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Reviews

Particle Engineering Strategies via Crystallization for Pulmonary Drug Delivery

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Abstract:

This review covers recent developments in the area of particle engineering via crystallization for pulmonary drug delivery. The past decade has witnessed a shift from empirical formulation efforts to an engineering approach based on a better understanding of the crystallization process. Microcrystals with nanoscale substructures can now be designed and their functionality has contributed significantly to the stability and efficacy of the particulate dosage form. This review provides concepts and a theoretical framework for particle design calculations. It reviews experimental research to identify variables that influence particle formation. It offers an explanation of how excipient properties in combination with process variables influence the morphology of the engineered particles. A wide range of pharmaceutical applications of large porous particles, particles with low surface energy, and particle aggregates, is discussed, with specific emphasis on the underlying crystal formation mechanism and design concepts.

1. Introduction

Particle engineering is a discipline that combines elements of microbiology, chemistry, formulation science, colloidal and interface science, heat and mass transfer, solid state physics, aerosol and powder science, and nanotechnology. It provides the theoretical framework for a rational design of structured microparticles. Particle engineering requires a deeper understanding of particle formation processes. Complex structured microparticles are difficult to design using an empirical approach alone because of the many process and formulation variables that need to be tuned correctly to achieve the desired result.

Recently, inhalation therapy has sparked considerable biomedical interest in the development of novel particle technologies for respiratory drug formulation. Introduction of new potent medicines in various therapeutic areas such as asthma, chronic obstructive pulmonary disease (COPD) and various infectious diseases has necessitated an accurate and consistent dosing using inhalation device. The inhalation dosage technology has primarily been focused on two parallel development pathways: fabrication of novel inhaler devices with enhanced efficiency and improvement of the existing inhalation formulations (1). Dry powder inhalers (DPIs) of the first generation, commonly exhibited a relatively low efficiency (10−15%) in terms of the fine particle fraction (*FPF*), and even more seriously, in the emitted dose (*ED*).[1,2] This is due to the sensitivity of the dose delivery from DPIs at low inhalation flow rates and particle adhesion and static charges, which are largely responsible for material retention in the delivery device. These problems can be resolved by developing optimized particulate formulations and more effective and user-friendly inhalers. The controlled production of drug particles in pure form or with carriers as composite materials of optimized size, morphology, and structure, can be broadly referred to as "particle engineering". The main goal of particle engineering is to ensure desirable attributes such as narrow particle size distribution, improved dispersibility, enhanced drug stability, optimized bioavailability, sustained release, and/or specific targeting.[3,4]

The present article is intended to provide a critical account of the current goals and technologies of particle engineering for the development of respiratory drug delivery systems. The complex nature of interparticulate interactions in the submicrometer-size scale affecting the aerodynamic behavior of particles in the inhaler devices and respiratory system have rendered such dosage form development one of the most challenging problems in pulmonary drug delivery. Microparticles can be manufactured by many different processing methods. This review focuses exclusively on different crystallization methods for production of inhalable particles. Spray freeze-drying, or supercritical fluid technologies, have also been widely used for particle engineering purposes and have been reviewed elsewhere.[5−9]

2. Terminology Used To Define Structured Microparticles

The purpose of this section is to define the terminology needed for a discussion of particle engineering. It will also

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provide equations that are used to describe particle properties, interparticulate interactions, surface energies, and particle dispersion.

2.1. Particle Morphology. Particle morphology can be described in terms of particle shape, internal structure, and surface properties.

2.2. Particle Aerodynamic Diameter. Aerodynamic diameter d_A is a physical property of a particle in a fluid such as air. In general, particles have irregular shapes and their actual diameters are difficult to measure. *Aerodynamic diameter* is an expression of a particle's aerodynamic behavior as if it were a perfect sphere with unit-density. Such a particle has the same terminal settling velocity as the original particle. This diameter is useful in defining the mechanism of particle deposition in the respiratory system. The aerodynamic diameter depends on the particle Reynolds number, *Re*, as well as the particle size, shape and density. It can be calculated numerically using semiempirical equations such as the one given by eq 1.

$$d_A(\text{Stokes}) \approx d_V\sqrt{\frac{\rho}{\chi\rho_0}} \quad (1)$$

where d_V is the *spherical-equivalent diameter*, ρ_0 is the unit *density* (of the spherical particle), ρ is the *true particle density* and χ is the *dynamic shape factor*, defined as the ratio of the drag force on the particle to the drag force on the spherical-equivalent particle at the same velocity. Thus, d_A can be decreased by one or a combination of the following: decreasing d_V and ρ and increasing χ.

