

Stabilizers used in nano-crystal based drug delivery systems.

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ABSTRACT

Nanocrystals have emerged as a viable tool to increase oral bioavailability of poorly water soluble oral solid dosage drugs. They also enable parenteral administration of these drugs as nanosuspensions. The high free surface energy, due to the large surface area to volume ratio of nanocrystals, makes them prone to aggregation, that can result in physical instability and loss of the previously increased solubility/dissolution. Stabilizers are incorporated into nanocrystalline formulations to prevent aggregation. These include excipients such as polymers and surfactants. They achieve stabilization through electrostatic repulsion and/or steric hindrance. This article focuses on aggregation in nanocrystal based formulations, stabilizers for the inhibition of aggregation, classification of stabilizers, properties of stabilizers and the mechanisms involved in stabilization. A review of stabilizers, drugs that have been stabilized, formulation types and methods for generating nanocrystals will be presented. Current challenges and future trends in the field of stabilizers will also be reviewed.

KEY WORDS: Nano-crystals, aggregation, physical stabilization, DLVO theory, stabilizer excipients

INTRODUCTION

It is estimated that approximately 70% of new chemical entities identified in drug discovery programs are poorly soluble in aqueous media (1, 2). This can pose significant challenges during drug discovery and the development phases (3, 4). It has, consequently, fuelled a

considerable amount of research into increasing the solubility of the drug, in order to be able to formulate them successfully. Attempts have been made to either increase oral bioavailability or use alternative routes of administration. Traditional approaches to increasing solubility include the use of prodrugs (5), salt formation (6), complexation (inclusion complexes) (7), co-solvents (8), emulsions (9), surface active agents (10), and solid-state manipulation (11). The last couple of decades have seen the emergence of various formulation approaches that are based on nanotechnology. These include micro/nano

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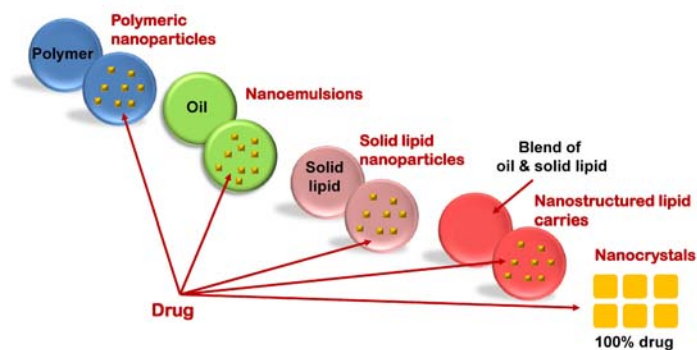


Figure 1 Nanotechnology based drug delivery systems. Reproduced with permission from Reference (16).

emulsions (12), polymeric nanoparticles (13), solid lipid nanoparticles (14), nanostructured lipid carriers (15) and nano-crystals (16) (Figure 1) and these approaches are reviewed here. As Table 1 shows there are already several medicinal drugs formulated with nano-crystals have been launched commercially.

Drug nano-crystals are crystals with a size in the nanometer range i.e., 10 to 1000 nm and are composed predominantly of a crystalline drug stabilized with excipients. Therefore, these particles have a very high drug load compared to nanoparticles consisting of a polymeric or lipidic matrix (17-21). A high drug load in nano-crystals significantly reduces the excipient load in the drug product.

Nano-crystals offer apparent solubility and dissolution rate benefits by virtue of particle size reduction and increased surface area (16). However, an increased surface area increases the surface free energy. In order to minimize the increase in surface free energy, nano-

crystals tend to aggregate spontaneously (16). Products based on nano-crystals are mostly formulated into solid dosage forms or nanosuspensions (see Table 1). The aggregation of particles poses significant challenges during nano-crystal based product development. The aggregation of nano-crystals can take place at various stages including: (i) during the generation of nano-crystals, (ii) the storage of nanosuspensions and, (iii) the dissolution of nano-crystal based solid dosage forms.

The inclusion of stabilizer(s) in the formulation is the most common strategy to overcome aggregation in colloidal systems. Nanosystems and colloidal systems discussed in this paper are synonymous, with the latter being the older term. Strategies for overcoming the problem of aggregation is based on conventional colloid science where capping agents or surface charges are used to provide stabilization of nano-crystals against aggregation (22). However, selecting a stabilizer is challenging due to the lack of a fundamental understanding of the

Table 1 Examples of nano-crystal products on the market.

TRADE NAME	THERAPEUTIC USE	APPLIED TECHNOLOGY	PHARMA COMPANY	ADMINISTRATION ROUTE AND DOSAGE FORM	APPROVAL DATE
Gris-PEG® (Griseofulvin)	Antifungal	Bottom up, coprecipitation	Novartis	Oral, tablet	1982
Verelan PM® (Verapamil)	Antiarrhythmic	Top-down, media milling	Schwarz Pharmaceuticals	Oral, sustained release capsule	1998
Rapamune® (Rapamycin, Sirolimus)	Immunosuppressive	Top-down, media milling	Wyeth Pharmaceuticals	Oral, tablet	2000
Focalin® XR (Dexmethyl-phenidate HCl)	Psychostimulant drug	Top-down, media milling	Novartis	Oral, extended release capsule	2001
Herbesser® (Diltiazem)	Antianginal	Top-down, media milling	Mitsubishi Tanabe Pharmaceuticals	Oral, extended release capsule	2002
Avinza® (Morphine sulfate)	Psychostimulant	Top-down, media milling	King Pharmaceuticals	Oral, extended release capsule	2002
Ritalin® LA (Methylphenidate HCl)	Psychostimulant	Top-down, media milling	Novartis	Oral, extended release capsule	2002
Zanaflex™ (Tizanidine HCl)	Muscle relaxant	Top-down, media milling	Acorda	Oral, tablet	2002
Emend® (Aprepitant)	Antiemetic	Top-down, media milling	Merck & Co.	Oral, capsules	2003
Tricor® (Fenofibrate)	Hypercholesterolemia	Top-down, media milling	Abbott Laboratories	Oral, tablet	2004
Triglide® (Fenofibrate)	Hypercholesterolemia	Top-down, high-pressure homogenization	SkyePharma	Oral, tablet	2005
Megace ES® (Megestrol acetate)	Antianorexic	Top-down, media milling	Par Pharmaceutical Companies Inc.	Oral, nanosuspension	2005
Cesamet® (Nabilone)	Antiemetic	Bottom up, coprecipitation	Eli Lilly	Oral, capsule	2005
Naprelan® (Naproxen sodium)	Anti-inflammatory	Top-down, media milling	Wyeth	Oral, sustained release tablet	2006
Theodur® (Theophylline)	Bronchial dilator	Top-down, media milling	Mitsubishi Tanabe Pharmaceuticals	Oral, extended release tablet	2008
Invega® Sustenna (Paliperidone palmitate)	Antidepressant	Top-down, high-pressure Homogenization	Johnson & Johnson	Intramuscular, nanosuspension	2009

