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EXPERT REVIEW

Application of Drug Nanocrystal Technologies on Oral Drug Delivery of Poorly Soluble Drugs

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ABSTRACT The limited solubility and dissolution rate exhibited by poorly soluble drugs is major challenges in the pharmaceutical process. Following oral administration, the poorly soluble drugs generally show a low and erratic bioavailability which may lead to therapeutic failure. Pure drug nanocrystals, 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 absorption. This is the most distinguished achievement of drug nanocrystals among their performances in various administration routes, reflected by the fact that most of the marketed products based on the nanocrystals technology are for oral application. After detailed investigations on various technologies associated with production of drug nanocrystals and their *in vitro* physicochemical properties, during the last decade more attentions have been paid into their *in vivo* behaviors. This review mainly describes the *in vivo* performances of oral drug nanocrystals exhibited in animals related to the pharmacokinetic, efficacy and safety characteristics. The technologies and evaluation associated with the solidification process of the drug nanocrystals suspensions were also discussed in detail.

KEY WORDS bioavailability · drug nanocrystals · oral drug delivery systems · redispersibility · solidification process

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ABBREVIATIONS

AN	aqueous nanosuspension
AUC	area under the blood concentration–time curve
BCS	biopharmaceutics classification system
CL	clearance rate
C_{max}	maximum plasma concentration
DDSs	drug delivery systems
EPAS	evaporative precipitation into aqueous solution
GIT	gastrointestinal tract
IVIVC	*in vitro–in vivo* correlation
MCC	microcrystalline cellulose
MRT	mean residence time
NSAIDs	non-steroidal anti-inflammatory drugs
P-gp	p-glycoprotein
T_{max}	time to maximum plasma concentration

INTRODUCTION

The oral route remains the first choice for drug administration due to its convenience, good patient compliance and low medicine production costs (1). These benefits lead to the fact that oral products account for nearly 70% of the value in the USA pharmaceutical market and 60% of the drug delivery systems (DDSs) used (2). Following oral administration, the drug is absorbed from the gut and enters into blood circulation, then distributed to various tissues (3). Drug absorption in the gastrointestinal tract (GIT) is considered to involve a dissolution step of the drug from formulation into aqueous luminal fluids followed by transporting the drug across the gastrointestinal epithelium. The dissolution is considered as the rate determining process in the oral delivery (4,5). Poorly soluble drugs, possessing very limited solubility and dissolution rate in the digestive juice, consequently display many biopharmaceutical issues (Table I). To make matters worse, the number of drugs and drug

Table I Formulation-Related Performance Issues in Oral Application of Poorly Soluble Drugs

- A low/variable bioavailability
- A high fed/ fasted variation
- A retarded onset of action
- Lack of dose proportionality
- A higher interpatient variation
- Local irritation for irritative drugs (e.g. NSAIDs)

candidates (new chemical entities) is steadily increasing. At present about 40% of the drugs being in the development pipelines are poorly soluble, even up to 60% of compounds coming directly from synthesis are poorly soluble (6). It was reported that 70% of the potential drug candidates were discarded due to low bioavailability related with poor solubility in water before they ever reached the pharmaceutics department (7). Hence, with such a large market share currently held by oral DDSs, development of biopharmaceutically acceptable oral formulations for poorly soluble drugs, mainly belong to the biopharmaceutics classification system (BCS) II and IV compounds, is a challenge.

Many different techniques have been developed to overcome the solubility problem of poorly soluble drugs, e. g. solubilization, solvent mixtures, inclusion compounds, complexation and so on. A basic problem is that these formulation techniques can only be used to a certain number of drugs exhibiting special features required to employ the formulation principle, for instance possessing sufficient solubility in oils or other hydrophobic mediums, having a suitable molecular size and shape to incorporate in the cyclodextrin ring (8). For drugs insoluble in both aqueous and organic media (drugs so-called 'brick dust drugs'), these approaches are often ineffective.

Recently, novel possibilities are offered by the rapidly emerging field of nanoscience. Drug nanocrystal technology has been undoubtedly the highlight in this stage. One of its major contributions is the benefits that can be gained by formulating poorly soluble drugs (9). This approach generally produces dispersions of drug nanocrystals in a liquid medium (typically water), which are called 'nanosuspensions'. Nanosuspensions consist essentially of pure drug nanoparticles (100–1,000 nm) and a minimum amount of surface active agents required for stabilization. At present, approaches developed to produce drug nanosuspensions mainly include 'bottom up' (precipitation) and 'top down' (media milling, high pressure homogenization etc.). The bottom up technology dissolves the drug in solvent, and then precipitates it by adding the solvent to a non-solvent. These techniques are not widely used because of some prerequisites, such as usage of organic solvents and the drug should be soluble at least in one solvent (10). The top down technologies are disintegration methods, and so can be employed for all insoluble drugs including 'brick dust drugs'. Moreover, another distinguished advantage of top down technologies is that they can not only process milligram quantities of compound in a rapid screening mode in early stage discovery stage, but also facilitate the possibilities of large-scale production for market (11–13).

For drug nanocrystals, according to Noyes–Whitney and Ostwald–Freundlich equations, particle size in nanometer range can lead to increased dissolution velocity and saturation solubility (14,15). Therefore drug nanocrystals display series of benefits in oral application of poorly soluble drugs, including improved oral absorption, higher bioavailability, rapid action onset, reduced fed/fasted state variability and reduced intersubject variability (16). All these advantages have tremendous impacts on promoting drug nanocrystals successfully from experimental researches to commercial products. Table II show that most of marketed products based on the drug nanocrystals technologies is for oral utilization.

The aim of this review is not to provide an extensive overview on all aspects of drug nanocrystals. The interested reader is referred to excellent reviews available in the field regarding to drug nanocrystals in their manufacturing technologies, *in vitro* physical and chemical properties and parenteral application (8–10,12,16–20). Our works mainly focus on the oral application of drug nanocrystals, including their effects on absorption, efficacy and safety as oral drug delivery systems. Finally, solidification techniques of aqueous nanosuspensions are also discussed in detail, focusing on the maintenance of the rapid dissolution properties of the nanocrystals after further downstream processing.

IN VIVO PERFORMANCES OF ORAL DRUG NANOCRYSTALS

Effects on the Pharmacokinetics

Many reports verify that drug nanocrystals have many positive effects on the oral absorption of poorly soluble drugs. Manifestations on the blood profiles are the changes of pharmacokinetic parameters, generally including increased maximum plasma concentration (C_{max}), reduced time to maximum plasma concentration (T_{max}), enhanced area under the blood concentration-time curve (AUC) and reduced fasted/fed variability (Table III). Increased oral bioavailability and reduced fasted/fed variation are major unanimous conclusions in most of the present studies, attributed to the large surface of the nanosized particles. However, there are still several controversial issues requiring further systemic investigations.

