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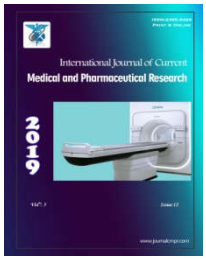

Research Article

CRITICAL VARIABLE PARAMETERS AFFECTING INDUSTRIALLY RELEVANT BOTTOM-UP TECHNIQUES OF NANOCRYSTAL FORMULATION

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ABSTRACT

The solubility dependent bioavailability problem has become a major hurdle in drug development processes. Drug nanocrystals have been widely accepted by the pharmaceutical industry to improve the bioavailability of poorly water-soluble drugs. Poorly water soluble drugs show many problems in formulating a dosage form. Micronization does not sufficiently to enhance bioavailability and so next step is nanocrystalization i.e. drug nanocrystals having size of 1-1000 nm whose main application is to enhance the saturation of solubility, rate of dissolution and bioavailability of poorly soluble or poorly permeable drugs. Pure drug nano crystals, generated by "bottom up" or "top down" technologies, facilitate a significant improvement on dissolution behavior of poorly soluble drugs due to their enormous surface area, which in turn lead to substantial improvement in oral bioavaibility**.** In this review article we are focusing on Bottom-up techniques due to its simplicity and more applicability towards industrial use. The final product from this process is a suspension of drug nanocrystals in a liquid stabilized by a surfactant or polymer so called nan-suspension and after solidification of nano-suspension, nano-crystals can be formulated. The main objective behind this review articles was to provide bottom-up techniques for the preparation of nano-crystals with various critical parameters which affects the process, the final particle size and stability of nanocrystal.

INTRODUCTION

Most of the APIs are identified through combinatorial screening program are poorly soluble in water. Enhanced solubility and dissolution, improved bioavailability and absorption, elimination of food effects, safe dose escalation, enhanced safety, efficacy and tolerability profiles are the inherited advantages of nanoparticles due to their size and surface features. Low bioavaibility of poorly soluble drug becomes obstacle for oral drug delivery system and to solve these problems, many approaches had been developed like solubilization by surfactants, complex formation, micronization of drugs and among those micronization method was more effective approach in which drug particle size ranges between 1 to 10 µm. Micronization leads to increased surface area and consequently leads to increased dissolution velocity up to certain extent this approach was helpful for increasing the surface area and thus dissolution velocity but it was not sufficient to overcome the bioavailability problems of very poorly soluble drugs. Micronization results in increase surface area of the particle which will ultimately going to enhance the dissolution rate but saturation solubility does not increases, thus bioavailability get affected. To overcome limitations of micronization, nanocrystal approach can be used. Drug nanocrystals (NCs) are the nanoparticles which offer an additional advantage of 100% drug loading since they are encapsulating-carrier free nanoparticles. The formulation simplicity and production scaling flexibility along with their intrinsic small particle size and large surface area make NCs stand a way unique not just among the pharmaceuticals but also among other nanoparticles. The consequent next step was to move from micronization to nanonization. Nanocrystals was more efficient approach to increase drug solubility and dissolution velocity.[1]

Nanocrystal are the crystals having size less than 1 µm. Smaller the particle size more will be the surface area which will enhance the solubility and bioavaibility of poorly soluble drug i.e. BCS class II or class IV, ultimately loading dose in the nanocrystals will reduced and patient compliance will increased. Nanocrystals leads to increase in dissolution rate and rapid absorption.

For preparation of the drug nanocrystal, first the nanosuspension is prepared and then it is converted into the nanocrystal. Conversion of liquid nanosuspensions into the solid dosage forms, such as capsules, tablets, granules and pellets, can help to avoid the potential physical instabilities (Ostwald ripening and agglomeration) and chemical instabilites (e.g. oxidation and hydrolysis). Also improve the commercialization of the product and allow better handling.[4] There are two methods for solidification of the nanosuspension into the nanocrystal. There are two types of drying