interactions within the colloidal systems and the lack of effective methods to screen stabilizers. This article will review stabilizers used in nano-crystal based formulations. Additionally the mechanisms involved in the aggregation of nano-crystals and their stabilization will be explained. Detailed insights will be provided on the problem of aggregation, collision mechanisms responsible for aggregation, theories of colloidal stability and mechanisms of stabilization. Methods used to screen stabilizers for nano-crystal based formulations will also be presented.

NANO-CRYSTALLINE DRUG DELIVERY SYSTEMS

Nano-crystals began to be developed in the early 1980s. The first medicinal product appeared on the market in 1982 followed by several other medicinal products from 1998 onwards (23). Nano-crystals are primarily formulated into oral solid dosage forms because of the potential of a larger commercial market and easier manufacturing. To a much lesser extent they are formulated into parenteral dosage forms. The various medicinal products on the market have been formulated using

different technology platforms for generating nano-crystals an overview of which are provided in Table 1.

Studies on the *in vitro* and *in vivo* performance of nano-crystals have shown that they provide biopharmaceutical advantages by increasing (i) dissolution rate (24), (ii) apparent solubility (25), and (iii) mucoadhesion (26). Enhancement of dissolution kinetics is governed by the Noyes-Whitney equation, while increased apparent solubility is governed by the Kelvin-Ostwald-Freundlich equation. Increased mucoadhesion is due to the enhanced penetration of nano-crystals in the gastric mucosa by virtue of reduced particle size. A diagram showing these mechanisms is shown in Figure 2. Interested readers are further referred to other reports on the mechanisms involved in formulating nano-crystals (16, 23-28).

Methods for producing nano-crystals

Methods for the production of nano-crystals can be broadly classified into 'bottom-up' (controlled precipitation/ crystallization) and

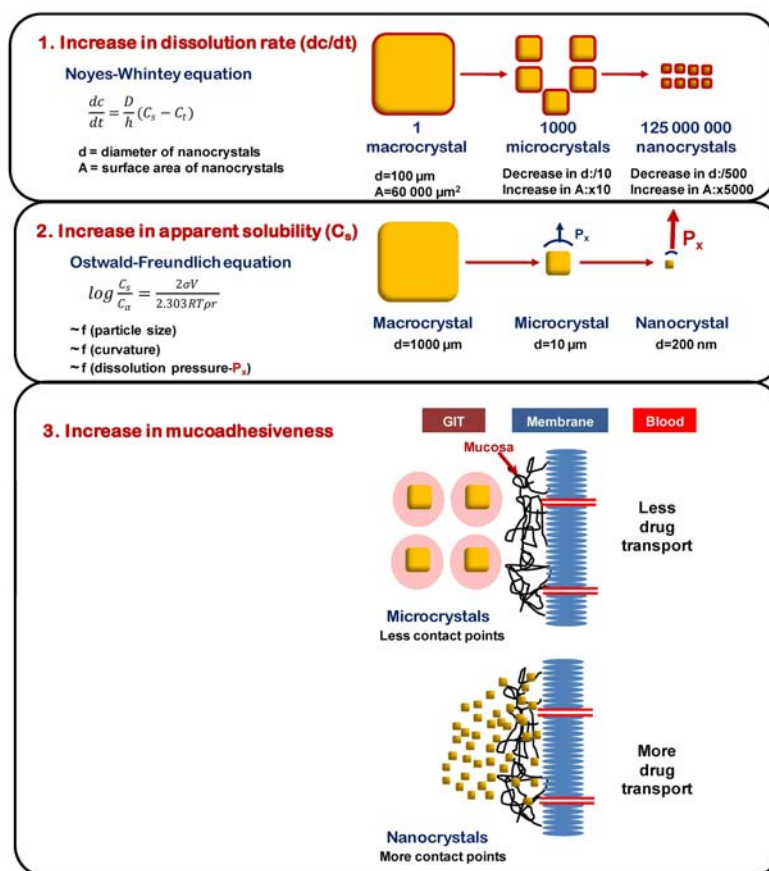


Figure 2 Features of nanocrystals: (1) increased dissolution rate due to increased surface area, (2) increased apparent solubility due to increased dissolution pressure of strongly curved small nanocrystals, and (3) increased mucoadhesiveness of nanocrystals due to increased contact area of small versus large particles.

‘top-down’ (particle size reduction by mechanical means). Combination methods involving precipitation followed by a size reduction step have also been used. Nanocrystals can also be generated through chemical synthesis. A classification of methods for the production of nano-crystals is outlined in Figure 3 (16, 23, 28).

Bottom-up methods

Bottom-up methods involve the controlled precipitation/crystallization of drug from its solution state. Process parameters are controlled to encourage crystal nucleation and allow crystal growth only upto the nanometer range. The preparation of nano-crystalline material using freeze drying was reported by De Waard *et al.*

(29). In this method, a drug dissolved in an organic solvent mixed with an aqueous solution of a cryoprotectant was immediately frozen and lyophilized. The freeze drying was performed at a relatively high temperature to induce the formation of nano-crystals (29). Nano-crystals have also been generated using spray drying. A solution of drug and excipient in a solvent or solvent mixture is spray dried to obtain discrete particles of micron size. Each particle consists of drug nano-crystals in a range of 10 to 1000 nm dispersed in the matrix of excipients (30). A nano spray dryer can be used to obtain nano-crystals directly through spray drying. Spray dryers that use piezoelectric driven actuator to generate nanosized porous particles are commercially available (31).