Enhanced Oral Bioavailability

The mechanisms contributed for the enhanced oral bioavailability of drug nanocrystals could be majorly summarized as

Table II Key Characteristics of Available Commercial Drug Products Based on Drug Nanoparticle Technology

Product/Company	Drug compound	Indication	Nano-sizing approach	Administration route	Approval date
Gris-Peg®/Novartis	Griseofulvin	Anti-fungal	Bottom up, coprecipitation	Oral	1982
Cesamet®/Lilly	Nabilone	Anti-emetic	Bottom up, coprecipitation	Oral	2005
Verelan PM®/Schwarz Pharma	Verapamil	Anti-arrhythmia	Top-down, media milling	Oral	1998
Rapamune®/ Wyeth	Sirolimus	Immunosuppressant	Top-down, media milling	Oral	2000
Focalin XR®/Novartis	Dexmethylphenidate hydrochloride	Anti-psychotic	Top-down, media milling	Oral	2001
Avinza®/King Pharm	Morphine sulfate	Anti- chronic pain	Top-down, media milling	Oral	2002
Ritalin LA®/Novartis	Methylphenidate hydrochloride	Anti-psychotic	Top-down, media milling	Oral	2002
Herbesser®/Mitsubishi Tanabe Pharma	Diltiazem	Anti-angina	Top-down, media milling	Oral	2002
Zanaflex™/Acorda	Tizanidine hydrochloride	Muscle relaxant	Top-down, media milling	Oral	2002
Emend®/ Merck	Aprepitant	Anti-emetic	Top-down, media milling	Oral	2003
Tricor®/ Abbott	Fenofibrate	Hypercholesterolemia	Top-down, media milling	Oral	2004
Megace® ES/Par Pharma	Megestrol acetate	Appetite stimulant	Top-down, media milling	Oral	2005
Naprelan®/ Wyeth	Naproxen sodium	Anti-inflammation	Top-down, media milling	Oral	2006
Theodur®/Mitsubishi Tanabe Pharma	Theophylline	Bronchial dilation	Top-down, media milling	Oral	2008
Tridlide®/Skye Pharma	Fenofibrate	Hypercholesterolemia	Top-down, high-pressure homogenization	Oral	2005
Invega Sustenna/Johnson & Johnson	Paliperidone palmitate	Anti-depressant	Top-down, high-pressure homogenization	Injection	2009

two points:i) improved solubility and dissolution rate; ii) bioadhesion to the intestinal wall. When drugs are administered as nanocrystal formulations, the increased saturation solubility and dissolution velocity lead to a high drug concentration gradient between GIT and blood vessel, and then contribute to improved absorption and a high bioavailability (Fig. 1). One classic example is danazol, a poorly soluble gonadotropin inhibitor (28). The absolute bioavailability of marketed danazol conventional microsuspensions in beagle dogs (200 mg, 10 μm) was only 5.2%. When administered as an aqueous nanosuspensions (200 mg, 169 nm), an absolute bioavailability of 82.3% could be achieved, meanwhile the T_{max} was reduced and the C_{max} was increased by 15 times. This study did not further investigate the possible differences of pharmacological effects between the two formulations. However, it is undoubtedly that for nanosuspensions the same efficacy equal to that of conventional formulation can be available at a much lower dose. It should be bear in mind that crystalline state is another factor affecting the dissolution behavior of drugs. Drugs in the amorphous state possess higher solubility and faster dissolution rate due to the higher inner energy (37,38). Therefore when dosed through the oral route, the drug nanosuspensions in amorphous rate would show more significant effects on enhancing bioavailability than the crystalline nanosuspensions provided the high energy state could be kept in the GIT (24).

It is generally known that nanoparticles possess general bioadhension to biological mucosa including gastrointestinal mucosa (39). This bioadhension effect also plays an important role in the enhancement of oral bioavailability. There are four general theories of mucoadhesion mechanisms of nanoparticles: the electronic theory (electrostatic attraction forces between the surfaces of particles and mucus), the adsorption theory (secondary forces such as hydrogen and van der Waals bonds between the surfaces of particles and mucus), the diffusion theory (interpenetration and physical entanglement of the protein of the mucus and polymer chains) and the trapping theory (retention of nanoparticles by the uneven mucosa surface). The profound reasons for these theories exceed the scope of this review, some other reviews could be referred to (40–42). Because of mucoadhesion to gastrointestinal mucosa drugs can be released exactly at the absorption sites. This leads to a higher concentration gradient and also a prolonged retention time (30). To strengthen the mucoadhesion, further processing or surface modification could be done. Incorporation of drug nanocrystals into a mucoadhesive polymer or modifying the surface with cationic polymers can facilitate stronger adhesiveness to the negative mucin on the mucosa surface (43–45).

Table III Changes of Pharmacokinetic Properties of Oral Drug Nanocrystal Formulations Compared with the Conventional Partners

Drugs	Nanosizing methods	Dosage form (mean particle size)	Control (mean particle size)	Comparison of the pharmacokinetic parameters	Animals	References
1,3-Dicyclohexylurea	Media milling	Aqueous nanosuspension (AN)(950 nm)	Unmilled dispersions (38.5 μm)	Peak plasma exposure increased over an order of magnitude	Rats	(21)
A BCS II substance	Media milling	AN (280 nm)	Microsized dispersions (4 μm)	1.7~2.3-fold increase in C_{max}, 1.6~2.0-fold increase in bioavailability	Rats	(22)
Aprepitant	Media milling	AN (120 nm)	Microsized dispersions 5 μm	No food effect at a dose of 2 mg/kg, 80 mg/kg and 125 mg/kg	Beagle dogs	(23)
AZ68	Media milling (crystalline) Precipitation (amorphous)	AN(125 nm) AN(200 nm)	Solution	Amorphous suspensions possessed higher C_{max} and smaller T_{max} compared with the crystalline nanosuspensions, the bioavailability was similar for the two formulations	Rats	(24)
BMS-488043	Media milling	AN(120 nm)	Microsized tablets (95% <7 μm)	2.6-fold increase in C_{max}, 2.5-fold increase in bioavailability	Beagle dogs	(25)
Cilostazol	Media milling	AN (220 nm)	Microsized dispersions (13 and 2.4 μm)	Fasted/fed ratios of the C_{max}, AUC, T_{max} and MRT were significantly reduced	Beagle dogs	(26)
Cyclosporine	High pressure homogenization	AN (962 nm)	Solid lipid nanoparticles (157 nm) and commercial microemulsion Sandimmun®	A very disappointing results, blood concentrations were in the range between 30 and 70 ng/ml over a period of 14 h.	Pigs	(27)
Danazol	Media milling	Aqueous nano-suspensions, (AN) (169 nm)	Unmilled suspensions (10 μm)	15-fold increase in C_{max}, 16-fold increase in bioavailability, speeded up the absorption	Beagle dogs	(28)
EMD 57033	Media milling	Spray-dried powder (4.5 μm)	HP-β-CD solution; Coground mixture of microparticles with lactose; Physical mixture of microparticles with lactose;	A very disappointing results, a lowest absolute bioavailability for spray-dried nanocrystal powders (26%), even lower than that of Coground mixture of microparticles (39%)	Beagle dogs	(29)
Fenofibrate	High pressure homogenization	AN (356 nm and 194 nm)	Micronized suspensions (5–10 μm) and coarse suspensions	1.8~12.5-fold increase in C_{max}, 1.7~17-fold increase in bioavailability, 1.3~2.3-fold reduction in T_{max}	Rats	(30)
Fenofibrate	High pressure homogenization	AN (340 nm)	Micronized fenofibrate (5 μm)	1.67-fold increase in C_{max}, 1.3-fold increase in bioavailability, 4.9-fold reduction in T_{max}	Rats	(31)
Itraconazole	Precipitation	AN (267 nm)	Sporanox pellets containing microparticles	1.2~1.8-fold increase in C_{max}, 1.5~1.8-fold increase in bioavailability; the fasted/fed ratio of AUC was markedly reduced.	Rats	(32)
Ketoprofen	Media milling	Pellets containing dried nanocrystals powder (265 nm)	Pellets containing microcrystalline powder (65 μm)	1.2-fold increase in C_{max}, 1.1-fold increase in bioavailability, 2-fold reduction in T_{max}	Dogs	(33)
Naproxen	Media milling	AN (270 nm)	Unmilled suspensions (20 μm)	1.5-fold increase in C_{max}, 1.25-fold increase in bioavailability, speed up the absorption	Rats	(34)