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technologies for solidifying the nanosuspensions to get the nanocrystals. First method is drying of the nanosuspension to a nanocrystal powder using oven drying, lyophilisation or spray drying, and then further formulation of the powder proceed to different dosage form such as capsules or tablets. The second method of solidification, includes pelletization or granulation, this method is the combination of shaping and drying processes together. The nanosuspension is used as layering dispersion on solid powder in a fluidized bed process or in a binder solution along with binder in the granulation processes.[4] Water or any other dispersion media is removed during the drying process from the nanocrystals suspensions by sublimation or evaporation and the motions of the stabilizer chains around the nanocrystals are decreased because of the possible partial crystallization or dehydration. The steric repulsion supplied by the stabilizer ceases and then the polymer chains adsorbed on the particles can entangle each other, which hinder particle separation and prompts particle aggregation. In addition, the contact points among nanocrystals are increased with water removal. In the drying process mechanical stresses (such as crystallization of ice, the stress of freezing and dehydration) and additional thermal stresses occurs, further nanocrystals are subject to reversible agglomeration or irreversible aggregation/fusion. The agglomeration/aggregation can compromise the advantages of nanocrystals based on the large surface area, e.g. decreased dissolution rate, the lag time in the beginning of the dissolution, and unpredictable variations in bioavailability.[5] Therefore, re-dispersibility of dried nanopowders in the dissolution medium with a short time (a few minutes) is an important standard to evaluate the drying step. The parameters of the drying procedure, the properties of drug compounds, the choice of dispersed matrix and stabilizer can affect the nanocrystal re-dispersibility. In freeze drying process, fast freezing and low API concentration are beneficial for re-dispersibility.[6] In spray drying process, high gas flow rate and feed temperature lead to formation of donut shaped particles.[7] Spray drying at high inlet temperature causes powders with higher lag time as well as lower dissolution rates.[5] Eerdenbrugh *et al.*[8] showed that regardless of the drying methods or other properties of drug compounds, such as molecular weight, melting point, solubility and density, the hydrophobicity of drug surface, which in general corresponds to log P values (partition coefficient) of the drug, is a key parameter for nanocrystal redispersibility. More hydrophobic compounds resulted in harder–to–disintegrate agglomeration. Chaubal *et al.*[5] showed that using a charged surfactant as a stabilizer and sugar as excipient can effectively prevent irreversible aggregation compared to non-charged polymeric surfactants and sugars. Excipients are usually added during the freeze drying process to protect the product from freezing stress (cryoprotectant) or drying stress (lyoprotectant).[9] The commonly used excipients include water-soluble sugars such as mannitol[10], sucrose[11], trehalose[12, 13] and water-insoluble microcrystalline cellulose (MCC) as a matrix-former to protect the nanocrystals from aggregation[14]. The results showed that the reconstituted nanocrystals dried with excipients had smaller mean particle size than the ones dried without added lyoprotectant. The preferable amount of cryoprotectant used is about 2%-25%, based on the total weight of the nanoparticulate suspension.[15] Sometimes dried powders without any excipients exhibit good re-dispersibility since stabilizers used in nanocrystal production act lyo/cryprotectant in a certain extent.

Bottom-Up Technique

Nanoprecipitation

There are two crucial steps included in the bottom-up process which grows nanocrystals from solution, these are nucleation and consequent crystal growth. Nucleation is very important to achieve uniform and small nanocrystal. In some cases nucleation rate may increase, which results in the increased number of nuclei formed from the supersaturated solution, leading to decreased supersaturation. If a large number of nuclei are produced in the nucleation stage, it results in a narrow particle size distribution.[17] Therefore, it is essential to promote homogeneous and rapid nucleation in the bottom-up process. Nucleation can be triggered by either mixing with removal of solvent or antisolvent.[17,18] The mixing of drug solution and anti-solvent can be done with use of any conventional mixing equipment, i.e. by using overhead mechanical stirrer or magnetic stirring.[19]

To promote the higher nucleation, sonication with the help of probe sonicator or bath sonicator can be used to provide cavitation effects, which is called as sonoprecipitation[20,21]. Some highly efficient mixing equipments can also be used to prepare nanocrystals, which includes static mixer,[22] confined impinging jet reactor[23–27] and multiple inlet vortex mixer[28] these instruments can be used for intense micro-mixing between the two fluids which can be fulfilled in some milliseconds only.[29-30] A homogeneous solution with high supersaturation can be achieved for the onset of nucleation, which favours small nanocrystals having narrow size distribution. Freeze drying and spray-drying are common methods to remove solvent. Recently, controlled crystallization during freeze-drying techniques[31] and spray-freezing into liquid[32–35] also has been developed to prepare nanocrystals by removal of solvent. Supercritical fluid (SCF) can be used to prepare nanocrystals by taking advantage of the unique physical properties of SCF, with solubilisation like liquid and diffusivity like gas. In addition, SCF can be removed very quickly and efficiently at low temperature without excessive drying and nanocrystals can be easily precipitated. Supercritical carbon dioxide (SCO_2) is the most favoured SCF due to the mild critical point (31^0C and 73.8bar) and low environmental impact. Depending on the solubility of a compound in SCO_2, nanocrystal preparation can be achieved by rapid expansion of SCO_2 from drug solution,[36] or by precipitation using SCO_2 as the antisolvent.[37–39]