2.3. Mass Median Aerodynamic Diameter (MMAD). The aerosol performance of precipitated particles in particle transport within the respiratory tract can be measured *in vitro* using liquid impinger. The cumulative mass of powder less than the stated size of each stage of the liquid impinger is calculated as percent of total mass recovered in the impinger against the effective cutoff diameter. Inhaled aerosols are typically described by the logarithm of the size distributions rather than the size itself because most aerosols exhibit a skewed distribution function with a long tail. Two important statistical properties of inhaled drug particles are the *mass median aerodynamic diameter (MMAD)* and the *geometric standard deviation (GSD)*. *MMAD* can be directly calculated as the size associated with a cumulative count of 50%. The *GSD* is obtained as:

$$\text{GSD} = (X/Y)^{0.5} \quad (2)$$

where X and Y are the sizes associated with a cumulative count of 84% and 16%,[10] respectively.

2.4. Fine Particle Dose (FPD). The 'fine particle dose' is the fraction of the loaded dose that is aerosolized in the respirable range. The *FPD* can be measured in an impactor directly if there is high and reproducible drug recovery.

2.5. Interparticle Interactions, Surface Energies and Particle Dispersion. In addition to the aerodynamic diameter of the particles, the dispersibility of the particles has to be taken into consideration for defining the overall particle size distribution and deposition during inhalation.[11−16] It should be noted that the magnitude of the interparticle forces may not adequately account for the dispersion phenomenon observed. This is because both the aggregate strength and aerodynamic forces generated, being dependent on the force per unit area (at contact point or particle projection), are more appropriately defined in terms of stress which is also a function of the aggregate structure.

Dispersibility of powders in the airflow is defined by the balance of the aerodynamic stress and the *aggregate strength*. When the aerodynamic stress exceeds a certain level, the primary particles forming an aggregate can disperse simultaneously and thus penetrate deeper into the respiratory system. The *aggregate strength*, (N/m^2), consisting of uniform primary particles, can be estimated using the following equation:

$$\sigma = 15.6\phi^4 W/d_V \quad (3)$$

where ϕ is the *packing fraction* expressed as the ratio of the powder *bulk density* to *true particle density* (ρ_B/ρ) and W (J/m^2) is the work of adhesion between particles. Thus, the strength of the aggregates should increase with adhesion but is strongly influenced by the *packing fraction*. It is assumed that the mean curvature of nonspherical particles increases in proportion to the *spherical-equivalent diameter*, d_V.[12]

The nature of interparticle forces (i.e., electrostatic, van der Waals, capillary/viscous, etc.) is complex. van der Waals force is assumed to be the most predominant interaction between aerosol particles. van der Waals forces constitute the major part of the dispersive component of the surface free energy which is calculated from the inverse gas chromatography, IGC, data on the basis of interaction with the nonpolar liquid probes.

For respiratory formulations, the strength of the aggregates is proportional to the *work of cohesion* (drug/drug interaction) or *work of adhesion* (e.g., drug/excipient interaction). Since the excipient and drug particles can differ in size by an order of magnitude, the effective interaction diameter can be taken as the harmonic mean of the drug and diameters.[17]

$$d_V = d_1d_2/(d_1 + d_2) \quad (4)$$

Electrostatic charges and associated Coulombic forces may also exert a significant influence on particle dispersion, and for certain materials, such interactions are comparable in strength to the van der Waals forces.[18,19] The *emitted dose*, ED, is strongly influenced by the electrostatic deposition in the inhalers and on the mouthpiece, and differently charged particles may promote particle agglomeration.

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Direct experimental determination of the aggregate strength is technically very difficult, and hence various theoretical approaches have been used to estimate this parameter on the basis of particle surface energy. However, it is also possible to take into account the total energy of interaction between particles of the same material, which consists of individual parts arising from (atomic) dispersion force, (molecular) permanent dipole−permanent dipole forces, and (molecular) hydrogen bonding using the *Hildebrand solubility parameters* δ_C and δ_A.[20] The following relationships describe this concept:

$$\sigma_C = 0.25\delta_C^2 \quad (5)$$

$$\sigma_A = 0.25\theta\delta_C\delta_A \quad (6)$$

where θ is the interaction parameter, which can also be determined by IGC.[21,22] The *work of cohesion or adhesion*, W_C or W_A, can be assumed to be directly proportional to the *strengths of the cohesive* (σ_C) and *adhesive* (σ_A) interactions, respectively.[5]

On the other hand, it is very important to distinguish the primary crystals from particle aggregates. Particle size measurements and scanning electron microscope can be used for this purpose. *Bulk density* can be defined as the mass of the sample powder divided by the undistributed volume in a graduated cylinder after filling. It is expressed in kg/m^3.

The following conclusions can be drawn from the above theoretical analysis:

(a) The aggregate strength decreases with an increase in d_V.

(b) Low powder *bulk density*, ρ_B, promotes loose and weak aggregates.

(c) Decreased particle surface energy (expressed through the parameters σ_C or σ_A) promotes dispersion.

(d) Particles with an irregular surface have a smaller aggregate strength than a smooth particle of the same d_V, because of reduced contact area and reduced interparticulate forces.

