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EXPERT OPINION

Inhalable nanocomposite microparticles: preparation, characterization and factors affecting formulation

Ibrahim Elsayed & Mohamed Hassan Hany AbouGhaly

Introduction: Nanocomposite microparticles are intelligent carriers utilised for pulmonary drug delivery. These carriers are composed of drug-encapsulated nanoparticles dispersed in microstructures of polysaccharides. Upon administration, the inhaled microparticles can penetrate and be deposited deeply in the lung due to their adjusted aerodynamic particle size. Subsequently, the nanoparticles are released into the lung and are retained there for a prolonged time due to their resistance to immunological opsonisation, engulfment and digestion.

Area covered: Nanocomposite microparticles may be prepared by spray drying, spray freeze drying, spray drying fluidised bed granulation or dry coating techniques. The selection of the included excipients, preparation technique and optimisation of the operational parameter play a significant role in the determination of the aerodynamic particle size, redispersibility of the nanoparticles, morphology, yield, moisture content, flowability and in vitro drug release. Moreover, the in vivo behaviour of this novel carrier may be optimised and traced by studying the lung deposition of the inhaled particles and the biological activity of the encapsulated drug.

Expert opinion: Nanocomposite microparticles have been found to be superior to both nanoparticles and microparticles and may represent a promising carrier for pulmonary drug delivery.

Keywords: aerodynamic particle size, lung deposition, nanocomposite microparticles, spray drying

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1. Introduction

Pulmonary drug administration is considered an attractive route for the delivery of a wide variety of locally and systemically acting drugs. For locally acting active ingredients, drug inhalation has achieved significantly higher concentrations in the lung compared with systemically injected formulations.[1–3] Moreover, inhalation is a non-invasive route of administration; thus, it is preferred over injections by almost all patients.[4,5] Furthermore, the lung is an ideal site for the absorption of peptide and protein drugs because it has negligible degrading enzyme activity.[6]

The optimization of inhalable drug delivery systems is a challenging issue due to interfering factors that affect drug deposition in the lung and the duration of action. Some of these factors are related to the drug itself, some to the carrier used for delivery and others to the used inhalation device.[7–10] All the factors should be

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optimized simultaneously to attain an ideal dosage form with reliable targeting efficiency and pharmacokinetic parameters.

The aerodynamic particle size of the inhaled powder is considered an important parameter affecting both lung deposition and retention.[11–14] For deep deposition in the lung, it was reported that the ideal aerodynamic particle size is in the range of 1 – 5 µm.[15,16] If the aerodynamic particle size is >5 µm, the coarse particles would suffer from early inertial impaction owing to the high velocity induced by gravity. Large particles become unable to change their direction with the inhaled air, leading to their collision with the lining of the mucosal membranes, especially at bifurcations.[17–19] On the other hand, if the inhaled powder size is <1 µm (within the nano range), particles may remain suspended in the respiratory system under the effect of Brownian movement and may be exhaled, rather than deposited in the lung.[20,21]

The duration of drug action is also significantly dependent on the aerodynamic particle size of the inhaled powder. It was found that particles with a size within the 1 – 5 µm range are highly susceptible to opsonization by immunological antibodies.[22–24] Consequently, they could be easily identified by macrophages as foreign bodies and undergo engulfment and digestion by intracellular enzymes. In contrast, particles in the nano-size range have a relatively higher chance to escape from antibody opsonization.[25–27] Therefore, those particles could be retained in the lung for a relatively longer time, thus producing more reliable drug pharmacological action with a reasonable duration. This review covers the latest advances in the field of nanocomposite microparticles for pulmonary delivery, including preparation, *in vitro* and *in vivo* evaluation.

2. Composition of nanocomposite microparticles

Pulmonary devices include aerosol, metered dose inhalers, dry powder inhalers and nebulizers.[28,29] These devices may carry conventional powders, solutions or suspensions as well as nanoparticles. Nanosystems have shown superior characteristics over conventional ones about controlled drug release and resistance to immunological clearance by macrophages.[30,31] As discussed in Section 1, the main limitation of the use of nanoparticles in pulmonary drug delivery is the lower efficiency of their deposition deeply inside the lung.[32–34] Incorporating drug-containing nanoparticles into a transient microparticle carrier, also called "nanocomposite microparticles," can overcome these drawbacks, as shown in Figure 1. This carrier is composed of a biodegradable matrix that rapidly dissolves in lung fluids. It has the potential to be deposited in the lung tissue and immediately disintegrates to release the incorporated nanoparticles.[35] Thus, nanocomposite microparticles could combine the advantages of micro- and nanoparticles and simultaneously avoid their disadvantages.

3. Preparation of nanoparticles

The first step in the formulation of nanocomposite microparticles is the preparation of the drug-loaded nanoparticles. Different techniques have been adopted for the preparation of the nanoparticles, as illustrated in Figure 2 and Table 1, and the choice of the preparation method depends on the nature of the used drugs and polymers as well as the target particle size.

One of the most commonly used polymers in the preparation of nanoparticles is the copolymer poly(lactic-*co*-glycolic

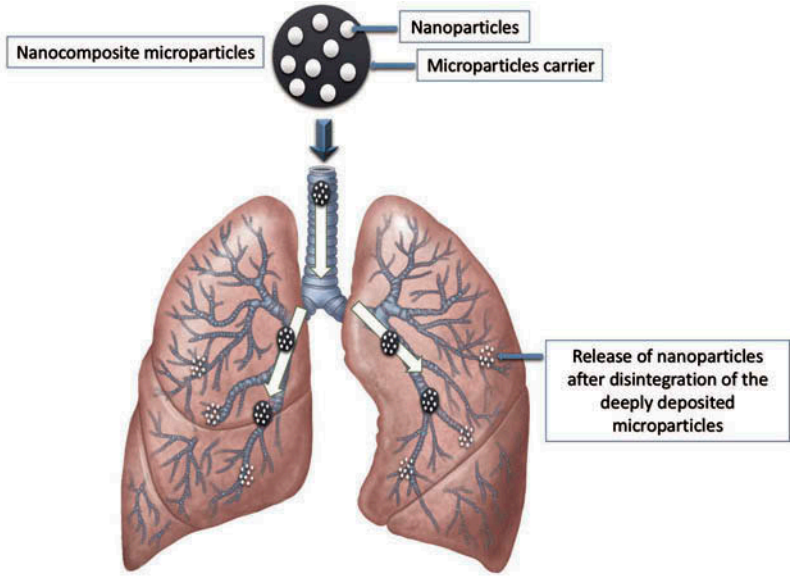

Figure 1. Nanocomposite microparticles composition and performance upon administration.

