Production and isolation of pharmaceutical drug nanoparticles

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ABSTRACT

Nanosizing of pharmaceutical drug particles is one of the most important drug delivery platforms approaches for the commercial development of poorly water-soluble drug molecules. Though nanosizing of drug particles has been proven to greatly enhance drugs dissolution rate and apparent solubility, nanosized materials have presented significant challenges for their formulation as solid dosage forms (e.g. tablets, capsules). This is due to the strong Van der Waals attraction forces between dry nanoparticles leading to aggregation, coagulation, and consequently poor flowability. In this review, the broad area of nanomedicines is overviewed with the primary focus on drug nanocrystals and the top-down and bottom-up methods used in their fabrication. The review also looks at how nanosuspensions of pharmaceutical drugs are generated and stabilised, followed by subsequent strategies for isolation of the nanoparticles. A perspective on the future outlook for drug nanocrystals is also presented.

1. Introduction

In the last decade, the US FDA has approved 100 nanomedicine applications and products (Etheridge et al. 2013), showing the importance of nanotechnology in today’s pharmaceutical and biomedical science. In the pharmaceutical industry, particles with sizes less than 1 µm are classified as nanoparticles (Dilnawaz et al. 2018). Nanotechnology plays a significant role in the field of medicine and drug delivery, mainly due to the major limitations and problems that affect conventional active pharmaceutical ingredients and finished dosage formulations.

Nearly 40% of the top 200 oral drugs marketed in the USA and Europe, and 90% of the new chemical entities which are in the drug development pipelines in the pharmaceutical industry, are poorly water-soluble (Tan et al. 2017). Thus, the low solubility of the drug at the site of administration leads to a low quantity of available diffusion, leading to insufficient drug concentration at the site of action and in-vivo failure (Siepmann and Siepmann 2013). According to the Biopharmaceutical Classification System (BCS), pharmaceutical drugs are divided into four categories based on their aqueous solubility and intestinal permeability: namely class I (high solubility and high permeability), class II (low solubility and high permeability), class III (high solubility and low permeability) and class IV (low solubility and low permeability) (Amidon et al. 1995). The aqueous solubility of a drug molecule plays an important role in the BCS system due to its essential role in the absorption of passively transported drugs across the gastrointestinal tract (Devalapally et al. 2007). According to the FDA guidelines (US FDA, 2017), a drug is considered a highly soluble drug if the highest dose strength of the drug can be dissolved in ≤250 mL of aqueous media at pH from 1 to 6.8, at a temperature of 37 °C ± 1 °C. Whereas if more than 85% of the administered drug is absorbed in the body it is considered a highly permeable drug (Charalabidis et al. 2019; Rautio et al. 2008). A detailed description of the BCS classes is presented in Fig. 1.

To date, the problem of low solubility has been tackled by reducing particle size of poorly soluble drugs using micronisation techniques (Aguirai et al. 2017). However, the demand for further improvements in drug dissolution has led to a shift from micronisation to nanosisation. In recent years, significant interest has developed in producing colloidal particles at the nanoscale with modified biological properties that allow modification of drug delivery and targeting.

Depending on the composition, function, and morphology, organic nanoparticles can be categorised as liposomal and lipid-based, polymeric and core-shell, micellar, dendrimer, and drug nanocrystals, as presented in Fig. 2.

Liposomes are biocompatible and can entrap hydrophilic pharmaceutical drugs in their internal water compartment and the hydrophobic pharmaceutical in the membrane. Thereby protecting the drugs from the inactivating effect of external conditions, without causing undesirable side reactions (Torchilin 2005). Liposomes are extensively studied in the drug delivery field due to their ability to increase the solubility and therapeutic index of chemotherapeutic agents (Torchilin 2005). The first nanomedicines approved for FDA clinical trials in the mid-1990s were based on the liposomal formulation of doxorubicin and amphotericin B.

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Polymeric nanoparticles/nanocapsules can be prepared using methods such as nanoprecipitation (Zili et al. 2005; Limayem Blouza et al. 2006), double emulsification (Jeong et al. 2008; Zhu et al. 2005), emulsion-diffusion (Guinebretière et al. 2002; Limayem et al. 2004), polymer coating (Calvo et al., 1997), emulsion coacervation (Lertsutthiwong et al. 2009) and layer-by-layer method (Chen et al. 2009; Agarwal et al. 2008). Polymeric nanomedicines are usually divided into two categories; (a) polymer-drug conjugate for increased half-life and bioavailability, and (b) controlled release application with degradable polymers leads to lower critical micelle concentration (CMC) and improved solubility due to increased surface area leading to increased solubility of a hydrophobic drug either by entrapping them in their intramolecular cavity or by conjugating to their external surface functional groups (Gupta et al. 2007; Devarakonda et al. 2005). Prios® was initially approved in 1996 that acted as an immunomodulatory in the treatment of multiple sclerosis (Johnson et al. 1998).

Polymeric micelles are self-assembled polymeric amphiphiles of AB diblock or ABA triblock copolymers in an aqueous environment tailored for controlled delivery of hydrophobic drugs. The use of block copolymers leads to lower critical micelle concentration (CMC) and enhanced stability in comparison to the surfactant-based micelles (Oerlemans et al. 2010). The hydrophobic core of the micelles is used to encapsulate poorly soluble drugs, thereby decreasing their elimination from the body, while the polar hydrophobic exterior surface allows dissolution in an aqueous medium (Hammad and Muller 1998). The core-shell structure of the micelles mimics the naturally occurring transport system in the body, also facilitating the absorption and distribution of entrapped drugs (Kwon and Okano 1996; Kabanov and Alakhov 2002). To date, estradiol (Estrasorb™) is the only FDA-approved micellar formulation in the market, used in the treatment of moderate to severe vasomotor symptoms of menopause (Bobo et al. 2016). Furthermore, many micellar formulations are in clinical trials. For example, BIND-104 has presented a significant improvement in prostate cancer therapeutics. It is a micellar formulation made up of Docetaxel encapsulated in degradable and hydrophobic polymeric core and hydrophilic PEG shell (Hrkach et al. 2012). Other examples of micellar formulations currently in clinical trials are CriPec® (Rijken et al. 2005) and CALAA-01 (Davis 2009).

Dendrimers are three-dimensional, hyper-branched nanoparticles that terminate with several external surface functional groups (Tomalia et al., 1990). Due to the high solubility (Soto-Castro et al. 2012) and bioavailability (Duncan and Izzo 2005) of dendrimers, they are used to increase the solubility of a hydrophobic drug either by entrapping them in their intramolecular cavity or by conjugating to their external surface functional groups (Gupta et al. 2007; Devarakonda et al. 2005). Priostar® (Swanson et al. 2007), Starburst® (Tomalia et al., 1990), Astra-mol® (Mark 2009), and Polylsine (Patton et al. 2006) are the commercially available dendrimers or dendrimer-based products.

Drug nanocrystals are another nanotechnology-based drug delivery platform. They are unique because they comprise 100% drug with improved solubility due to increased surface area leading to increased saturation solubility, and decreased retention in the mucosal layer. Nanocrystals also provide improved dose-bioavailability, reproducibility of oral absorption, and increased patient compliance via a reduction in the number of dosages (Müller et al. 2001; Rabinow 2005). Drug nanocrystals not only serve as a remarkable oral drug delivery system (Hanafy et al. 2007; Mauludin et al. 2009) but can also be injected intravenously as aqueous nanosuspensions (Rabinow 2004). Generally, a nanocrystal formulation contains drug nanoparticles and one or more stabilisers dispersed in an aqueous or non-aqueous medium.

**Fig. 1.** Biopharmaceutical Classification System (BCS) characterisation of drugs based on their solubility and permeability along with measures to improve the pharmacokinetic properties of each class.
Nanocrystal-based formulation Emend® was the fastest to appear on the market within 10 years of the first patent filing, compared to the first lipid-based pharmaceutical formulation which took about 25 years to appear on the market (Muller et al., 1999). Drug nanoparticles can be produced by either top-down techniques (diminution approach), or bottom-up techniques (precipitation method). Both approaches will be discussed in this review.

Despite several advantages of nanoparticles as mentioned earlier, some drawbacks include complex manufacturing (Blagden et al. 2007; Kipp 2004), nanotoxicity (Fischer and Chan 2007), and stability (Rabinow 2004; Patravale et al. 2004). Stability is one of the critical aspects of ensuring the safety and efficacy of drug products. Stability can be categorised as physical or chemical stability. Sedimentation, agglomeration, crystal growth, and recrystallisation are generally physical stability issues. Whereas, degradation of functional groups such as hydrolytic degradation of esters and amides, and oxidative degradation of amino groups are chemical stability issues. Unlike the physical stability issues, which are commonly observed, chemical stabilities, are drug-specific. One example of agglomeration or crystal growth related to intravenously administered nanosuspensions is the formation of larger particles (>5 µm) which can lead to capillary blockage and embolism (Patravale et al. 2004). Physical stability affects the development stages of the pharmaceutical product such as manufacturing, storage, and shipping. These physical stability issues also influence the rheological properties of drugs. Nanocrystals can exhibit poor flowability and compressibility due to their charged nature thereby driving them towards agglomeration or crystal growth. This leads to an inefficient drug formulation and hence drug failure during the clinical stages. Hence, there is a great demand in the pharmaceutical sector to isolate drug nanoparticles by uniformly capturing/coating them onto other micron-sized carrier particles, or encapsulating them in polymer matrices, thereby, improving rheological behaviour in drug product.

This article reviews the following topics related to nanoparticles:
- Bottom-up and top-down approaches to produce dry drug nanoparticles or nanosuspensions
- Drug nanoparticle-based products currently on the market and in clinical trials
- Existing methodologies for the isolation of nanoparticles.

### 2. Generation of API nanoparticles and nanosuspensions

The pharmaceutical industry has used several particle-engineering technologies to control the size/size distribution, crystallinity, porosity, and purity of micro and nano-sized crystals. The preparation techniques of API nanocrystals or nanosuspensions can be broadly classified into three main categories, namely (1) bottom-up techniques involving controlled precipitation/crystallisation, (2) top-down techniques involving reduction of particle size using mechanical attrition, and (3) combination methodologies comprising of bottom-up and top-down methods. The above-mentioned approaches are selected based on the physicochemical properties of the drugs such as solubility and hardness of the material (Keck and Müller 2006).