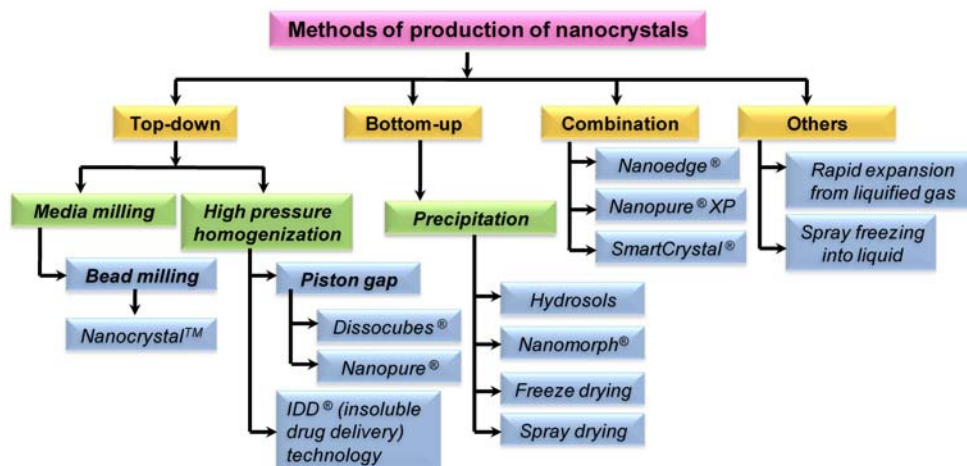


Figure 3 Methods for generating nanocrystals.

Top-down methods

Top-down methods involve breaking down larger crystals into smaller particles using milling techniques. The Nano-crystals[®] technology by Élan corporation uses bead milling to achieve particle size reduction. Milling media, dispersion medium containing stabilizer together with a drug are charged into a milling chamber. Shear forces generated by the milling media generate nano-crystals. The Nanomill[®] system is a lab scale version of this technology (Élan Drug Discovery, PA, USA). High pressure homogenization involves Micro-fluidizer technology (IDD-PTM technology), or piston-gap homogenization in aqueous media (Dissocubes[®] technology, SkyePharma), or in non-aqueous media (Nanopure[®] technology, Abbott Laboratories). The drug particles are reduced in size by cavitation (32).

Combination of methods

The Nanoedge[™] technology by Baxter uses a precipitation step followed by annealing using a high energy process such as high pressure homogenization. The annealing step prevents the growth of the precipitated nano-crystals. In this method, annealing has been defined as the process of converting a thermodynamically unstable matter into a more stable form by

single or repeated application of energy, followed by thermal relaxation. Another combination technology is smartCrystal[®], developed by Abbott Laboratories. It combines a number of different processes which either accelerate production by reducing the number of passes through the homogenizer or result in very small nano-crystals below 100 nm (16).

Nano-crystals on the market

Gris-PEG[®], the first nano-crystal product to be launched commercially was developed using the bottom-up co-precipitation method (33). Later, more products have been commercialized and as shown in Table 1. As can be seen, the majority of the products are for oral administration. There are currently several medicinal products using nano-crystals in advanced stages of clinical trials (34).

AGGREGATION IN DISPERSED SYSTEMS

A dispersed system is defined as a heterogeneous biphasic system in which the internal phase is dispersed within the continuous phase (35). Dispersed systems are classified as suspensions and emulsions based on the physical state of the constituent phases. Another classification system is based on the size of the dispersed particles within the

dispersion medium. The particle size of the dispersed phase varies considerably ranging from atomic/molecular dimensions to $>1\mu\text{m}$. Dispersed systems, based on size, are classified as molecular, colloidal or coarse dispersions (Table 2) (35).

Table 2 Various pharmaceutical colloidal systems

CATEGORY	PARTICLE SIZE	CHARACTERISTIC OF SYSTEM	EXAMPLES
Molecular dispersion	$< 1 \text{ nm}$	Particles invisible by electron microscopy; Pass through semi-permeable membrane	Oxygen molecules dissolved in water
Colloidal dispersion	$1 \text{ nm}-1000 \text{ nm}$	Particles are visible by electron microscope; pass through filter paper but not through semi-permeable membrane	Colloidal silver sols, surfactant micelles in an aqueous phase, pharmaceutical nanodispersions
Coarse dispersion	$>1000 \text{ nm}$	Particles visible by ordinary microscope; do not pass through filter paper and semi-permeable membrane	Pharmaceutical emulsion and suspension

Molecular dispersions are homogeneous in nature and form true solutions. Colloidal dispersions are intermediate in size between true solutions and coarse dispersions. A colloid is generally defined as a system comprised of discrete particles in the range of 10 to 1000 nm, dispersed in a continuous phase. Dispersions containing larger dispersed phases, 10 to 50 μm in size, are called coarse dispersions (23). Nano-crystals, either during processing, during performance, or both, are present in a suspended state. These nanosuspensions fall under the category of colloidal dispersions by virtue of their size from 10 to 1000 nm.

The aggregation of dispersed particles and the resulting instabilities such as flocculation and sedimentation in suspensions represent major challenges in formulating nano-crystal based pharmaceutical dispersed systems. Particle aggregation refers to the formation of clusters in a colloidal suspension and is the most frequent reason for destabilization. During this process, which normally occurs within seconds to hours or over a longer time, as well as, particles dispersed in the liquid phase stick to each other, and form aggregates. Flocculation refers to the process by which destabilized particles conglomerate into larger aggregates.

The latter are loosely bound clusters with an open structure. Sedimentation is the tendency of particles in a suspension to settle under gravity in the fluid in which they are entrained (36-38).

Aggregation is caused by the mutual attraction between particles through van der Waals forces or chemical bonding. This is a fundamental growth process for all dispersions of particulate matter (39). Aggregation in drug delivery systems such as nanosuspension is responsible for other issues including settling, crystal growth, dose inconsistency and most importantly loss of the solubility/dissolution advantage (40). Aggregation plays an important role in defining the physical instability and performance of nano-crystalline drug delivery systems.

AGGREGATION AND COLLISION MECHANISMS

Three different mechanisms account for the aggregation of particles: (i) perikinetic aggregation, (ii) differential sedimentation, and (iii) orthokinetic aggregation. These mechanisms are shown in Figure 4.

Perikinetic aggregation

The rate of aggregation is in general determined by the frequency of collisions and the probability of cohesion during collision. If the collisions are caused by Brownian motion, the process is called perikinetic aggregation and is solely driven by diffusion. Small particles in colloidal suspensions continuously undergo Brownian motion. These particles collide with each other and stick together due to attractive forces acting between them. Multiple collisions can lead to the formation of multi-particle aggregates (41, 42).