The first effect is well-known for porous or hollow particles which have a relatively large d_V value compared with their d_A. The low powder bulk density has been observed for some nonspherical irregular particles produced by spray freeze-drying (SFD), and this may partially explain their enhanced performance in the cascade impactor tests.[5]

3. Requirements of Respiratory Particles

The overall efficiency of any inhalation system is expressed mathematically by the product of the fraction of *emitted dose* (*ED*), dose delivered to the lung (i.e., *fine particle fraction*, *FPF*), and lung bioavailability. Emitted dose, in percentages, is the fraction of the loaded dose (drug dose in the dosage form) that leaves the inhaler in the form of aerosol. *Fine particle fraction* is the fraction of the drug dose in the aerosol cloud that is in a predefined range, usually based on a cutoff diameter in a cascade impactor. The *FPF* is normally determined *in vitro* using a multistage cascade impactor (MSCI)[11] and is predominantly governed by both the particulate properties and inhaler design. The *FPF* is measured as the mass of particles (with reference to the *ED*) below a certain cutoff diameter, e.g., 4.7 μm, which is below the Andersen Cascade Impactor stage 2.[11] The bioavailability is influenced not only by the nature of the drug, its *in vivo* molecular permeability and metabolism, but also by the drug's particle size and shape through their effects on the dissolution rate and phagocytic clearance in the lung.[3]

Solid particles engineered for pulmonary delivery can exhibit markedly different aerosolization behaviors, depending on the complex nature of the interparticulate interactions. The type of formulation, inhalation device, inhalation flow rate, and breathing pattern are the physical variables influencing the therapeutic performance of respiratory formulations. The desirable product characteristics include high *FPF* and *ED*, high deposition in the respiratory tract, and independence of the type of device and inhalation flow rate. Thus, apart from the correct aerodynamic particle size (often expressed as the *mass median aerodynamic diameter (MMAD)*, the particles should have a relatively narrow particle size distribution (*PSD*) and be readily aerosolizable at relatively low aerodynamic dispersion forces.[12−14] The *span*, as an index of the width of the distribution relative to the *median diameter* D_{50}, was obtained from eq 7.[15]

$$\text{span} = (D_{90} - D_{10})/D_{50} \quad (7)$$

where D_{10}, D_{50}, and D_{90} are diameters at which the cumulative masses of the particles are under 10, 50, and 90%, respectively. A small '*span index*' indicates a narrow size distribution.[15] The *polydispersity* of the primary powder affects the *impaction loss*, which is defined as the mass fraction of particles collected in the mouth and throat. With a larger *span index*, there is the potential of some larger particles impacting the throat. Larger particles could also act as carriers with "binding sites" onto which the smaller particles adhere. In comparison with the smaller *span index* powder, the impaction loss for larger *span index* powders is much higher, and the particles are flow dependent, as impaction loss is proportional to the air flow and the square of particle size.

4. Drug Micronization

4.1. Spray Freeze-Drying (SFD) as a Tool for Preparation of Hollow Porous Microparticles. Microparticles can be prepared by spray-drying of a drug solution. However, spray-drying of a drug solution leads to the production of thermodynamically active, amorphous particles showing a higher tendency to recrystallize or degrade and thereby alter the product characteristics of the inhaled product. Spray freeze-drying (SFD) is preferred for heat-sensitive drugs. A typical SFD technique involves the atomization of an aqueous drug solution or suspension of drug and/or polymeric materials via a two-fluid or an ultrasonic nozzle into a spray chamber filled with a cryogenic liquid (liquid nitrogen) or halocarbon refrigerant such as chlorofluorocarbon or fluorocarbon.[23] Organic solvents and hydro-organic mixtures that have suitable freezing points may

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also be used to prepare drug and excipient solutions or suspensions. The spraying process can be performed beneath (spray-freezing into liquid) or above the surface of the cryogenic liquid, depending on the position of the nozzle.[24] It is also possible to use a nozzle arrangement for introducing liquid nitrogen directly into the spraying solution,[25] although the application of such a method for inhaled particles has not been discussed in the literature. Since the level of the cryogenic liquid will inevitably drop due to evaporation, continuous addition of fresh cryogenic liquid is required, especially when a lengthy atomization process or a large spray volume is used. Upon contact with the cryogenic medium, the liquid droplets solidify rapidly (in milliseconds time scale) because of the high heat-transfer rate.[26] Rapid solidification of freeze-dried material is likely to be caused by a higher cooling rate occurring during spraying into the cryogenic medium. During spray freeze-drying the surface area available for heat transfer is much larger than conventional freeze-drying, thus increasing the rate of heat transfer. Stirring of cryogenic liquid may be required to prevent the possible aggregation of newly formed frozen particles. Once the spraying process is completed, the whole content can be lyophilized, as with conventional freeze-drying. Spray freeze-dried particles can be engineered to the desired respiratory size range <5 μm[27−29] or even down to nanoscale.[7,29−32] The most significant operating parameter governing particle size is the mass flow ratio of atomized nitrogen to liquid feed.[33] A decrease in particle size can be achieved by an increase in mass flow ratio,[33,34] while the addition of excipients (e.g., trehalose, ammonium sulfate) may lead to an increase in particle size.[35] Further modification of the spray freeze-drying process has been proposed; instead of spraying the drug solution into the cryogenic medium, the drug solution is atomized and frozen simultaneously by mixing with a liquefied gas or supercritical fluid, such as supercritical CO_2 (SFD-CO_2 method).[6,7,13]