#### 2.1. Top-down approaches

Top-down techniques involve high-energy approaches to reduce the particle size of pharmaceutical drugs down to the nanoscale. The two main groups of top-down approaches are media milling and high-pressure homogenisation (HPH), as presented in Fig. 3. Both these techniques are based on collisions, stress, attrition, and shearing forces to reduce the particle size (Lim Chin et al. 2014; Al-Kassas et al. 2017; Moschewitz 2013; Gao et al. 2013). Media milling, also known as a mechanochemical process, includes both dry and wet media milling (Rasenack and Müller 2004; Merisko-Liversidge and Liversidge 2011). This technique was developed by Liversidge et al. in 1992 and is popularly known as Nanocrystal® (Moschewitz 2013). Dry media milling reduces the particle size by high energy particle-particle or particle-wall collision under the influence of opposing high-velocity jet of compressed air (Louey et al. 2004). On the contrary, the wet milling technique uses a low energy approach in which the drug is dispersed in the aqueous or non-aqueous medium along with a small amount of stabiliser and then milled using hard glass, ceramic, or stainless steel balls/beads (Leleux and Williams 2014; Li et al. 2016). Milling techniques have been extensively studied in the literature to reduce the particle size of drugs to increase their pharmacokinetic properties. Jia et al. produced a nanosuspension of carbendazim (an anticancer drug) with an average particle size of 280 ± 32 nm using Netzsch media mill at 2000 rpm for 2 hr, with water as milling media. The relative
bioavailability of nanoparticle carbendazim was enhanced by 166% compared to regular carbendazim (Jia et al. 2003). Jinno et al. studied the effect of particle size on the dissolution of cilostazol (synthetic antiplatelet agent with vasodilating effect) by preparing three different suspensions, one with a hammered mill, the second using jet milling, and third by forming a spray dried powder. The Nanocrystal technology improved the bioavailability better than the milling techniques without compromising the drug absorption (Jinno et al. 2006). Onoue et al. developed the novel respirable powder of tranilast, an antiallergic agent, which has been clinically used in asthma treatment, using a nanocrystal solid dispersion. Nanocrystals of tranilast were prepared using the wet-milling technique to achieve a mean particle size of 122 nm. These nanocrystals presented improved dissolution behaviour and lower systemic concentration, thereby providing an alternative to oral therapies for the treatment of asthma (Onoue et al. 2011).

High-pressure homogenisation (HPH) is the second class of the top-down approach. The three existing variations of HPH are as follows:

1. IDD-P™ (insoluble drug delivery-particle) technology, microfluidizer technology based on jet stream principle (Harold 1991). Small particles are generated by the frontal collision of two jet streams arranged in a Y-type or Z-type chamber under high pressure up to 1700 bar. Recirculation of particles between 50 and 100 times is needed to achieve the desired particle size.

2. Dissociubes® technology, piston-gap homogenisation in water (Muller et al., 1999). Drug particles dispersed in an aqueous medium with some stabilisers are forced by a piston under high pressure up to 4000 bar. This motion of aqueous suspension results in an increase in

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**Fig. 3.** Top-down and Bottom-up approaches used to generate API nanosuspensions or dry nanoparticles (Padrela et al. 2018).
Dynamic pressure which is compensated by a reduction in the static pressure below the vapour pressure of the aqueous phase leading to the boiling of water. This creates gas bubbles that implode upon leaving the homogenisation gap. The high-energy shock wave generated from the cavitation process breaks the drug particles (Shegokar and Müller 2010).

3. Nanopure® technology, piston-gap homogenisation in reduced water, or non-aqueous media (Helmut et al., 2000). This is an advanced version of the Dissocubes® technology using a non-aqueous medium with homogenisation happening at room temperature. Size diminution occurs due to shear force, particle collisions, and turbulence (Shegokar and Müller 2010).

Top-down techniques mostly use water as a non-solvent to disperse poorly soluble drugs, making these techniques more eco-friendly. In general, because of the more streamlined process flow, controlled particle size, and the non-solvent feature of top-down methods, most of the marketed and developmental nanosuspension-based pharmaceutical formulations have been produced by top-down methods. Hanafy et al. enhanced the bioavailability of poorly soluble fenofibrate by forming its nanosuspension using DiSSoCube and comparing it with three other formulations, one solid lipid nanoparticle and two suspensions of micronized fenofibrate (Hanafy et al. 2007). Ganta et al. prepared dry nanoparticles (133 ± 22 nm) of Asulacrine using HPH techniques followed by freeze drying that improved physical and chemical stability. The nanosuspension of asulacrine nanoparticles presented a lower plasma concentration compared to its solution state which increases its half-life to 6 h from 2.7 h (Ganta et al. 2009). Mitri et al. prepared a nanosuspension of Lutein using the HPH approach with a particle size of 429 nm presenting a 26.3 fold increase in the saturation solubility compared to the coarse powder. In-vitro permeation through a cellulose nitrate barrier also increased 14-fold (Mitri et al. 2011).

2.2. Bottom-up approaches

Typically in bottom-up approaches, nanoparticles of a drug are precipitated in crystalline or amorphous form from a supersaturated solution (aqueous/organic) (Al-Kassas et al. 2017), by evaporation of solvent or by use of an antisolvent (Sinha et al. 2013; Salazar et al. 2012). The bottom-up approach is broadly classified into two categories namely, precipitation methods and evaporation methods, as shown in Fig. 3. The liquid antisolvent precipitation method is the most effective method to produce drug nanoparticles. Mixing of drugs, previously dissolved in a solvent, with an antisolvent (e.g. water) results in increased supersaturation leading to the formation of a large number of small particles. This is an easy, cost-effective, and scalable process compared to other methods of nanosizing. This technique has been widely used in the literature to produce nanoparticles of fenofibrate (Tierney et al. 2017), mfenamic acid (Bodnár et al. 2017), valsartan (Kumar et al. 2019), atorvastatin (Zhang et al. 2009), taxifolin (Zu et al., 2014b), donazol (Zhao et al. 2009), dalteparin (Bodnár et al. 2020), and amorphous amphotericin B (Zu et al., 2014a).

Sonoprecipitation is another approach to produce uniformly dispersed nanoparticles working on the cavitation principle. The size of the final product depends on the ultrasound frequency, duration, intensity, and horn length. Amorphous nanoparticles of cefuroxime axetil were produced using this technique with a particle size of 130 nm which further decreased to 80 nm on doubling the amplitude of sonication (Dhimal et al. 2008). Another bottom-up nanosizing approach is high gravity antisolvent precipitation, where the drug dissolved in a solvent is mixed with antisolvent in a rotating packed bed chamber. The mixture is subjected to high gravity due to centrifugal force allowing the mixture to pass through the packing thereby leading to the formation of uniform size nanoparticles (Sinha et al. 2013). Nanoparticles of cefuroxime axetil with a particle size of 300 nm were produced without any stabiliser, and with a four-fold increase in surface area compared to the commercial formulation (Chen et al. 2006).

The use of supercritical CO2 is another approach to reduce the particle size of drugs. Dissolving drugs in supercritical CO2 and then expanding the solution into a low-pressure area using a narrow nozzle orifice of <100 μm results in the formation of nanosized drug particles. This process is known as the rapid expansion of supercritical solutions (RESS) (Padrela et al. 2018; Matson et al. 1986; Matson et al. 1987). Nanoparticles of raloxifene (19 nm) (Keshavarz et al. 2012), theophylline (85 nm) (Uchida et al. 2015), magedrol acetate (102–516 nm) (Samei et al. 2012), naproxen (560–820 nm) (Türk and Bolten 2010), and phenytoin (75–120 nm) (Thakur and Gupta 2006) have been successfully produced using RESS and its derivative approaches. Additional supercritical CO2-based processes include the supercritical antisolvent (SAS) and the Gas antisolvent (GAS) processes, which use supercritical CO2 as an antisolvent, with the drug previously dissolved in a solvent (Padrela et al. 2018; Rodrigues et al. 2009; Rodrigues et al. 2011; Padrela et al. 2017). The particle size of the precipitated drug depends on the solvent/antisolvent ratio, drug concentration, degree of mixing and rate of antisolvent addition (Reverchon 1999; Reverchon et al. 2007). In addition, supercritical assisted spray drying (SASD) process have also been successfully used both to generate nanoparticles and control the polymorphic form of carbamazepine, risperidone, ketoprofen (Verma et al. 2020; Long et al., 2019a, 2019b), and carbamazepine-saccharin cocystal (Padrela et al. 2019).

Spray drying is a well-known evaporative method to produce microparticles of pharmaceutical drugs. The nanospray drying method, Buchi B-90, was developed by Buchi Labortechnik AG, Switzerland to produce nanoparticles of pharmaceutical drugs (Bürki et al. 2011) (Heng et al. 2011). The working principle of the nanospray dryer is thoroughly explained by Kassas et al. (Al-Kassas et al. 2017). Nanoparticles of valdagipltin (445 nm) (Harsha et al. 2015) and calpain inhibitor (300 nm) (Baba and Nishida 2012) have been successfully produced using the nanospray dryer. Onoue et al. developed a respirable powder formulation of cyclosporine A using spray dried O/W emulsion. A mixture of erythritol and O/W emulsion of cyclosporine A, polyvinylpyrrolidone, and glyceryl monooleate (an emulsifier) was spray dried and mixed with the lactose carrier. The nanomulsified particles generated with a mean diameter of 317 nm, exhibited a 4500 fold increase in the dissolution rate of cyclosporine A (Onoue et al. 2012). Another evaporative method to produce nanoparticles is spray freeze drying, where the drug solution is atomised into the cryogenic liquid resulting in the freezing of formed particles which are later lyophilised. This process generally produces amorphous nanoparticles with high surface area (Singh and Van den Mooter 2016). Hu et al. produced 100 nm amorphous particles of diazox using a spray freezing approach which exhibited high surface area and solubility (Hu et al. 2004).

Nanoparticles below 100 nm have proven to possess novel physical properties and improved permeation through various biological barriers, although this size range has proven difficult to form by either top-down or bottom-up methods (Sinha et al. 2013). Therefore, to enhance the efficacy of both the approaches and to achieve ultrafine nanoparticles, combination approaches are being studied. Combination techniques involve a pre-treatment step involving bottom-up techniques followed by a high-energy top-down technique (Möschwitzer 2013). Nanoedge® by Baxter Inc. was the first reported combination technique involving antisolvent precipitation generating amorphous microparticles that are later converted to crystalline nanoparticles using high-pressure homogenisation (Kipp James et al. 2001). Xu et al. reported producing ultrafine particles of beclometasone dipropionate for dry powder inhalation using this process (Xu et al. 2012). This approach was also used to prepare nanosuspensions of itraconazole and isradipine with a mean particle size below 500 nm (Shelar et al. 2013; Robinow et al. 2007). The SmartCrystal® platform of techniques commercialised by Abbott labs includes combination technologies such as H42 (spray drying followed by HPH), H69 (flow precipitation followed by HPH), H96 (lyophilisation followed by HPH) (Salazar et al. 2014). A detailed
description of all the combination technologies is discussed by Salazar et al. in their review article (Salazar et al. 2014).

