et al. Alternative matrix formers for nanosuspension solidification: Dissolution performance and X-ray microanalysis as an evaluation tool for powder dispersion. *Eur J Pharm Sci*. 2008;35:344–53
91. Kumar S, Gokhale R, Burgess DJ, Sugars as bulking agents to prevent nanocrystal aggregation during spray or freeze-drying. *Int J Pharm* 2014;471:303–11
- * Study on the role of sugars as matrix formers to prevent irreversible aggregation of nanocrystals during drying processes.**
92. Bosch WH, Ostrander KD, Cooper ER. Aerosols comprising nanoparticle drugs. WO00/27363 (1999)
93. Hong JN, Van Oort MM. Aggregate nanoparticulate medicament formulations, manufacture and use thereof. EP2627317A2 (2013)
94. Malamataris M, Somavarapu S, Bloxham M, Buckton G. Nanoparticle agglomerates of indomethacin: the role of poloxamers and matrix formers on their dissolution and aerosolisation efficiency. *Int J Pharm* 2015;495:516-26
95. Salem HF, Abdelrahim ME, Abo Eid K, Sharaf MA. Nanosized rods of agglomerates as a new approach for formulation of dry powder inhaler. *Int J Nanomedicine* 2011;6:311-20
96. Konkel J, Myerson AL. Empirical molecular modelling of suspension stabilisation with Polysorbate 80. *Mol Simul* 2009;34:1353-7
97. Verma S, Huey BD, Burgess DJ. Scanning probe microscopy method for nanosuspension stabiliser selection. *Langmuir* 2009;25:12481-7
98. Verma S, Lan Y, Gokhale R, Burgess DJ. Quality by design approach to understand the process of nanosuspension preparation. *Int J Pharm* 2009;377: 185-98

Table 1 Advantages of nanosuspensions

General advantages	
	<ul style="list-style-type: none">• ↑ Dissolution rate• ↑ Saturation solubility• ↑ Bioavailability• ↑ Chemical stability• ↑ Drug loading• ↓ Excipient-related toxicity
Route of administration	Advantages
Oral	<ul style="list-style-type: none">• ↓ Food effect• ↑ Gastrointestinal residence time• Quantitative & easy administration to preclinical animal studies• Extension of the life cycle of off- patent drugs
Pulmonary	<ul style="list-style-type: none">• Delivery of high drug concentrations to the lungs• No need for co-solvents• Ease of dosing using syringe in animals (intratracheal instillation)

Table 2 Commercially available and developmental nanocrystalline formulations [13,25]

Trade name, Company	Active substance	Indication	Particle size reduction technology	Status (FDA approval)	Route	Dosage form	Solidification technique
Rapamune® , Wyeth	Sirolimus	Immunosuppressant	Elan NanoCrystals®	Marketed (2000)	Oral	Suspension Tablet	- Tablet coating
Emend® , Merck	Aprepitant	Antiemetic	Elan NanoCrystals®	Marketed (2003)	Oral	Hard gelatin capsule containing pellets	Bead coating
TriCor® , Abbott	Fenofibrate	Antihyperlipidemic	Elan NanoCrystals®	Marketed (2004)	Oral	Tablet	Granulation
Triglide® , First Horizon Pharmaceuticals	Fenofibrate	Antihyperlipidemic	SkyePharma IDD®-P	Marketed (2005)	Oral	Tablet	Spray drying
Megace ES® , PAR Pharmaceutical	Megestrol acetate	Appetite stimulant	Elan NanoCrystals®	Marketed (2005)	Oral	Suspension	-
Invega® Sustenna™ , Janssen & Johnson	Paliperidone palmitate	Antipsychotic	Elan NanoCrystals®	Marketed (2009)	Intramuscular	Suspension in prefilled syringe	-
Semapimod , Cytokine PharmaSciences	Guanylylhydrazone	TNF- α inhibitor	Self developed	Phase II	Oral	-	-
Theralux™ , Celmed Biosciences	Thymectacin	Anticancer	Elan NanoCrystals®	Phase II	Intravenous	-	-
NPI 32101 , Nucryst	Silver	Antimicrobial	Magnetron sputtering	Phase II	Topical	-	-
Zolip® , Solvay Pharmaceuticals (now Abbott)	Fenofibrate/ Simvastatin	Antihyperlipidemic	Elan NanoCrystals®	Phase III	Oral	Tablet	n/a
Budesonide , Sheffield Pharmaceuticals	Budesonide	Antiasthmatic	Elan NanoCrystals®	Phase III	Pulmonary	Suspension administered via nebulizer	-

Figures

Figure 1. Schematic representation of the various solidification techniques for the production of solid oral nanocrystalline dosage forms.