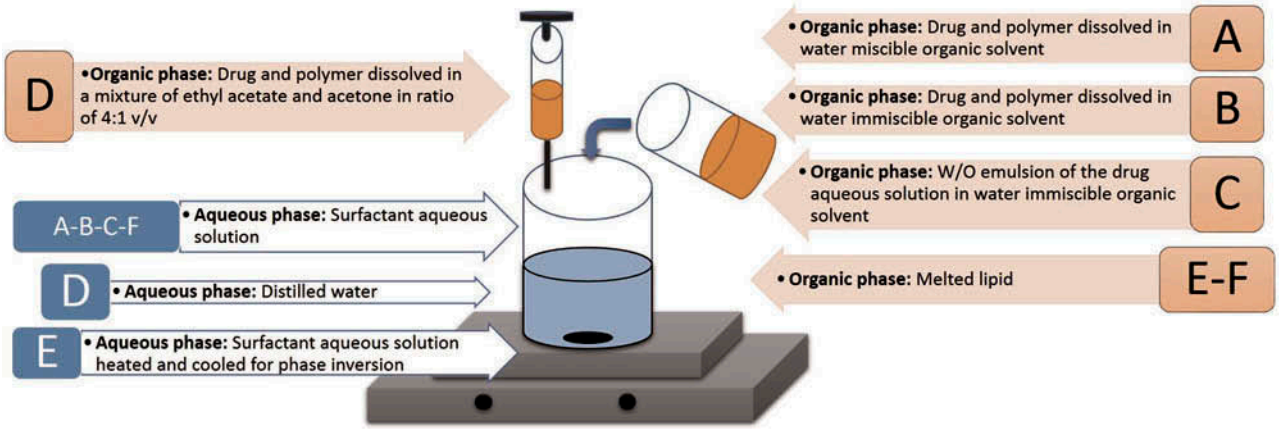

Figure 2. Illustrations of different methods used for the preparation of nanoparticles before loading into microparticles. **A**. Emulsion solvent diffusion. **B**. Emulsion solvent evaporation. **C**. Double emulsion solvent evaporation. **D**. Modified solvent displacement. **E**. Solvent-free phase inversion. **F**. Melt dispersion.

acid) (PLGA).[36–39] For the preparation of nanoparticles, PLGA with a monomer ratio of lactic acid/glycolic acid of 75/25 is predominantly used. The molecular mass of the PLGA used ranges between 10 and 40 kDa.[40,41] Different emulsifiers are used to facilitate particle size reduction and to stabilize the formed nanoparticles. Polyvinyl alcohol is typically used at percentages ranging between 1 and 5% w/v. Other surfactants have been tested, such as Tween, sodium cholate, Kolliphor HS 15 and soybean lecithin.[42]The use of surfactants and organic solvents in inhaled products must be within certain limits to avoid irritation and toxicity.[43,44]

For the preparation of PLGA nanoparticles, emulsion techniques are usually adopted. For instance, the emulsion solvent diffusion or emulsion solvent evaporation techniques are chosen according to the type of solvent and whether it is miscible with water. The immediate precipitation of the polymer occurs during the addition of the water-miscible solvent to the aqueous phase in case of solvent diffusion.[45] On the other hand, the addition of a water-immiscible solvent to the aqueous phase leads to the formation of an oil-in-water emulsion with continuous stirring. Upon the evaporation of the organic phase, the precipitation of the polymer occurs in the formed nanoparticles.[46] These methods have the disadvantage of using organic solvents and surfactants, which could lead to toxicity and irritation, respectively. Moreover, the encapsulation of hydrophilic drugs in the prepared nanoparticles is very limited owing to the escape of the water-soluble drug by diffusion from the organic phase to the aqueous surroundings.[47]

The double emulsion solvent evaporation technique (w/o/w) is used to increase the encapsulation efficiency of water-soluble drugs, such as water-soluble small interfering RNA (siRNA), inside prepared nanoparticles.[48–51] Practically, the encapsulation efficiency of hydrophilic drugs could be improved by the double emulsion solvent evaporation technique, but it remains limited and needs further improvement. Moreover, drug instability during the encapsulation process is critical if the drug is liable to hydrolysis.[52] Another method is the surfactant-free solvent displacement technique, which helps avoid the irritation caused by interactions between surfactants and the mucous membranes of the lung.[43,53] In this method, a PLGA solution in a mixture of ethyl acetate and acetone at a ratio of 4:1 v/v was injected dropwise into ultra-purified water kept under stirring at 500 rpm. Stirring should be continued for 3 hours after the addition of the organic phase to allow the complete evaporation of the used volatile solvents. The formed surfactant-free nanoparticles are stabilized by incorporation into the nanocomposite system. This method may be superior to both solvent diffusion and emulsion solvent evaporation techniques owing to its avoidance of surfactant use, but it still has their other disadvantages.

The benefits of coating PLGA nanoparticles with chitosan were investigated by Guo *et al.*[41] Coating was simply performed by incubating the preformed PLGA nanoparticles with a chitosan solution. These coated nanoparticles are designed to increase the adhesion and retention capacity in the lung tissues owing to the presence of the cationic mucoadhesive chitosan. Moreover, chitosan significantly increased the cellular uptake of the coated nanoparticles by cancer cells and thus facilitated the intracellular delivery of the used gene. Another modification of the PLGA nanoparticles was achieved by incorporating dioleoyl trimethyl ammonium propane (DOTAP) into the formulated nanoparticles.[49] DOTAP is a liposomal transfection reagent that enhances the cellular uptake of negatively charged biomolecules. It provided more efficient gene-silencing ability to the PLGA-encapsulated siRNA compared with nonmodified PLGA nanoparticles.[48] Moreover, DOTAP was able to translocate siRNA into

Table 1. Summary of the previous trials for preparation and optimization of different inhalable nanocomposite microparticles formulations.