3. API nanoparticle-based drugs in the market

The global nanomedicine market is anticipated to reach USD 350.8 billion by 2025, according to a new report by Grand View Research, Inc. (“Nanomedicine Market Size Worth $350.8 Billion By 2025 | CAGR: 11.2%” (Available at: https://www.grandviewresearch.com/press-release/global-nanomedicine-market”) 2017) The key players operating in this industry include Pfizer Inc., Ablynx NV, Nanotherapeutics Inc., Nanoviricides Inc., Abraxis Inc., Arrowhead Research Inc., Celgene Corporation, Bio-Gate AG, and Merck. Doxil® was the first FDA-approved liposome-based nano-drug (1995), which was administered intravenously (Barenholz 2012). Since then the FDA has approved more than 50 nano drugs mostly based on liposomes, polymers, and nanocrystals for various therapeutics (Caster et al. 2017; Bobo et al. 2016). Whereas, Rapamune® was the first nanocrystal-based FDA-approved drug by Wyeth in 2000. The 370 mg tablet contains only 1–3 mg of sirolimus. The low drug loading eliminated the tablet compression problem such as nanocrystal agglomeration, which is a common feature of nanocrystal products. Also, the bioavailability of the nanocrystal tablet was 21% higher than that of the nanosuspensions, showing that the kinetic saturation solubility is greater than the saturated solubility in solution (Shegokar and Müller 2010). Rapamune provided a constantly generated the maximum revenue of $1.8B in the year 2015 making it the most successful nanocrystal-based formulation (Havel 2016). Most of these formulations are manufactured using the nano-milling technology pioneered by Elan (now Alkermes), generating nanocrystals of less than 400 nm in size which was stabilised successfully to pass the FDA regulations (Liversidge et al. 1992).

Several nanocrystal-based drugs are currently in clinical trials, as presented in Table 2. All these drugs are poorly soluble and have minimal bioavailability. Semapimod® (guanylylhydrazone) acts as an immunomodulator, preventing the reduction of TNF-α (a pro-inflammatory cytokine) during Phase I study in cancer patients. Paxceed® (paclitaxel), an anti-inflammatory that could potentially reduce hypersensitivity in cancer-treated patients. Theralux (temafolin) can be potentially used to treat autoimmune diseases and cancer. PanzemNCD (2-methoxy estradiol) has shown promising antiproliferative and antiangiogenic properties in clinical trials (Jarvis et al. 2019), but did not proceed beyond Phase II and all its clinical developments were suspended due to its clinically insignificant study in treating prostate cancer (Harrison et al. 2011). A recent review by Anselmo and Mitragotri, reviews the current landscape of nanoparticle drug delivery systems and provides an update on the various types of nanomedicines in clinical trials (Anselmo and Mitragotri 2019).

4. Why there is a need to capture drug nanoparticles?

Despite the benefits of nanoparticles and the relative ease of administration, nanoparticles provide special challenges in the design of nanosuspension formulations, as well as nano-powder formulations. Though nanoparticles exhibit a characteristic nonlinear increase in kinetic solubility upon nanosizing as described by the Ostwald-Freundlich equation (Equation (1)), the stability of nanosuspensions is still a big issue.

\[ S_{NP} = S_0 \exp\left(\frac{2V_m \gamma}{RT}\right) \]  

Where \( S_0 \) is the solubility of nanoparticles with radius \( r \), \( S \) is the solubility of bulk material, \( V_m \) is the molar volume, \( \gamma \) is the interfacial tension, \( R \) is the gas constant and \( T \) is the temperature. The effect of particle size on drug dissolution increases exponentially by changing the particle size from 1 \( \mu \)m to 100 nm. The increased dissolution is advantageous for non-targeted delivery of oral drugs but it becomes a drawback for intravenous delivery of nanosuspensions. The enhanced solubility at the non-targeted site for a targeted delivery system reduces its efficacy as a drug due to the reduced circulation time of the nano-systems. This further reduced the efficacy and utilisation of stabilising

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Table 1

| Active ingredient | Trade name | Dosage form | Technology | Developer | Approved date (FDA) |
|-------------------|------------|-------------|------------|-----------|---------------------|
| Griseofulvin       | Gris-Peg®  | Tablet      | Coprecipitation | Novartis  | 1982                |
| Sirolimus          | Rapamune®  | Tablet      |            | Wyeth     | 2000                |
| Naprelone          | Cesamet®   | Tablet      |            | Lilly     | 2005                |
| Vanepamal          | Verelan PM® | Tablet      | Media milling | Schwarz Pharma | 1998                |
| Megestrol acetate  | Megace® ES | Nano suspension |         | Par Pharm  | 2001                |
| Morphine sulfate   | Avisina®   | Capsule     |            | Pfizer    | 2002                |
| Methyl phenidate HCI | Ritalin LA | Capsule |            | Novartis  | 2002                |
| Tizanidine HCI     | Zanaflex®  | Tablet      |            | Acorea    | 2002                |
| Diltiazem          | Herbesser® | Tablet      |            | Mitsubishi Tanabe Pharma | 2002                |
| Aprepitant         | Emend®     | Capsule     |            | Merck     | 2003                |
| Fenofibrate        | TriCor®    | Tablet      |            | Lupin Atlantic | 2004                |
| Dextromethaphidate HCI | Focalin XR | Tablet/ Capsule | | Novartis  | 2005                |
| Naproxen sodium    | Naprelan®  | Tablet      |            | Wyeth     | 2006                |
| Theophylline       | Theodur®   | Tablet      |            | Mitsubishi Tanabe Pharma | 2008                |
| Paliperidone       | Invega®    | Tablet      |            | Janssen Pharmaceuticals | 2009                |
| Diclofenac         | Zorvolex®  | Capsule     |            | Iroko Pharmaceuticals | 2016                |
| Fenofibrate        | Triglide®  | Tablet      | High pressure homogenisation | SkyPharma | 2005                |
| Paliperidone Palmitate | Invega® Sustenna® | Nano suspension | Janssen Pharmaceuticals | 2014                |
| Paliperidone Palmitate | Invega® Trinza® | Nano suspension | Janssen Pharmaceuticals | 2015                |
| Dantrolene Sodium  | Ryanodex®  | Nano suspension | Eagle Pharmaceuticals | 2014                |
and/or targeting agents (Peltonen and Hirvonen 2018). Shegokar et al. provided evidence of an increase in bioavailability of nanoparticles of sirolimus by 21% compared to that of the nanosuspension of the same, presenting that the kinetic saturation solubility is greater than the saturated solubility in solution (Shegokar and Müller 2010). Furthermore, the stabiliser used to stabilise the nanosuspension could also lead to toxicity.

The market for oral administration is enormous and, since the product is primarily composed of the drug and can be incorporated with GRAS-approved stabilizers and excipients, the regulatory approval process for nanocrystal drug products is easier (Jarvis et al. 2019). The special challenge with nanocrystals is the design of oral solid drug formulations. The strong Van der Waals attraction force between the dry nanoparticles leads to aggregation, cohesion, and consequently poor flowability. Hence, to improve the stability, handling, and flowability of powder formulations, APIs micro/nano particles are often combined with large carrier/excipient particles. However, drug content uniformity and further aggregation in downstream processing steps remain a concern (Han et al. 2013). Nanocrystal agglomeration remains a bottleneck issue in the pharmaceutical industry and because of this, nanocrystal formulations struggle to reach clinical trial stages. Therefore, the isolation of these nanocrystals is the prime focus in the pharmaceutical community, to enhance the stability of nanocrystals in future medicines.

5. Isolation methodologies of drug nanoparticles

Most of the nanoparticle isolation methodologies converge to either the coating of carrier particles (host) with the drug nanoparticles (guest) or encapsulation of drug nanoparticles (guest) with the carrier particles (host). This host-guest interaction presents a feasible way to immobilise nanoparticles on a larger surface. This improves material handling throughout the manufacturing line and further facilitates the delivery of formulation to the patient (Matos et al. 2020). The coating of nanoparticles onto carrier particles could be discrete or continuous depending on the experimental requirements. The discrete coating is produced when the nanoparticles attach in small pockets onto the surface of the carrier particle. While the continuous coating is produced as a monolayer on the surface of carrier particles. Moreover, a continuous coating is preferred as it produces content homogeneity in the batch. This uniform coating mostly depends on two factors, surface charge and drug loading, which are interrelated. Similarly, encapsulation also aims to capture individual drug nanoparticles, which are subsequently coated by carrier particles.

Different technologies have been used to either coat a carrier with nanoparticles or to encapsulate nanoparticles in a carrier matrix. For coating, carriers can be either microparticles like excipients or solid forms as metallic medical devices. Advantages of coating/encapsulating nanoparticles onto/by the carrier particle are as follows:

- Operation at a lower temperature compared to hot melt extrusion, and hence with thermally labile core and coating material.
- Using organic solvent in the processing of APIs without risk of explosion.
- Coating onto water-soluble API at near ambient temperatures without the risk of API degradation.

Nanoparticle isolation processes can be further subdivided based on the number of steps required. The generation of dry nanoparticles and isolating them in the same step with a coupled technology is a single-step process. While the generation of nanosuspensions and then drying these into nanoparticles coupled with isolation, the technique is a multi-step process. Fig. 4 presents the different isolation techniques involved in single-step and multi-step isolation processes of encapsulation and coating.

5.1. Single-step nanoparticle isolation processes

In single-step nanoparticle isolation processes, the API solution is converted into nanoparticles which are isolated by either encapsulation or coating in the same step. Broadly it involves three techniques namely, supercritical CO₂ processing, nanospray drying, and electrospaying.

5.1.1. Supercritical CO₂ processes

5.1.1.1. Encapsulation of drug nanoparticles using supercritical CO₂ processes

Various supercritical CO₂ processes have been used to isolate nanoparticles in the past either by encapsulation or by coating approaches, as presented in Fig. 5. In 2000, Benoit et al. (Benoit et al. 2000) developed a supercritical fluid technique that assisted the API encapsulation process, where API microparticles are encapsulated with lipids or polymers. Niu et al. (Niu et al. 2012) reported the encapsulation approach to encapsulate caffeine-loaded microcrystalline cellulose (MCC, 1–1.4 mm) beads with ethyl cellulose using the Supercritical Antisolvent process integrated with Wurster type coater (SAS-WTS). This resulted in the delayed release of the caffeine from MCC beads, demonstrating the ability of the process to prepare controlled-release formulations. Another similar process, Supercritical Antisolvent Precipitation with Enhanced Mass transfer (SAS-EM) was used to form PLGA-circumin nano-capsules with an average size of 63 nm and the drug loading of 38%. The average size of the nano-capsules could be further decreased to 40 nm, while the drug loading was reduced to 36%, by increasing the ultrasonic power from 210 W to 350 W (Zabih et al. 2014). To increase the loading efficiency, the Suspension-Enhanced Dispersion by Supercritical Fluid (SpEDS) process was used to prepare microencapsulation of methotrexate and Fe₃O₄ by spraying Fe₃O₄ suspended in an organic solution of methotrexate. These microcapsules were further suspended in a PLLA-PLG-PLLA polymeric solution, which was sprayed again into scCO₂ (supercritical CO₂) generating a second layer of microencapsulation. This process increased the loading up to 8.9 wt% and loading efficiency up to 60.8% (Chen et al. 2012). The limitation of this encapsulation process is the ability of supercritical fluid to dissolve the encapsulating material but not the API particles.