Figure 2. Schematic representation of the various solidification approaches for the preparation of inhalable micron-sized nanoparticle agglomerates.

Figure 3. Scanning electron microscopy images of (a) spray dried salbutamol sulfate from 10% w/v aqueous solution, taken from [53] with permission and (b) spray dried nanosuspension of cyclosporine A containing mannitol (1:0.5 mass ratio), taken from [56] with permission.

Figure 4. Scanning electron microscopy images of (a,b) spray freeze-dried nanosuspensions of phenytoin and PVP (1:1 mass ratio) at different magnifications, taken from [62] with permission and (b) freeze dried nanoparticle agglomerates of nifedipine, taken from [68] with permission.

Figure 5. Schematic representation of the matrix former influence on redispersibility of solidified nanosuspensions upon their rehydration.

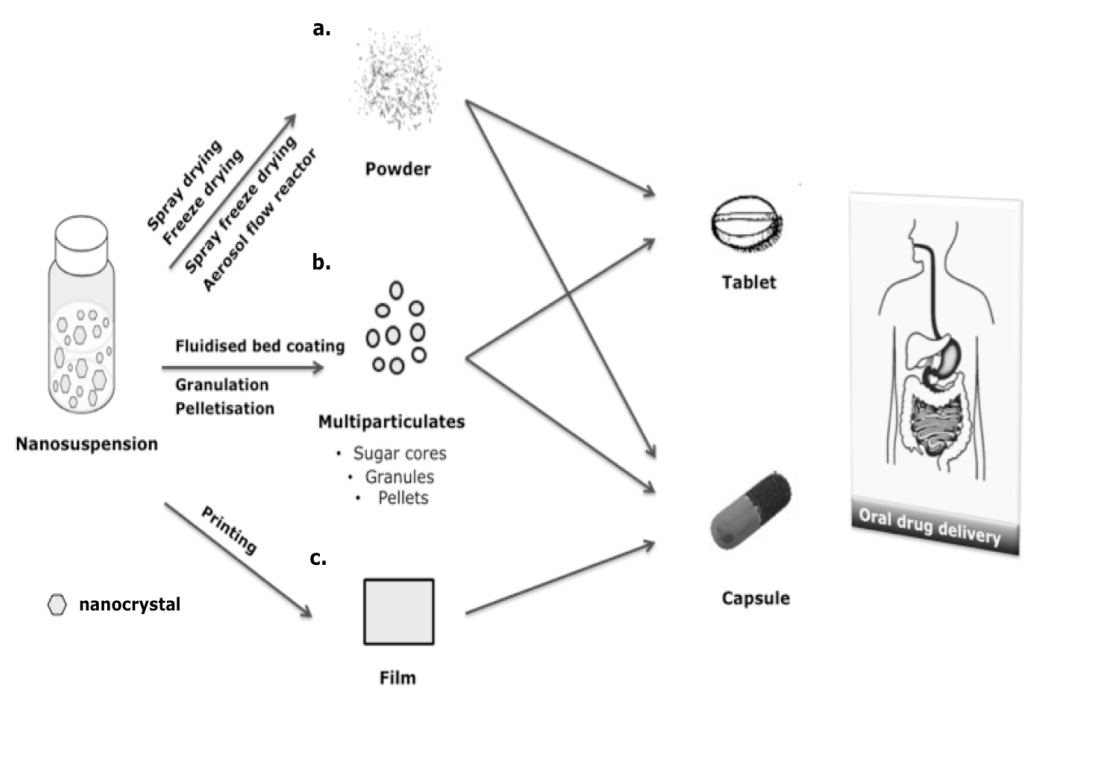


Figure 1. Schematic representation of the various solidification techniques for the production of solid oral nanocrystalline dosage forms.