Table III (continued)

Drugs	Nanosizing methods	Dosage form (mean particle size)	Control (mean particle size)	Comparison of the pharmacokinetic parameters	Animals	References
Spironolactone	High pressure homogenization	AN(400 nm)	Microsized dispersions (1~5 μm)	3.5~4.5-fold increase in C_{max}, 3.3~5.1-fold increase in bioavailability	Rats	(35)
UG558	Media milling	AN(190 nm)	Microsuspensions (12 μm)	3.5~3.7-fold increase in C_{max}, 3.6 ~4.5-fold increase in bioavailability	Rats	(36)

Reduced Fasted/Fed Variation

Poorly soluble drugs often exhibit increased or accelerated absorption when they are administered with food. This can be attributed to the enhancement of the dissolution rate in the GIT caused by many factors such as delayed gastric emptying, increased bile secretion, larger volume of the gastric fluid, increased gastric pH (for acidic drugs), and increased splanchnic blood flow (26). For example, it was reported that a standard high fat breakfast increased both the rate and extent of cilostazol absorption in human after oral administration of 100 mg tablet (46), suggesting that the oral bioavailability of cilostazol could be enhanced by food effects. The fasted/fed variation will be dangerous for drugs with a narrow therapeutic window. When poorly soluble drugs are formulated as uniform nanosuspensions, this variation may be minimized. The reason is that the dissolution rate of nanocrystals is fast enough even under the fasted condition. Then, the absorptions both in fasted and fed state might be a permeability-limited progress, and the absorption difference resulting from variable dissolution between the two conditions will be eliminated (Fig. 2). Studies by Jinno *et al.* showed that the fasted/fed variation in C_{max}, AUC, t_{max} and MRT were almost eliminated when cilostazol nanocrystals dispersions (220 nm) were given in dogs (26). Fenofibrate is another drug vulnerable to food effect. The extent of absorption varies from 30% to 50% when the traditional fenofibrate tablets in the fasting state to 60–90% when it is given after a meal (31). When tablet formulation containging fenofibrate nanoparticles were given, the food effect is absent in human (47).

Arguments and Further Focuses in this Field

Transcellular Uptake of Nanoparticles. Some researchers believe that transcellular uptake of polymeric nanoparticulates through epithelial cells is another reason for the enhancement of oral bioavailability (44,48–50). However, there has been no research on the evidence of direct uptake of drug nanocrystals. Moreover, even for polymeric nanoparticulate the reported data are conflicting and confusing, mainly due to two reasons. Firstly, the factors controlling intestinal absorption of particles are two complicate—size, nature of the polymer, zeta potential, vehicle, coating materials or other adhesion factors, presence of nutrients have been determined as critical factors influencing particle uptake. Secondly, a major source of confusion may lie in the large variety of analytical methods and models that have been employed to investigate particle uptake (48). Transcellular uptake of nanoparticles majorly occurred through two types of intestinal cells, enterocytes and M cells of Peyer's patches. However, because of the limited transcytotic capability of enterocytes and small proportion (~1%) of M cells in total

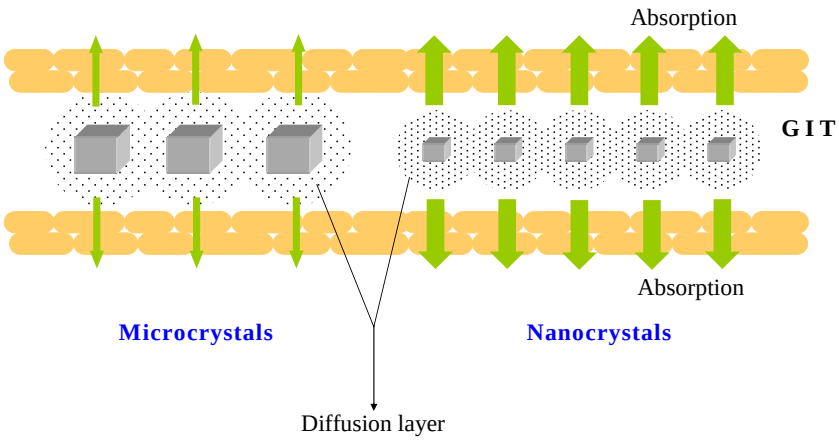

Fig. 1 Drug nanocrystals form a high drug concentration gradient due to the increased saturation solubility and dissolution velocity in digestive juice, and lead to a significant improvement on absorption.

intestinal surface, the level of uptake and to what extent it helps the oral absorption are still suspected (51). Studies by Ponchel *et al.* found that the body distribution of ^{14}C–labelled poly (lactic acid) nanoparticles 1 h after administration showed that 97% of radioactivity was localized in the GIT. Only 3% was recovered in other organs, supporting the particle translocation through the mucosa is a limited process (39). For drug nanocrystals lots of works should be done to investigate whether the evidences exist for direct uptake pathway, and if they exist, to what extent this pathway contributes to the enhancement of bioavailability.

The Role of P-gp. Some authors speculated that the ability of drug nanocrystals to enhance bioavailability should partly attributed to the inhibition effects of coated surfactants on the efflux function of the P-glycoprotein (P-gp) which located in the apical membranes of intestinal absorptive cells (31,52,53). Indeed, many studies have demonstrated that some surfactants, such as Tween 20, Tween 80, Pluronic L61, Vitamin E TPGS and so on, can enhance the membrane transport by modulating the intestinal P-gp function (54–56). However, all of the results are obtained from experimental data in models of Caco-2 cells, everted gut sac, *in situ* perfusion and rats and so on. There are still no related reports in humans. For nanosuspensions, two points should be questioned before disputing the action of added surfactants on the P-gp function. First, is the poorly soluble drug indeed a P-gp substrate? Secondly, are the amounts of surfactants added for stabilization sufficient to inhibit the P-gp's function?