5.1.1.2. Coating of drug nanoparticles using supercritical CO₂ processes

The combination of fluidised bed coating and supercritical fluid-based precipitation has been reported for many years. These techniques are schematically presented in Fig. 5. Tsutsumi et al. (Tsutsumi et al. 1995) coated paraffin nanoparticles (500 nm) precipitated using Rapid Expansion of Supercritical Solvents (RESS) onto the surface of microspheroidal catalyst particles (56 µm) in a circulating fluidised bed process. Subramaniam et al. (Subramaniam et al. 1998) demonstrated the use of fluidised bed technology to coat micron-sized carrier particles with nano/micron-sized drug particles. In this method, a dense CO₂ is
used to fluidise the carrier particles while the drug dissolved in a solvent is sprayed with the help of this dense CO$_2$ onto the fluidised carrier particles. The dense CO$_2$ also acts as a drying gas for the nano/micro particles by selectively extracting the solvent. Integration of supercritical CO$_2$ process with the fluidised bed process to capture the spray dried API particles onto carrier particles, reduced the product transferability between processes and lowers the risk of operator’s exposure to the drug nanoparticles. Further to avoid drug-drug particle agglomeration, Sathigari et al. also used the supercritical antisolvent-drug excipient mixing (SAS-DEM) process to precipitate micro-particles of itraconazole (0.73–1.73 µm, 6–50 wt% loading) in the presence of surfactants (sodium dodecyl sulfate and poloxamer 407) and successfully coated them onto lactose powder (~100 µm) (Sathigari et al. 2011). Hence, this confirms that the supercritical CO$_2$ process is an efficient way to coat drug nanoparticles onto micron-sized carrier/excipient particles. Furthermore, improvement in drug release suggested a reduction in particle-to-particle agglomeration.

In 2014, Leeke et al. (Leeke et al. 2014) used the ability of sc-CO$_2$ to act as a solvent by adapting the Rapid Expansion of Supercritical Solutions (RESS) process in combination with the Wurster coater (RESS-WTS) and the fluidised bed (RESS-BFB). In both processes, the jet of sc-CO$_2$ saturated with the API is expanded in an upward direction in a bed of excipient particles. Table 3 presents different supercritical CO$_2$ processes that are coupled with a fluidised bed system to coat API nanoparticles onto carrier particles. Nanoparticles (<30 nm) of vitamin K3, benzoic acid, and other organic compounds were precipitated onto microcrystalline (MCC, 300 µm), close to the point of generation of nanoparticles, thereby avoiding particle agglomeration. A uniform layer of API nanoparticles was formed onto the surface of MCC particles without compromising MCC flowability. Though the processing yield was between 14 and 74%, the API loading was poor (0.01–0.54 wt%) due to the poor solubility of API in sc-CO$_2$ making this process less feasible to be adapted in the pharmaceutical industry.

Considering the limitation of sc-CO$_2$ to freely dissolve APIs, the same group used Supercritical Antisolvent (SAS) process integrated with a fluidised bed (SAS-FB) to coat API nanoparticles onto carrier particles in a single step. Nanoparticles of naringin (<200 nm) were precipitated onto MCC particles (100–500 µm) with a drug loading of 2.5 wt%. The faster dissolution rate of naringin was obtained when compared to the dissolution rate of naringin precipitated using a conventional fluidised bed process (Li et al. 2017). A similar approach was used by Matos et al. (Matos et al. 2018; Matos et al. 2020) to precipitate curcumin nanoparticles onto α-lactose monohydrate (125–145 µm), MCC (175 µm), corn starch (15 µm), and lactose (5 µm). The drug yield was between 71 and 93% and the drug loading was between 1 and 6 wt%. Due to efficient mixing in the SAS-FB process, smaller and less aggregated nanoparticles of curcumin were precipitated compared to the particles obtained from the SAS process. To achieve uniform coatings using a fluidised bed process integrated with the sc-CO$_2$ process, the CO$_2$ flow rate should be high enough to efficiently fluidise all the carrier particles.

5.1.1.3. Supercritical CO$_2$-assisted dynamic bed coating process. The
supercritical CO2 process such as supercritical antisolvent (SAS) has been used in combination with fluidized bed technology to coat micron-sized carrier particles with drug nanoparticles (Li et al. 2017). Different experimental approaches involve spraying of organic solute onto fluidised carrier particles, resulting in the low loading of <4% and poor processing yield. (Li et al. 2017; Leeke et al. 2014). Recently, Verma et al. (Verma et al. 2020) reported a novel production and isolation method for drug nanoparticles using a ‘top spray dynamic bed coating’ process (as shown in Fig. 6), which uses CO2 spray as the fluidizing gas. Nanoparticles of poorly soluble APIs such as carbamazepine, ketoprofen, and risperidone were produced and captured onto micron-sized microcrystalline cellulose carrier particles that were located on the top of the steel mesh inside the drying chamber, thereby improving the rheological properties of poorly soluble drug nanoparticles. The size distribution of the API nanoparticles was in the range of 90–490 nm, while a variable API loading of 10% to 20% was successfully achieved. Further, dissolution of risperidone was enhanced by 6-times compared to as-received risperidone particles, while the dissolution of ketoprofen and carbamazepine was increased 3-fold and 2-fold, respectively.

5.1.2. Nanospray drying equipped with electrostatic collector

Conventional spray drying has long been a useful technique to control the size and morphology of micron-sized particles of pharmaceutical drugs. However, the increased efficacy of nanoparticles compared to microparticles, increased the need to extend the capabilities of existing spray dryers into the nanometer regime (Heng et al. 2011; Al-Kassas et al. 2017; Baba and Nishida 2012). Buchi Labortechnik has developed the Nano spray dryer B-90 (shown in Fig. 7) to deliver particle sizes within the range of 300 nm to 5 µm, for milligram sample quantities at high yield, with minimal activity loss (Heng et al. 2011). The spray drying process is generally divided into three major phases: atomisation, solvent evaporation, and particle collection. Briefly, ultra-fine droplets are produced using vibration technology. The piezoelectric actuator is driven at an ultrasonic frequency of 60 kHz resulting in the vibration of the steel membrane (4–7 µm), thus ejecting ultra-fine droplets. These droplets dry to form nanoparticles while traversing through the drying chamber with the inert carrier gas. The drying gas escapes from the bottom of the chamber. Dried nanoparticles are collected using electrostatic precipitators, as they have the advantage to collect lower size particles especially 50 nm. The cyclone used in the conventional spray dryers is unable to collect small particles less than 2 µm efficiently (Heng et al. 2011). The organic solvents used to dissolve poorly water-soluble drugs are mainly ethanol, methanol, acetone, ethyl acetate, and dichloromethane. With highly diluted solutions containing 0.1–1% (w/

![Diagram of drug nanoparticle isolation using supercritical CO2 coating and encapsulation processes.](image)

**Table 3**

| Techniques | API | Excipient | Reference |
|-----------|-----|-----------|-----------|
| Supercritical antisolvent process (SAS) | Curcumin | α-lactose monohydrate (125–145 µm) | (Matos et al. 2018) |
| Supercritical antisolvent process (SAS) | Curcumin | Microncrystalline cellulose (175 µm)Corn starch (15 µm) | (Matos et al. 2020) |
| Supercritical antisolvent process (SAS) | Naringin | Microncrystalline cellulose (100–200 µm, 200–355 µm, & 355–500 µm) | (Li et al. 2017) |
| Rapid expansion of supercritical solution (RESS) | Vitamin K3 & Benzoic acid | Microncrystalline cellulose (300 µm) | (Leeke et al. 2014) |
v) solid concentrations, fine solid particles down to 50 nm can be obtained by nanospray drying. Comprehensive use of the nanospray dryer is well reported in the literature (Arpagaus et al. 2017; Arpagaus et al. 2018).

The nanoparticles produced tend to agglomerate upon isolation hence some researchers used polymers to encapsulate these drug nanoparticles. Table 4 presents a summary of the APIs isolated from the solution using the nanospray dryer. Recently, Harsha et al. (Harsha et al. 2015) prepared nanospheres (445 nm) of vildagliptin with aminated gelatin using the Buchi nano spray dryer B-90. The drug content and percentage yield of the nanospheres were found to be 76.2 ± 4.6% and 83 ± 2%, respectively. The nanospheres also presented good flowability as the angle of response value was 33.5° (cut-off is 40°). The same research group also prepared amoxicillin-loaded carbopol nanospheres with particle size in the range of 280–320 nm for the controlled release of amoxicillin. The drug content and percentage yield were 85.3% ± 0.7% and 92.8% ± 0.9%, respectively. Nanospheres allowed a 19% dissolution in 30 min compared to the 90% dissolution of amoxicillin nanosuspension. The nanospheres also presented good flowability as the angle of repose was <32.4° (Sree Harsha et al. 2013). Sithole et al. (Sithole et al. 2018) prepared the acyclovir-loaded semi-synthetic biopolymer complex (SSBC) nanoparticles with an average particle size of 257.92 nm. The SSBC nanoparticles improved the solubility of acyclovir by 30% and the ex-vivo permeation by 10% compared to the conventional acyclovir formulations, thereby improving its bioavailability. The nanoparticles generated displayed either amorphous or semi-crystalline phases. Recently, Baghdan et al. (Baghdan et al. 2018) used the nanospray dryer B-90 to coat medical implants with curcumin nanoparticles to improve the biocompatibility of the implant as well as avoid tissue generation on the implant. The average particle size of the coated nanoparticles was 500 nm.

![Fig. 6](image-url) Schematic representation of (A) top spray dynamic bed coating process, (B) enlarged view of the drying chamber consisting of a co-axial nozzle at the top, carrier particle holder in the middle, and CO₂ outlet at the bottom part of the chamber. (Adapted from Verma, Ryan, and Padrela 2020)

![Fig. 7](image-url) Schematic of the nanospray dryer (Buchi B-90) that uses an electric field to isolate encapsulated drug nanoparticles.

| API        | Excipient                  | Nanoparticle size | Yield        | Reference       |
|------------|----------------------------|-------------------|--------------|-----------------|
| Amoxicillin| Carbopol-934P              | 280–320 nm        | 92.8% ± 0.9% | (Harsha 2012)   |
| Vildagliptin| Carbopol-934P              | 355.8 nm          | 96 ± 0.4%    | (Sree Harsha et al. 2013) |
| Vildagliptin| Aminated gelatin           | 445 nm            | 83 ± 2%      | (Harsha et al. 2015) |
| Acyclovir  | Semi-synthetic biopolymer complex | 257.92 nm       | 85%          | (Sithole et al. 2018) |
| Curcumin   | Medical Implant            | 500 nm            | –            | (Baghdan et al. 2018) |
Another advantage of this process is the isolation of nanoparticles by the coating process without the need for additives to stabilise the nanoparticles.

