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Review

Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods

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Abstract

Objectives Wet milling is a common technique to produce drug nanocrystals. Stability of the nanocrystals is a critical question, and different kinds of stabilizers, e.g. polymers, celluloses, surfactants and lipids, have been tested for various drugs. Still, the question about how to select the best stabilizer to a certain drug material and also to a selected process is open.

Key findings Many different factors, such as surface energy, hydrophobicity, solubility, viscosity and functional groups, affect the stability of the formed nanosuspensions. Affinity of the stabilizer to the particle surfaces seems to be the most important parameter. This affinity is partly related to the surface energy and hydrophobicity of the surfaces and stabilizers.

Summary In this review the most important factors affecting nanocrystal formulation and efficacy of stabilizers are presented. In order to widen understanding of the milling process, the most important variables related to milling techniques and particle fracturing processes during the milling are briefly presented.

Keywords nanocrystals; stability; wet milling

Introduction

Drug nanocrystals are nanoparticles containing 100% drug without any matrix material. A stabilizing agent is situated on the surface of the drug particles. The drug delivery route and its limitations should be taken into account in the selection of the stabilizer. According to the definition of nanoparticles, the mean particle size is below 1 μm, but typically nanocrystals are sized between 200 and 500 nm. Depending on the purpose, the preferred particle size of the end product may differ.[1–3]

Nanocrystallization techniques are used to increase the dissolution rate and thus the bioavailability of poorly soluble drug materials. The dissolution rate is increased by decreasing the particle size. Many different techniques exist for the production of nanocrystals, the most commonly used of which are precipitation,[4–6] pearl milling[7–9] and high pressure homogenization.[2,10,11] So far, four products, namely Rapamune (sirolimus, Wyeth), Emend (aprepitant, Merck), TriCor (fenofibrate, Abbott) and Megace (megestrol acetate, Par Pharmaceutical) have been commercialized.[12] All these techniques utilize Elan's NanoCrystal technology, which is a media milling technique. Invega Sustenna (paliperidone palmitate, Janssen), also based on NanoCrystal technology, has been approved by the US Food and Drug Administration. One commercial product, Triglide (fenofibrate, Skye Pharma), is based on a high-pressure homogenization technique.[13]

Although the products formed by the different techniques are nanosized particles covered with a stabilizer, the amount of a stabilizer and the efficiency of stabilization of a system differ considerably when using the same materials in different processes.[14,15] This indicates that the stabilizing method and attachment of the stabilizer on the particle surfaces is important in the particle formation processes. Accordingly, it is very important to understand the particle formation process in order to be able to select proper stabilizer(s) for successful nanoparticle formulations. Differences exist even between various top-down or bottom-up techniques. This means that the stabilization mechanisms are not dependent on whether the particles are formed by reducing the particle size or building up structures molecule by molecule. For example, in precipitation techniques, the basics of which are

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closely related to emulsion formation, the hydrophilic–lipophilic balance (HLB) value of the stabilizer correlates well with the stabilization of the formed nanocrystals.[15] In high pressure homogenization, the nanocrystal size resulting from the process depends not only on the hardness of the drug material, but also on the homogenization pressure and cycle number. It does not, however, depend on the type of stabilizer.[11] Thus, the efficiency of the stabilizer – whether or not it is able to prevent aggregation of the particles – is shown after processing and during storage. However, in this process, the stabilizing effect is related to the affinity of the stabilizer for the particle surface. Accordingly, it is extremely important to understand the phenomena involved in particle formation in the particular process used.

Stabilization of nanocrystals is based on two basic mechanisms: steric stabilization and charge or electrostatic stabilization. It is possible to combine chemical functionalities within the same molecule to achieve both steric and electrostatic stabilization, also referred to as electrosteric stabilization. Electrosteric stabilization can also be provided by the use of a combination of two different stabilizers. Combination of more than one stabilizer has sometimes been preferred for enhanced long-term stability.[1,16] If the stabilization is based purely on the electrostatic effect, the eigenvalue of the zetapotential of the nanocrystals should be at least 30 mV. Nonionic materials (surfactants, polymers and so on) stabilize nanocrystals by the steric effect while ionic surfactants and polymers stabilize the system by electrostatic action or, depending on the molecular weight (chain length), by electrosteric action.

The particle size of a drug usually decreases to a steady state value over time, depending on the kind of stabilizer and also the drug material. Materials with high crystal energy (high melting point) and high molecular weight are the best candidates for nanocrystallization, as they benefit the most from the decreased particle size.[16,17] Materials that benefit the most from nanocrystallization are those with solubility less than 200 μg/ml.[18]

It is clear that different drugs require different stabilizers. This has also been briefly pointed out in the excellent earlier reviews on the various aspects of nanomilling.[16,19–21] Still, the questions of how to select the best stabilizer and preparation process for a certain drug remain open. Lack of systematic understanding on how to select a proper stabilizer (and the amount of stabilizer) means that researchers and the pharmaceutical industry still use the trial-and-error method. This review presents the most important findings related to these questions. Also, the principal phenomena behind particle fracturing during the process of nanomilling and particle formation are presented.