Differential sedimentation

Another important collision mechanism arises whenever particles of different sizes and density settle from a suspension. Particles of different diameters settle at different velocities

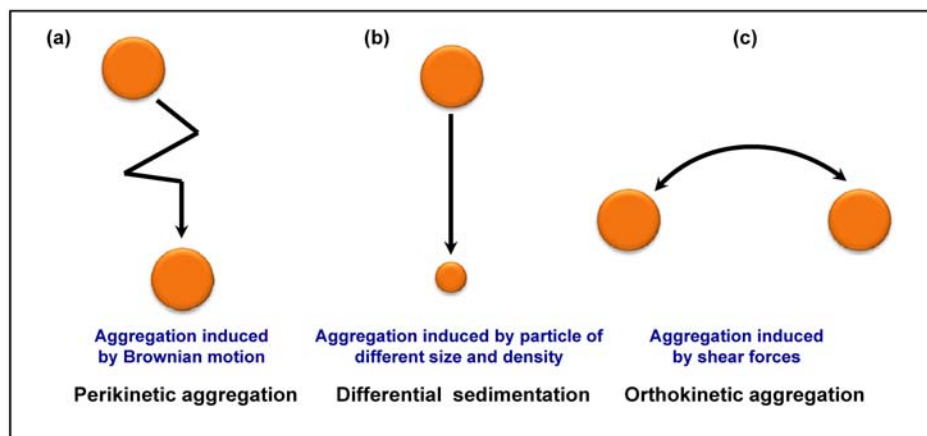


Figure 4 Schematic representation of (a) perikinetic aggregation (b) differential sedimentation and (c) orthokinetic aggregation.

causing the faster moving particles to collide with the slower moving particles resulting in aggregation. Even for an initially uniform suspension of equal particles, aggregates of different size are formed which settle at different rates. It is often at the later stages of flocculation that floccule growth becomes significant in sedimentation (43, 44).

Orthokinetic aggregation

Particle transportation brought through fluid motion can cause an enormous increase in the rate of inter-particle collisions, and aggregation brought about in this way is known as orthokinetic collision. Such collisions occur under conditions of shear, either by stirring or by flow. Orthokinetic aggregation is dependent on the initial particle size and velocity gradient, but is independent of temperature. For instance, stirring does not enhance the aggregation rate of small particles until they grow to a size of about 1 μm . Once particle size reaches 1 μm , stirring must be provided to promote further aggregation (45, 46).

INHIBITION OF AGGREGATION IN NANOCRYSTALLINE SYSTEMS

In both aqueous and non-aqueous media, electrostatic repulsion and steric stabilization are the two major mechanisms involved in the stabilization of colloidal nanosuspensions.

Electrostatic stabilization occurs through an electrical double layer surrounding the colloidal particles, and steric stabilization is achieved when polymeric molecules are adsorbed or attached chemically (47-51). It is also possible to combine chemical functionalities within the same molecule to achieve both steric and electrostatic stabilization known as electrosteric stabilization (52, 53). Ionic-polymers impart electrostatic repulsion from surfactant properties and steric stabilization from polymeric properties. Electrosteric stabilization can also be provided by using a combination of two different stabilizers, an ionic surfactant and a polymer, respectively (54). Electrostatic, steric and electrosteric stabilization mechanisms are shown in Figure 5.

Electrostatic stabilization

Electrostatic stabilization involves the adsorption of ionic charges on the particle surface resulting in mutual repulsive forces between the particles (55-57). The ionic strength of the medium significantly influences the repulsive forces. For a given colloidal system, an increase in the ionic strength reduces the thickness of electrical double layer which leads to a decrease in the repulsion potential of the particles (58, 59). Thus, other factors remaining the same, coalescence of electrically stabilized dispersed particles can be observed with increasing ionic strength of the

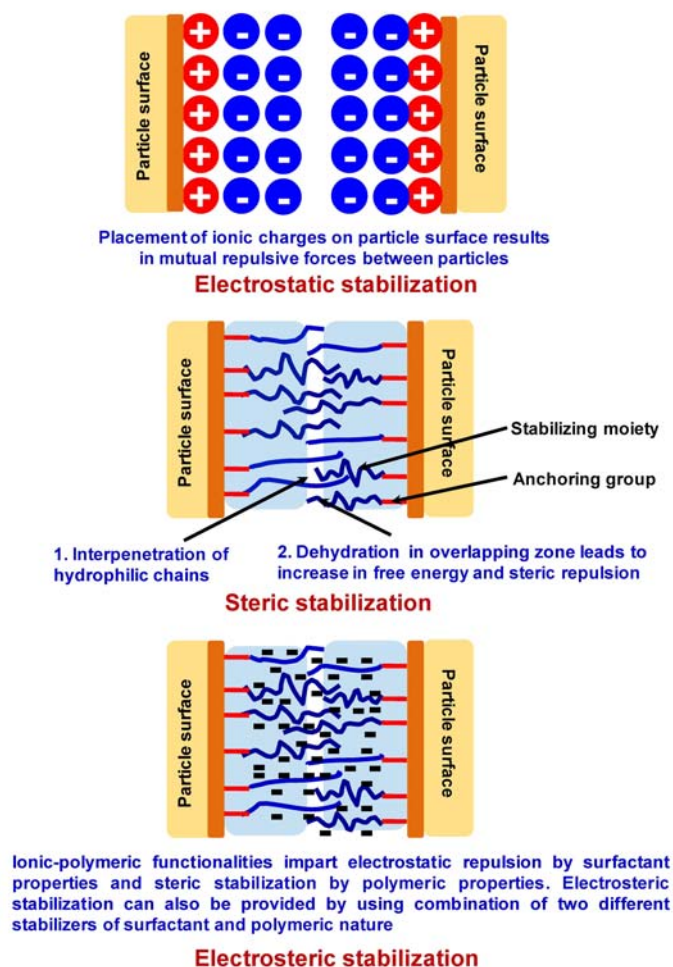


Figure 5 Schematic illustration of electrostatic, steric and electrosteric stabilization.

dispersion medium.

Moorthi *et al.* prepared nano-crystals of curcumin using a nanoprecipitation method (60). The nano-crystals were coated using either sodium lauryl sulphate (SLS) or poloxamer 188 for stabilization. Both stabilizers produced very small nano-crystals. The study concluded that the anionic nature of sodium lauryl sulfate (SLS) provided a higher zeta potential and provided higher electrostatic repulsive force between particles (60-62). This overcame the van der Waals attraction and gravitational force preventing nano-crystal aggregation resulting in narrow sized and stable curcumin nano-crystals (60). Similarly, Vergote *et al.* developed a nano-

crystalline dispersion of ketoprofen using Nano-crystal[®] technology (63). SLS and Cremophor RH40 as stabilizers. SLS stabilized the system through electrostatic repulsion (63-66).