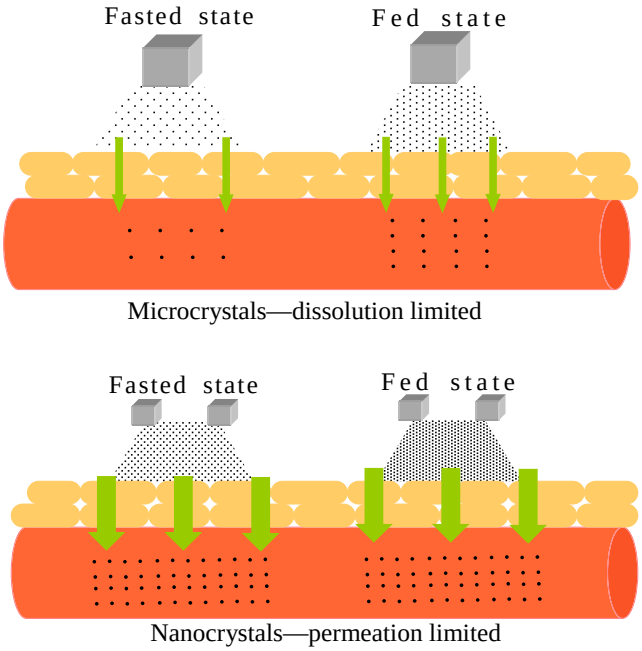

Fig. 2 Oral absorption of microcrystals of poorly soluble drugs generally is a dissolution-limited process in both fasted and fed state; absorption of drug nanocrystals generally is a permeation-limited process. Therefore the fasted/fed variation may be eliminated.

Effects on the Therapeutic Efficacy

For poorly soluble drugs (mainly belong to BCS II and/or IV), investigations on pharmacological effects are usually not easy. Sometimes these drugs can exhibit visible *in vitro* pharmacological effects after dissolving in non-aqueous solvents. But *in vivo* experiments are difficult to obtain the similar results, mainly due to the drug recrystalisation caused by the very limited solubility in physical liquids. Meanwhile the interference of organic solvents used on the pharmacological effects to the body can not be ignored. The advent of drug nanocrystal technology is significant for new drug discovery. With this approach *in vivo* data can be obtained early in the discovery process by utilizing non-ideal, prototype compounds prior to making a major investment in the search for efficacious drug-like compounds (21). In addition, many *in vivo* studies have proved that oral administration of drug nanocrystals can effectively improve the local or systemic therapeutic effects compared with conventional formulations.

Due to the bioadhesion properties, drug nanocrystals can bring marked improvement on local pharmacological effects

due to sufficient local drug concentration gradient on mucosa surfaces. For example, regarding the pathophysiological situation of Cryptosporidium, the localization of the pathogen in the epithelial membrane of the GIT will be of advantage for applying mucoadhesive nanosuspensions, which directly interact with the pathogen coating of the entire infected GIT (43). Oral drug nanocrystals can also be effective on improving systemic effects. It is majorly credited to their altered pharmacokinetic properties compared to the conventional formulations. In most cases, an equilibrium constant of drug distribution exists between diseased tissues and systemic circulation. So drug concentration in diseased tissues would increase or reduce in proportion to the systemic exposure. Since drug nanocrystalas can improve the systemic absorption and lead to an increase in AUC, more drug molecules will distribute into diseased tissues resulting in improved therapeutic efficacy (57,58). For example, Kayser *et al.* found that when administered as nanosuspensions, oral absorption of amphotericin B was significantly improved compared to conventional commercial formulations such as Fungizone®, AmBisome®, and micronized amphotericin B. Accordingly oral amphotericin B nanosuspensions significantly reduced parasite numbers in the liver of infected female Balb/c mice by 28.6%, whereas other formulations did not show any curative effect at all upon oral administration (59). Another studies by Ghosh *et al.* also demonstrated that oral 1,3-dicyclohexylurea nanosuspensions could raise antihypertensive efficacy due to an increased bioavailability (21).

Safety of Oral Nanocrystal Formulations

Safety is a primary issue for medicines, thus the toxicity assessment is the most important data for registration of a new medicine. Oral delivery is safe in most cases as a non-invasive route. For oral application of the poorly soluble drugs, however, the high and prolonged local concentration may be a problem, especially for the irritative drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) (60). The tolerability of drug nanosuspensions has been widely evaluated in animals for injection delivery, but up to now no systematic investigations have been published related to the oral nanocrystal products, although they have been on the pharmaceutical market for more than 10 years. Good tolerability can be presumed because each drug crystal orally taken will reduce in size during its dissolution in GIT and undergo a progress of being in nanometer range prior to its complete dissolution. This has been utilized by mankind lives for centuries. Due to the fine particle size, drug nanocrystals can even reduce irritancy to cell layers, e.g. the gastric wall, by increasing the distribution uniformity in the gastrointestinal fluid and avoiding the high and prolonged local concentration. Nanocrystals of naproxen were shown to decrease the gastric irritancy compared to the bulk drug powder (34). Studies on SU5416 and UG558 nanocrystals administered orally also verified a good tolerability even at a high dose (36,61).

On the other hand, as mentioned in "Effects on the Pharmacokinetics" section, oral nanocrystals normally change the pharmacokinetic profile of actives (i.e. higher C_{max}, shorter t_{max}). This may lead to some adverse effects associated with a higher drug exposure in the blood. For example, the nephrotoxicity of Amphotericin B is mainly related with the higher concentrations of free Amphotericin B for Amphotericin B injectable solution in comparison with the Ambisome® liposomes (59). Hence, there is still a need for further closer examination of tolerability of drug nanocrystals and offer experimental data to adjust their oral dose in order to avoid an excessively high blood peak.

SOLIDIFICATION OF NANOSUSPENSIONS

It should be bear in mind that most drug nanocrystal formulations used in the *in vivo* experiments are aqueous dispersions (Table III). When it comes to the clinical application, solid dosage forms are usually more acceptable for long-term stability (especially for the labile compounds), controllable drug release and patient convenience reasons (62,63). However, technical knowledge in the solidification process of nanosuspensions is still in the beginning, although most of the marketed formulations based on the drug nanocrystals technology are solid forms (Table II). In comparison with the redundant reports on the nanosuspension techniques, up to now few studies is associated with the solidification process of nanosuspensions, especially with the shaping process.

Solidification Technologies

Mainly two technologies have been utilized to transform the aqueous nanosuspensions into solid dosage forms including tablets, capsules, granulations and pellets. The first technology consists of two steps. In the first step, the aqueous nanosuspensions were powdered through some drying process, such as the lyophilisation, spray drying, oven drying and so on (64–67). Then, the resultant powders, blended with other excipients, are further processed into solid dosage forms through shaping handling, such as direct compression and capsule filling (68,69). In the second technology, drying process and shaping process are combined in a single step, briefly the aqueous nanosuspensions were used as granulating liquid in the granulation process or as layering dispersion in a fluidized bed process (60,70). No matter what kind of solidification technology is chosen, aggregation of the drug nanoparticles is a phenomenon that has been reported to be able to profoundly impact the properties of products

intended for a diversity of applications (71). If irreversible aggregation occurs, the benefits that can be gained from large surface of the original nanometer-sized particles would be greatly compromised since the surface area advantage of nanosuspensions would be lost.