5.1.3. Electrospraying to encapsulate and isolate drug nanoparticles

Electrohydrodynamic atomisation (EHDA) is a process to convert liquid jets breaks up into fine droplets under the influence of an external electric field, as shown in Fig. 8. EHDA is classified into electrospinning and electrospraying. In the electrospraying process, a conical-shaped ‘Taylor cone’ is formed at the tip of the needle due to two electrostatic forces namely electrostatic repulsion of induced like charges in the liquid and columnic force of external electric field liquid droplets. A fine jet of the charged polymeric solution is broken into fine droplets which move towards the collector to overcome the surface tension of the liquid. The solvent gets evaporated forming solid particles during the flight path between the tip and the collector (Anu Bhushani and Anantharamakrishnan 2014). Generally, low solution concentrations are used for the electrospraying process. Droplet size is further influenced by conductivity, surface tension, viscosity of the solution, and flow rate (Chen et al. 1995; Xie et al. 2006; Jaworek 2007). A detailed overview of the electrospraying process and the influence of the process parameter on the droplet formation is well documented in the literature (Peltonen et al. 2010; Pawar et al. 2018).

Electrospraying is a potential method for producing polymeric nanoparticles and for encapsulating both hydrophilic and hydrophobic drugs efficiently in them. Table 5 summarises the API nanocrystals isolated from solutions using the electrospraying technique. Xie et al. (Xie et al. 2006) produced paclitaxel-poly(D, L-lactide-co-glycolide) (PLGA) nanoparticles with an average particle size of 250 nm. Similarly, poly lactide (PLA) was used to produce polymeric nanoparticles of beclometasone dipropionate and salbutamol sulfate with an average nanoparticle size of 200 nm (Valo et al. 2009). In a related study, Arya et al. (Arya et al. 2009) optimised the electrospraying process parameters to produce ampicillin-loaded chitosan nanoparticles with an average particle size of 520 nm and encapsulation efficiency of 80.4%. The drug-loaded nanoparticles showed an initial burst release followed by a sustained release for 5 days, thereby making these particles the most suitable for sustained nasal and gastrointestinal tract delivery. A different approach by Wu et al. (Wu et al. 2009) was used as an electrospray to construct bioresponsive peptide-based particulates (300–400 nm) encapsulating doxorubicin using genetically engineered elastin-like polypeptides (ELPs). Therefore, electrospraying is an efficient and flexible method for generating stimuli-responsive drug particles. Electrospraying has also been used to generate spherical nanoparticles (average particle size of 400 nm) of an anti-bacterial drug, curcumin, encapsulated in gelatin. Curcumin-loaded gelatin nano-spheres showed improved anti-bacterial properties (25-fold) compared to raw curcumin (Gómez-Estaca et al. 2017). Further, electrospraying can also be used to produce core-shell nanoparticles (280–450 nm) for sustained release applications by loading the drug into a pH-sensitive polymer, thereby reducing the side effects and improving the therapeutic effect of the drug (Hao et al. 2014). In a similar study, Cui et al. (Cui et al. 2014) successfully prepared core-shell nanoparticles (average diameter of 530 ± 80 nm) of polystyryl-lactone (PVP) and shellac with ferulic acid for controlled release formulation. Recently, Garjani et al. (Garjani et al. 2018) were able to produce nanobeads (mean size of 82.9 nm) and

| API              | Stabiliser | Matrix     | Average Nanoparticle size (nm) | Reference                  |
|------------------|------------|------------|-------------------------------|----------------------------|
| Paclitaxel       | DTAB       | PLGA       | 250                           | (Xie et al. 2006)          |
| Beclometasone dipropionate and salbutamol sulfate | Ammonium hydroxide | PLA | 200                           | (Valo et al. 2009)         |
| Ampicillin sodium | –          | Chitosan   | 520                           | (Arya et al. 2009)         |
| Doxorubicin      | –          | Elastin-like polypeptides | 300–400 | (Wu et al. 2009)            |
| Curcumin         | –          | Gelatin    | 400                           | (Gómez-Estaca et al. 2017)|
| Aspirin          | –          | Eudragit   | 280–450                       | (Hao et al. 2014)          |
| Propranolol hydrochloride | –          | Eudragit   | 80–400                        | (Garjani et al. 2018)      |
| Ferulic acid     | –          | PVP and shellac | 350–900                       | (Cui et al. 2014)          |

![Fig. 8. A typical electrospraying setup presenting isolation of encapsulated drug nanoparticles.](image-url)
nanofibres (mean particle size of 232.3 nm) by modulating the drug-polymer solution concentration.

5.2. Multi-step nanoparticle isolation processes

Nanosuspensions can be stabilized using suitable stabilizers but their prolonged storage is associated with chemical instability resulting in their hydrolysis, chemical reactivity of the drug, or leakage of the drug. Conversion of nanosuspensions to solid nanoparticles can improve the physical and chemical stability of nanocrystals (Van Eerdenbrugh et al. 2008; Bose et al. 2012). Generally, the two-step nanoparticle isolation process is used in the pharmaceutical industry to isolate the dry nanoparticle from nanosuspensions. These isolated nanoparticles are either coated on the carrier material or encapsulated in the matrix, thus improving the physicochemical stability and the rheological properties of the nanoparticles. The API nanosuspension is transformed into solid products using established isolation approaches namely, drying, filtration, and coating. The dry powder obtained can be either filled in capsules or compressed into tablets.

5.2.1. Encapsulating drug nanoparticles using a multi-step isolation process

The solvent removal techniques that are frequently used in the pharmaceutical industries to remove water from nanosuspensions include spray drying, freeze drying, electrospaying, vacuum drying, oven-drying, and fluidised bed drying. This review article focuses on spray drying, freeze drying, and electrospaying; as these techniques can efficiently encapsulate drug nanoparticles, as summarised in Table 6.

5.2.1.1. Encapsulation of drug nanoparticles using a freeze drying process

Freeze drying of nanosuspensions to generate dry nanoparticles (as presented in Fig. 9) is an established method in the pharmaceutical sector. To maintain the primary objective of the nanoparticulate system i.e. rapid dissolution, the disintegration of solid forms, and redispersion of individual nanoparticles, matrix former is often added to the nanosuspension (Abdelwahed et al. 2006). The matrix formers used are typically water-soluble sugars such as mannitol, sucrose, and lactose, as adapted from freeze drying (Kesisoglou et al. 2007). Mauludin et al. (Mauludin et al. 2009) freeze dried sodium docetyl sulfate (SDS) stabilised rutin nanocrystals that were previously produced via a high-

| Drying technique         | Nanosuspension technique | API                  | Stabiliser       | Nanoparticle size (nm) | Carrier particle | Reference                          |
|--------------------------|--------------------------|----------------------|------------------|------------------------|-----------------|------------------------------------|
| Freeze drying            | High-Pressure Homogenisation (HPH) | Rutin | SDS or Tween 80 | 721 | – | (Mauludin, Müller, and Keck 2009) |
|                          |                          | Ortidion            | Pluronic F68 and Lecithin | 103.3 ± 1.5 and 897.2 ± 14.2 | Mannitol | (Gao et al. 2008) |
|                          |                          | Paclitaxel          | PSS, GC, SA, and Tween 80 | 235 and 200 | Mannitol | (Sharma et al. 2015) |
|                          |                          | Bexarotene          | Poloxamer 188, Lecithin, PVP K30 | 323.5 ± 12.7 | Mannitol | (Chen et al. 2014) |
|                          |                          | Lutein              | Decyl glycolide | 435 | Trehalose | (Mitri et al. 2011) |
|                          |                          | Ascorbyl Palmiante  | Tween 80         | 365 | Trehalose | (Tornmachaideekul et al. 2008) |
|                          |                          | Pirorxicam          | Poloxamer 188    | 501.7 ± 21.1 to 723.7 ± 14.5 | Xanthan gum, PEG400, and maltodextrins | (Lai et al. 2011) |
| Media milling            |                          | Danazol             | PVP               | 400 | sucrose | (Liversidge, Phillips, and Candy 1994) |
|                          |                          | Loviride            | Tween 80 and Poloxamer 188 | 264 ± 14 | Sucrose | (Van Eerdenbrugh et al. 2007) |
|                          |                          | Itraconazole        | TPGS               | 337 ± 74 | MCC | (Sofie et al. 2008) |
|                          |                          |lovastatin           | HPMC and SDC      | 503.2 ± 20.4 | Mannitol | (Guo et al. 2015) |
|                          |                          | Naproxen            | HPC                | 100–200 | Sucrose, lactose, mannitol and PEG | (Lee et al. 2009) |
| Ionic gelation           |                          | Vancomycin          | TPP                | 150–650 | Glycerol, mannitol, and sucrose | (Cerchiara et al. 2015) |
| Sonoprecipitation        |                          | Lovastatin          | PVP K30 and Lutrol® Poloxamer 188 (Fo68) | 500.6 ± 21.0 | Mannitol and glucose | (Gao et al. 2015) |
| Antisolvent precipitation|                          | Erlotinib           | SLS                | 260 ± 4.8 | Mannitol, sorbitol, sucrose, and trehalose dehydrate | (Thakkar, Sharma, and Misra 2018) |
| Electrospaying           |                          | Doxorubicin         | –                  | 800 | Silk fiber and PVA | (Cao et al. 2017) |
|                          |                          | Budesonide          | Tween-20          | 154 ± 19 to 884 ± 154 | PCL | (Mildahan et al. 2011) |
| Spray drying             | High-pressure homogenisation (HPH) | Itraconazole Poloxamer 188 and SDC | 500 | Mannitol | (Chaubal and Popescu 2008) |
|                          |                          | Nifedipine          | HPMC               | 339 | Mannitol | (Heiq et al. 2005) |
|                          |                          | Gliostazol          | HPMC               | 300–1300 | Mannitol | (Miao et al. 2011) |
|                          |                          | Nifrendipine        | PVA                | 190 | Lactose, mannitol | (Qian et al. 2011) |
| Media milling            |                          | Allisartan          | SDS                | 250–550 | Mannitol | (Iou et al. 2017) |
|                          |                          | Isoproxiol          | SDSL, PVP, Poloxamer 188 and HPMC | 134 | Mannitol | (Nekkanti et al. 2009) |
|                          |                          | Candesartan cilextil | SDSL, PVP, Poloxamer 188 and HPMC | 630 | Mannitol | (Niwa, Miura, and Danjo 2011) |
|                          |                          | Phenytion           | SDSL and PVP      | 553 | Lactose and Mannitol | (Hu et al. 2011) |
|                          |                          | Fenofibrate         | HPMC and SDS      | 247 | Lactose, Alginolate | (Hadiinoto and Yang 2014) |
|                          |                          | Bicalutamide        | –                  | 330 | Lactose, HPMC, arabia gum, and MCC | (Li et al. 2011) |
| Electrospaying           | Antisolvent precipitation | Erlotinib          | SLS                | 329 ± 5.2 | PVA and Mannitol | (Thakkar, Sharma, and Misra 2018) |
pressure homogenisation (HPH) process. Nanocrystals of lyophilised rutin could be re-dispersed completely in water with an average particle size of 721 nm compared to the rutin nanosuspension of average particle size of 730 nm. The dissolution velocity of the rutin nanocrystal-loaded tablet (100% dissolution in 30 min) was superior compared to rutin microcrystal-loaded (71% dissolution) and a marketed tablet (55% dissolution). Similarly, Gao et al. (Gao et al. 2008) produced and isolated two different sizes (103.3 ± 1.5 nm (suspension A) and 897.2 ± 14.2 nm (suspension B)) of oridonin nanocrystals prepared using the HPH process, using mannitol as the matrix. The dissolution rate of freeze dried suspension A was so fast that 93.2% dissolution was achieved in 5 min, while only 35% dissolution was observed for freeze dried suspension B particles due to large particle size. Similarly, Tween 80 and low molecular weight synthetic polymer sodium polystyrene sulfonate (PSS) stabilised paclitaxel nanosuspension prepared via the HPH process with an average particle size of 235 nm and 200 nm respectively was transformed to dry powder via a lyophilisation process in the presence of mannitol as the matrix. The oral in-vivo pharmacokinetic studies demonstrated that paclitaxel nanocrystals exhibit a ~10 fold increase in area under the curve (AUC) compared to plain paclitaxel crystals (Sharma et al. 2015).