Electrostatic stabilization is widely used due to its simplicity and low cost (56). Nonetheless, shortcomings are associated with this method. It is effective only in aqueous medium and is less effective after drying the formulation, as the ionized state is no longer maintained (56). It is sensitive to changes in the ionic strength of the dispersion medium and is difficult to apply to multiple phase systems because different solids develop different electric potentials (67-72). Electrostatic stabilization is

also sensitive to processing such as heat sterilization. However, if the components of formulation are heat labile, alternative strategies like filtration sterilization can be used (55, 73).

Steric stabilization

Steric stabilization involves the adsorption or attachment of non-ionic amphipathic polymers on the particle surface thus preventing aggregation. The stabilizing moieties are mutually repulsive in order to effectively keep the particles at a distance from each other. They have to be attached, partially absorbed or adsorbed to the particle strongly enough so that they are not affected by Brownian collisions of particles. Complete surface coverage also helps prevent escape. Polymers having specific affinity for the surface are generally used for steric stabilization. They are adsorbed in such a way that segments of polymeric chains extend to some distance from the surface into the dispersion medium. As particles approach sufficiently close, the adsorbed layers come into contact resulting in interpenetration of the hydrophilic chains (74). Since these chains are hydrated, overlap of these layers causes some dehydration and an increase in free energy (75-77). This results in repulsion between the particles thus stabilizing the nanosuspensions.

Polymeric stabilization offers several advantages over electrostatic stabilization, including (i) the particles stabilized by steric method are usually re-dispersible (78-80) (ii) a very high concentration of nano-crystals can be accommodated and the dispersion medium can be completely removed (81-83) (iii) it is not sensitive to the addition of electrolytes below their 'salting out' concentrations (52, 84-86), and (iv) it is suitable for multiple phase systems (85).

Sterically stabilized dispersions are usually sensitive to temperature changes. Flocculation may occur upon heating or cooling, or both, but is reversible. However, if the ratio between the hydrophilic to hydrophobic parts of the polymer is such that their cloud points exceed

the applied temperature variations, sterically stabilized dispersions exhibit stability towards temperature changes (32, 51, 87-91).

The most useful steric stabilizers are povidone (PVP), hypromellose (HPMC), hydroxypropyl cellulose (HPC), block and graft co-polymers (23, 51, 87-91). They generally comprise of an anchor group and a stabilizing moiety. The anchor group connects the polymer to the surface of the colloidal particle (47, 85, 92, 93). The stabilizing moiety is soluble in the dispersion medium and provides steric repulsion. Polymeric stabilizers are effective only when they have affinity for the surface of the particle and are adsorbed in such a way that segments of polymeric chains extend to some distance into the dispersion medium (94). The dispersion medium should be a good solvent for the adsorbed polymer. The main drawback associated with steric stabilization is the constant need to tailor the anchoring segment according to the particular drug of interest.

CLASSIFICATION OF STABILIZERS

Figure 6 shows a classification of commonly used stabilizers for pharmaceutical colloidal systems. These include synthetic linear polymers, synthetic co-polymers, semi-synthetic non-ionic polymers, amphiphilic amino acid co-polymers, celluloses, ionic surfactants and non-ionic surfactants (95, 96). Apart from these conventionally used stabilizers, the development and discovery of new stabilizers is underway for nanosuspension technology. The mechanism of stabilization depends on the physico-chemical nature of the stabilizer.

It has been reported that food biopolymers such as zein and polylactic acid, and food proteins such as soybean protein isolate, whey protein isolate and β -lactoglobulin can be used as stabilizers for colloidal systems (97, 98). Various amino acid co-polymers can also be used for stabilization (99-101). For example, naproxen nanosuspensions were stabilized with phenylalanine and leucine (32). Block co-

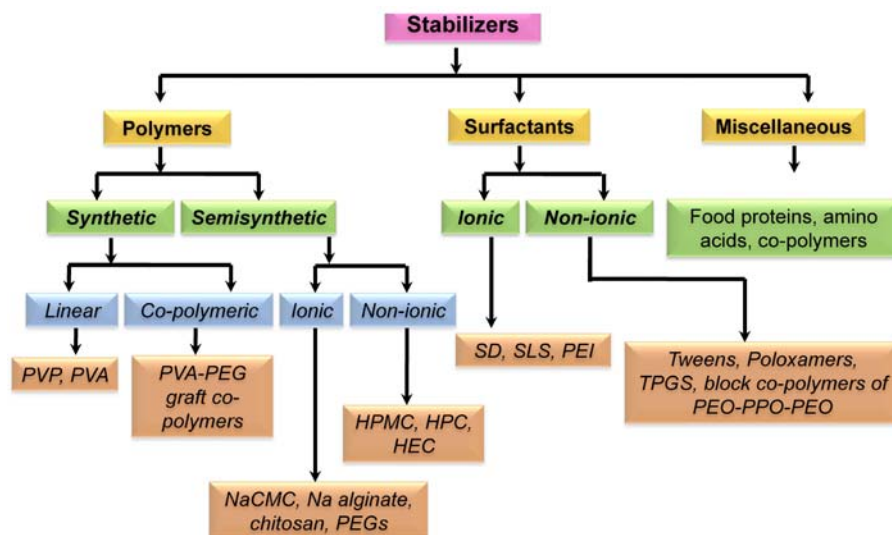


Figure 6 Various types of the stabilizers used for stabilization of nanoformulations. PVP (Povidone), PVA (Polyvinyl alcohol), PEG (Polyethylene glycol), HPMC (Hypromellose), HPC (Hydroxypropyl cellulose), HEC (Hydroxyethyl cellulose), NaCMC (Carboxymethylcellulose sodium), SD (Docusate sodium), SLS (Sodium lauryl sulfate), PEI (Polyethylene imine), TPGS (D- α -tocopheryl polyethylene glycol succinate), PEO (Polyethylene oxide) and PPO (Polypropylene oxide).

polymers such as poloxamers have been extensively used in a variety of pharmaceutical formulations (102-106). Stability of the drugs during processing can be maintained by polymeric stabilization as the crystal structure of drug particles is not affected by the polymers. In contrast, conventional small molecular weight surfactants such as SLS lack this property (107). Table 3 shows a list of stabilizers used in nanocrystalline formulations together with the mechanism of stabilization used.