Process variables in nanomilling

Nanomilling is an efficient way to produce nanosized drug materials. It is also very cost-efficient; with modern mills the milling times may be only a few minutes (Figure 1) and scale-up is also possible. Most of the nanocrystalline products that have reached the market so far are produced by the wet-milling technique.[21,22]

During nanomilling, high energy and shear forces are generated as a result of impaction of the milling medium with the drug. This provides the necessary energy input to disintegrate microparticulate drug particles into nanosized particles. In the process the milling chamber is loaded with the milling medium, water or a suitable buffer, the drug and the stabilizer. The milling medium and milling pearls/beads are rotated so as to produce a very high shear rate. Once the formulation and the process are optimized, very little batch-to-batch variation is observed in the quality of the dispersion.[23]

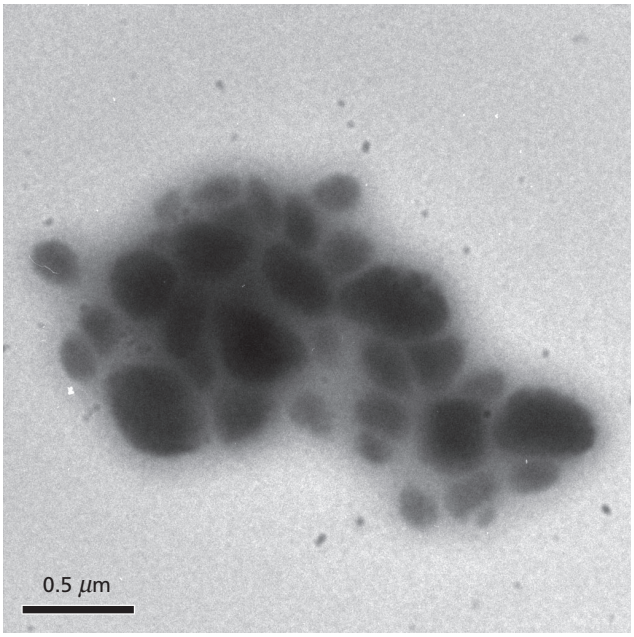

Figure 1 Transmission electron microscopy figure of wet-milled indometacin after total milling time of 12 min. Milling was performed by Fritsch Pulverisette 7 in aqueous Tween 80 solution with 1100 rpm.

A fine nanocrystalline nanosuspension can be obtained through pearl milling progressing for periods ranging from hours to several days, depending on the drug hardness, quantities and the requested particle size for different administration routes.[24] The progress can be performed in either batch or recirculation modes. Critical parameters for nanomilling to obtain optimal products have been found to include drug amount, number and size of the milling pearls, milling speed, milling time and temperature (Table 1).

The values for these critical parameters may vary considerably. Typically, the amount of drug in the milling chamber is rather low, from 2 to 30%(wt),[9,17,18,25–29] while the number/volume of the milling pearls/beads is rather high, 10–50% of the weight/volume of the slurry. The size of the nanomilling pearls is constant, between 0.5 and 1.0 mm.[9,17,25–27,29,30] The milling times and speeds required to obtain nanocrystals of the desired size range vary considerably. Nanocrystals are obtained either by low milling speeds (80–90 rpm) and long milling times (1–5 days)[7,9,18,28,29] or high milling speeds (1800–4800 rpm) and short milling times (30–60 min).[17,25,26,30,31] Thus, depending on the mill type and batch volume, the nanomilling process may last from hours to several days and up to a week.[32] This obviously may limit the utilization of the

Table 1 Parameters investigated to obtain optimal nanocrystal formulations by wet ball milling

Parameter	Range	References
Drug amount	8%(wt)	[17,25,26]
	1 g in 5 ml	[9,27]
	16%(wt)	[28]
	2%(wt/vol)	[18]
	15–30%(wt/vol)	[29]
Amount of milling pearls	10%(wt) of the slurry	[17,25,26]
	15 g in 5 ml	[9,27]
	50%(vol) of the slurry	[28]
	50%(wt/vol)	[29]
Size of milling pearls	0.5–1.0 mm	[30]
	0.5 mm	[17,25,26]
	0.5 mm	[9,27]
	0.6–0.8 mm	[29]
Milling speed	2000 rpm	[30]
	4800 rpm	[25]
	1800–4400 rpm	[31]
	7 out of 10 in intensity scale	[27]
	84 rpm	[28]
	57% of critical speed	[18]
	80–90 rpm	[29]
Milling time	30 min	[17,25,26]
	40–60 min	[31]
	24 h	[9]
	5 days	[28]
	Up to 4 days	[18]
	3–4 days	[29]

methodology because very long milling times may lead to increased contamination risks, unwanted degradation or stability problems during milling, and increased costs.

Benefits of nanomilling include the facts that poorly soluble drugs (in both aqueous as well as organic media) can be easily formulated into nanosuspensions. The process is easily scaled-up, providing little batch-to-batch variation (narrow size distribution of the final product).[23] Major concerns of nanomilling include possible generation of residues of the milling media in the final product due to erosion. In particular, it is important to take into account contamination problems due to longer milling times. Because of this, milling devices, especially the milling pearls/beads, need to be manufactured from highly resistant materials. Milling beads can also be coated in order to avoid erosion.