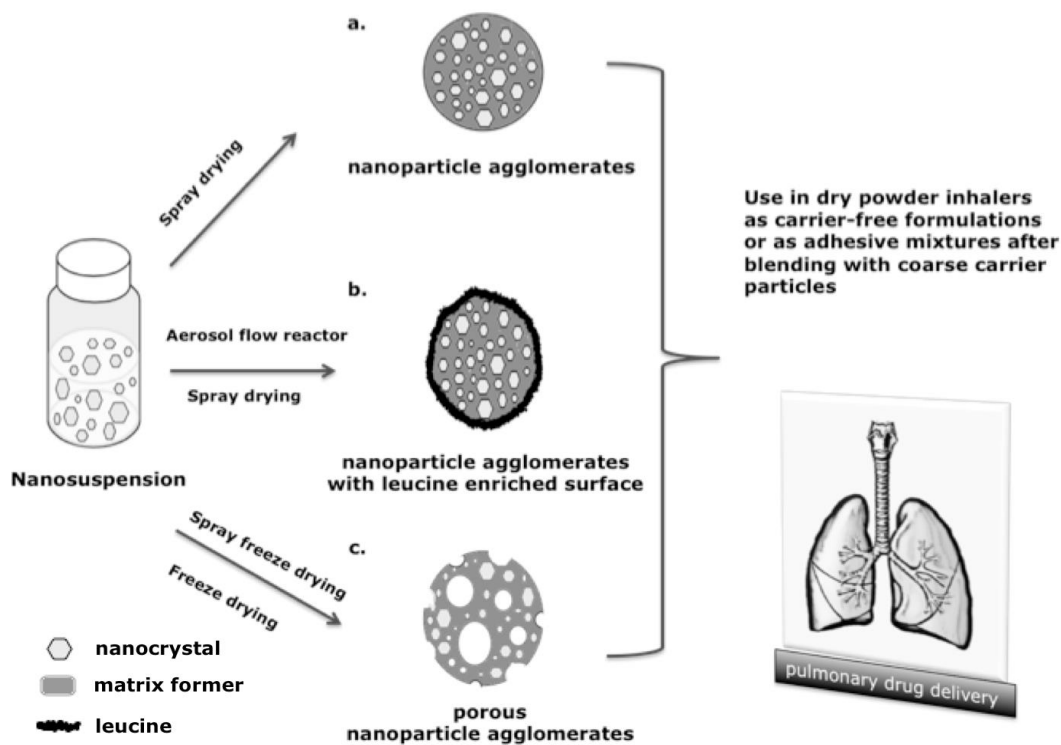


Figure 2. Schematic representation of the various solidification approaches for the preparation of inhalable micron-sized nanoparticle agglomerates.

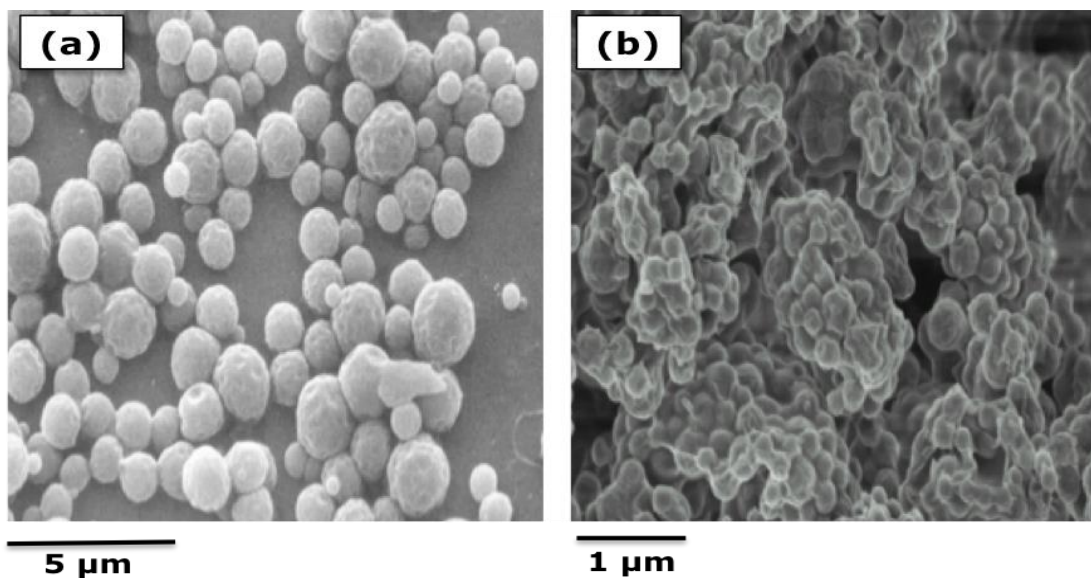


Figure 3. Scanning electron microscopy images of (a) spray dried salbutamol sulfate from 10% w/v aqueous solution, taken from [53] with permission and (b) spray dried nanosuspension of cyclosporine A containing mannitol (1:0.5 mass ratio), taken from [56] with permission.