PRACTICAL CONSIDERATIONS FOR THE SELECTION OF STABILIZERS

Studies focusing on developing empirical relationships between stabilizer efficacy and their properties have been published to aid in stabilizer selection (110, 133-139). These properties are summarized in Figure 7. A large number of research papers focusing on pharmaceutical nanosuspensions are available in the literature (87). This review focuses mainly on the studies that provide directions for selection of stabilizers for nanosuspensions.

Drug related parameters

Zeta potential

Zeta potential (ζ) is the electrokinetic potential of a colloidal system. It is the potential difference between the dispersion medium and the stationary layer of fluid surrounding the dispersed particle. Zeta potential is the potential at a hydrodynamic shear plane and is calculated from electrophoretic mobility (140). The zeta potential provides a measure of the magnitude of the interaction between colloidal particles (141). The zeta potential caused by a stabilizer is affected by the pH of the dispersion medium (142), conductivity (142), valency of ions (142, 143), concentration of drug in dispersion (144), and electrokinetic effects such as electrophoresis and electro-osmosis (145). Electrostatic stabilization gives rise to a mobile, charged, colloidal particle whose electrophoretic mobility can be measured (146).

The square of the zeta potential is proportional to the force of electrostatic repulsion between charged particles. The zeta potential is,

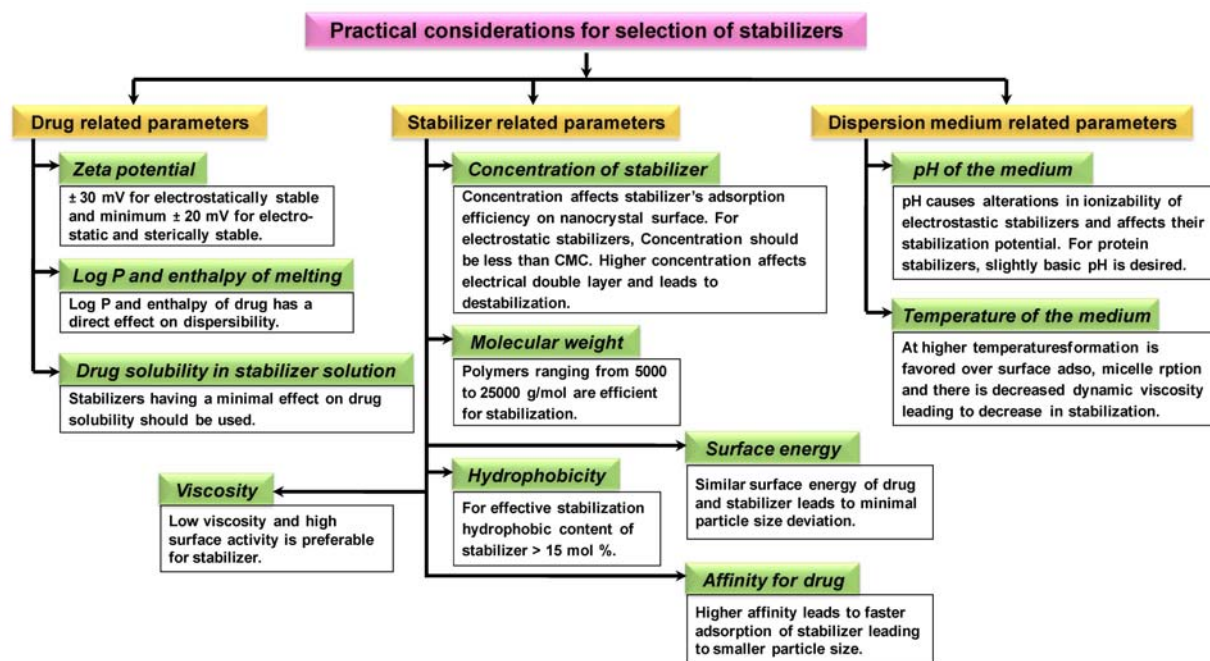


Figure 7 Practical considerations for the selection of stabilizers.

therefore, a measure of the stability of a colloidal systems. Increasing the absolute magnitude of the zeta potential increases the electrostatic stabilization. As the zeta potential approaches zero, electrostatic repulsion becomes small compared to the ever-present van der Waals attraction. Eventually, instability increases, which can result in aggregation followed by sedimentation and phase separation. Generally, for a suspension to be electrostatically stable, a zeta potential of ± 30 mV is required, whereas, for a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable (61, 140-144, 147).

Log P and enthalpy of melting

George *et al.* identified the correlation between drug/stabilizer properties and critical quality attributes of nanosuspension formulation prepared by wet media milling (148). A combination of a drug and a stabilizer with a similar log P value formed a stable nanosuspension. This was due to the affinity between the drug and the stabilizer resulting in

the adsorption of the stabilizer onto the drug surface (149). Drugs with low enthalpy of melting have been observed to form the least stable nanosuspensions regardless of the stabilizer used (87, 148, 150). Van Eerdenbrugh *et al.* reported no significant co-relation between physicochemical properties (molecular weight, melting point, log P, solubility and density) with the ease of nanosuspension stabilization (151). They demonstrated that surface hydrophobicity of the drug candidates was the driving force for nano-crystal agglomeration, thus reducing the success rate of producing nanosuspensions.

Drug solubility in a stabilizer solution

The solubility of a drug in a stabilizer solution impacts significantly the selection of the stabilizer. Ostwald ripening refers to the growth of larger particles at the expense of smaller particles. It occurs due to the faster rate of the solubilization of smaller particles because of their larger surface area and smaller curvature relative to the larger particles.