Changes in the physical form or amorphization are also possible during the milling.[33,34] Mechanical pressure above certain critical pressure values increases lattice vibrations, which destabilize the crystal lattice. The number of defects increases, and transformation into an amorphous state occurs above a critical defection concentration, where the amorphous state is more stable than the disordered crystals.[35] Thus, the formation of amorphous regions during the milling is closely related to the properties of individual drug(s), stabilizer(s), possible interactions between them and process parameters. However, unlike in dry milling processes, during wet milling of crystalline drugs the water may behave as an inhibitor of the formation of amorphous material due to the reduced glass-transition temperature (Figure 2).[33] Formation of amorphous regions in nanocrystals is often undesirable because they cause poor stability. The number and location of amorphous regions is also very difficult to control. However, in some formulations amorphous regions have been utilized successfully by stabilizing these regions in a carrier system, e.g. inside a polymeric network.[36]

The nanomilling process is carried out under controlled temperature conditions, which are crucial, especially for thermolabile drugs and drugs with a low melting point.[24] During milling, the temperature in the milling chamber is increased. Accordingly, temperature-related stress relaxation by intraparticle crack formation, following crack propagation and later particle fracturing, has been proposed as one possible mechanism for the diminished particle size.[37]

Particle fracture during nanomilling

During milling, two opposite processes are interacting in the milling vessel: fragmentation of material into smaller particles and particle growth through interparticle collisions.[30] The occurrence of these two opposite phenomena is dependent on the process parameters.[38] Often after a certain time point the particle size has achieved a constant level and continuing the milling does not further decrease the particle size. In some cases an increase in grinding time may even lead to a gradual increase of particle size and heterogeneity of the material, while decreased particle sizes are achieved with decreased milling speeds (Figure 3).[17,39]

Particle surfaces can be mechanically activated during milling.[40] Crystal defects are also shown to be formed due to the disordering of the crystal surfaces.[39,41,42] Local amorphous regions formed during milling increase the surface energy of the system. These high-energy surface areas may result in both physical and chemical instability of the end product during storage.[15]

Energy consumption for efficient fracturing is related to the hardness and particle size of the milled material and the type of applied stress. Crystal lattices are cleaved at the weakest sides, which have the lowest attachment energy.[43] When the particle size is decreased, the hardness of the material is increased and the number of crystal defects, which are often the brittle parts of the particles, is diminished. Crystal defects can be caused by, for example, dislocations or impurities. Crystal defects affect the dissolution rate of the material and, hence, the properties of the end product.[44] Dissolution of crystal faces starts from emerging dislocation lines, which are thermodynamically unstable areas and form the core of distorted regions in the crystal lattice.[45]

In (nano)milling, a typical assumption is that the rate of break-up of particles is proportional to the rate of collision of the particles with the milling beads/pearls.[30] The collision rate of particles in dilute systems is often described by the kinetic theory of gases. However, in (nano)milling, the number of beads/particles does not represent a dilute system, and this affects the fragmentation rate of particles, the 'collision efficiency' or the fraction of collisions resulting in fragmentation. When a particle breaks, two or more fragments are formed, with mass (and hence volume) being conserved. The volume of the fragments is therefore the same as the volume of the parent

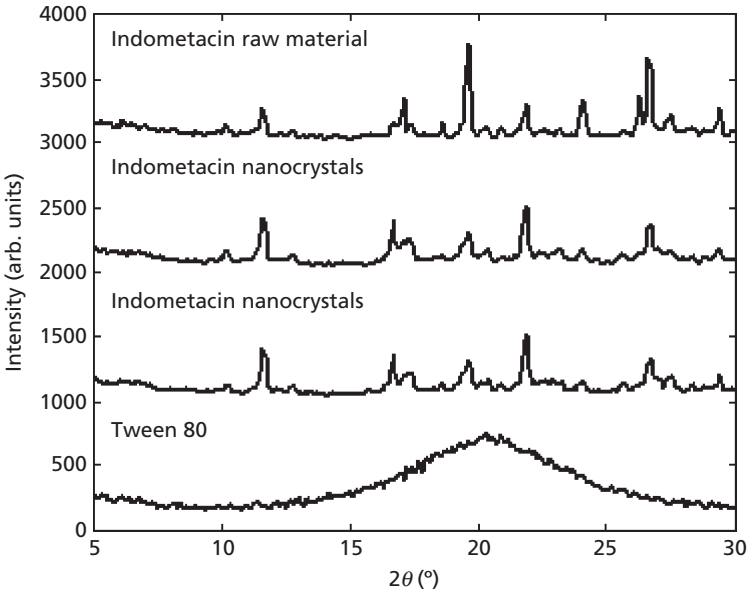

Figure 2 X-ray powder-diffraction patterns from nanocrystalline indometacin. The crystalline form of indometacin remains unaltered after the nanomilling. Milling was performed in aqueous Tween 80 solution by Fritsch Pulverisette 6. X-ray diffraction patterns were measured using an X-ray powder diffraction (XRPD) theta-theta diffractometer (Bruker axs D8, Germany). The XRPD experiments were performed in symmetrical reflection mode with CuK_α radiation (1.54 Å) using Göbel mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. The angular range was from 5 to 30° with steps of 0.1° and the measuring time was 5 s/step.