	Nanoparticles			Microparticles		
Drug	**Polymer**	**Surfactant**	**Technique**	**Sugar**	**Technique**	**Reference**
Nile red and tetra methyl rhodamine	PLGA with a weight average molecular mass of 12 kDa	Free	Modified solvent displacement	2% (w/v) Mannitol, lactose and α-cyclodextrin	Spray drying	[43]
siRNA	PLGA with molar ratio of 75:25, molecular mass: 20 kDa	2% (w/v) PVA	Double emulsion solvent evaporation	1–3% (w/v) Mannitol, lactose and trehalose	Spray drying	[48]
siRNA	PLGA with molar ratio of 75:25, molecular mass: 20 kDa + dioleoyl trimethyl ammonium propane	2% (w/v) PVA	Double emulsion solvent evaporation	Mannitol	Spray drying	[49]
6-Coumarin	PLGA with molar ratio of 75:25, molecular mass: 20 kDa	3% (w/v) PVA	Emulsion solvent diffusion	20% (w/v) Mannitol	Agglomaster™	[81]
TAS-103 (model anticancer drug)	PLGA with molar ratio of 75:25, molecular mass: 10 kDa	2.0% (w/v) PVA	Emulsion solvent evaporation	1% (w/v) Trehalose	Spray drying	[40]
Rifampicin	PLGA with molar ratio of 75:25, molecular mass: 10 kDa	4.5% w/v PVA	Emulsion solvent evaporation	Trehalose (with weight ratio 0–1 to the nanoparticles)	Spray drying	[96]
Salmon calcitonin	PLGA with molar ratio of 75:25, molecular mass: 20 kDa	2.5% w/v PVA	Emulsion solvent diffusion	Pharmatose325M (lactose)	Mechanofusion™	[85]
Salmon calcitonin	PLGA with molar ratio of 75:25, molecular mass: 20 kDa	2.5% w/v PVA 403 and 0.5% (w/v) chitosan in acetate buffer	Emulsion solvent diffusion	Mannitol	Mechanofusion™ or Agglomaster™	[85]
Rifampicin	PLGA with molar ratio of 75:25, molecular mass: 10 kDa	2% (w/v) PVA	Emulsion solvent evaporation	Trehalose dehydrate and lactose monohydrate	Spray drying	[71]
Plain	Iron oxide	Free	Precipitation reaction	Mannitol	Spray drying	[68]
Plain	Polymeric nanoparticles: PLGA with molar ratio of 50:50, ethyl cellulose or Eudragit RL PO Lipid nanocapsules: triglycerides	0.1% (w/v) sodium cholate, 0.1% (w/v) tween or 1% (w/v) polyvinyl alcohol Kolliphor HS15 and soya been lethicin	Emulsion solvent evaporation Solvent-free phase inversion	5% (w/v) maltodextrin or trehalose + 5% (w/v) polyvinyl pyrrolidone 5% (w/v) trehalose + 5% (w/v) polyvinyl pyrrolidone	Spray drying or spray freeze drying	[42]
2-Methoxyestradiol	PLGA with molar ratio of 50:50, molecular mass: 38.5 kDa	1% (w/v) PVA	Emulsion solvent evaporation	Lactose, leucine and poloxamer 188	Spray drying	[41]

PLGA, poly(lactic-*co*-glycolic acid); PVA, polyvinyl alcohol; siRNA, small interfering RNA.

the oily phase during preparation; thus, it could help increase the encapsulation efficiency. This result might be due to electrostatic interactions between the positively charged head groups of DOTAP and the negatively charged phosphate groups available in the structure of the siRNA.

Other than PLGA, lipid nanocapsules and nanoparticles are formulated and prepared using solvent-free phase inversion and melt dispersion techniques, respectively.[42] The phase inversion technique is based on mixing the oily phase with small amount of water and surfactants, followed by the repeated heating and cooling of the formed mixture to enhance phase inversion.[54–56] Then, cold water is added with stirring for 10 min to form the dispersed lipid nanocapsules. Melt dispersion is another solvent-free technique performed by melting the lipid, followed by adding it to the preheated aqueous phase under stirring.[57,58] Lipid nanoparticles are formed upon cooling and can be collected after complete congealing. The limitations of this method include the likely thermosensitivity of the drug and/or the used excipients.[59] Nanostructured lipid carriers (NLC) are also used for drug delivery to the lung. They are prepared using the conventional thin-film hydration method, in which the drug and the used lipids are dissolved in a suitable organic solvent.[60] Then, the organic solution is evaporated under reduced pressure using a rotary evaporator. The produced film is then dispersed into an aqueous solution, forming the NLC. However, due to its lipophilic nature, NLC is not usually used for proteins and peptides because they tend to yield low encapsulation efficiencies. Several attempts have been used to increase the lipophilicity of the proteins by complexing them with different materials, such as phospholipids [61] and bile salts.[62]

Another polymer used in the preparation of nanoparticles for later inclusion in nanocomposite microparticles is chitosan. Being a mucoadhesive polymer, chitosan can increase the residence time of the prepared particles and their ability to promote drug permeation through mucosal membranes.[63] For this reason, this system has been used for the pulmonary delivery of various proteins, including insulin [35,64] and salmon calcitonin.[65]

Chitosan nanoparticles are usually prepared through the ionotropic gelation of chitosan using tri-poly phosphate (TPP).[66] The general procedure includes the preparation of two separate solutions for chitosan and TPP. Then, after the TPP solution is added to the chitosan solution under mild stirring, nanoparticles form spontaneously. The drug is included in the TPP solution. The size and drug entrapment efficiency can be controlled by changing the chitosan-to-TPP ratio. Typically, chitosan is used at a higher proportion than the TPP to form nanoparticles; increasing the amount of chitosan leads to larger particle sizes if the effect of the added drug is excluded.[66] However, this is not always the case; sometimes, a higher proportion of TPP is needed for the formation of nanoparticles.[67] High concentrations of TPP are usually used when the pH of the chitosan solution is adjusted to a low value. In this case, the high proton concentration in the chitosan solution reduces the effect of the negative charge on the TPP, which makes the formation of electrostatic crosslinks with the chitosan amine groups less efficient at low TPP concentrations.