4.2. Jet Milling. Jets in fluidized beds can cause intense interparticle collisions, and this has been used successfully for milling of materials for producing very fine products. The fluidized particles may suffer some degree of surface damage, leading to generally undesirable breakage. Jet milling provides only limited opportunity for the control of important product characteristics such as size, shape, morphology, surface properties, and electrostatic charge.[36] In addition, the jet-milled powders exhibit a broad particle size distribution.[37] Surfaces in mechanically micrometerized powders are not naturally grown as the crystals cleave at the cleavage plane with the lowest attachment energy.[38] The micrometerization process using mills is described as extremely inefficient due to the high-energy input which decreases crystallinity[39] and can enhance chemical degradation.[40,41]

As a thermodynamically activated surface[42,43] is created, the surface properties and thus the drug substance properties are altered. The conversion of crystalline solid surfaces into partially amorphous solid surfaces leads to a "dynamic nature" of the micrometerized drug.[44] Thus, disordered structures in the material influence the performance in formulations[45,46] and processing properties such as powder flow, as micrometerized powders with a higher energetic surface show poorer flow properties.[47,48] As mechanically micrometerized powders show high particulate cohesion forces,[49] the drug may be delivered less effectively from a DPI. This was shown for micrometerized (median size = 1.6 μm) and milled (7.2 μm) nedocromil sodium.[50] Due to the association with active sites of a carrier or within the micrometerized drug, the dispersibility decreases.[51] Milling techniques, in general, show several disadvantages. However, the main research effort (represented by the number of patent applications) in the pulmonary drug delivery area is focused on the development of devices.[52] New techniques which produce the drug directly in the required small particle size are desirable, and more research is needed. Microcrystallization is a technique with high potential.

5. Crystallization As a Tool for Preparation of Inhalable Drug Particles

5.1. Microcrystallization of Proteins Using the pH-Controlled Method. Crystalline protein particles have been found to be more stable than their amorphous counterparts.[53,54]

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The higher stability arises from the fact that, unlike crystalline phases, amorphous materials consist of a disordered arrangement of molecules and therefore possess no distinguishable crystal lattice.[55] Thermodynamically, the absence of crystallinity causes energy content higher than that of the crystalline state, leading to lower stability and higher reactivity.[55] Amorphous protein particles are cleared rapidly from systemic circulation and are more susceptible to hydrolytic and enzymatic degradation because of their higher reactivity.[56] These factors make crystalline protein desirable as a fine pharmaceutical ingredient. Crystallization of proteins results in high-purity product. Crystallization can improve protein handling during processing, storage, and delivery. It can also offer sustained release of the therapeutic agent for an effective duration by changing the dissolution characteristics.[57] Despite these advantages, apart from insulin, few crystalline forms of proteins have been used as pharmaceutical active ingredients, even though over 90% of pharmaceutical products that contain a drug in particulate form are in crystalline form.[58] This is because proteins, being large molecules with a high degree of orientational freedom as a result of their sheer size, are difficult to crystallize.[56] Furthermore, crystallization can lead to particles with a wide size distribution. Milling has been applied to reduce the size of crystallized protein to respirable size, but milling may lead to a high energy input, leading to particles of reduced crystallinity with disordered regions and reduced stability.[59]

The concept of microcrystallization has been used to overcome milling-induced disorder in crystalline powders. Lee et al.[56] have succeeded in producing microcrystals of α-lactalbumin, a 16 kDa glycoprotein. α-Lactalbumin was dissolved in 0.1 N acetic acid containing PEG-8000 (PEG) as a stabilizer. The pH of the protein solution was adjusted to about 3.0. Solution of 10 N NaOH was added rapidly to the protein solution to adjust pH to about 4.0 (at this pH, the isoelectric point, the protein had the lowest solubility). The supersaturated protein solution was stored at 16 °C. The microcrystals formed roughly spherical particles with diameters between 1 to 2 μm. These spherical crystals have been used for pulmonary delivery.[60]

5.2. Crystallization of Proteins Using a Seed Zone Method. Pulmonary delivery of systemically acting drugs presents challenges. The most adequate drugs for pulmonary systemic delivery need to be selected first. The therapeutic peptides and proteins represent particularly interesting candidates because inhalation is currently the only needle-free route of administration capable of delivering macromolecules with bioavailabilities as high as 10%, without the use of physical or chemical enhancers.