In the aqueous nanosuspensions, the stabilizer molecules attached on the surface of nanocrystals can prevent aggregation of individual nanocrystals, by offering ionic or steric repulsion. However, during the removal of the water between nanoparticles, the ionized groups and ceaseless motion of molecular chains are not maintained, and then entanglement of polymers and particle fusion are induced (72). This coincidence might be a reason why the use of the small amounts of stabilizers in aqueous nanosuspensions is not sufficient for further drying process. Therefore, often a significant amount of dispersants is added to the suspensions prior to drying operation. The dispersants would fill the gaps between drug nanocrystals after the removal of water and form a continuous matrix, and the fillers physically block the entanglement between the stabilizer molecular chains on nanocrystal surfaces and particle fusion (73). By optimizing the category and amount of the dispersants and the parameters of solidification process (such as the freeze rate and spraying temperature and so on), the particle aggregation may be prevented, or at least reversible particle aggregation, which can rapidly reconstitute to the individual nanocrystals when dispersed in an aqueous medium, can be realized (74,75). It has been reported that a slight aggregation does not yet impair the dissolution velocity, but pronounced aggregation will decrease the dissolution velocity seriously (68) Fig. 3.

Drying Process

Most examples of drying process of aqueous nanosuspensions are based on lyophilization or spray-drying technology (Table III). Each of the two techniques has its own advantage. Lyophilization is chosen in most cases for its ability to retain the structure of nanosuspensions and its applicability to the thermolabile compounds (70). Yet the spray-drying is often welcomed for its time and cost saving and less residual moisture content of products (76). Both of them need the addition of a significant amount of dispersants such as sugars and polymers (usually more than drug content) for effective structure preservation. For lyophilization, the quality of the products mainly relies on the kind and amount of cryoprotectants and freezing rate. Waard and Lee (77,78) respectively investigated the importance of the freezing rate applied as well as the drug/cryoprotectants ratio of the nanosuspension. They proved that a higher freezing rate and a lower drug/cryoprotectants ratio resulted in higher nucleation rate and smaller crystals, and then less agglomerated products. Studies on freeze-drying of danazol (sucrose and mannitol), loviride (sucrose), AZ68(mannitol) and oridonin (mannitol) all showed that the inclusion of sugars leaded to a good redispersion characteristics of final dried products (Table IV). There are also exceptions reported. Studies by Kumar *et al.* found that sugars alone (25~250 wt.%) can not be effective to prevent aggregation of albendazole nanosuspensions. When 12.5 wt.% HPMC or 2.5 wt.% carbopol was additionally used, aggregation could no longer be observed (82). Van Eerdenbrugh *et al.*

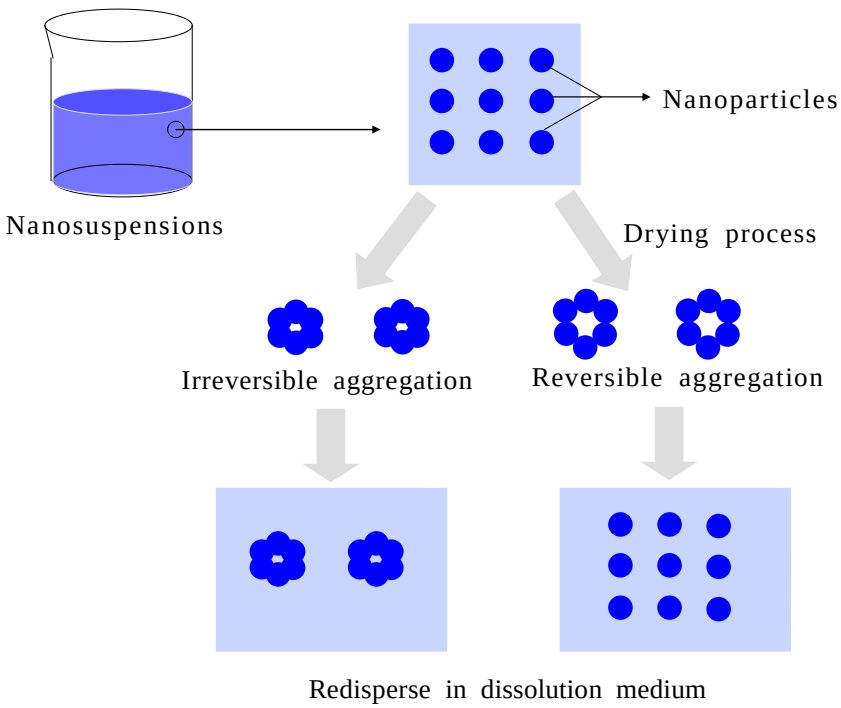

Fig. 3 Reversible aggregation can rapidly reconstitute into the individual nanocrystals when dispersed in an aqueous medium; irreversible aggregation can hardly redisperse into individual nanocrystals.

reported that although the cryoprotective effect could be observed for freeze-drying of itraconazole nanocrystals, aggregation occurred during the last phase of the drying step, which was more pronounced when using higher sucrose amounts. Yet the water-insoluble microcrystalline cellulose (MCC) proved to be a better matrix former in this study, where the inclusion of MCC resulted in fast dissolution that increased with increasing amounts of MCC (63). In addition, characteristic of the drug itself is also a factor affecting the amount of dispersants used for a good redispersibility. Study by Kim *et al.* showed that in the freeze drying process naproxen required a minimum carrageenan concentration of 0.5 wt.%, itraconazole required a carrageenan concentration of 3 wt.%, 6 times of the amount for naproxen (73).

As for the spray drying process, the inlet suspension concentration and feed temperature are seen to be important parameters for effective drying (72). Although a higher suspension concentration and feed temperature generally favors a faster drying process, the two parameters should be appropriately adjusted. Too high feed temperature, on the one hand, may be unfavorable to the stability of the drugs and forms fusion of the sugars. On the other hand, it may also lead to a loss of structural stability of the droplet and then formation of fused or donut shaped particles during spray drying of crystalline nanoparticles (91,92). High concentration sugars may be problematic due to the sticking of the product to the wall of the drying chamber (87). Glass transition temperature rather than melting point of sugars is a better predictor for spray drying performance. Sugars with lower glass transition temperatures results in sticky powders (e.g. dextrose and sucrose, Tg=62°C and 31°C, respectively). Lactose (Tg=101°C) and mannitol (Tg=87°C) on the other hand provides easily flowable powders (72). Transformation of the crystalline state of sugars should also be taken into account, which may affect the residual moisture content of dried products. For example, during spray drying the lactose may transfer into its monohydrate form, resulting in higher moisture content than the mannitol-based dried powders (72). Apart from the examples of using sugars, such as nifedipine (mannitol) and EMD 57033 (lactose), other examples of polymers used in spray-drying of nanosuspensions have also been reported, such as microcrystalline, PVP and so on (Table IV). When the polymeric dispersants are used in the spray drying, their aqueous solutions can be physically gelled during the removal of water, which then restrict motions of drug nanoparticles and chain entanglement of the stabilizer molecules on the surface of drug nanocrystals (73). Because of the rapid solvent evaporation, amorphous state is often obtained after spray drying of nanocrystal suspensions (93). Although conversion to the amorphous state can markedly improve solubility and dissolution characteristics, the amorphous state may revert to a lower energy state, typically crystalline form during storage. Unfortunately the timeframe of such conversions is not easy to predict (85).