Targeted magnetic nanoparticles were prepared and investigated by Stocke *et al.*[68] The precipitation of the magnetic nanoparticles was achieved by the reaction of ammonium hydroxide with hydrated ferrous and ferric chlorides dissolved in deionized water and heated to 85°C. The precipitated magnetic nanoparticles were washed with ethanol, recovered by magnetic decantation and dried under vacuum.

There are many obstacles facing the large-scale production of polymeric nanosystems. For example, the high cost of the used polymers affects their cost-effectiveness.[69] Moreover, the stability of most biodegradable polymers, such as polylactic acid and polylactic co-glycolic acid, is affected by temperature and the presence of water. Thus, they could be susceptible to degradation during preparation and/or storage.[70]

4. Preparation of nanocomposite microparticles

4.1 Excipients used in the preparation of nanocomposite microparticles

Prepared nanoparticles (polymeric, lipidic or magnetic) were processed to be dispersed in polysaccharide microparticles. Several polysaccharides were investigated, such as lactose, mannitol, trehalose, cyclodextrin and maltodextrin, with variable concentrations ranging between 1 and 20% w/v, as presented in Table 1.[42,43,71] Polysaccharides are freely dissolved and easily absorbed in lung fluids. Lactose is best avoided if the patient suffers from or is susceptible to lactose intolerance.[72] After the polysaccharides dissolve inside the lung, they release the loaded nanoparticles, which are able to avoid opsonization and engulfment by the mononuclear phagocyte system.[73]

Stabilizers have been used in association with the polysaccharide during the formation of nanocomposite microparticles in some studies. Polyvinyl pyrrolidone (PVP) was found to increase the mechanical stress of the prepared nanocomposite microparticles during handling and spraying.[42] Moreover, PVP could act as a crystallization inhibitor, maintaining both the nanoparticles and the polysaccharide in amorphous state and facilitating the reconstitution of the formed nanocomposite microparticles. Leucine, a branched-chain α-amino acid, has also been used as a physical stabilizer.[41] Being amphiphilic, it could align on the liquid–gas interface during the spray drying process, which is thought to decrease the inter-particulate friction. Further, poloxamer 188 was added to the mixture of the polysaccharide and leucine to increase the powder's fluidity and decrease its hygroscopicity.

4.2 Preparation techniques

4.2.1 *Spray drying*

Spray drying is the most common technique used for the preparation of nanocomposite microparticles. As shown in Figure 3A, the liquid (solution or suspension) is dispersed by an atomizer as fine droplets into forced hot air, where the liquid solvent is immediately evaporated and dried. Spray drying is a quick technique with a very short drying time (2 – 20 s).[74–76] Thus, it is suitable for the commonly used thermosensitive polymer PLGA. Various conditions have been investigated to optimize the formed microparticles, prevent their aggregation and control their aerodynamic particle size. The inlet temperature ranged between 45 and 150° C.[48,68] The diameter of the nozzle orifice was 0.7 mm in most cases. The feed rate was adjusted to a minimum of 0.3 ml/min and a maximum of 1.7 ml/min.[43,49] Finally, the air flow rate ranged between 470 and 750 l/h.

4.2.2 *Spray freeze drying*

Spray freeze drying is another intelligent technique suitable for extremely thermosensitive materials and those with very low melting points.[77–79] This technique is composed mainly of three steps (spraying, freezing and lyophilization).[42] The liquid is sprayed through a two-fluid nozzle system into an extensively cooled tower by means of a liquid nitrogen jacket, as demonstrated in Figure 3B. The temperature inside the tower is adjusted to −130°C with a feed rate of 2 ml/min. The sprayed droplet undergoes immediate freezing into small frozen spheres. Later, these spheres are lyophilized to produce nanocomposite microparticles. In this technique, the nanoparticles are protected from different stresses (freezing and dehydration) through immobilization in the glassy matrix of the used polysaccharide. However, a crystallization inhibitor is used to maintain both the nanoparticles and the polysaccharide in an amorphous state. Finally, spray freezing techniques have advantages over spray drying, especially for lipid nanoparticles that are unable to withstand the high temperature during the conventional drying process, even for a short time.[80]

4.2.3 *Spray drying fluidized bed granulation*

Spray drying and fluidized bed granulation represent two different drying techniques. They are combined in a device called the Agglomaster™, which is simplified in Figure 4A.[81–83] Spray drying fluidized bed granulation is capable of drying the sprayed nanosuspension and particle growth of the dried nanoparticles to form nanocomposite microparticles. Particle growth may result from the agglomeration of particles by solvent bonding before drying. After solvent evaporation, the solvent bonding is transformed into solid bonding. In this case, the agglomerates are irregular and cluster-like. On the other hand, particle growth may be produced through layering. In this case, the solvent is evaporated before particle collision, and the formed particles are uniformly rounded and have an onion-like layered structure.[84] The size of the produced particles can be modulated by controlling the operating conditions. The nanoparticle suspension could be sprayed from the bottom of a cylindrical vessel containing hot air for drying. Moreover, the circulation of the hot air allows the fluidization of the dried fine particles. After drying, the fluidized particles gradually start to agglomerate to form nanocomposite microparticles. During drying

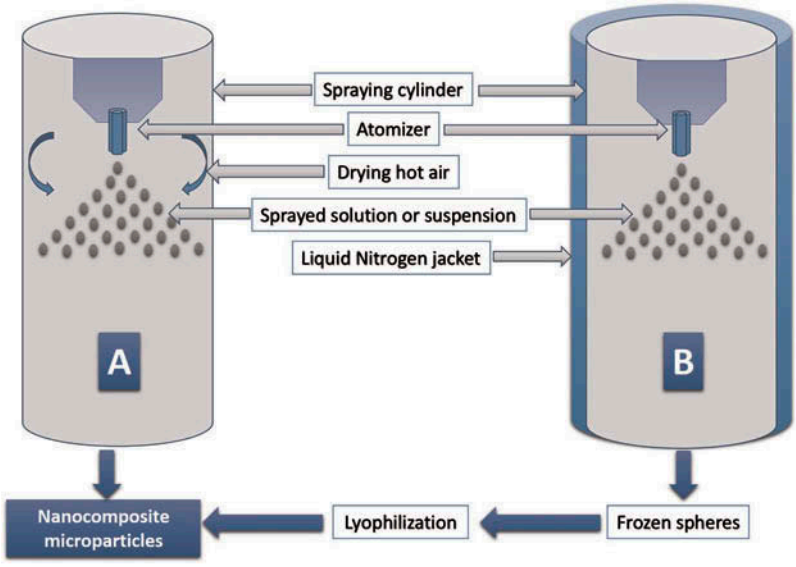

Figure 3. Simplified diagrams for the preparation of nanocomposite microparticles by A spray drying and B spray freeze drying techniques.