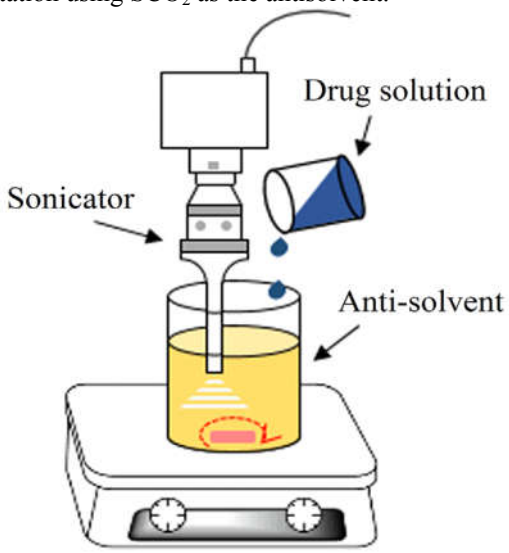

Figure 1 Diagram showing process of nanoprecipitation[16]

Critical Variable Parameters Affecting Nanoprecipitation

Effect of the drug concentration

Optimum drug concentration is required to get the desired smaller particle or nanocrystal size. Concentration above the optimum level results in generation of lager particle size. Higher drug concentration is responsible for higher super saturation which results in a higher rate of nucleation and thereby smaller size particles are obtained but, at the same time, the growth of nuclei is also increased due to the higher super saturation. Second, due to increase in the drug concentration, viscosity gets increases, which will hinder the diffusion between anti solvent and solvent, which leads to slower nucleation rates, non-uniform super saturation and increased particle agglomeration, and hence, larger size nanocrystals are obtained.[40-42]

Ying Lu *et al* have developed paclitaxel nanocrystal of size less than 300nm in that process different trial batches have been taken with different concentrations of paclitaxel in the solvent which is shown in the table (Table 1) along with the average particle size, In the optimization process he found that 3 mg/ml paclitaxel in ethanol is the optimum concentration required to meet the minimum requirements. By using the drug concentrations above this level results in increase in the particle size. This increase in the particle size had been observed as the drug concentration in solvent increased which was due to the higher level of supersaturation which reduces the diffusion rate of crystal nuclei between solvent and antisolvent and increases the particle growth. So the data generated due to these trial batches as shown in the table 1 supports the above statement[43].

Table 1 effect of paclitaxel concentration on average particle size of nanocrystal[43]

Paclitaxel concentration	Average particle size (nm)
1 mg/ml	296 ± 12
3 mg/ml	268 ± 8
5 mg/ml	1670 ± 89
9 mg/ml	4421 ± 144
12 mg/ml	4358 ± 103

Solvent to Anti-solvent ratio

The relative ratio of anti-solvent to solvent have major effect on the particle size property of nanosuspension or nanocrystal. Particle size gets decreases with an increasing ratio of antisolvent. Increased ratio of antisolvent to solvent, results in higher or increased degree of super saturation which increases the rate of nucleation and results in a decreased particle size. Once the nuclei are formed, particle growth occurs which is partially hindered at higher anti-solvent volumes as the diffusion distance for the growing species increases and the process becomes diffusion limited.[40-42,44]

Ying Lu *et al.,* have developed paclitaxel nanocrystal formulation of size less than 300nm in that process different trial batches have been taken with different solvent to antisolvent ratio as shown in the (table 2) with the average particle size obtained. An antisolvent to solvent ratio of 20:1 was found to be necessary in case of paclitaxel to get nanocrystals that met the requirements. Below this ratio the nanocrystal size got increased significantly. The explanation for that was given by Ying Lu *et al* that at a same drug content in the formulation, the degree of supersaturation got increased when there was increased ratio of antisolvent to solvent. A higher degree of supersaturation results in a greater nucleation rate, which causes smaller particle size. In case of low antisolvent to solvent ratios, the rate of nucleation was higher but the effect is demolished due to the increase in the growth due to higher solvent phase ratio, and the kinetics favour overall particle growth. Above the ratio of 20:1 of antisolvent-to-solvent, there was no further decrease in nanocrystal size was seen. This was most likely due to the equilibrium between nucleation and growth processes.[43]

Table 2 effect of solvent to Anti-solvent ratio on average particle size (nm)[43]

Antisolvent–solvent ratio	Average particle size (nm)
40:1	291 ± 10
20:1	287 ± 8
10:1	518 ± 19
5:1	746 ± 25