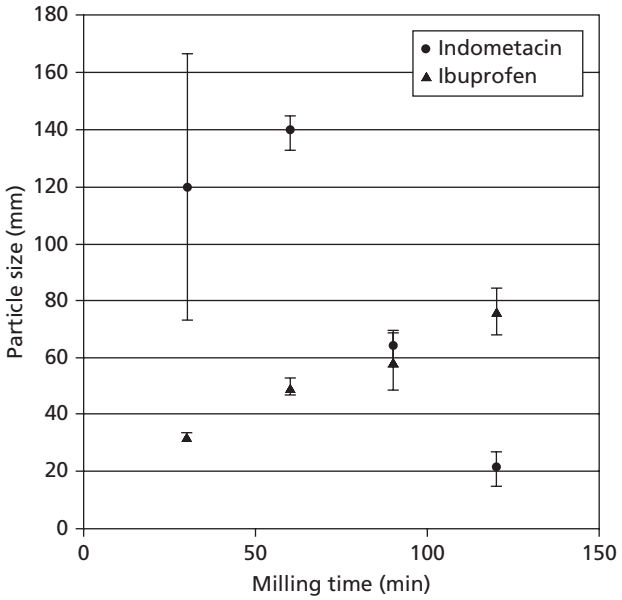

Figure 3 Effect of milling time on particle size of ibuprofen and indometacin. Longer milling times slightly increased the particle size of ibuprofen, but indometacin benefited from longer milling. Before size analysis by photon correlation spectroscopy, samples were filtered through a 0.45 μm filter. Milling was performed by Fritsch Pulverisette 6. Milling speed was 600 rpm and milling was performed in aqueous Tween 80 solution ($n = 4$).

particle. The uniform probability distribution allows fragments of all sizes to be formed with equal probability. The physical interpretation of this is that the larger the particle is, the larger the number of potential fragment sizes it can break into and, therefore, the smaller the probability of occurrence of each fragment size.[30] A reduction in mill speed reduces the rate of size reduction. At smaller bead sizes, the rate of size reduction is increased, an observation that is attributed to increased collision frequency of the small beads with the particles due to the larger number of beads.

Friction and fracture processes contribute (at maximum) to the transformation of 84–93% of the mechanical energy into dislocation and distortion energy of the crystalline lattice elements.[40] Crystal imperfections further impair the material under mechanical stress. Hüttenrauch *et al.*[40] describe two categories of instability and disorder: highly excited short-living states (10^{-7}–10^{-3} s), which cause the activation and destruction of solids by fracture, and longer lasting (10^{-3}–10^{6} s) metastable activation states with lower energies to provide, for example, vacancy healing. The magnitude and importance of the two mechanisms depend on the material and reaction (milling, tabletting, etc.) conditions.

The state of activation is characterized by the thermodynamic enthalpy and entropy of the material(s) and conditions. The ability of the system to absorb mechanical energy seems to saturate over time.[40,46] With advancing disorder (diminished drug particle size in the case of nanomilling), the creation and storage of distortion and dislocation energy input is increasingly difficult to achieve, and a stage will be reached at which no further energy transfer takes place.

Stabilizers used in nanomilling

Nanoparticles are controlled by surface forces and if the particles are not stabilized, they may coagulate because of the high particle mobility. Stabilization is achieved by tailoring the particle surfaces, for example through repulsive

double-layer forces.[47] During nanomilling in stirred ball mills, the mean particle size can be controlled by either the mixing intensity or the surface charge density of the particles. In stirred media mills, particles as small as 10 nm can be achieved by stabilizing the particles appropriately.

Surfactants (nonionic and ionic) as well as polymers have been used as common stabilizers (Table 2). The stabilizer effect of ionic surfactants is due to the formation of surfaces with a charge sufficient for stabilization. However, the presence of other charged materials can lower the surface charge of the particles, and decreased electrostatic repulsion may lead to agglomeration.[48]

Nonionic stabilizers do not have charges associated with them and according to the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory do not provide a significant repulsive barrier against agglomeration. Instead, their function as a stabilizer is based on steric effects: physical barriers on the particle surfaces prevent close contact between particles and further hinder the van der Waals' attractive forces between particles. Polymeric stabilizers also form steric barriers. Compared to polymers, nonionic surfactants typically have higher adsorption potential than polymers with equal chain lengths.[62]

Polymeric steric stabilization does not usually destroy the crystal structure of drug particles, unlike the action of conventional small molecular weight surfactants like sodium dodecyl sulfate (SDS),[17,25] where the drug is solubilized by micelle formation. Steric stabilization is also more sensitive to temperature fluctuations than electrostatic repulsion. This may be problematic if the product needs to be sterile.

Nanocrystal suspensions have been sterilized by gamma irradiation[3,27,63,64], sterile filtration[54] and steam