To be well absorbed in the lung, a compound needs to be delivered to the alveolar region. Increased fraction of drug absorbed from the alveoli results from their large epithelial surface area and the very thin diffusion path to the bloodstream. Yet, in spite of the high efficiency of new-generation inhaler systems, pulmonary bioavailabilities do not exceed 10%, in general, indicating that clearance mechanisms within the respiratory tissue operate effectively. On the basis of this, two strategies could be undertaken to enhance pulmonary absorption. The first strategy would involve increasing the rate of molecular transport across airway and/or alveolar epithelia, while the second would consist in decreasing the rate of specific degradation pathways. Many methods have been investigated to accelerate drug transport across respiratory epithelia and decrease exposure time to degradation processes. Viable and recent strategies include the use of low-molecular weight amino acid analogues that interact with proteins to convert them into partially unfolded structures, which are more easily transported across epithelia.[61,62]

Recently, a unique insulin microcrystallization process using a seed zone method was developed. Insulin in acetate buffer conditions can form a stable zone (pH 10.5 ± 0.5) characterized by an excess of seeds. Upon the introduction of supersaturation conditions, the seeds grow into microcrystals suitable for pulmonary delivery. Insulin zinc crystals have been used as long-acting injection formulations for glucose control, but crystals used in these formulations (up to 20 μm in diameter) are too large to be inhaled deeply into the lungs. The smaller insulin microcrystals (approximately 3 μm in diameter) prepared by a seed zone method may be a better model formulation to test the long-acting effect of crystals as a pulmonary formulation.[60]

Alveolar macrophages were found to comprise a major barrier to the transport of macromolecules from the lung into the bloodstream, particularly for large proteins. Using intratracheal instillation of liposome-encapsulated dichloromethylene diphosphonate, the rat lung was depleted of alveolar macrophages and several-fold enhancement in pulmonary absorption of IgG (150 kDa) and human chorionic gonadotropin (39.5 kDa) followed the elimination of alveolar macrophages. Lowering the rate of endocytic uptake by alveolar macrophages might, therefore, lead to increased pulmonary bioavailability. IgG transport across respiratory epithelia takes place via saturable neonatal Fc receptor (FcRn)-mediated transcytosis and decreases the dose of IgG delivered to the lung. This was shown to favor transport of IgG from the lung lumen into the systemic circulation relative to local degradation. PEGylating peptides and proteins with an appropriately sized PEG can protect the macromolecule from clearance mechanisms (probably proteolytic degradation) in the lung, and thereby increase bioavailability following inhalation. Additionally, systemic activity can be prolonged owing to increased plasma half-life of the PEG derivative. Covalently attaching PEG of 750 or 2000 Da

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molecular weight to insulin increased bioavailability and duration of action several fold following dry powder inhalation in dogs. Yet, attachment of larger PEGs (5−12 kDa) to insulin or to recombinant human granulocyte colony stimulating factor (18.8 kDa) impedes transport across respiratory epithelia and significantly decreases bioavailability following pulmonary delivery in rats, emphasizing the importance of optimizing PEG size.[63]

Microencapsulation using a biodegradable polymer has been also proposed for prolonging insulin absorption in the lung, but problems are perceived with the accumulation of these polymers in the lung[6] and loss of insulin activity during the preparation of microspheres.[64] Recently, a unique insulin microcrystallization process using a seed zone method was developed.[60] Insulin microcrystals with a mean diameter of 3 μm were prepared using this seed zone method. SEM micrograph showed the microcrystals to be of a homogeneous rhombohedral with some rhombus forms, without aggregates. Following the administration of 32 U/kg of the microcrystal suspension to streptozotocin-induced diabetic rats by intratracheal instillation, the blood glucose levels were reduced, and hypoglycemia was prolonged over 13 h as compared to the normal insulin solution.[60] It was suggested that the sustained release effect was due to the decreased solubility of the microcrystals. Insulin microcrystals can be prepared by the seed zone method involving pH adjustment of the crystallization medium.[60] In contrast to the rapid onset and short duration of action with spray-dried amorphous insulin, the crystalline insulin particles produced by the seed zone method persistently reduced the blood glucose level in diabetic rats for over 7 h.[60]

5.3. Production of Inhalable Microcrystals by Direct Controlled Crystallization. Controlled crystallization of hydrophobic drugs in the respirable size range can be attained by an antisolvent precipitation technique using growth-retarding stabilizing additives such as hydroxylpropylmethylcellulose (HPMC).[65−67] Schematic diagram of controlled crystallization process is presented in Figure 1. Controlled crystallization was carried out using the solvent change method by instantaneously mixing two liquids in the presence of stabilizing agent as described by Steckel et al.[68] Higher additive concentrations usually yield smaller particles. The precipitated drug crystals (e.g., budesonide, predonsiolone, fluticasone, and disodium cromoglycate) have been shown to exhibit a higher *FPF* than jet-milled samples.[65,68,69] The amorphous content of such particles is lower than that of the mechanically treated micrometerized materials, thus affording better physical stability. Figure 2 shows the SEM micrographs of jet-milled and in situ-micrometerized disodium cromoglycate.[69] Jet-milled disodium