Shaping Process

Through a shaping process, the drug nanosuspensions are finally converted into "macro-particles"—tablets, pellets, capsules and so on, which are suitable for oral administration of patients. However, they are still expected to be reconstituted into individual nanocrystals in the digestive juice to fully display their effects on the improvement of bioavailability. In general, solid dose forms from shaping processes exerting lower energy are often more easily reconstituted into primary nanoparticles. For example, making pellets by extrusion instead of compression minimized risk of nanocrystals aggregation and leaded to a faster dissolution (16). Another study by Heng *et al.* demonstrated that when a tablet formulation of cefuroxime axetil nanocrystals was adapted to a capsule dosage form with the same composition, the dissolution was markedly speeded up (76). For the same reason, many researchers preferred fluid bed process, where the nanosuspension was generally layered onto water-soluble carriers (usually sugars) (Table IV). Apart from the aim of time saving, to obtain an easily hydrated solid intermediate of nanosuspensions is more important for this process (70). For the layering process of nanosuspensions, a binder, often mucoadhesive polymer such as chitosan, is necessary (88). It works not only as a binder in the layering process but also as physical stabilizer for the nanosuspensions, due to the increased viscosity of the dispersion medium.

As early as in 1967, Aguiar *et al.* reported that tabletting process could potentially aggravate the aggregation behavior of particles in solution (94). It is well known that for powdered pharmaceuticals, drug nanoparticles have a large number of contact points because of the high surface area available for binding (95). Therefore, the punch of the die in the tablet production of drug nanocrystals might lead to the irrespersible aggregation (8; 35). A large amount of more matrix formers can significantly decrease the contact points among the drug nanocrystals and subsequently particle aggregation during tablet compression will be reduced (Fig. 4). Waard *et al.* found that higher amount of mannitol in the fenofibrate tablet resulted in a higher dissolution rate (77). Similar findings were reported by Vergote *et al.*, the dissolution rate of matrix pellets containing nanocrystalline ketoprofen increased with the increase of amount of starch derivatives (89). In addition, because of the stronger interparticular interactions and interactions between nanoparticles and fillers, tablets of nanoparticles are harder than that of microparticles at a given compaction force (88). Therefore, much lower compaction force may be needed for tabletting of nanoparticles to prevent excessive hardness of

Table IV Solidification of Aqueous Nanosuspensions Reported in Literature

Solidification Technology	Nanosizing Technology	Compound	Dispersants/Fillers (wt% relative to drug)	Evaluation and results	References
Lyophilization	High pressure homogenization	Azithromycin	None	Little aggregation. Dissolution compared to micronized powder: 65% *vs*. 20% dissolved after 5 h. (Still very poor dissolution characteristics)	(79)
Lyophilization	High pressure homogenization	Oridonin	Mannitol (100 wt.%)	No aggregation. Dissolution compared to micronized powder: 98% after 24 min *vs*. 40.3% after 2 h.	(80)
Lyophilization	High pressure homogenization	Oridonin	Mannitol (20 wt.%)	No aggregation. 103.3 ± 1.5 nm: 93.2%, 99.9% dissolved after 5 min, 10 min, respectively. 897.2 ± 14.2 nm: 35.4%,75.2% dissolved after 5 min, 10 min, respectively.	(81)
Lyophilization	Media milling	Itraconazole	None Sucrose (50,100 and 200 wt.%) Avicel® PH 101 (50, 100 and 200 wt.%)	63.2% dissolved after 42.0 ± 6.9 min 63.2% dissolved after 22.7 ± 8.7, 40.1 ± 8.3 and 209.2 ± 178.9 min. (Dissolution decreased with the increase of sucrose amount) 63.2% dissolved after 10.5 ± 0.7, 6.4 ± 1.2 and 3.1 ± 0.5 min, respectively. (dissolution increased with the increase of MCC amount)	(63)
Lyophilization	Media milling	Naproxen	None Carrageenan/Gelatin (2.5~125 wt.%)	Aggregation. No aggregation, reconstituted particle size reduced with the increase of dispersants.	(73)
Lyophilization	Antisolvent sonoprecipitation	Itraconazole	None Avicel® PH101, Aerosil® 200 (100 wt.%)	Aggregation, 20% dissolved after 20 min No aggregation, 40.29%~67.42% and 66.37~81.53% dissolved after 10 min for Aerosil® 200 and Avicel® PH101, respectively.	(64)
Lyophilization	High pressure homogenization	Piroxicam	PEG4000(40 wt.%) DE 39 (800 wt.%) Xanthan (10 wt.%)	No aggregation Dissolution compared to micronized powder: 65.07 ± 5.10% *vs*. 17.82 ± 2.31% dissolved after 1 h.	(65)
Lyophilization	High pressure homogenization	Albendazole	Mannitol (25, 125, 250 wt.%) Sucrose (250 wt.%)	Without additional HPMC or carbopol: Aggregation With additionally 12.5 wt.% HPMC or 2.5 wt.% carbopol: No aggregation	(82)
Lyophilization	Media-milling	AZ68	None Mannitol (2632 wt.%)	Aggregation No aggregation	(24)
Lyophilization	High pressure homogenization	Clofazimine	Mannitol (280 wt.%) Mannitol (280 wt.%) and Trehalose (100, 400 wt.%) Mannitol (400 wt.%)	No aggregation (after manual shaking of 2 min) Reconstituted particle size compared to original size: 532~649 nm *vs*. 601 nm	(83)
Lyophilization	Media-milling	Loviride	None	Aggregation	(84)

Table IV (continued)

Solidification Technology	Nanosizing Technology	Compound	Dispersants/Fillers (wt% relative to drug)	Evaluation and results	References
				Only 58.1 ± 26.3% dissolved after 15 min	
			Sucrose (100 wt.%)	No aggregation	
				Complete dissolution within minutes	
Lyophilization	Media-milling	Naproxen	None	No aggregation (after a short sonication)	(66)
Lyophilization	Media-milling	Undisclosed	None	Aggregation upon redispersion: Aggregation was suppressed above a critical freezing rate.	(78)
				Critical freezing rate increases with concentration	
Lyophilization	Melt emulsification	Ibuprofen	None	Reconstituted particle size compared to original size: 849.4 ± 10.5 nm *vs* 317.2 ± 12.9 nm.	(60)
				Dissolution compared to micronized powder:	
				65% *vs.* 15% dissolved after 10 min.	
Spray-drying	Media milling	3 compounds	Avicel® PH101(100 wt.%)	No aggregation for cinnarizine, dissolution of 90% after 5 min; aggregation for itraconazole and phenylbutazone, dissolution of 42% and 67% after 5 min, respectively.	(71)
			Aerosil®200(100 wt.%)	No aggregation for 3 compounds, dissolution of 90% after 5 min.	
			Inutec®SP1(100 wt.%)	No aggregation for 3 compounds, dissolution of 90% after 5 min.	
			Fujicalin®(100 wt.%)	Slight aggregation for phenylbutazone, dissolution of 88% after 5 min, aggregation for itraconazole and cinnarizine, dissolution of 56% and 66% after 5 min, respectively.	
Spray-drying	Microprecipitation–homogenization	Itraconazole	None	Aggregation	(72)
			Mannitol (2.5, 5%w/v) [a]	Without additionally Sodium deoxycholate, aggregation	
				With additionally Sodium deoxycholate, no aggregation	
Spray-drying	Media milling	EMD 57033	Lactose (100 wt.%)	No aggregation Dissolution compared to micronized powder:	(85)
				60% *vs.* 20% dissolved after 30 min.	
				However, a poor absolute bioavailability (Table II)	
Spray-drying	Media milling	Cilostazol	NR [b]	Dissolution in water, FaSSIF and FeSSIF: Completely dissolved within 1 min for spray died nanocrystal powder, only ca. 70% and 20% dissolved after 10 min for Jet-milled, Hammer-milled product, respectively.	(26)
Spray-drying	High pressure homogenization	Nifedipine	None	Aggregation 20% dissolved after 2 min.	(86)
			Mannitol (100 wt.%)	No aggregation	
				75% dissolved after 2 min.	
Vacuum drying	High pressure Homogenization/ EPAS [d]	Quercetin	None	No aggregation. Dissolution of HPH nanocrystals: 73.2% after 20 min.	(37)
				Dissolution of EPAS nanocrystals: 92.9% after 20 min.	
				Dissolution of bulk drugs: 61.5% after 24 h.	