Table 2 Examples of stabilizers used for the production of nanocrystals

API	Stabilizer	Method	Reference
RMKP22	Tween 80	Pre-milling/high pressure homogenization	[49]
Nifedipine	HPMC, Tween 80, poloxamer, SDS	High pressure homogenization	[50]
ucb-35440–3	HPMC, MC, PVA, SDS, acacia gum, poloxamer	High pressure homogenization	[51]
Buparvaquone	Poloxamer, PVA, glycerol	High pressure homogenization	[52]
Hesperetin	Poloxamer, Inutec SP1, Tween 80, Plantacare 2000	High pressure homogenization	[11]
Indometacin, simvastatin	PVP, Poloxamer	High pressure homogenization	[33]
Crystalline API	HPC	Wet milling	[28]
Cinnarizine, griseofulvin, indometacin, itraconazole, loviride, mebendazole, naproxen, phenylbutazone, phenytoin	D-α-tocopherol polyethylene glycol 1000 succinate	Wet milling	[53]
Cinnarizine, griseofulvin, indometacin, itraconazole, loviride, mebendazole, naproxen, phenylbutazone, phenytoin	HPMC, HPC, HEC, CMCNa, sodium alginate, PVP, PVA, Kollicoat IR, Poloxamer, D-α-tocopherol polyethylene glycol 1000 succinate, Tween 80	Wet milling	[9,27]
Piposulfan, camptothecin, etoposide, paclitaxel	Tween 80, Span 80, Pluronics	Wet milling	[18]
Iodipamide	Pluronic	Wet milling	[54]
Ethyl diatrizoate	Poloxamine	Wet milling	[29]
Ibuprofen, naproxen, prednisolone acetate, nifedipin, hydrocortisone acetate, itraconazole, anthracene	HPC, PVP	Wet milling	[25]
Naproxen	Amphiphilic amino acid copolymers	Wet milling	[55]
Tamoxifen, paclitaxel	PAH, PSS, poly(dimethyldiallylamide ammonium chloride)	Ultrasonication, layer-by-layer coating	[56]
Ibuprofen	Poloxamer, PVP, Tween 80, SDS, PVA	Melt emulsification	[14]
Ibuprofen	Tween 80, PVP, Pluronics, HPMCs	Microfluidization, precipitation	[15]
Progesterone, betamethasone valerate, carbamazepine, oxcarbazepine	Gelatine, HPMC, Lipoid S75, poloxamer, PEG	Precipitation	[57]
2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide	–	Reprecipitation	[58]
Naproxen	PVP, poloxamer	Antisolvent precipitation	[59]
Itraconazole	Poloxamer	Antisolvent precipitation	[60]
Mitotane	Tween 80, caprylyl-capryl glucoside, lecithin	Solvent quenching	[4]
Indometacin	Cyclodextrin	Emulsion solvent diffusion	[61]

API, active pharmaceutical ingredient; CMCNa, carboxymethylcellulose sodium; HEC, hydroxyethylcellulose; HPC, hydroxypropylcellulose; HPMC, hydroxypropyl methylcellulose; MC, methylcellulose; PAH, poly(allylamine hydrochloride); PEG, polyethylene glycol; PSS, poly(styrene sulfonate); PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; SDS, sodium dodecyl sulphate.

sterilization.[29] With steam sterilization, problems may be caused by warming up above the cloud point of the stabilizing surfactant leading to an increased particle size. Irradiation has been noted as causing aggregation of the stabilizing surfactant due to the decrease in zeta potential value.[64]

Polymeric stabilizers have many sites in their chains that have an attraction to particle surfaces, which are hence bound to numerous surface sites.[65] If the affinity of individual parts of the chain to the surface is weak, the polymer chain as a whole can still be strongly attached to the surface. New drug crystal surfaces formed by fracturing during milling attract these polymer chains if the entropy loss by physical surface adsorption is less than the related enthalpy gain. Hence, the behavior of stabilizers in solution and the physical and chemical properties of the newly formed surfaces play key roles. It has to be emphasized also that different surface characteristics of drug materials require different stabilizer properties.

Ionic stabilizers are effective in aqueous environment, but during the drying they may become less effective because the ionized state is not maintained in dry material.[65] Ionic stabilizers are also sensitive to changes in pH and ionic strength. The advantages of steric stabilization over electrostatic stabilization are relative insensitivity to electrolyte additions, equally efficiency in both aqueous and nonaqueous environments and the existence of high solid concentration forms in relatively low viscosity systems. It has to be remembered that high viscosity in nanomilling may prolong the process times, although the high viscosity enhances the stability of the end product.

Molecular weight is an important factor for polymeric stabilizers. The chain length should be high enough that the polymer chains have an optimum length to overcome the van der Waals forces of attraction. With short chains the steric barrier is too thin and aggregation is promoted, while thick layers may also cause particle bridging. Usually polymeric chains from approximately 5000 to 25 000 g/mol are long enough for steric repulsion and thus stabilization of nanoparticles.[55]

A very interesting technique for stabilization of nanocrystals is the layer-by-layer (LbL) coating technique,[56] although the technique has not yet been utilized in nanomilling (particles are formed by ultrasound fracturing). Sequential electrostatic LbL adsorption of oppositely charged polyelectrolytes on a charged surface forms ultrathin coating layers with tailored properties. Originally the technique was applied to flat surfaces,[66] but it has since also been used to coat polymeric drug nanoparticles.[67] The principle of the LbL coating is electrostatic adsorption of the polyelectrolytes on charged surfaces. The highly charged polymeric layer that is formed on the drug particle surface prevents particle aggregation. Lower molecular weight polyelectrolytes have been observed to have higher affinity for charged surfaces.[68]

When selecting the stabililizer, other processes should also be taken into account. For example, the low melting point of a stabilizer may limit the selection of a drying method. With Tween or poloxamer stabilizers, particles may aggregate during spray drying due to the low melting point. Addition of specific stabilizers for the drying process may be necessary for the preparation of stable products.[28,53,69]

Stabilization of nanocrystals in nanomilling

The most important problem in processing drug nanoparticles is how to compensate for the extra free energy of the newly created surfaces. The degree of compensation required is related to the interactions between the drugs and stabilizers. For effective stabilization and a reasonable processing time, strong and fairly fast adsorption is necessary, with full coverage and slow desorption. In drug nanocrystallization the weight ratio of drug to stabilizer is commonly from 20 : 1 to 2 : 1. If the amount of the stabilizer is too low, particles tend to aggregate, while concentrations that are too high promote Ostwald ripening (Figure 4).