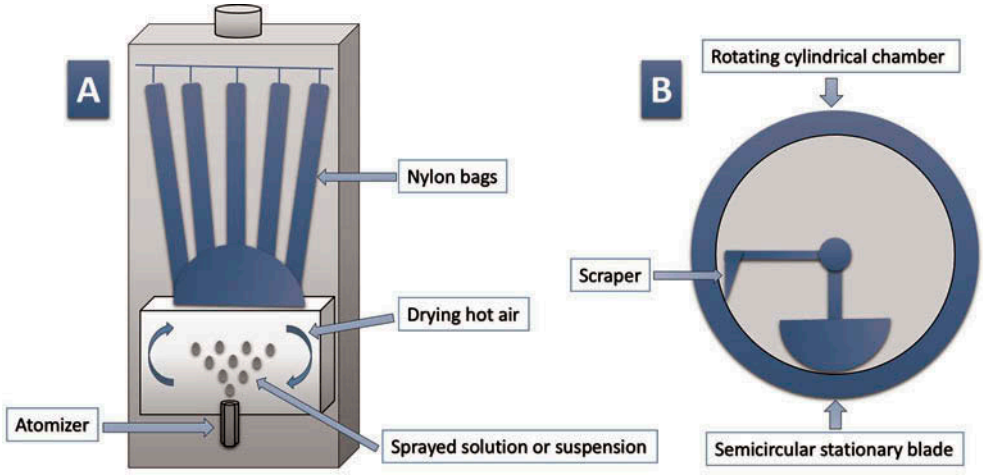

Figure 4. Simplified diagrams for the preparation of nanocomposite microparticles by A spray drying fluidized bed granulation and B dry coating techniques.

and granulation, powders sticking to the nylon bags are returned back to the granulation zone through timed air pulses. The resultant aerodynamic particle size can be controlled by adjusting the feed rate, spray pressure, inlet temperature and frequency of the washing pulsed-air jets.

4.2.4 *Dry coating technique*

In this technique, the prepared nanoparticles are lyophilized and then introduced into the Mechanofusion™ apparatus chamber simultaneously with lactose (Pharmatose 325M™).[85–87] This apparatus consists of a rotating chamber (200 – 1600 rpm) and a stationary blade and scrapper, as illustrated in Figure 4B. The clearance between the chamber wall and blade is controlled to monitor the stress exerted on the powder and the granule size produced.[88] This shear generates heat capable of fusing guest particles to the surface of larger host particles. The scraper removes any cake formed on the inner wall of the chamber. This process has the disadvantage of being a batch process. On the other hand, the applied force generates heat sufficient for the production of strong physical and/or chemical bonds, which help with the efficient dry coating. It is operated under the conditions optimized by Yamamoto (rotor speed: 372 rpm, operation time: 30 min and clearance space: 5 mm).[89] Under these conditions, the lyophilized cake of nanoparticles undergoes milling and composing processes. Nanoparticles are adsorbed onto the surface of lactose, and the formed nanocomposite microparticles can be easily decomposed inside the lung, releasing the adsorbed nanoparticles.

4.2.5 *Michael addition crosslinking during (water-in-oil) emulsion*

Microgels entrapping nanoparticles were prepared by crosslinking through a Michael addition.[90] Equimolar amounts of disulfhydryl peptide, a peptide with the sequence cysteine-glycine-arginine-glycine-glycine-cysteine, and poly(ethylene glycol) acrylate (10 kDa) were crosslinked through a Michael addition reaction while being stabilized in a water-in-oil emulsion. Briefly, solutions of the two materials were mixed together and then emulsified in a liquid paraffin-containing surfactant mixture of span and Tween. Then, the mixture was allowed to react at 37°C for 2 hours, during which the emulsion was gradually transformed into a suspension of the enzyme-sensitive microgel in the oil phase. The formed gel was subsequently subjected to the proper washing steps. For the preparation of the nanocomposite gel, the nanoparticles were mixed with the gel-forming materials in the first step. The parameters for microgel-size optimization were the homogenization time and speed as well as the surfactant concentration. The variables for the reaction optimization were the pH and type of buffer used as well as the reaction temperature.

5. Characterization of nanocomposite microparticles

The prepared particles have been characterized by measuring the geometric particle size of the nanoparticles and the aerodynamic particle size of the final nanocomposite microparticles. Moreover, the redispersibility of the formed microparticles has been measured to ensure immediate nanoparticle release after reaching the lung. Moreover, yield has been measured to compare the validity of different preparation techniques. Moisture content has been characterized to indicate the flowability of the prepared particles, and *in vitro* drug release studies have been done to predict their *in vivo* behavior after administration. *In vivo* studies have included cellular uptake, cytotoxicity, clearance by macrophages, lung deposition and biological activity.

5.1 Factors affecting particle size

Nanoparticle size should be determined twice before and after the formation of the nanocomposite microparticles.[40] This enables a comparison and evaluation to determine whether any fusion has occurred between the nanoparticles due to the used technique. In this case, the technique must be optimized to avoid the aggregation of nanoparticles and to maintain the original nano-size after their release in biological lung fluids.

The particle size of the loaded nanoparticles is also critical and should be <200 nm (ideally, <100 nm) to avoid immunological opsonization by antibodies [91] so that the nanoparticles can remain hidden from the local macrophages present in the lung, allowing them to deliver the active ingredient over an extended time. Dynamic laser light scattering (Zetasizer™) is used to determine the particle size of nanoparticles. Moreover, it is also used for the determination of the polydispersity index (PDI) and the zeta potential of the nanodispersion. The PDI provides an indication of the particle size distribution and variation, whereas the zeta potential indicates the physical stability of the dispersed nanoparticles.[73,92]

Aerodynamic particle size is the most significant physicochemical factor that should be controlled for the efficient delivery of nanocomposite microparticles deeply into the lung. It has been reported that coarse particles are unable to reach the lung and collide with the upper air pathway. On the other hand, ultrafine powders (nanoparticles) remain suspended inside the alveoli and are exhaled without deposition by gravity.[93] An aerodynamic diameter <5 μm may be ideal for powder deposition inside the lung. Cascade impactors, Marple–Miller impactors and multistage impingers are used to determine the aerodynamic particle size distribution by calculating the percentage of each particle size fraction to indicate the deposition in the lung, including the fine particle fraction (FPF).[94] The formula with the highest FPF could be considered the optimal formula able to penetrate deeply into the lung.