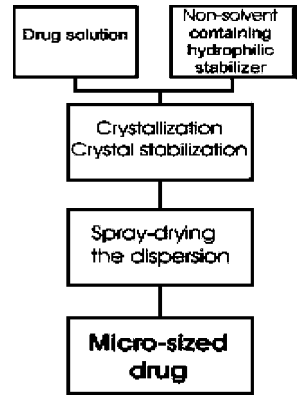

Figure 1. **Flowchart of the in situ-micrometerization process, ref 68.**

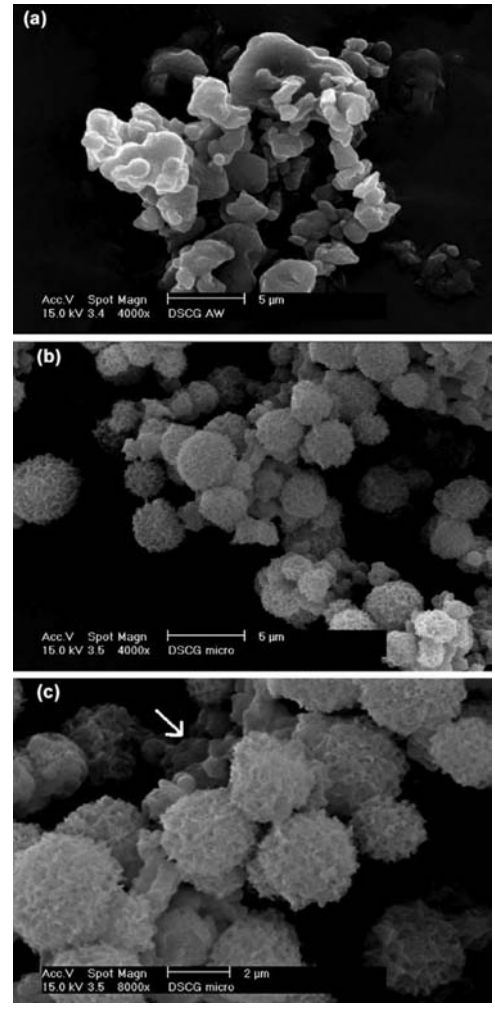

Figure 2. **SEM photographs of (a) jet-milled and (b,c) in situ-micrometerized disodium cromoglycate crystals, ref 69.**

cromoglycate shows nonhomogeneous particle shapes and a broad particle size distribution as a typical jet-milled product. On the other hand, the in situ-micrometerized drug powder shows more uniform small particles smaller than 1 μm.

Zinc-free insulin crystals in the inhalation size range of 0.2−5 μm have also been prepared by the antisolvent addition (antisolvent precipitation) method.[70] The precipitated insulin crystals were shown to be more stable than powders of

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essentially the same composition prepared by spray drying, freeze-drying, vacuum drying, or oven drying.[70]

In addition direct crystallization of spherical agglomerates has been utilized in pulmonary formulations. This technique involves antisolvent of drug solution in a water-miscible organic solvent, followed by addition of a bridging solvent, which is immiscible or partially miscible with water. For example, introduction of ethyl acetate into the water/acetone crystallization medium resulted in formation of spherical agglomerates (200−300 μm) composed of primary crystals in the respirable range (d_{50} = 1.3−2.7 μm).[71,72] The agglomerated crystals could deagglomerate readily into primary particles upon mixing with lactose carrier for 2 min or more, and the adhered primary crystals can easily detach from the lactose during inhalation with substantial enhancement in inhalation efficiency, i.e., 2−3 times higher *FPF* compared with micrometerized materials.[71−74] Spherical crystallization can also be achieved for certain drug materials by quenching of a hot organic or aqueous organic solution of the drug with a cold organic or aqueous organic solvent. The quench solvent should be miscible with the drug solvent. For instance, spherical accretions of microcrystals of salmeterol xinafoate, a long-acting anti-asthmatic agent, can be readily produced by adding a hot solution of the drug in 2-propanol to a chilled quench solvent.[75] The resulting agglomerates are free-flowing, friable, and readily micrometerizable to a material suitable for inhalation delivery.