From the point of view of end product properties, it is also important to notice possible relaxation and its effect on particle size after the milling process. Deng and coworkers[31] studied interactions between drugs and polymeric stabilizers, and they noticed relaxation behavior after the milling. Due to the relaxation, particles agglomerated and formed clusters; the maximum particle sizes were achieved within the first 24 h. Within a few days, the clusters were relaxed and dissociated into primary particles and the suspension was stable at the sub 100 nm level. Milling parameters, like milling time, speed and amount of stabilizer, affected the relaxation behavior.

Adsorption characteristics of stabilizers are affected by differences in their crystal faces. In milling processes, it is very probable that different crystal faces are formed depending on the raw material and process parameters.[42,70] These crystal faces can possess various solubility properties.[71–73] Regarding the properties of raw materials, factors like impurities, solvent inclusion, number of mechanical deformations, and crystal habit impact on the properties of the end product.[45,74] Excipients may also show preferred adsorption or they may adsorb only to certain faces of the

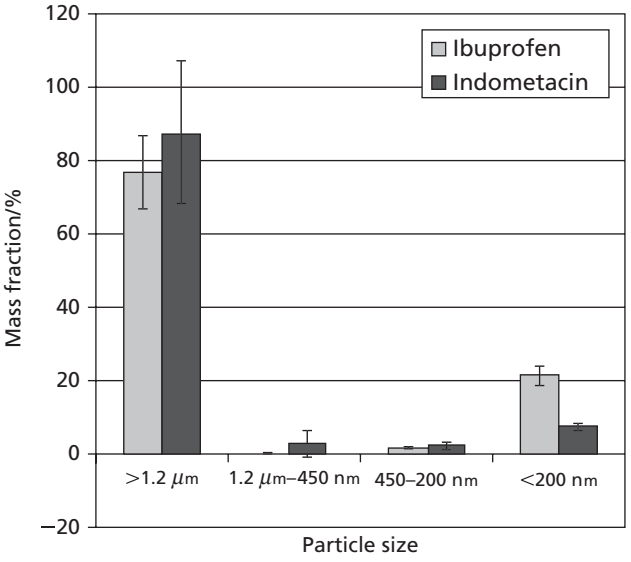

Figure 4 Fractionated particle size distributions (indometacin and ibuprofen) measured after filtration separation. Milling time was 40 min and speed 600 rpm. Milling was performed by Fritsch Pulverisette 6 in aqueous Tween 80 solution ($n = 7–9$).

crystals,[75–77] depending on the affinity of the excipient for the newly formed surfaces. With micron-sized particles it has been observed that it is also possible that the stabilizer does not cover the whole surface.[77]

Surface energy

Surface energy determinations may help to find a proper stabilizer for a nanosuspension.[78] A rough approximation of the surface energy can be calculated from the static contact angle measurements. If the surface energy of the stabilizing polymer is similar to the surface energy of the drug, the drug may be prepared in the form of nanoparticles with narrow particle size deviation.[17] The addition of small molecular weight surfactants can still decrease the particle size in some cases – and the size deviation.

Usually, the addition of surfactant is more beneficial where the surface energy difference between the drug and the polymer is high. This addition of another surfactant probably changes the surface energies of the system to make them more favorable to each other. Still, the role of the surface energy is not totally clear and the importance of the hydrophobic nature of the stabilizer and strong adsorption should also be highlighted (Table 3).

Choi *et al*[25] studied hydroxypropyl cellulose and polyvinylpyrrolidone (PVP) as stabilizers for seven drug nanocrystals produced by the wet-milling technique. The surface energies of the drug materials differed considerably. Correlations between the surface energies of the stabilizers and drugs were looked for, but, for example, with hydroxypropyl cellulose no good correlation was found. With PVP, the smallest particles were formed when the surface energy of the drug and stabilizer were close to each other, but there was also one exception. Accordingly, the role of surface energy is important, but not totally clear.

Effect of molecular weight

As mentioned above, polymers from approximately 5000 to 25 000 g/mol are usually efficient for the stabilization of nanoparticles. When considering the molecular weight it is important to also consider the effect of chain length on the viscosity. When comparing the different molecular weights of the same polymer, the higher viscosity of the higher molecular weight polymers may cause slower adsorption during milling, with a need for longer process times.[17] However, in a study with the poorly soluble anti-cancer agents, piposulfan, camptothecin, etoposide and paclitaxel, the most effective stabilizers were the higher molecular weight polymeric stabilizers from the same polymers.[18] For piposulfan only a mixture of Span and Tween was successful in producing drug nanocrystals, and this was concluded as being due to the fact that the surfactant mixture adequately wetted the drug substance but still provided steric stabilization.