Several factors can affect the aerodynamic particle size of the formulated nanocomposite microparticles, as listed in Table 2. The preparation technique is one of the factors with a significant effect on aerodynamic particle size. It was found that the dry coating technique produced relatively large particle sizes. The D_{90} of the produced particles ranged from 50 to 90 μm, with only 10% of the particles smaller than 10 μm; thus, only a small percentage of the produced particles were efficiently delivered to the lung.[85] This result might be due to the large diameter of the Pharmatose 325 M (50 μm). On the other hand, the resultant particle size diameter of the nanocomposites produced by the spray dryer and spray drying fluidized bed granulation could be easily controlled to be <10 μm by controlling the processing variables. The most critical variable is the inlet temperature, which should be optimized to control the produced diameter, decrease fusion between the nanoparticles and improve their redispersibility.[71] At high inlet temperatures, the nanosuspension dries within a very short period, leading to the shrinkage and collapse of the dried materials and the production of smaller microparticles. In contrast, low inlet temperatures allow the wetted sugar to maintain the droplet size without collapse or a decrease in size. Finally, it was found that increasing the concentration of the dispersed nanoparticles and dissolved polysaccharide in the processed suspension could lead to increases in the produced aerodynamic particle size.[48] This finding might be due to the presence of a larger amount of dry material in each droplet after atomization.[95]

5.2 Factors affecting the redispersibility of nanoparticles

The redispersion of the loaded nanoparticles after lung deposition was affected by the inlet temperature, molecular mass of PLGA and the used excipients, as demonstrated in Table 2. Increasing the inlet temperature above the PLGA

Table 2. Factors affecting particle size and redispersibility of the prepared nanocomposite microparticles.

Parameters	Particle size	Redispersibility	Reference
Molecular mass of PLGA used in the preparation of the nanoparticles	Increasing the PLGA molecular mass increases both particle size and redispersibility		[81]
Method of preparation of the nanocomposite microparticles	Mechanofusion: relatively large particle size (10% of the produced particles was < 10 μm) Agglomaster: small particle size (<10 μm)	–	[85,98]
Concentrations of dispersed nanoparticles and dissolved polysaccharides	Increasing the concentration decreases the particle size	–	[48]
Presence of polysaccharide	–	Presence of polysaccharides increases redispersibility	[96]
Type of polysaccharide	α-Cyclodextrin is superior over lactose and mannitol		[43]
Presence of chitosan	–	Presence of chitosan decreases redispersibility	[85]
Inlet temperature utilized in the spray drying and Agglomaster techniques	Increasing the inlet temperature increases both particle size and redispersibility		[71]

PLGA, poly(lactic-*co*-glycolic acid).

glass transition temperature (T_g: 45°C) led to the transformation of the polymer into a soft, rubbery material. As a result, adhesion occurred between the sprayed nanoparticles, which was followed by fusion. These physical consequences could make the redispersibility and release of nanoparticles from the carrier microparticles more difficult.[71] On the other hand, the presence of polysaccharides could decrease the possibility of adhesion and increase the resistance of the nanoparticles to high temperatures. In some cases, the nanoparticles were found to be completely redispersed despite being subjected to high temperatures (70 – 90°C) owing to the protective role of the used sugar.[96] Moreover, the T_g of PLGA is dependent on its molecular mass; increasing the molecular mass raises the T_g and thus increases the tolerance of the nanoparticles to temperature increases.[81] However, unfortunately, increasing the PLGA molecular mass could negatively affect other physicochemical characteristics, such as the aerodynamic particle size and the drug release rate. Thus, these physicochemical factors must be optimized simultaneously.

The presence of certain excipients, such as chitosan, might decrease the nanoparticle redispersibility. Chitosan is a mucoadhesive, positively charged polymer. It is used to increase nanoparticle retention inside the lung through its mucoadhesion properties.[41] In a test of the redispersibility of nanocomposite microparticles containing chitosan, the latter could not dissolve in a neutral dispersion medium. Instead, it hydrated and swelled to form an adhesive gel that enhanced nanoparticle aggregation and hindered redispersibility.[85]

The type of polysaccharide used in the formulation of the nanocomposite microparticles also has an impact on redispersibility. In a comparison of mannitol, lactose and α-cyclodextrin, the latter was found to be superior because it yielded the best redispersibility after spray drying and the aerodynamic particle size remained under 200 nm.[43] This result might be due to the small crowns of α-cyclodextrin that covered the nanoparticles, producing a rigid coat through hydrogen bond formation with nanoparticle surface molecules. This phenomenon prevented the adhesion and fusion of nanoparticles during the preparation of microparticles, producing better redispersibility. Moreover, α-cyclodextrin was especially selected and preferred over β-cyclodextrin owing to its very small cavity (0.57 nm for α-cyclodextrin and 0.78 nm for β-cyclodextrin).[97] This could protect the drug included in the formula from forming an inclusion complex with the used cyclodextrin.

5.3 Yield, moisture content and bulk density

The yield is the amount produced after the collection of the product and is calculated as a percentage of the total raw materials used in this process. One of the most important goals of optimization is to increase the yield of the product. Jensen *et al*. studied factors that affect the yield percentage of nanocomposite microparticles.[48] It was found that increasing the concentration of the feed, including the dissolved polysaccharide and the suspended nanoparticles, could increase the yield percentage. This finding might be due to an increase in the total produced nanocomposite microparticles, thus reducing the loss as calculated as a percentage of the total weight.