Table 3 List of stabilizers together with their mechanism of stabilization

STABILIZING AGENT(S)	MECHANISM OF STABILIZATION	DRUG COMPOUND	FORMULATION TYPE	TECHNIQUES FOR MAKING NANO-CRYSTALS	REF.
POLYMERS (SYNTHETIC)					
PVP K15	Steric	Danazol	Nanosuspension	Ball milling	(108)
PVA	Steric	Nitrendipine	Nanosuspension	Precipitation-ultrasonication	(109)
POLYMERS (SEMISYNTHETIC)					
HPMC	Steric	Ibuprofen	Nanosuspension	Precipitation under sonication followed by microfluidization	(110)
HPMC	Steric	Nifedipine	Freeze dried nanosuspension	High pressure homogenization	(24)
HPMC	Steric	Spironolactone	Nanosuspension	Antisolvent precipitation	(111)
HPMC	Steric	Naproxen	Nanosuspension	Media milling	(112)
Plantacare® 2000 (decyl glucoside)	Steric	Lutein	Cream and gel containing Freeze dried nanosuspension	High pressure homogenization	(113)
SURFACTANTS (IONIC)					
SLS	Electrostatic	Curcumin	Nanosuspension	Nanoprecipitation	(60)
SLS	Electrostatic	Ketoprofen	Matrix Pellet containing nano-crystals	Bead milling followed by spray drying and melt pelletization	(63)
SURFACTANTS (NONIONIC)					
TPGS	Steric	Itraconazole	Freeze dried nanosuspension	Ball milling followed by freeze drying	(114)
Tween 80	Steric	Spironolactone	Nanocapsules	Nanoprecipitation	(115)
Tween 80	Steric	Baicalein	Nanosuspension	Antisolvent recrystallization followed by high pressure homogenization	(116)
Tween 80	Steric	Quercetin	Nanosuspension	Bead milling	(117)
Poloxamer 188	Steric	Simvastatin	Nanosuspension	Sonoprecipitation	(118)
Poloxamer 188	Steric	Piroxicam	Orally disintegrating tablet	High pressure homogenization	(119)
Poloxamer 188	Steric	Diclofenac	Nanosuspension	High pressure homogenization	(120)
Poloxamer 188	Steric	Naproxen	Nanosuspension	Milling	(121)
Poloxamer 338	Steric	Azithromycin	Freeze dried nanosuspension	High pressure homogenization	(122)
Poloxamer 407	Steric	Paclitaxel	Nanosuspension	Stabilization of nano-crystal method	(123)
Poloxamer 407	Steric	Cyclosporine	Nanosuspension	Media milling	(124)
COMBINATION OF STABILIZERS					
Capryol 90 and Solutol HS 15	Steric	Atovaquone	Freeze dried nanosuspension	Microprecipitation followed by high pressure homogenization	(125)
PVP K30 and SLS	Electrosteric	Celecoxib	Nanosuspension and tablet	Emulsion diffusion (solvent exchange) followed by spray drying	(126)
Lutrol F127 and SLS	Electrosteric	Itraconazole	Nanosuspension	High pressure homogenization	(127)
HPMC and SLS	Electrosteric	Miconazole	Nanosuspension	High energy milling	(128)
Tween 80, Poloxamer 188 and Sodium cholate	Electrosteric	Amphotericin B	Nanosuspension	High pressure homogenization	(129)
Lecithin and Tyloxapol	Electrosteric	Budenoside	Nanosuspension	High pressure homogenization	(130)
Poloxamer 188 and Lecithin	Electrosteric	Oridonin	Nanosuspension	High pressure homogenization	(131)
Poloxamer 188, Phospholipon 90 and Sodium cholic acid	Electrosteric	Clofazimine	Nanosuspension	High pressure homogenization	(132)

As the smaller particles dissolve and crystallization occurs around large particles, the particle sizes of the latter grow over time. Many small crystals disappear, and the larger ones grow at the expense of the small crystals. The

smaller crystals act as fuel for the growth of large crystals. Molecules on the surface are energetically less stable than the ones already well ordered and packed in the interior. As the small particles dissolve and large particles grow,

the free energy of the dispersion is reduced (87, 110, 152-154).

A study of ibuprofen nanosuspensions demonstrated greater apparent aqueous solubility of ibuprofen in the presence of stabilizers such as SLS, Tween 80 and poloxamer 407 (155). Further, Ostwald ripening was observed through the increase in particle size. In contrast, stabilizers such as PVP K30 and HPMC minimally affected the intrinsic aqueous solubility of ibuprofen nanosuspension. Thus, stabilizers having minimal effect on apparent drug solubility should be used for the preparation of nanosuspensions (155).

Stabilizer related parameters

Concentration of stabilizer

The concentration of stabilizer in the dispersion media is important for the stabilization of colloidal systems. The amount of the stabilizer contributes to the stability of the suspension by influencing absorption affinity of the stabilizer on the surface of drug particles (155). An optimum concentration of stabilizer is required, since an inadequate amount of stabilizer may not provide a complete coverage of the drug surface, thus compromising the steric repulsion between the particles (156-159). The effective concentration of the surfactant required for stabilization depends on its molecular structure. It has been observed that a lower molar concentration is required for surfactants having a longer hydrophobic chain and a larger hydrophilic head, as these impart better steric hindrance and thus reduce the tendency to agglomerate (31, 160-162).

Van Eerdenbrugh *et al.* generated drug nano-crystals using wet media milling. The success rate in producing nanosuspensions using polysaccharide based stabilizers was limited by the high viscosity of these polymeric stabilizer solutions. Increasing the concentration of these stabilizers was not helpful for the generation of nanosuspensions. In contrast, the other

stabilizers such as PVP K30, PVA, poloxamer 188, polyvinyl alcohol–polyethylene glycol graft copolymer (K-IR), Tween 80 and TPGS did not show the dependency of nanosuspension formation on viscosity. The formation of nanosuspensions using PVP K30, PVP K90, F68 and K-IR was highly dependent on their concentration, where higher concentrations increased the stabilizing effect significantly (155, 163). Some literature reports suggest that, the use of a stabilizer in a concentration above the critical micelle concentration (CMC) could destabilize the nanosuspension (123, 164, 165). However, colloidal science is mostly empirical at this stage and the effect of the stabilizer concentration on nanosuspension stability depends on the properties of the drug, the stabilizer, the dispersion media and overall system.

Molecular weight

Polymeric stabilizers with higher molecular weight are generally effective steric stabilizers (166). The chain length of the polymers should be large enough to overcome the van der Waals forces of attraction. Short chain lengths offer a thin barrier to steric repulsion and promote aggregation. Polymers ranging from approximately 5000 to 25000 g/mol are usually suitable for the stabilization of nano-crystals (123, 167, 168).

Hydrophobicity

Lee *et al.* investigated amphiphilic amino acid co-polymers as stabilizers for the preparation of naproxen nano-crystal dispersions (169). The use of different amino acids imparted different hydrophobicity to the amino acid co-polymers. The hydrophobicity of the co-polymers was quantified using the hydrophobicity scale developed by Black and Mould (170). It was concluded that co-polymers with higher hydrophobicity successfully produced stable nano-crystals of hydrophobic drugs in comparison to those with lower hydrophobicity. This was attributed to the strong

polymer adsorption onto the hydrophobic drug surfaces (169). Thus, it can be concluded that, the drug and polymer hydrophobicity have a combined effect the formation of nanosuspensions, and their stability (87, 148, 150, 151, 169).