Polymer adsorption and the related steric stabilization may be disrupted if the drug material has a low melting point, due to the melting of the surfaces of drug nanocrystals. It is therefore important to remember the size dependence of the melting point.[79] Although polymeric stabilizers are efficient in the suspension state, one has to remember that once dried the polymer chains solidify and stabilization is no longer efficient.[28] So far, the role of molecular weight is not clear, and one needs to take into account the concomitant co-effect of viscosity.

Table 3 Factors affecting the stability of nanocrystals produced by nanomilling

Property	Importance	Reference
Viscosity	Should be taken into account	[9]
Physicochemical properties of the drug	Interactions between surface functional groups important	[9,28]
Surface energy	Important	[9,28]
Surface hydrophobicity, wettability, HLB value	Important	[9,15,18,53]
Affinity of the stabilizer to the particle surface	Important	[11]
Solubility to used stabilizer	Not clear	[15]

HLB, hydrophilic–lipophilic balance.

Viscosity

Rheologic parameters are important during milling.[80] Factors like milling speed, number and size of the balls, milling volume, solid concentration, temperature, pH and particle size distribution affect the end product. Solid content is important for determining the specific breakage rate of the material. On the other hand, the medium is important in order to prevent the aggregation of newly formed surfaces.[81] High viscosity media may require longer processing times.[55] Small changes in the molecular weight seem not to be important, although steric repulsion is known to be related to the molecular weight (chain length) of the polymer.

Van Eerdenbrugh *et al*[9] examined surface stabilization during the production of drug nanocrystals using 13 different stabilizers and 9 different drugs. They used both polymers (synthetic and semisynthetic) and surfactants. Overall, the surfactants gave the best results in stabilizing the nanosuspensions. This was explained as being caused by the low viscosity and high surface activity of the surfactants. The celluloses studied (hydroxypropyl methylcellulose, methylcellulose, hydroxypropylcellulose, hydroxyethylcellulose, carboxymethylcellulose sodium), in combination with sodium alginate, made the viscosity of the media so high that the concentrations obtained with these materials were very low and their stabilizing effect poor. A high viscosity medium is inefficient for production of small particles, although according to the Einstein equation, the diffusion velocity is lower in high viscosity media, promoting nanosuspension stability during storage. With the surfactants and synthetic linear polymers studied (Table 2), high viscosity was not a problem. Accordingly, much higher concentrations compared to the polymers could be used. Also, the effect of rheology on particle size has been explained by correlating the degree of pseudoplasticity to particle size distribution.[80] The Bingham effect should also be considered with polymer solutions because the relationship between shear stress and viscosity is not necessarily linear. In conclusion, rheology needs to be

taken into account, but it is not the main factor behind stabilization of nanosuspensions.

Functional groups

Possible interactions between the functional groups of a stabilizer and drug materials always need to be considered before selecting the drug–stabilizer pair. Many drugs have structures containing functionalities like phenols, amines, hydroxyl groups, ethers or carboxylic acid groups, which are capable of interactions. Strong ionic interactions, hydrogen bonding, dipole-induced forces, and weak van der Waals or London interactions may enhance or disturb particle formation. So far, the effect of functional groups on the stability of wet-milled nanocrystals has been considered only in few studies.[25,55,82,83]

With hydroxypropyl cellulose as a stabilizer, drugs having functional groups (in this case hydroxyl groups also existing in the structure of the polymer) did not reduce particle size successfully.[25] Specific interactions, e.g. hydrogen bonding, between the functional groups of the drug and polymer interfered with the stabilization activity of the polymer, and particle size reduction was hindered. The same kind of behavior has been noted with stabilization of different polymorphic forms of drug materials:[82] hydrogen bonding between excipients and carbamazepine was explained as being one factor behind the inhibition of particular polymorphic changes. In the case of PVP the situation was different.[25] PVP does not have any strong hydrogen bonding groups in the structure. It was concluded that both the PVP and hydroxypropyl cellulose work the best with surfaces without polar functional groups.

Even stronger interactions, ionic interactions, are possible. With materials of very poor aqueous solubility this is not a problem but, for example, in the case of widely studied non-steroidal anti-inflammatory drugs with carboxylic acid groups, some problems may exist. For example, with amphiphilic amino acid copolymers as stabilizers, possible ion pairing between lysine and the functional carboxylic acid groups of naproxen were suggested as being the reason for the wide size variation observed with the product.[55] During dissolution testing, diclofenac sodium has been shown to be capable of forming ionic complexes with ionized amino groups of chitosan polymers, a process which hinders dissolution.[83] Accordingly, interactions between the functional groups need to be taken into account before selecting the drug–stabilizer pair.

Hydrophobicity

The stabilizer needs to adsorb on the particle surfaces in order for proper stabilization to be achieved. Furthermore, the adsorption should be strong enough to last for a long time. Adsorption of the stabilizer may occur by ionic interaction, hydrogen bonding, van der Waals or ion–dipole interaction or by hydrophobic effect.[84] The surface of wet-milled drug nanocrystals is hydrophobic. High affinity of the stabilizer on the hydrophobic surfaces is thus important, but the role of the hydrophobic nature of the stabilizers has been stressed in only few studies so far.[9,55]

In the screening study by van Eerdenbrugh *et al*.,[9] the hydrophobic nature of the formed nanocrystal surfaces was analyzed by determining the absorption of hydrophobic D-α-tocopherol polyethylene glycol 1000 succinate on the particle surfaces. Although many other properties were tested too, it was concluded that surface hydrophobicity is the most important driving factor for the aggregation of nanoparticles.