5.4. Reactive Crystallization/Reactive Precipitation. Reactive crystallization involves reaction between two homogeneous liquid phase reactants that leads to the formation of a sparingly soluble crystalline product. In reactive precipitation, however, the supersaturation created as a result of the reaction between the homogeneous reactants is so high that the product molecules do not have sufficient time to arrange themselves in a crystalline form and therefore, the product is often amorphous. Precipitation, may also occur in the absence of a chemical reaction, e.g. by the addition of an antisolvent or change of pH in the precipitation of protein particles by isoelectric precipitation.[76,77] In addition, ultrafine particles of sumatriptan succinate (in the range of 630−679 nm) could be synthesized by reactive precipitation without any additives followed by spray drying at the optimal parameters, and it was detected having the same physicochemical properties as the standard product. Furthermore, it was found to be propitious for the pulmonary delivery since the *FPF* value was as high as 50.6% ± 8.2%.[77]

6. Challenges in Using Crystallization

The challenge of particle size control in all crystallization methods is that most small molecules tend to form relatively large crystals. This is due to a competition between the nucleation and growth mechanisms, which normally yields particles within the 10−100 μm size range, even under ideal mixing conditions.[78] Different types of mixing between the drug solution and nonsolvent can be employed, including fast agitation,[79] high-velocity mixing jets in coaxial or impinging configurations, which is a natural choice for particle production in a continuous manner,[78,80] and also precipitation with ultrasound.[81] A more exotic mixing technique involves the use of high gravity in rotating packed beds.[82] In the ultrasonic crystallization method, the particle size can be altered not only by the sonic-induced mixing but also by the influence of cavitation on supersaturation and nucleation, which renders the particles more uniform. In order to decrease the mean particle size, high concentrations of growth-retarding or stabilizing excipients are usually required. These growth inhibitors are compound-specific in terms of their interaction with the crystal surfaces. The following tailor-made non-volatile additives have been used: polysorbate 80, polysorbate 20, hydroxypropylmethyl cellulose, gelatin, poloxamer 188, sodium alginate, and L-leucine. They are molecularly designed to inhibit crystal growth and can be highly restrictive due to stringent purity control and toxicity issues. Finally, it should be noted that, although the possibility to obtain well-defined, crystalline particles with narrow size distribution is quite feasible with controlled liquid crystallization, a major drawback of such a process is the necessity to remove the residual solvents and to dry the material completely. The latter processing steps are not at all straightforward and may result in powder caking, impure sample, and reduced powder *dispersibility*.

7. Challenges and Possible Solutions on Stabilization of Inhalable Proteins: The Use of Supercritical Fluid Technology

Formulating protein powders for inhalation for aerosol delivery requires not only *flowability* and *dispersibility* of the powders but also biochemical stability of the protein molecules. Proteins have secondary and higher-order structures. During powder production, removal of water from the proteins can cause significant molecular conformational damage, which can lead to further protein degradation such as aggregation, deamination, and oxidation during storage. Amorphous glassy excipients, mainly carbohydrates, have been widely employed to stabilize proteins for inhalation, e.g., lactose for recombinant human deoxyribonuclease (rhDNase),[83] trehalose, lactose, and

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mannitol for recombinant humanized anti-IgE monoclonal antibody (rhuMAbE25).[84]

To satisfy better protein dispersibility, proteins are usually formulated in amorphous glasses, which are physically unstable and tend to crystallize with interparticulate bond formation and loss of powder *dispersibility*. The choice of the excipients is thus critical. Sodium chloride is co-spray dried with rhDNase to increase the *dispersibility*. In this particular case, the *FPF* of rhDNase increased linearly with the sodium chloride content and powder crystallinity. Scanning electron microscope revealed the presence of sodium chloride crystals on the surface of the protein particles.[85] The *dispersibility* enhancement can be attributed to decreased cohesion as a result of changes in surface energy and morphology of crystalline particles when the protein−salt composition changes. On the other hand, inhalable protein particles can be also obtained by precipitation from aqueous solution using nonsolvents. In recent years, supercritical fluids (SCFs) are increasingly used for this application. For example, insulin precipitated from dimethyl sulfoxide (DMSO) has been structurally stable for two years.[86] However, DMSO is toxic, and residual solvent can be a major concern. To overcome this limitation, water-based protein solutions can be used. A special coaxial nozzle has been used to enhance mixing of water-based protein solution with supercritical CO_2.[87] More recently, Foster and co-workers developed another approach by using high-pressure CO_2 modified with ethanol, which has successfully been employed as an antisolvent to precipitate rhDNase and insulin from aqueous solutions.[88] A potential problem of using CO_2 is its acidic nature, but solution pH can be adjusted to minimize protein degradation.

8. Polymorphism

Polymorphism is the tendency of a substance to crystallize into more than one crystal structure.[55] This is a potential problem for the crystallization of biopharmaceuticals for pulmonary delivery as regulatory bodies only approve a specific crystal structure or polymorph.[89] During crystallization, different polymorphs of a compound may be formed.[55] The more stable polymorph has the higher molecular packing density and has the lower values of Gibb's free energy, vapor pressure, and thermodynamic activity, dissolution rate per unit area in any solvent and rate of reaction, and decomposition rate.[55] For thermodynamic reasons, the less stable polymorph has the tendency to convert to the stable one. The phase transition can occur through different mechanisms: solid−solid transition, melting, and solution mediated.[90] The kinetics of the phase transitions can be influenced by typical environmental parameters (temperature, pressure, relative humidity), the presence of crystalline defect, impurities, and mechanical stress.[55]