Moisture content can be determined using Karl Fisher equipment or a thermogravimetric analysis. Low moisture content is highly desirable to decrease cohesion between particles and improve flowability through the upper respiratory system and redispersibility in the lung. Moreover, even a low moisture content may be a critical threat to moisture-sensitive drugs. Thus, controlling the moisture present in the drying chamber is very important to optimize the produced particles. Moisture can be reduced by increasing the inlet temperature or decreasing the feed rate.[81] Further, the type of excipients used can affect the moisture content. For example, mannitol was found to be superior over lactose and trehalose because it produced particles with the lowest moisture content.[48]

Density is a key connector between aerodynamic and geometric particle size values. Particles prepared with the spray freeze drying technique possess a geometrically larger diameter but a smaller aerodynamic diameter.[42] This is due to the porosity and low density of the prepared particles compared with the powders produced by the conventional spray drying technique.

5.4 Effects of preparation technique on morphological characters

The shape of the prepared nanocomposite microparticles is greatly affected by the preparation technique. The spray drying process usually leads to the formation of nonporous, rough, collapsed particles with clear deformations.[43] In other cases, the yielded nanocomposite microparticles are spherical with a smooth surface after spray drying.[49,68] This result might be related to the effect of the inlet temperature on the drying behavior of the particles, as previously discussed. In the presence of high inlet temperatures, the particles tend to collapse and become irregular in shape, whereas in cases of low inlet temperatures, the polysaccharide keeps the size and shape of the original sprayed droplets. Further, inlet temperature may affect the morphology of the formed particles. It was found that increasing the inlet temperature could lead to the formation of agglomerates of smaller unit particles.[81]

On the other hand, the spray freeze drying technique produced porous spherical particles.[42] In contrast, the spray drying technique yielded Pharmatose 325M microparticles coated with flakes similar to nanoparticles.[98] Finally, spray drying fluidized bed granulation produced a soft matrix of aggregated particles able to decompose easily upon redispersion.[85]

5.5 *In vitro* drug release

Drug release from nanocomposite microparticles can be characterized using direct dissolution or dialysis techniques. In the case of direct dissolution, each sample is centrifuged at very high speed for a long time to separate a clear supernatant, a tedious and time-consuming step.[99] Moreover, any removed precipitate contains mostly undissolved active ingredients, yielding inaccurate dissolution percentages. On the other hand, the dialysis method of dissolution overcomes the previously mentioned disadvantages. However, the latter method has its own limitation, which is strongly related to the sink conditions that should be achieved during drug dissolution.[100–103] Phosphate buffer (pH 7.4) was used as a dissolution medium at volumes ranging between 5 and 20 ml.[40,41] Surfactants may be used to achieve sink conditions.[104–106] A horizontal shaker was used at a speed of 50 strokes/min.[107,108] Further, the paddle over disc method has been used, in which the particles were kept in a disc or watch glass and covered by a membrane filter. A membrane holder was used to fix the prepared disc inside a USP II dissolution apparatus with the release surface facing upward.[109,110] Moreover, the prepared particles can be stored in a membrane filter placed in a Franz diffusion cell containing the dissolution medium. The powder is stored in the interface between the air and the dissolution medium.[111,112] Further, a modified flow-through dissolution technique has been developed for the dissolution of inhaled powder. In this technique, the prepared powder is retained within a membrane filter covered on both sides by a metal sieve support. This simple device is stored in a small flow-through cell where the dissolution medium flows uniformly.[113,114] A sustained release profile was observed for several days, depending on the drug properties and other formulation parameters. This sustained release was mainly due to the former nanoparticle matrix, the PLGA, being very hydrophobic and able to control the drug release for several days, even if the drug is water soluble.[115,116]

5.6 Cellular uptake, cytotoxicity and clearance by macrophages

Nanocomposite microparticles based on PLGA possess a high safety profile owing to the well-established biodegradation of the used polymer into lactic and glycolic acids, which are by-products of metabolic reactions already present in the human body.[117,118] Thus, research has focused on the cellular uptake of the loaded drug through the endocytosis of the nanoparticles after the dissolution of the used polysaccharide. Pulmonary delivery produces immediate drug action, similar to intravenous injection, and this action is difficult to stop or reverse if side effects occur; therefore, human studies are limited to inhalation drug products. Animal studies represent an alternative; nonetheless, they cannot provide perfect predictions for actual human cases owing to differences in anatomical and physiological features. A549 or SPC-A1 cells were used in cell culture through incubation with prepared formulae containing the drug. In some studies, a drug-free formula was used as a control; maximal viability (100%) was observed in that case.[40] In other studies, the drug solution was used as a control to evaluate alterations in cytotoxicity and cellular uptake in the presence of nanoparticles or nanocomposite microparticles.[41] Cells were incubated with the test formulae, and viability was then measured. This evaluation was performed either by spectrophotometrically measuring (at 570 nm) the uptake of 0.1% crystal violet by living cells or by examining the fluorescence generated by the hydrolysis of calcein AM into fluorescent calcein by intracellular esterase under fluorescence microscopy.[40,68] Moreover, Sulforhodamine B or tetrazolium dye assays can be used.[119,120] It was found that nanoparticles (within 200 nm) were engulfed by endocytosis, which increased the cellular uptake and cytotoxicity of anticancer drugs.[40,121] Moreover, chitosan-coated nanoparticles loaded with 2-methoxyestradiol showed a more significant increase in the cellular uptake of the drug than uncoated nanoparticles. Finally, the inclusion of nanoparticles into nanocomposite microparticles had no significant effect on cellular uptake owing to their rapid disintegration into the originally loaded nanoparticles in the culture medium.[41]

On the other hand, the safety of magnetic nanocomposite microparticles was investigated by Stocke *et al.*[68] Increasing concentrations of the blank formulae were incubated with A549 cells, and viability was determined on the basis of the fluorescence intensity of the living cells. Slight decreases in cell viability were reported with the higher concentrations of the magnetic nanocomposite microparticles, indicating the moderate cytotoxicity of the investigated formulae.