Mannitol also formed the matrix for Poloxamer 188 and lecithin stabilised nanosuspension of bexarotene, to achieve an average particle size of 235.5 ± 12.7 nm after the freeze drying process (Chen et al. 2014). The AUC of bexarotene nanocrystals doubled while the maximum saturation concentration (Cmax) was reduced to half, compared to a bexarotene nanosuspension indicating a significant increase in the bioavailability of bexarotene and also a decrease in undesirable side effects. Trehalose has also been successfully used as a matrix former to isolate decyl glycoside-stabilised nanosuspensions of lutein (Mitt et al. 2011) and a Tween 90 stabilised nanosuspension of ascorylb palmitate (Teeranaachidekul et al. 2008). The saturation solubility of lutein nanocrystal increased by > 20 fold and permeation through the cellulose nitrate membrane was higher (14-fold) compared to the coarse powder. Lai et al. (Lai et al. 2011) prepared nanocrystals of piroxicam polymorphs using the HPH process and stabilised the nanosuspension using Poloxamer 188. Nanocrystals were isolated from the nanosuspension via a freeze drying process using Xanthan gum, PEG400 and maltodextrins as cryoprotectants. X-ray diffraction confirmed the polymorphic transformation of piroxicam form I to form III and monohydrate, due to high energy associated with HPH process. The increased surface to volume ratio of piroxicam nanocrystals resulted in the increased dissolution rate compared to coarse piroxicam.

Nanosuspensions produced via media milling, another top-down approach, have also been transformed into a solid product using the freeze drying method. Liversidge et al. (Liversidge et al. 1994) patented the solidification of polyvinylpyrolidone (PVP) stabilised danazol nanosuspension via the freeze drying process using sucrose as the matrix former. The average nanocrystal size of the danazol particle was 400 nm. Sucrose was also used as a cryoprotectant and matrix former for the solidification of loviride nanosuspension (mean size of 264 ± 14 nm) prepared via a media milling approach using zirconium beads (0.5 mm diameter) as a milling agent (Van Eerdenbrugh et al. 2007). The fast-dissolving sucrose matrix enabled loviride nanopowder to dissolve 100% in 15 min compared to 64.7% dissolution of the pure untreated loviride at the same time. On the contrary, Sofie et al. (Sofie et al. 2008) experienced that a higher amount of sucrose compromised the dissolution of itraconazole due to agglomeration. Henceforth, they used microcrystalline cellulose (MCC) as the matrix former to obtain itraconazole nano powder with faster dissolution. In a related study, Guo et al. (Guo et al. 2015) isolated the solid nanospheres (503.2 ± 20.4 nm) of lovastatin in the presence of mannitol as the matrix. With the success of the use of matrix formers in stabilising and improving the solubility of poorly soluble APIs, Lee et al. (Lee et al. 2009) studied the effect of the cryoprotectant concentration on the mean size of naproxen nanocrystals. Different concentrations of sucrose, lactose, mannitol and polyethylene glycol (PEG) were studied with the conclusion that a higher concentration of all the cryoprotectants and slow freezing rate can more effectively protect the nanoparticles.

Recently, Freeze drying has also been used for the solidification of nanosuspensions produced via ionic gelation (Cerchiara et al. 2015), sonoprecipitation (Guo et al. 2015), antisolvent precipitation (Thakkar et al. 2018), and electrospraying techniques (Cao et al. 2017; Midhun et al. 2011). Chitosan-loaded vancomycin nanoparticles were produced using ionic gelation techniques which were solidified later using the freeze drying process. Sucrose, mannitol, and glycerol were used as cryoprotectants with an average particle size of 603.4 ± 70.8 nm, 562.5 ± 71.1 nm, and 187.1 ± 22.3 nm respectively. Despite the smaller particle size, the release of vancomycin was poor with only 2% release in 6 hr (Cerchiara et al. 2015). On the contrary, Guo et al. (Guo et al. 2015) isolated nanorods (500.6 ± 21.0 nm) of lovastatin produced via a sonoprecipitation technique and stabilised using poloxamer 188 and PVP. Lovastatin nanorods exhibited higher dissolution compared to the lovastatin nanospheres due to the larger surface area of nanorods compared nanospheres. The in-vivo pharmacokinetic study demonstrated that nanorods were superior to nanospheres. The AUC0–24h and Cmax of lovastatin for nanorods were about two and three-fold respectively that of marketed lovastatin capsules (Junning®). A different approach was used by Thakkar et al. (Thakkar et al. 2018) to produce nanocrystals of erlotinib with the aid of sodium lauryl sulfate (SLS) as a surfactant in the antisolvent precipitation method. Nanocrystals (260 ±
4.8 nm) were transformed into solids via a freeze drying process using mannitol, sorbitol, sucrose, and trehalose dehydrate as cryoprotectants and matrix formers. The physicochemical properties such as particle morphology, pore-volume, solid-state, and particle size revealed that the lyophilised powder was superior compared to the electrosprayed powder. On the contrary, Cao et al. (Cao et al. 2017) produced a core-shell structure with PVA core encapsulating doxorubicin and silk fibroin shell with more than 90% encapsulation efficiency using electrospraying, which was later freeze dried to obtain a dry powder. The silk fibroin coating acted as a barrier limiting the initial burst release of the drug. Further, at low pH, silk fibroin lost their overall acidic surface properties thus decreasing the electrostatic interactions between doxorubicin and silk fibroin, thereby enabling the release. Similarly, Midhun et al. (Midhun et al. 2011) successfully achieved monodisperse nanobeads of budesonide encapsulated PCL for sustained drug-release applications. Thus, the above-mentioned literature demonstrates that electrospraying coupled with freeze drying is a viable method to generate dry powder of a drug entrapped in nanoscale biopolymer matrices.

5.2.1.2. Encapsulation of drug nanoparticles using the spray drying process. Spray drying has been increasingly used as a drying procedure for nanosuspension produced by either bottom-up or top-down methods. Fig. 10 is a schematic representation of a multi-step approach to encapsulate drug nanoparticles to obtain dry powder using the spray drying process coupled with top-down or bottom-up approaches. Nanosuspension of itraconazole stabilised using poloxamer 188 and sodium deoxycholate (SDC) were produced via high-pressure homogenisation (HPH) techniques and transformed into dry powders using spray drying. The absence of stabilisers resulted in particle agglomeration thereby limiting the dissolution. Mannitol was added as a matrix former, which also provided the most desirable particle morphology and enhanced the flowability of the final powder. Spray dried nanoparticles dissolved much more rapidly as compared to the micronized drug (Chaubal and Popescu 2008). In a related approach, nanosized nifedipine prepared by HPH was spray-dried along with mannitol to isolate a solid product (Hecq et al. 2005). The d50 of the re-dispersed spray dried particles was 339 nm compared to the 291 nm particle before spray drying. Therefore, the carrier particles prevented the nifedipine nanoparticle agglomeration during the spray drying process. Similarly, cilostazol nanocrystals were also isolated as dry solid by spray drying the cilostazol nanosuspension in the presence of dissolved mannitol (Miao et al. 2011). The saturation solubility of the nanosized cilostazol crystalline powder increased by 5-fold, while the dissolution rate was enhanced by 4-fold compared to the raw cilostazol powder. In a related approach, Quan et al. (Quan et al. 2011) also used mannitol as the matrix former to encapsulate nanosized nitrendipine crystalline powder (average particle size of 190 nm) by spray drying the nitrendipine nanosuspension produced via the HPH process. The in-vivo testing demonstrated that the Cmax of the nanocrystals was approximately 15-fold and 10-fold greater than that of the physical mixture and commercial tablet, respectively. Besides, the AUC0-24 of the nanocrystals was approximately 40-fold and 10-fold greater than that of the physical mixture and commercial tablet, respectively.

Spray drying of media milled nanosuspensions is also a well-documented route in the literature to obtain a dry powder. Dry nanoparticles of allisartan isopropil were produced by spray drying the media milled nanosuspension of allisartan isopropil. The optimised formulation gave a drug loading of 61.7% and high redispersity. It was also observed that the nanocrystal size of the re-dispersed formulation decreases with increasing stabiliser concentration. In addition, the bioavailability of the optimal formulation increased by 4.73 times compared to the crude drug (Hou et al. 2017). Similarly, a milled nanosuspension of candesartan cilexetil nanoparticles produced using a wet bead milling technique was converted to a solid intermediate via a spray drying process. The redispersity of the spray dried particles was high with an average particle size of 134 nm compared to the nanosuspension size of 127 nm. Tablets prepared using spray dried drug nanoparticles of candesartan cilexetil presented significantly higher dissolution compared to its micronized and commercial formulations. Systemic exposure studies in rats indicated a significant increase in the rate and extent of drug absorption with a 2.51-fold increase in area under the curve (AUC50) and a 1.77-fold increase in the plasma concentration (Nekkanti et al. 2009). With the success of mannitol as a matrix former in freeze drying processes, Niwa et al. (Niwa et al. 2011) used it to encapsulate dry nanoparticles of phenytoin produced via the milling process. The dried nanosuspension of the phenytoin product could be spontaneously redispersed in water, transforming into a nanosuspension, and displayed immediate release behaviour in both acidic and neutral media.