Table IV (continued)

Solidification Technology	Nanosizing Technology	Compound	Dispersants/Fillers (wt% relative to drug)	Evaluation and results	References
Vacuum drying	Media milling	Naproxen	None	Aggregation	(73)
			Carrageenan/Gelatin/Alginic acid (2.5~125 wt.%)	When amount of Carrageenan, Gelatin, Alginic acid were less than 12.5%, 50% and 25%, respectively, aggregation occurred.	
Oven drying	Antisolvent sonoprecipitation	Itraconazole	None	Little aggregation, 35.76~60.44% dissolved after 10 min.	(64)
Lyophilization –Direct tabletting	Precipitation	Fenofibrate	Cryoprotectants: Mannitol (150, 233, 400, 900 wt.%)	Dissolution compared to tablets of micronized powder: 80% dissolved after 10 min *vs*. 50% dissolved after 120 min.	(77)
			No fillers.	Higher freezing rate resulted in a smaller crytal size and higher dissolution rate.	
				Higher amount of mannitol leaded to a higher dissolution rate.	
Spray drying- Direct tabletting	Emulsion-diffusion	Celecoxib	Dispersants in drying process: none	Dissolution compared to tablets of micronized powder:	(87)
			Fillers: MCC [c] (300 wt.%)	80% dissolved after 20 min *vs*. 80% dissolved after 90 min.	
Fluidized bed process (nanosuspensions were used as a granulating liquid)	Melt emulsification	Ibuprofen	Lactose (7000 wt.%) MCC (3000 wt.%)	Dissolution compared to granulations of micronized powder: 79% dissolved after 10 min *vs*. 6% dissolved after 10 min.	(60)
Fluidized bed process (nanosuspensions were used as a layering liquid)	high pressure homogenization	hydrocortisone acetate	Sugar sphere (ca. 9900 wt.%) (710–850 μm)	Dissolution of pellets of nanocrystals was faster than that of microsized powder.	(88)
			Eudragit L30 D-55(Enteric layer) (10% and 20% of the total weight)	Dissolution of pellets with 10% coated layer was faster than that with 20% coated layer.	
Fluidized Bed Processes-Tabletting (nanosuspensions were used as a layering liquid)	Media-milling	Ketoconazole	Granulation core: lactose particles (151.5 wt.%)	Redispersion of granules: Reconstituted particle size compared to original size: 126 nm *vs* 121 nm.	(70)
				Dissoluition of tablets:	
			Fillers:	Dissolution compared to tablets of micronized drugs:	
			Sodium Starch Glycolate (36 wt.%)	65% *vs*. 45% dissolved after 60 min	
			Corn Starch (36 wt.%)	Stability:	
			Crosspovidone (36 wt.%)	There was no impact of tablet physical and dissolution properties upon 3-months storage at accelerated condition (40°C/75% RH)	
Lyophilization -direct compression	High pressure homogenization	Rutin	Cryoprotectants: None	Redispersion of freeze-dried powder: Reconstituted particle size compared to original size: 721 nm *vs*. 727 nm.	(68)
			Fillers in tablets	Dissolution compared to tablets of micronized drugs:	
			Avicel PH 101 (80 wt.%)	80% *vs*. 40% dissolved after 5 min.	
Spray drying-melt pelletisation	Media-milling	Ketoprofen	Dispersants in drying process: none	Redispersion of spray-dried powder: Reconstituted particle size compared to original size: 240 nm *vs*. 230 nm	(89)
				Dissolution:	

Table IV (continued)

Solidification Technology	Nanosizing Technology	Compound	Dispersants/Fillers (wt% relative to drug)	Evaluation and results	References
			Microcrystalline wax (233 wt.%) and starch derivatives (33.3~333 wt.%)	Dissolution rate increased with the increase of amount of starch derivatives.	
Vacuum oven drying-direct compression/capsule filling	Antisolvent precipitation	Cefuroxime axetil	Dispersants in drying process: none	Dissolution compared to tablets of micronized drugs: 72.3 ± 0.7% *vs*. 36.5 ± 1.0% dissolved after 10 min.	(76)
			Fillers in tablets and capsules:	The dissolution rate of the drug was improved at higher concentrations of disintegrants.	
			Croscarmellose sodium (16.7 wt.%)	Higher levels of surfactants, smaller compression force promoted much faster disintegration times	
			Mannitol and MCC (48.3 wt.%)	77.0 ± 1.4% dissolved in 10 min for capsules, significantly faster than tablets.	
Extrusion and Spheronization-capsules filling	High pressure homogenization	Lutein	Lactose powder (500 wt.%)	Reconstituted particle size compared to original size: 429 nm to 679 nm. Dissolution compared to capsules of bulk lutein: 74.6% *vs*. 23.31% dissolved after 30 min.	(90)
Lyophilization –capsules filling	High pressure homogenization	Lutein	None Trehalose (3%, w/w) [a]	Reconstituted particle size compared to original size: None: 532 nm *vs*.429 nm Trehalose: 435 nm *vs*. 429 nm	(90)

[a] Concentration in the solution

[b] *NR* No report

[c] *MCC* Microcrystalline cellulose

[d] *EPAS* evaporative precipitation into aqueous solution

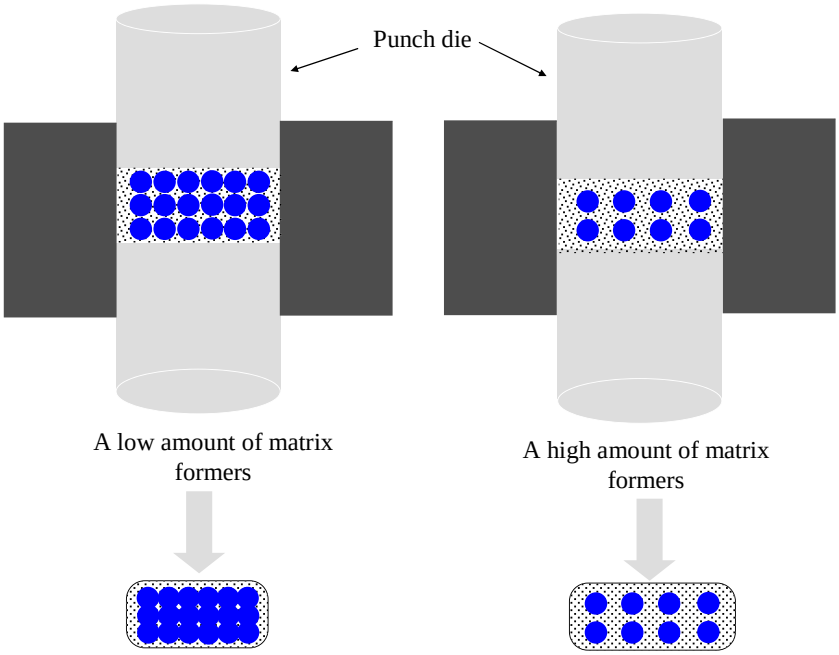

Fig. 4 More matrix formers can significantly decrease the contact points among the drug nanocrystals and lead to less particle aggregation during tablet compression.