The opsonization and engulfment of micro- and nanoparticles by macrophages can be controlled by several factors, including the particle size and surface characteristics of the nanocomposite microparticles, which were compared with conventional PLGA microparticles as a reference. The polymer in both formulae was fluorescently labeled, and the formulae were individually incubated with the U 937 cell line, which imitates alveolar and bronchial macrophages.[43] The nanoparticles arising from the nanocomposite microparticles showed lower affinity and better resistance to engulfment by the used cells. These results were in accordance with previously reported findings that particles <200 nm are not actively taken up by macrophages.[122,123]

5.7 Lung deposition and biological activity

The deposition of the inhaled particles deeply inside the lung is a very critical factor in judging the credibility of nanocomposite microparticles as an efficient drug delivery system. Nanocomposite microparticles were administered to Wistar male rats using a syringe,[81,85,98] dry powder inhaler [40] or insufflator.[41] It was found by Yamamoto *et al.* that 80% of the particles were deposited into the bronchioles and alveoli;

more specifically, 50% were detected in the alveoli by Yang *et al.* [81,85,98] Furthermore, Tomoda *et al.* reported a 300-fold increase in the drug concentration inside the lung compared with the plasma concentration after the inhalation of nanocomposite microparticles containing the anticancer model drug 6-([2-(dimethylamino) ethyl] amino)-3-hydroxyl-7 H-indeno [2,1-c]quinolin-7-onedihydrochloride.[40]

During the preparation of nanocomposite microparticles, certain conditions may affect the stability and activity of the encapsulated drug, such as the temperature required for the spray drying process. The heat-sensitive and delicate siRNA integrity and biological activity were preserved after spray drying. This result was attributed to the protection exerted by the PLGA matrix or the excipient used during the spray drying process.[48]

Insulin-loaded nanocomposite microparticles yielded sustained pharmacological action lasting more than 12 hours with high bioavailability.[81] This finding might be due to the low release profile of insulin from the deposited nanoparticles and the protection of the protein drug from peptidases and other catabolic enzymes. On the other hand, calcitonin-loaded nanocomposite microparticles showed a persistent hypocalcemic effect for 24 hours, whereas nanospheres produced limited action for 12 hours only.[98] Moreover, chitosan coating of the nanoparticles loaded into the inhaled microparticles caused a significant increase in the hypocalcemic effect of calcitonin compared with the uncoated formulations.[85] This result could be due to the capability of chitosan for mucoadhesion and enhancement of transmucosal transport.[124]

No clinical trials involving nanocomposite microparticles have been done. The approval of an IND by the FDA requires that all the used excipients must be approved for administration by inhalation. Lactose and mannitol are approved for inhalation, whereas PLGA is approved only for administration through the intravitreal and intramuscular injection routes.[125] PLGA is the main polymer used in the formulation of nanocomposite microparticles; therefore, this could be considered a substantial obstacle to the application of clinical trials for nanocomposite microparticles.

6. Conclusions

Nanocomposite microparticles are smart delivery systems that smoothly carry a drug and efficiently deliver it to the lung. Several techniques have been developed to produce the primary nanoparticles and the final microparticle carriers. These varieties have broadened the allowable range for the inclusion of drugs with different physicochemical characteristics. *In vitro* evaluations have showed the feasibility of optimizing each character by carefully selecting preparation techniques and fine-tuning the used conditions. Furthermore, *in vivo* evaluations have demonstrated the pulmonary deposition and retention of prepared nanocomposite microparticles in the lung and the significant sustainment of the drug for prolonged periods. Finally, nanocomposite microparticles may be considered a promising carrier for drugs targeted to the lung.

7. Expert opinion

It is clear from the discussion that the formulation of drugs as nanocomposite microparticles can be highly beneficial for the pulmonary targeting of drugs. Nanocomposite microparticles combine the advantages of micro- and nanoparticles. Nanoparticles can evade the immune system and thus increase the time that drugs are retained in the lung. Further, PLGA nanoparticles have the ability to prolong drug release for extended periods of time. Thus, they can decrease the frequency of application of already sophisticated inhalers and, as a result, increase patient compliance. PLGA nanoparticles can be used for local and systemic drug administration, serving as a non-invasive alternative to injectable dosage forms. Nanocomposite microparticles can be administered using dry powder inhalers, which is superior to liquid preparations in terms of stability, ease of use and the lack of propellants. On the other hand, there are limitations, such as patient dependence actuation and protection from atmospheric humidity. Several techniques can be used for the manufacturing of nanoparticles, but the intelligently developed free surfactant and solvent techniques are considered the best for avoiding the tissue irritation and toxicity that can be caused by incorporated surfactant or residual organic solvents. The incorporation of nanoparticles into polysaccharide microparticles improves the aerodynamic properties of the nanoparticles, allowing them to penetrate deeply into the lung. Moreover, the presence of polysaccharides helps to avoid aggregation and enhances the redispersibility of the nanoparticles after administration. The other significant advantage of nanocomposite microparticles is their preparation techniques. Most of these techniques are common procedures that can be scaled up, such as spray drying, which helps such products reach the market without substantial manufacturing complications. The spray freeze drying technique is superior to spray drying because it is suitable for thermolabile components (either active ingredients or excipients). Furthermore, it produces spherical and porous microparticles, which may have better aerodynamic characteristics. However, this technique includes an extra step, the lyophilization of the frozen beads. On the other hand, the spray drying fluidized bed granulation technique is able to produce smaller microparticles than dry coating, although it still includes heating, which might make it unsuitable for thermolabile substances, such as lipid nanoparticles. Inlet temperature is the most critical preparation factor to be controlled during the preparation of

nanocomposite microparticles. Although increasing that temperature decreases the size of the produced particles, unfortunately, it also decreases the redispersibility of the nanoparticles within the lung owing to the possibility of adhesion between the processed nanoparticles. The used polysaccharides are able to act as cryoprotectants and physical barriers to protect both the drug from degradation and the nanoparticles from adhesion. However, further advances may be introduced in the preparation of nanocomposite microparticles to combine the production of both nanoparticles and microparticles into a single step to save time and ensure that the nanoparticle size is maintained without increases due to storage. In addition, the stability of such dry powder must be tested to ensure its stability and expiry date under common storage conditions to allow these formulations to be marketed. Moreover, several studies could be done to increase the lung-targeting efficiency of this recently developed carrier, for example, the use of mucoadhesive agents to increase the nanoparticle residency inside the lung, such as the previously used chitosan. It would also be advisable to incorporate nonionic mucoadhesive polymers rather than the positively charged chitosan to increase the stealth effect and avoidance of the immunological opsonization of the deposited nanoparticles.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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* **Prepares nanocomposite microgels**

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** **Compares two preparation techniques of nanocomposite microparticles (dry coating and spray drying fluidized bed granulation)**

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