The spray drying process can also be used with nanosuspension produced by the antisolvent precipitation method (Thakkar et al. 2018; Hu et al. 2011; Hadinoto and Yang 2014; Li et al. 2011). A dry powder of fenofibrate nanoparticles (553 nm) was produced by immediately spray drying the nanosuspension. Particle growth and aggregation were minimised by adding lactose or mannitol as re-dispersant, and hydroxypropyl methylcellulose (HPMC) and sodium dodecyl sulfate (SDS) as stabilizers. The spray dried nanof ormulation quickly disintegrates into acidic and neutral media.

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granule of nanocrystal stabilised using sodium lauryl sulfate (SLS). The particle size of erlotinib nanocrystals with the electrospraying process to produce mannitol-loaded erlotinib was readily compacted into tablets, therefore further enhancing the sustainability of the overall oral solid dosage form preparation. Similarly, Li et al. (Li et al. 2011) produced lactose-loaded bicalutamide nanoparticles with an average size of 330 nm and narrow size distribution via a combination of antisolvent precipitation and spray drying methods. The lactose–bicalutamide formulation achieved a 2-fold increase in the dissolution rate in 10 min compared to the commercial bicalutamide tablets. Further, the antisolvent precipitation approach was combined with the electrospraying process to produce mannitol-loaded erlotinib nanocrystals stabilised using sodium lauryl sulfate (SLS). The particle size, surface area, pore-volume, and % drug release for the re-dispersed nanocrystals were 329 ± 5.2 nm, 0.341 ± 0.05 m²/g, 0.0009 cc/g, and ~78% in 600 min respectively (Thakkar et al. 2018). Therefore, coupling antisolvent precipitation with a spray drying process has huge potential to achieve continuous industrial-scale production of pharmaceutical nanof ormulations.

5.2.2. Coating drug nanoparticles onto carrier particles using a multi-step isolation process

The multi-particulate drug delivery system such as API coated onto microparticles/pellets offer many advantages in comparison to single-unit dosage forms, for example, uniform drug dispersion in the gastrointestinal (GI) tract leading to minimal variability in the bioavailability (Schmidt and Bodmeier 2001) and more predictable gastric emptying (Efentakis et al. 2000). The combination of the mucoadhesion principle and nanonisation of the drugs is an effective dosage form with enhanced efficiency (Kayser 2001). Mucoadhesion is commonly defined as the adhesion between the two materials. The materials adhere because of the forces acting between two chemical structures which could be either physisorption or chemisorption based on the interaction (Shaikh et al. 2011). The mucoadhesive polymers possess numerous hydrophilic groups such as hydroxyl, amidic, carboxyl, and sulfate, which leads to the adhesion of pharmaceutical drugs. Among all the nanocrystal-based drugs on the market, only Emend® by Merck is a multi-particulate dosage form containing drug nanocrystals.

5.2.2.1. Coating drug nanoparticles onto dry carrier particles using a fluidised bed process

Fig. 11 is a schematic representation of a multi-step approach to coat carrier particles with drug nanoparticles to obtain a dry powder using a fluidised bed process coupled with top-down or bottom-up approaches. Literature discussing the coating of drug nanoparticles onto carrier particles using a fluidised bed process in a multi-step process is summarised in Table 7. Vergote et al. (Vergote et al. 2001) were the first to discuss the incorporation of nanocrystalline ketoprofen as a spray dried powder into controlled release matrix pellets. Later on, other researchers also used the mucoadhesion principle to coat drug nanocrystals onto sugar and cellulose-based excipient particles. Nanosizing of the drug particles is achieved by either top-down or bottom-up approaches while adhesion to the excipient is controlled by the fluidised bed process. Nanocrystals of hydrocortisone acetate (908 ± 3 nm) produced using high-pressure homogenisation (HPH) process and stabilised using poloxamer 188 and chitosan chloride were coated onto sugar beads (710–850 μm) via a fluidised bed process equipped with a Wurster column and bottom spray nozzle (Moschwitzer and Muller 2006). Drug release could be tailored according to the pH by applying an enteric coating on the nanocrystal sugar beads. Similarly, Bourezg et al. (Bourezg et al. 2012) successfully coated nanoparticles of Stearoyl macrogol-32 glycerides (SMG) and Glyceryl dibehenate (GB) lipids containing dissolved spironolactone onto sorbitol and mannitol carrier particles. The particle size of SMG and GB nanosuspension was 82.7 nm and 254.6 nm respectively. After the successful fluidisation process, the coating efficiency and drying yield of SMG and GB nanoparticles were (93.54% and 56.6%), and (98.37% and 86.7%) respectively. The coating process enhanced the dissolution and stability properties of spironolactone. Therefore, it was shown that a fluidised process is an ideal technique to produce granules but is also time-consuming and requires prolonged high temperatures that may also lead to product degradation.

In another approach, a fluidized bed pellet coater with a Wurster insert was used to coat the naproxen and cinnarizine nanosuspensions prepared by media milling on sugar beads (710–850 μm) (Kayara et al. 2011). Coating of cinnarizine nanosuspensions resulted in complete dissolution in 15 min, compared to only 11% in 1 h for the unmilled powder. Naproxen also dissolved three times faster when formulated on

![Fig. 11. Schematic representation of multi-step approach to coat carrier particles with drug nanoparticles to obtain dry powder using fluidised bed process coupled with top-down or bottom-up approaches.](image-url)
Table 7

Coating of drug nanoparticles onto carrier particles using fluidised bed process in a multi-step process. The nanosuspension of drug nanoparticles was generated either by high-pressure homogenisation, media milling, or emulsion diffusion. (SMP, sucrose mono palmitate; TPGS, D-α-tocopherol polyethylene glycol succinate; SDS, sodium dodecyl sulfate; HPMC, hydroxypropyl methylcellulose; SLS; sodium lauryl sulfate; HPC, hydroxypropyl cellulose; PLGA, poly(lactic-co-glycolic acid); PVA, poly(vinyl alcohol)).

| Nanosuspension technique | API | Stabilisers | Nanoparticle size (nm) | Excipient (size) | Reference |
|--------------------------|-----|-------------|-----------------------|------------------|-----------|
| High-pressure homogenisation (HPH) | Hydrocortisone acetate | Poloxamer 188 and chitosan chloride | 908 ± 3 | Sugar beads (710–850 μm) | (Mochwitzer and Muller 2006) |
| Media milling | Spironolactone | Poloxamer 188; SMP TPGS and hypermellose | 50–250 | Sorbitol & Mannitol | (Bourezg et al. 2012) |
| | Naproxen & Cinnarizine | | 380 and 410 | Sugar beads (710–850 μm) | (Kayaert, Anne, and Van den Mooter 2011) |
| | Itraconazole | SDS and HPMC ES | 200 | Sugar beads (1000–1400 μm & 177–250 μm) & Cellulose sphere (1000–1400 μm & 210–355 μm) | (Tan et al. 2017; Parmentier et al. 2017) |
| | Ketoconazole | SLS and HPMC | 150–250 | Lactose monohydrate | (Basa et al. 2008) |
| | Azodicarbonamide | SLS and HPMC | 250 | Lactose monohydrate | (Bhakay et al. 2014) |
| | Fenofibrate & Itraconazole | SLS and HPMC | 200 | Gralnulac® 200 (<50 μm) & PrismaLac® 40 (<300 μm) | (Azad et al. 2016) |
| | Proprietary Novartis compound | TPGS and HPMC | 200-400 | Mannitol (150 μm) & Spray dried lactose monohydrate (150 μm) | (Bose et al. 2012) |
| | Naproxen & Proprietary Novartis compound | SLS and HPMC | 300 | Mannitol (150 μm) | (Figueras and Bose 2013) |
| Emulsion diffusion | PLGA | PVA | 198 ± 5 | Lactose (40-100 μm) & Microcrystalline cellulose (100-150 μm) | (Horster et al. 2019) |

A bead. Cinnarizine showed re-agglomeration upon release from the coating, limiting the dissolution behaviour which was solved by using a higher stabiliser concentration. Hence, a higher concentration of stabiliser may be required to keep the drug nanocrystals dispersed in the dissolution medium even if that concentration is not required to stabilise the nanosuspension (Kayaert et al. 2011). Similarly, an iraconazole nanosuspension was coated onto sugar beads (1000–1400 μm & 177–250 μm) & cellulose spheres (1000–1400 μm & 210–355 μm). HPMC was used as a coating polymer while PEG 400, was used as a plasticiser dissolved with the iraconazole nanosuspension to avoid agglomeration of the iraconazole nanocrystals, for immediate release (Parmentier et al. 2017). The iraconazole layered beads were mixed with another excipient, microcrystalline cellulose (MCC) to access the tabletability of the layered beads. The compressed tablets presented adequate tensile strength and a fast dissolution rate with a release of 99.0% (±1.0% SD) within 10 min. Hence, the compaction of nanosuspension-layered beads is a suitable process for processing an iraconazole nanosuspension into a solid dosage form, such as a compacted tablet, without compromising on drug release (Tan et al. 2017).

Basa et al. (Basa et al. 2008) prepared a ketoconazole nanosuspension using a media-milling technique, which was layered onto the watersoluble carrier (lactose monohydrate) using a fluid bed processor to obtain a ketoconazole nanoparticle formulation with enhanced bioavailability and proper systemic exposure. The tablets compressed with nanosuspension-layered carrier particles presented < 0.1% friability indicating good mechanical strength. The rate and extent of drug dissolution from tablets incorporating ketoconazole nanoparticles was significantly higher, 65% as compared with 45% for micronized and 37% for commercial formulation at the end of 60 min. Besides, there were no impact on the tablet dissolution upon storage for three months at accelerated (40 °C/75% RH) and room temperature conditions (25 °C/60% RH). Similarly, lactose monohydrate was also used as carrier particles to prepare dispersible fast-dissolving dry nanocomposite microparticles with high drug loading (40–49%) of azodicarbonamide nanocrystals (Bhakay et al. 2014).