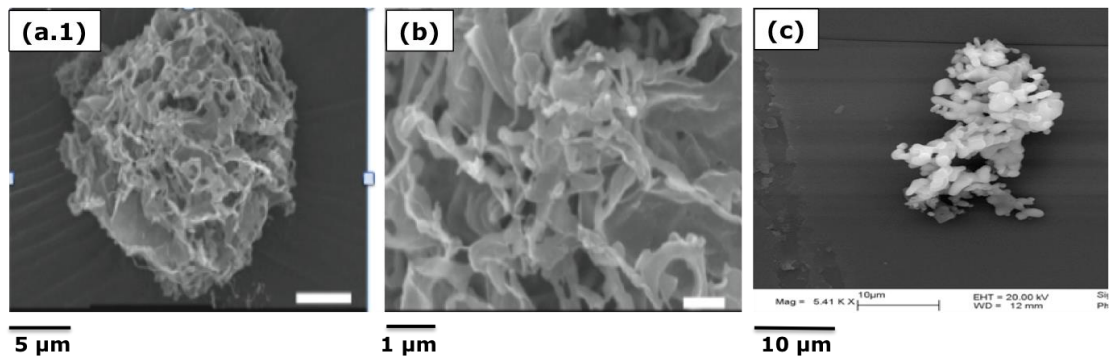


Figure 4. Scanning electron microscopy images of (a,b) spray freeze-dried nanosuspensions of phenytoin and PVP (1:1 mass ratio) at different magnifications, taken from [62] with permission and (b) freeze dried nanoparticle agglomerates of nifedipine, taken from [68] with permission.

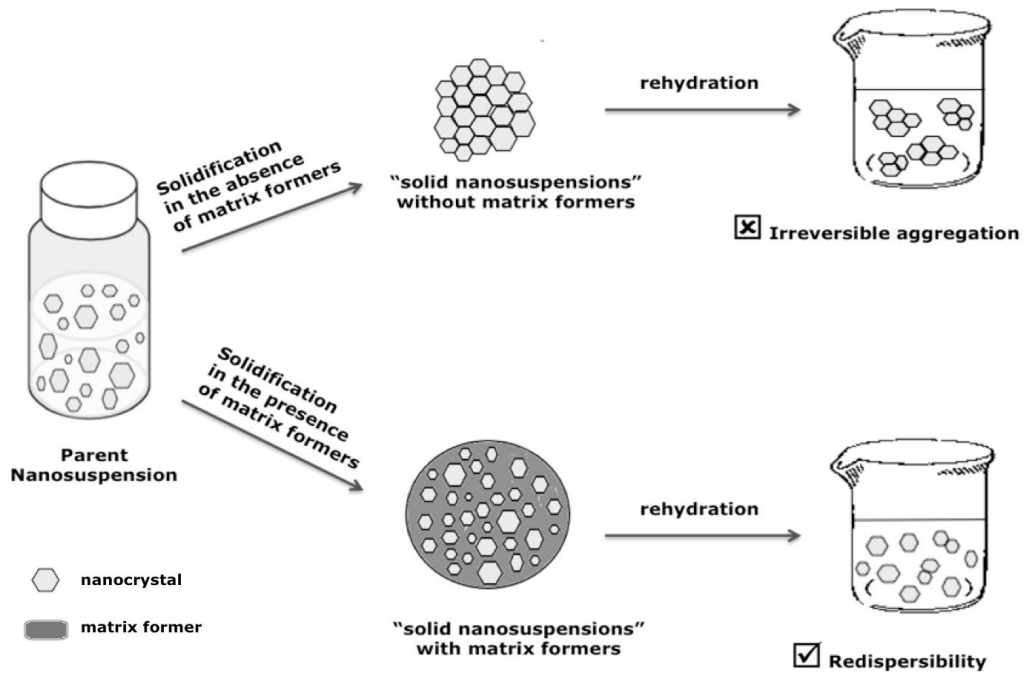


Figure 5. Schematic representation of the matrix former influence on redispersibility of solidified nanosuspensions upon their rehydration.