Polymorph purity is therefore an important parameter to consider in a drug product since the presence of differing crystal phases can accelerate the conversion process by lowering the relevant activation energy barrier.[91] The phase transformation could lead to a different polymorph with unwanted physicochemical properties. For this reason, regulatory authorities have long recognized the need to limit polymorphic impurities in pharmaceutical materials.[91]

Characteristic morphologies associated with different crystalline polymorphs have also been exploited in improving deep lung delivery. Solid-state transformation from one polymorph to another can be induced by agitating the powdered material in a liquid medium. For instance, the α form (mean diameter = 2.2 μm) of the steroid KSR-592 can be converted to the acicular β-form (1.8 × 41 μm) by agitation in hexane/ethanol (95:5) mixture, followed by hexane washing, oven drying, and deaggregation by a blender.[92] The resulting particles show substantial aerosolization improvement because of the increased aerodynamic shape factor. Furthermore, it was possible to transform the original plate-like crystals (α-form) of KSR-592 to needle-like crystals (β-form). The β-form crystals in the DPI formulation were separated from carrier lactose effectively on inhalation, because of their decreased force of adhesion to the carrier particles, leading to significantly improved *RF* and *FPF* values compared with those of the original crystals. The β-form crystals liberated from the carrier lactose particles were preferentially crushed into fine crystals through the milling function, which could be deposited on a deeper (lower) stage of the cascade impactor on inhalation. The above inhalation performance of β-form crystals depended on their particle size in DPI formulation (i.e., finer crystals decreased the *FPF* value owing to their increased adhesion to carrier lactose particles).[72]

9. Polymorph Selection

The identification of stable polymorphs with desired physical properties is very important for product development. Before a drug is submitted for review to the regulatory authorities, it is important to identify the most stable polymorph of the drug. The solid phase must be monitored, via X-ray diffraction, especially after processes that may cause modification in the solid-state properties of the components.[55] For instance, during micrometerization processes such as milling, spray drying, and spray freeze-drying, the substance is usually exposed to mechanical stress, contact with solvents, or heating−cooling cycles that can often lead to alteration of the solid phase, such as new polymorph formation, dehydration, or melting mechanism.[55] The supercritical fluid process has proved to be an effective technique in obtaining pure polymorphs of different drugs based on different operating conditions and crystallization kinetics.[93] The rapid drying and cooling experienced in the process of solution-enhanced dispersion of supercritical fluids (SEDSF) resulted exclusively in a polymorph of a drug.[55]

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10. Conclusions

The emergence of advanced particle engineering techniques coupled with the modification of the traditional methods such as milling has contributed to the increased possibility of formulating biopharmaceuticals for pulmonary delivery. Particles with aerosol properties suited for deep lung delivery have been engineered without destroying the biological activity of these sensitive molecules. The wide range of techniques currently available or that are being developed, coupled with the increasing knowledge of excipients used for the protection of biopharmaceuticals, allow a diverse range of biopharmaceuticals to be processed for use in inhalation delivery devices. Microcrystallization offers a viable technique for the preparation of respiratory drugs. Other techniques such as spray drying, spray freeze-drying, and jet milling have certain disadvantages. Micrometer-sized particles can also be produced by precipitating the drug in supercritical gas phases as shown for steroids and proteins.[94,95] These techniques require specialized equipment and scale-up into the kilogram scale.

LIST OF ABBREVIATIONS

COPD	Chronic obstructive pulmonary disease
DMSO	Dimethyl sulfoxide
DPI	Dry powder inhaler
ED	Emitted dose
FPD	Fine particle dose
FPF	Fine particle fraction
GSD	Geometric standard deviation
HPMC	Hydroxylpropylmethylcellulose
IGC	Inverse gas chromatography
MMAD	Mass median aerodynamic diameter
MSCI	Multistage cascade impactor
PEG	Polyethylene glycol
PSD	Particle size distribution
Re	Reynolds number
rhDNase	Recombinant human deoxyribonuclease
rhuMAbE	Recombinant humanized anti-IgE monoclonal antibody
SCF	Supercritical fluids
SEDS	Solution enhanced dispersion of supercritical fluids
SFD	Spray freeze drying

LIST OF LATIN SYMBOLS

d_A	Aerodynamic diameter (m)
δ_C and δ_A	Hildebrand solubility parameters ($J^{1/2}/m^{3/2}$)
ρ_B	Powder bulk density (kg/m^3)
D_{50}	Median diameter (m)
d_V	Spherical-equivalent diameter (m)
ø	packing fraction
X	Size associated with a cumulative count of 84% (m)
Y	Size associated with a cumulative count of 16% (m)
θ	Interaction parameter
ρ	True particle density (kg/m^3)
ρ_0	Unit density (of the spherical particle) (kg/m^3)
σ_A	Strength of the adhesive interactions (J/m^3)
σ_C	Strength of the cohesive interactions (J/m^3)
χ	Dynamic shape factor
W_A	Work of adhesion between particles (J/m^2)
W_C	Work of cohesion (J/m^2)

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