In a similar approach, core-shell nanocomposites of fenofibrate and iraconazole were prepared for a well-stabilised high drug-loaded nanosuspension using a fluidised bed coating process (Azad et al. 2016). Both fine carrier particles (Granulac® 200 (<50 μm) and large carrier particles (PrismaLac® 40 (>300 μm)) were used for the coating process. The resulting fine carrier particles were freely flowing, had high bulk density, and had much faster, immediate dissolution of the poorly water-soluble drugs, in particular for iraconazole. This is attributed to a much higher specific surface area of the carrier and corresponding thinner coating layer for fine carriers as opposed to those for large carrier particles (Azad et al. 2016). Similarly, Figueras et al. (Figueras and Bose 2013) converted naproxen and a proprietary Novartis compound into nanosuspensions through wet media milling and successfully spray dried onto mannitol (150 μm). In-vivo studies in beagle dogs with the proprietary Novartis compound showed no significant difference between the liquid and the dried forms of the nanosuspension in terms of overall area under the curve (AUC). However, differences were observed in the maximum time (tmax) which correlated with the rank ordering observed from the in vitro dissolution profiles. This makes spray granulation an eye-catching option for the production of powders with desired processing and handling properties without compromising the enhanced pharmacokinetic profiles of the active compound in nanoparticle form. The same group also used another proprietary Novartis compound and converted its nanosuspension into a solid dosage form using a spray granulation process. Granulation with 10% drug loading resulted in a dissolution rate similar to the nanosuspension while the dissolution rate was compromised with 20% drug loading due to particle agglomeration. An in-vivo pharmacokinetic study in beagle dogs showed an 8-fold increase and a 6-fold increase in the AUC0-48 of the nanosuspension and dried nanosuspension formulations respectively compared to the coarse suspension. Also, the nanosuspension and dried nanosuspension formulations showed a 12-fold and 8-fold increase in the maximum concentration (Cmax) respectively compared to the coarse suspension (Bose et al. 2012).

In a different approach, Horster et al. (Horster et al. 2019) produced poly(DL-lactide-co-glycolide) (PLGA) nanosuspension using the electrospraying process which was later converted into the solid product using a fluidised bed coating technique with lactose (40–100 μm) and microcrystalline cellulose (100–150 μm) as the carrier particles. Fluid bed granulation with aqueous PLGA nanoparticle suspensions and soluble carriers was shown to be a simple and high yield process for drying the nanoparticles. The granules were compressed to tablets without impairing the nanoparticle diameter and the highest nanoparticle release was achieved in the presence of comparable high amounts of the filler MCC in combination with a low tablet breaking strength.

5.2.2.2. Coating drug nanoparticles onto insoluble carrier particles. In the last decade, researchers have been able to successfully produce nanosuspensions with several different active ingredients. By controlling the
rate of nucleation and growth and by employing rapid mixing, a narrow particle size distribution of particles can be achieved particularly in the nano range. Some of the above sections extensively described the processes for generating and isolating nanoparticles. The main bottleneck associated with nanoparticles is stabilisation due to their small size thereby leading to higher surface area but reduced stability (Tierney et al. 2017; Kumar et al. 2019; Kumar et al. 2020). Hence, to preserve the small particle size of the individual nanoparticles, certain stabilizers are used to control the growth of preformed nanoparticles. Stabilisers can be classified as non-ionic polymers (eg: polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polyethylene glycol, cellulose, and its derivatives, etc.), anionic polymers (eg: phosphates, albumins, alginates, etc.), cationic polymers (eg: chitosan and its derivatives, etc.), surfactants (eg: tween, span, phospholipids, etc.) (Wu et al. 2011). Stabilisers mainly wet the nanoparticles by adsorbing on the particle at a specific adsorption site thereby preventing Ostwald ripening and agglomeration of nanoparticles. This adsorption disrupts the interaction with the adjacent nanoparticle and thus eliminating crystal growth. Adsorption of the additive on the nanoparticles is facilitated either by non-covalent interactions such as hydrogen bonding, hydrophobic/hydrophilic interaction, or by electrostatic/ionic interactions (Van Eerdenbrugh et al. 2008; Liu 2013).

For long-term stabilization and convenience during oral administration, drug nanoparticles are often isolated into the solid state for incorporation into solid dosage forms such as tablets. Nanoparticle isolation methodologies as described above-comprising solvent evaporation techniques such as spray drying, freeze drying, and electrospaying, or coating techniques such as fluidised bed coating process. Though these techniques can isolate drug particles in their nano form, they can also be at times consuming, expensive, difficult to scale up, and can adversely affect the particle size (Chaubal and Popescu 2008; Matteucci et al. 2006; Abdelwahed et al. 2006). In 2014 Khan et al. (Khan et al. 2014) published the first report to isolate pharmaceutical nanocrystals using an insoluble carrier. They successfully produced the nanosuspension of ibuprofen and glibenclamide using the antisolvent precipitation process and then used dibasic calcium phosphate as an insoluble carrier particle to isolate the drug nanocrystals using the filtration approach. However, the process was notably limited by a low maximum drug loading of 0.35%, restricting its application to high potency drugs. A schematic of the production and isolation of API nanoparticles using insoluble carrier particles is presented in Fig. 12.

Filteration techniques have been largely used in the pharmaceutical industry, but can be problematic when dealing with sub-micron or nano-sized particles. Hence the adsorption/adhesion of nanoparticles to microparticles can facilitate the filtration of drug nanoparticles. As discussed earlier, the coating of drug nanoparticles onto 1–5 µm microparticles facilitates the pulmonary delivery systems by preparing effective dry powder inhaler formulations with improved rheological properties of nanoparticles (Smyth and Hickey 2005; Frijlink and De Boer 2004). Therefore, the adsorption/adhesion of drug nanoparticles to the surface of a carrier particle with a subsequent filtration step could be an efficient way to isolate drug nanoparticles with improved rheological properties.

Table 8 summarises the nanocrystals isolated from nanosuspensions using insoluble carrier particles. After the isolation of ibuprofen and glibenclamide using dicalcium phosphate by Khan et al. (Khan et al. 2014), other researchers also used a similar approach to isolate other drug nanoparticles with increased loading. Tierney et al. (Tierney et al. 2017) used protamine-modified montmorillonite (MMT) clay as carrier particles to isolate nanoparticles of BCS class II drugs, fenofibrate, and mafenamic acid with a maximum loading of 9.1% and 4.8% respectively with significantly improved dissolution. Protamine was used as a charge modifier, to modify the charges on the MMT surface to achieve a uniform coating of drugs. Protamine modification of MMT not only increased the fenofibrate loading onto the MMT surface from 4.8% to 9.1% but also increased the speed of filtration. The filtration speed of the fenofibrate system increased by 10-fold while 6-fold for the mafenamic acid system, compared to non-modified MMT. Hence, Tierney et al. (Tierney et al. 2017) developed a novel one-step, carrier-mediated method for preparing, stabilising, and isolating fast-dissolving, solid-state drug nanoparticles of poorly water-soluble BCS Class II drugs with increased drug loading. Later on, the same group extended the study to isolate nanoparticles of curcumin, clozapine base, and valsartan (Kumar et al. 2019) with enhanced loading of 21.8%, 28%, and 33.3% respectively using protamine modified MMT as a carrier (Kumar et al. 2020). Fig. 8 provides a schematic path to produce and isolate valsartan nanoparticles from the nanosuspension using MMT or protamine modified MMT as carrier particles. Further, they also isolated dalcetrapib (Bodnár et al. 2020) and carbamazepine (Kumar et al. 2020) nanoparticles with a higher drug loading of 20.9% and 33.3% without modifying MMT with protamine. The Hudson research group founded a correlation between the zeta potential of API nanoparticles and the surface properties of the carrier particles to obtain high drug loading onto carrier particles. They concluded that the API nanoparticles with zeta potentials more negative than ca. – 25 mV require a carrier particle surface modified with protamine to obtain a uniform coating of nanoparticles onto the carrier surface and to maintain the fast dissolution from nanoparticle-carrier composite at higher API loadings (Kumar et al. 2020). Hence, this nanoparticle generation and isolation process provides huge potential to produce a fast dissolving solid dosage form of poorly soluble APIs.

Fig. 12. Schematic of the production and isolation of API nanoparticles using insoluble carrier particles.
6. Conclusions and future perspective

The goal of this review is to provide a summary of existing technologies to produce and isolate dry nanoparticles. A range of different one-step and multi-step techniques has been shown effective to generate and stabilise nanoparticles of pharmaceutical drugs. The isolation of nanocrystals has been achieved by either coating or encapsulation with the aid of GRAS (generally regarded as safe) approved carriers or matrix materials. The nanocrystal isolation approaches developed largely ignore the processing steps of granulation, bead layering, and tabletting. Further research is needed to deepen our understanding of the downstream processing of isolated drug nanocrystals, which can greatly influence the contribution to the future application of drug nanocrystals for oral delivery purposes.

Nanosizing means achieving a particle size < 1 µm (Junyaprasert and Morakul 2015). It is well understood in the literature that a decrease in the particle size of drugs increases its surface area and thus proportionally an increase in the dissolution rate and the saturation solubility. Drug nanocrystals with increased dissolution also improve the bioavailability and pharmacokinetic properties of poorly soluble drugs that are administered through various administration routes such as oral, ocular, dermal, pulmonary, and buccal (Al-Kassas et al. 2017). In addition to improving solubility, nanocrystal formulations also offer decreased toxicity and increased efficacy because of passive and active targeting (Allen and Cullis 2004).

Nanosuspension technology has offered a viable approach to formulate poorly soluble drugs with increased bioavailability leading to a decrease in the dose and subject-to-subject variability. However, due to the potential risk of physical instability (such as agglomeration and Ostwald ripening) and chemical instabilities (such as hydrolysis and oxidation), liquid nanosuspension is converted to solid dosage forms such as tablets, capsules, pellets, and granules. This further improves the sampling and handling of the produced nanopowders, which are particularly challenging downstream issues. While the nanocrystal technology is attractive due to ease of formulation and macroeconomic values, it also has the potential to overcome the biggest challenges of drug development. The factors influencing the clinical efficacy of nanocrystal drugs include size, morphology, surface charge, degree of dispersability, and site-specific targeting. Therefore, meticulously studying the above-mentioned factors will enable nanocrystal drugs to open up new frontiers in the field of therapeutics. Developing crystalline products with a size below 100 nm will provide an add-on advantage towards avoiding renal clearance and sequestration by the mononuclear phagocytic system. Thus, nanocrystals bear the potential to become a benchmark therapeutic platform for cancer and other diseases. In addition, the ability to develop nanocrystal products of the off-patent drugs for clinical use will provide a competitive edge for pharmaceutical companies in the market.

Despite the impressive methods and nanosizing technologies available to the pharmaceutical industries, only a few formulations containing drug nanocrystals have been able to reach clinical trial phases or even be approved by regulatory agencies. One of the many reasons is that the nanosizing principle is a complex process in comparison to providing simple pre-clinical formulations, and many pharmaceutical companies lack sufficient in-house capabilities in terms of technology and expertise to develop nanocrystals for clinical studies. Typically, nanosizing of poorly soluble APIs needs to be outsourced to specialised contractors, who often own IP on the production technology and have the necessary know-how in the manufacture of such nanoformulations. The other reason is that nanosized formulations have to compete with products of other enabling technologies that can be easily produced using in-house facilities that are already established (Müller and Keck 2012). Other reasons are linked to poor rheological properties of nanoformulations that still need to be addressed to design novel nanomedicines (Bobo et al. 2016). This could potentially be managed by a synergistic interaction and communication between researchers with different backgrounds.

CRediT authorship contribution statement

Vivek Verma: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. Kevin M. Ryan: Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. Luis Padreia: Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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