When using amphiphilic amino acid copolymers as stabilizers, the total hydrophobicity of polymers seems to be a more important parameter than the distribution of hydrophobic moieties in the polymer chains.[55] The hydrophobicity affects the physical adsorption, while chain morphology has an impact on the conformation of the adsorption and the achieved steric repulsion. The hydrophobic moiety content should be more than 15 mol-% in the polymer structure for efficient stabilization.

In a binary mixture of nonionic and ionic stabilizers (SLS, Brijs, Spans, Symperonics, Tweens) the stability of the dispersion was increased with decreasing HLB number of the nonionic surfactant.[62] This was explained by the fact that materials with lower HLB values (i.e. more hydrophobic) escape more readily from the aqueous phase to the particle surfaces than materials with higher HLB values (i.e. more hydrophilic). Also the efficiency of the chain length as a steric stabilizer depends on the properties of the chain, e.g. lengthening of the ethylene oxide or alkyl chain decreases the stability of dispersion, although it was earlier thought that longer chains should provide better stabilization due to the thick steric barrier.[85,86] Stabilities with longer chains are generally poor because they pack densely and lose the conformational entropy of the surfactant layer.[86,87] Accordingly, surfactant mixtures have been used to increase the disorder of the surface layer and hence to enhance nanodispersion stability.[88]

When the hydrophobicity of the surfactant is increased, the driving force for adsorption on the hydrophobic particle surface is also increased and, because of this, the more hydrophilic surfactant with theoretically higher steric stabilization capability does not adsorb sufficiently. As a whole, it seems that in most of the studies the level of hydrophobicity of the stabilizer is one of the main factors in stabilizing drug nanosuspensions.

Amount of stabilizer

The concentration level of the stabililizer is also important. Average adsorption per unit surface area is similar for both nano- and microparticles.[28] In other words, the adsorption per unit area is a surface property that does not usually depend on particle size. As the adsorbed amount correlates to the surface area, this means that the total amount of stabilizer is directly related to the particle size. Adsorption of polymer molecules onto the particle surfaces takes place when the free energy reduction due to the adsorption compensates the accompanying entropy loss.[89] Because steric stabilization is based on adsorption/desorption processes, process variables such as the concentration of the stabilizer, particle size, solvent, etc. are important factors for the effectiveness of the stabilizer. It is also important to remember that although the steric stabilization of polymer chains may be sufficient in aqueous suspensions, in the dry form it may no longer

effectively maintain steric repulsion. Also, in a suspension flocculated particles may lead to irreversible aggregation as a function of time. When particles are close to each other, it is possible that the stabilizer is slowly diffused away from the high energy zones between two separated particles, and the stabilizing effect is lost.

During nanomilling of miconazole, the minimum concentration of SDS stabilizer for efficient formulation of nanocrystals was 0.0125–0.05%.[90] At this concentration SDS enhanced particle wetting but, according to the zetapotential measurements, stabilization via electrostatic repulsion was not achieved. With higher amounts of SDS the particle size did not decrease any more, but the electrostatic stabilization was increased. The amount of hydroxypropyl cellulose required for efficient nanocrystal formation was higher (3.125–5%). In this case the particle suspension is stabilized not only by the adsorption of hydroxypropyl cellulose on the particle surfaces (steric stabilization), but also by increased viscosity, which increases the shear energy for breaking up the particles. Nanomilling is efficient only if the viscosity of suspension is above 50 mPas.

With binary mixtures, there is also competition between hydroxypropyl cellulose and SDS for adsorption site: SDS has higher affinity for the particle surfaces than hydroxypropyl cellulose. The concentration above which the SDS adsorption onto the particle surfaces increased markedly is close to the critical aggregation concentration (CAC) of SDS in the presence of hydroxypropyl cellulose.[91,92] Above the CAC the polymer layer was replaced by an SDS layer.

There is also an upper limit for the amount of stabilizer. Micelles formed by excess surfactants may act as adhesives between the particles and promote formation of aggregates in some suspensions with higher surfactant concentrations.[62] Accordingly, the amount of stabilizer affects the stability and needs to be tailored to the appropriate particle size.

Solubility

The solubility of drug material in the aqueous stabilizer solutions may also play a role. In microfluidization processes, the higher solubility of ibuprofen in the presence of stabilizer caused increased Ostwald ripening and increased particle sizes.[15] According to the Lifshitz–Slyozov–Wagner theory, Ostwald ripening is directly correlated to the concentration of the dispersed phase in the system. Aqueous surfactant solutions increase the solubility of small molecular weight materials by forming micelle-like structures and thus they result in the destruction of crystallinity. These systems may therefore face stability problems later.

Conclusions

Stability of nanocrystalline suspensions is closely related to the formation process. In nanomilling, particle formation is based on particle fracturing during milling and stabilizers are needed throughout the process. One single factor cannot explain the efficiency of stabilizers, but it seems that the affinity of the stabilizer to hydrophobic surfaces, which is related to the hydrophobicity of the material, is important. In addition, the surface energy difference between the drug and stabilizer has an important role. Functional groups of the drug material and surfactant need to be taken into account in advance. Higher viscosity increases the stability of the end product, but highly viscous materials are difficult to mill.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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