tablets with markedly decreased dissolution rate. Moreover, lower forces also contribute to slighter deformation and less elastic behavior of tablets after the compacting force is removed (87). Dolenc *et al.* found that tablets with micronized celecoxib need to be compacted with higher forces to reach the same hardness as tablets with nanoparticles. Elastic recovery was higher in the case of tablets compacted with micronized celecoxib than nano-sized (87). In most experiment of tabletting of drug nanosuspensions, direct compression was chosen to minimize aggregation of nanocrystal during longer processing by traditional granulation (Table IV). Microcrystalline cellulose (MCC), Ac-Di-Sol and Explotab are excipients usually used for direct compression for their good compressibility and flowability (96,97).

Evaluation of the Redispersibility

For oral formulations, the transit time of the particles in the GIT typically ranges from <1 to 8 h. Hence, the reversible aggregations formed during solidification process should rapidly redisperse so that the original particle size is regained within a short period of time, and the particle dissolution would occur in a short time frame to facilitate availability of the drug for absorption. This depends on good wetting, disintegration and redispersibility characteristics of the products upon addition of the solid dosage form to water (98). Generally the redispersibility of solid formulations can be evaluated related to the reconstituted particle size of the nanocrystals in water, disintegration and/or dissolution rate (Table IV). The reconstituted particle size is often measured after addition of the solid forms of nanocrystals to water (this can be speeded up by manual shaking or sonication of a short time), and compared to the original particle size of the primary nanosuspensions. It is the reconstituted particle size in water but not the particle size of dried powders determining the dissolution characteristics. Chaubal *et al.* demonstrated that, although the dry particle size of the itraconazole microsuspension and spray dried itraconazole nanosuspension were similar, the dissolution rate of the latter was 20-fold higher than that of the microsuspensions (72). There is no evaluation standard, in general the solid formulations with a reconstituted size remaining in the nano-range can be considered as possessing a good redispersibilitly (98). More attention should be paid into the evaluation on disintegration and dissolution rate, for variability in disintegration and dissolution times due to the presence of aggregates can cause unpredictable variations in bioavailability. In order to better distinguish the discrimination between dissolution of unaggregated nanosuspensions and aggregated products, a poor sink conditions is often more beneficial, which can be offered by a medium where the drug solubility was only 150% of the amount of the drug added (71). It is well known that the disintegration time and dissolution rate of solid formulations are correlated with the surface hydrophobicity of drugs. For compounds with more hydrophobic surfaces, solidification process may be more devastating for the disintegration and dissolution of the solid nanocrystal formulations (98,99). However, for a given drug, the dissolution rate of solid nanocrystal formulations is mainly associated with the velocity to reconstitute to the individual nanocrystals. Discrimination between dissolution rate of nanocrystal formulations and that of microparticles formulations and original nanosuspensions will offer an evaluation criterion (Table IV).

The redispersion progress of a solid formulation containing drug nanocrystals in the GIT is more complex. Physiological factors (including pH variation, compositions of the digestive juice and GI peristalsis, etc.) affecting dispersion of nanocrystals are more complicated (100). Hence, sometimes a good *in vitro* dissolution behavior can not ultimately ensure a good *in vivo* performance. Studies on EMD 57033 showed that spray-dried nanoparticular EMD 57033 failed to show an improved bioavailability in animals, in spite of an exciting result in the *in vitro* dissolution studies (85). Another disappointing results were reported by Müller *et al.*, who found that the oral bioavailability of cyclosporine nanocrystals was much low, although a complete dissolution could be observed within 5 min *in vitro* (27). In general nanocrystals of basic drugs are more easily affected by pH variation in the GIT. For weak bases, a nanometer-sized drug formulation will dissolve fast and more efficiently in the low stomach pH environment. During transit from stomach to duodenum the rise in pH may illicit uncontrolled precipitation of drug substance (101). To prevent the *in vivo* dysfunction of nanocrystal formulations with a good *in vitro* dissolution behavior, firstly, the stabilizer type should be screened by monitoring the change of particle size after reconstitution in different pH media (102). In general ionic stabilizers are sensitive to changes in pH and ionic strength when the solid dosage forms redisperse in the GI fluid (103). On the contrary, in most cases the polymer and non-ionic surfactant stabilizers can be effective to support sufficient steric repulsion in GI fluid, given that the amount of stabilizers is enough (104). Secondly, results of *in vivo* bioavailability evaluation will be the ultimate judgement on screening of the solid formulation of drug nanocrystals. In addition, the establishment of an *in vitro-in vivo* correlation (IVIVC) is an essential part for oral formulations. For the Class II drugs dissolution is a rate-limiting step in the GIT, so in general they have a good IVIVC result (105). When they are processed into nanocrystal formulations, an IVIVC should be reevaluated again since their dissolution velocity has been markedly enhanced. In the other hand, the IVIVC data also help modulate the process and the amount of matrix in the solidification progress of nanosuspensions. However, research on the IVIVC of nanocrystal formulations has not been reported, but we believe it will be the next focus in this field.

CONCLUSIONS

Drug nanocrystals are a promising formulation technology for oral delivery of poorly soluble drugs. For marketed products having low and erratic bioavailability due to the poor solubility, reformulation into nanocrystal dosage forms can offer the possibility of adding new life to old compounds by improving oral bioavailability as well as efficacy and safety. The industrial applicability is also confirmed by the short time between invention and the first products on the market, less than 10 years. What is more important is that the number of products is keeping growing during the last decade as shown in Table II, very remarkable for such a relatively young technology. However, oral drug nanocrystal may not be versatile for all the poorly soluble drugs. If a molecule is too rapidly metabolized and or exhibits limited bioavailability as a result of poor permeation, particle size reduction may not be of value (74). Furthermore, some issues have still not received adequate attention in literature, including: to what extent intercellular uptake and stabilizers with P-gp inhibitory effects enhance the bioavailability; as for nanosuspension solidification, shaping process as granulation, pelletisation and tabletting have been infrequently investigated until now. In addition, the boost in solubility and consequently blood peak due to the particle size reduction raise the demand to obtain a controllable drug dissolution rate. We believe that with the market-oriented advancement of the nanocrystal technologies, more attentions would be paid into these issues in the future.

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