Stabilizer

It is the key challenge in the nanoprecipitation process is to retain the nano size of the particles in nanosuspension. In Ostwald ripening phenomenon, it is stated that, smaller particles are more soluble than large ones that's why material transfer occurs from the fines or smaller particle size to the coarse or larger size particles, coarse particles grow and fine particles redissolve. Moreover during the antisolvent precipitation, surface Gibbs free energy of the newly formed nanoparticles gets increased and due to which the particle undergoes aggregation to reduce Gibbs free energy which compromises with the advantage of higher saturation solubility and bioavailability, and faster rate of dissolution.[44-46]

Stabilizers gets adsorbed on the surface of the newly formed particles which prevents the Ostwald's ripening and agglomeration of nanocrystals in the nanosuspension and gives a physically stable formulation by providing ionic or steric barrier. The amount and type of stabilizer has a major effect on in vivo behaviour of nano suspension and physical stability. Steric and/or electrostatic techniques are the common approaches for stabilization of nanosuspension. In electrostatic stabilization, ionic surfactants (such as sodium lauryl sulfate (SLS) and soya lecithin gets adsorbed on the particle surface of nanoparticles and this electrostatic repulsion and surface charge prevents the aggregation of Nano sized particles. Steric stabilization can be achieved by adsorbing polymers (for example hydroxyl propyl b cyclodextrin (HP-b-CD), hydroxyl propyl methyl cellulose (HPMC) and D-a-tocopherol polyethylene glycol 400 succinate (TPGS)) or nonionic surfactants (for example Poloxamer 188) on the surfaces of drug nanoparticles to give a dynamically rough surface to prevent coalescence by repulsive entropic forces.[44-46]

In some cases use of surfactants or hydrophilic polymers may improve physical stability of nanocrystals. In most cases due to presence of polymers and surfactants in the antisolvent phase causes in an increased size of the nanocrystals as compared to the pure antisolvent. Presence of surfactants and polymers in the antisolvent increased the solubility of the drug in water. The solubility enhancing property of surfactants and polymers has been commonly seen in the formulation of poorly water-soluble drugs. The enhancement of drug solubility in the antisolvent causes reduction in the solubility difference between the drug in the antisolvent and the solvent phases, which associated with a decreased rate of precipitation. In addition, increased solubilization could play a role in enhancing nanocrystal particle size growth by Ostwald ripening.[47]

Ying Lu *et al* have developed paclitaxel nanocrystal formulation with the aim of formulating the nanocrystal of size less than 300nm in that process different batches have been taken with different stabilizers which resulted in different size of nanocrystals as shown in the table 3. Ying Lu *et al* tried 8 different stabilizers including HPMC, PVP, PEG400, Pluronic F68, Pluronic F127, Tween 20, Tween 80, and SDS with the same concentration and he had observed a lot of variation in the average particle size as shown in the table 3.[47]

Table 3 Effect of different stabilizers at same concentration on average particle size (mm)[47]

Stabilizer	Average particle size (nm)
HPMC	490 ± 19
PVP	514 ± 23
PEG400	303 ± 11
Pluronic F68	330 ± 18
Pluronic F127	278 ± 10
Tween 20	353 ± 14
Tween 80	290 ± 12
SDS	379 ± 21

Effect of the stirring speed

Stirring speed is the important critical parameter which usually affects the particle size. Increase in the stirring speed of solution results in decreased particle size, which may be due to higher intensity of micro mixing. High micro mixing causes increased mass transfer between the multiphase and rate of diffusion and thus high homogenous super saturation in less time is achieved which causes rapid nucleation and resulting into generation of smaller particles size. Also the growth of the particle is also reduced at high stirring speed. Thus smaller and narrow particle size distribution is achieved at higher stirring speed.[40]

Zhengxi Zhu et al have formulated Polyelectrolyte Stabilized b-Carotene Nanoparticles via Flash Nanoprecipitation, during formulation development he has taken trial batches at different stirring speed and from these studies he has concluded that as the stirring speed gets increases the particle size gets decreases up to a certain speed of stirring which is called as critical stirring speed but increase in the speed above the critical do not causes to further decreases in the particle size, and particle size remains same.[47]

CONCLUSION

The formulation of poorly soluble drugs is a challenging problem which can be solved by nanocrystal or nanosuspension formulation. Nanosuspension formulation is most of the times not stable due to Ostwald ripening or agglomeration. These stability problems can be solved by conversion of nanosuspension formulation into nanocrystal formulation by oven/tray drying, spray drying or lyophilisation. The drug NCs being unique nanoformulation options with notable advantages, the researches encompassing scalable formulations/methods for the production of drug NCs open a new vista and pave a new platform for the design of these nanopharmaceuticals. In the present article, so many critical variable parameters affecting nanocrystal formulation by bottom-up process are mentioned. By controlling these critical variable formulation parameters, desired size of nanocrystals can be formulated with less variation per batch with highest robustness.

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