HLB (Hydrophilic Lipophilic Balance) is an empirical expression for the relationship between hydrophilic ("water-loving") and hydrophobic ("water-hating") groups of a surfactant (171). It is a measure of the degree to which a surfactant is hydrophilic or lipophilic. HLB numbers are low for hydrophobic surfactants and high for hydrophilic surfactants. HLB values of surfactants can serve as a rough, but quick, tool to assess their efficacy in stabilizing drug nano-crystals (172-179).

Viscosity

Van Eerdenbrugh *et al.* studied 13 stabilizers at 3 different concentrations to stabilize 9 drug compounds (32). Both surfactants and polymeric stabilizers were used. Overall, surfactants gave the best results in stabilizing the nanosuspensions as they exhibited low viscosity and high surface activity. Polysaccharide based stabilizers such as HPMC, methylcellulose, HEC, HPC, NaCMC and Na alginate had a negative effect during the formation of nano-crystals by media milling. These polymers were not able to produce sub-micron sized particles because of the high viscosity of these polymeric solutions. A high viscosity medium was inefficient in producing sub-micron size particles.

However, high viscosity can have a positive influence on the stability of colloidal particles (97). According to the Stokes-Einstein equation, a high viscosity medium leads to reduced diffusion velocity and ensures colloidal stability. The Stokes-Einstein equation (1) is as follows:

$$D = \frac{kT}{6\pi\eta r} \quad \text{Eq. 1}$$

where, D is the diffusion coefficient, K is the Boltzmann's constant, T is the absolute temperature, η is the viscosity and r is the radius of the spherical particle (180-183).

Rachmawati *et al.* investigated the influence of five stabilizers on the particle size in curcumin nanosuspensions (184). The smallest particle size was obtained from the TPGS stabilized system. The particle size increased in the order of TPGS < SLS < PVP < PVA < NaCMC. TPGS has a hydrophilic tail (polyethylene glycol) and hydrophobic portion (tocopherol), as well as, a large surface area. The combination of low viscosity and high surface activity makes it a superior stabilizer for nanosuspensions (14, 184).

Surface energy

Knowledge of the surface energy can be used to assist in the selection of an appropriate stabilizer (55, 110, 138, 155). Static contact angle measurements can provide a rough approximation of the surface energy. Lee *et al.* investigated the effectiveness of stabilizing polymers as a function of the similarity between the surface energies of the stabilizer and the drug (139). Correlations between the surface energies of the stabilizers and drugs were taken into account. The study concluded that nano-crystals with minimal particle size deviation could be prepared when the surface energy of the stabilizing polymer is similar to that of the surface of drug. However, no general trend between surface energy and particle size reduction was observed in a separate study carried out with the stabilizers HPC, PVP K30, poloxamer 407, poloxamer 188, PEG, SLS, benzethonium chloride and eleven model drugs (139). Thus, it is important to properly determine the role of surface energy when selecting a stabilizer. This will require carrying

out further experimental studies to obtain pertinent information.

Particle-stabilizer affinity

The affinity of the stabilizer for the particle surface regulates its adsorption kinetics (94, 185-188). Higher affinity leads to faster adsorption of the stabilizer. For effective stabilization, stronger and faster adsorption of stabilizer at full coverage is required. Possible interactions between the functionalities of the stabilizer and dispersed particles strengthen the adsorption of stabilizer (189). Hydrophobicity of a drug and a stabilizer generally serves as an indicator of a possible affinity between them (190).

Dispersion medium related parameters

pH of the dispersion medium

The pH of the dispersion medium plays an important role in electrostatic stabilization and steric stabilization using ionizable polymers (56, 86). The pH of an aqueous medium affects stabilizer performance. He *et al.* prepared indomethacin nanosuspensions using food proteins (whey protein and soybean protein isolate and β -lactoglobulin) as novel stabilizers (191). The isoelectric points (pI) of these denatured food proteins are in the range of 4-6. At the pI, a limited stabilization effect was obtained as the net charge on proteins was zero. Thus, colloidal instability was significant at the isoelectric point and resulted in aggregation. At pH values, above or below the pI, food proteins exhibit amphiphilic properties and thus could act as effective stabilizers. However, at highly acidic or highly basic pH, monomers are generated compromising the effectiveness of the stabilization of food proteins. A slightly basic pH is considered beneficial for food protein stabilizers (191).

Temperature of the dispersion medium

Temperature affects the affinity of the stabilizer for drug nano-crystals which may result in the

destabilization of the system (55, 123). As stated earlier, reversible flocculation may occur upon heating or cooling in sterically stabilized dispersions if the cloud point of polymer is lower than the applied temperature variations (32, 51, 87-91). Furthermore, an increase in temperature can alter the dynamic viscosity and the diffusion coefficient (180-183). Kakran *et al.* observed the effects of temperature on the stability of quercetin nanosuspensions. They found that, nano-suspensions stored at 40°C were unstable, compared to those stored at 25°C and 4°C (192).

TECHNIQUES FOR SCREENING STABILIZERS

The total surface area of the particles in a nanosuspension is typically extremely high compared to a conventional suspension. This encourages particle aggregation and therefore the selection of the type and quantity of stabilizer is critical for the development and subsequent physical stability of the nanosuspension. The selection of stabilizers for the successful formation of drug nano-crystals is influenced by the processing methods. Thus, it is very important to understand the process of particle formation to enable the selection of optimum stabilizer(s). Top-down or bottom-up methods rely on completely different mechanisms for the generation of nano-crystals and therefore the criteria for the selection of stabilizers for these two methods also differ. For example, for the bottom-up precipitation method, the HLB value of the stabilizer is critical to enable stabilization of the formed nano-crystals. In contrast, for the top-down high pressure homogenization method, the nano-crystal size depends, not only on the hardness of the drug material, but also on the homogenization pressure and number of passes. Larger particles are broken down into smaller ones thus generating new surfaces. In such cases, successful nano-crystal formation is based on the kinetics of stabilization of the newly formed surfaces. Therefore the affinity of a stabilizer for the particle surface is critical for the success of the process.

So far only a limited number of studies of a systematic selection of stabilizers for nanosuspensions have been reported. The reported methods for these selections are reviewed further below.

Selection based on surface free energy

The greatest challenge during processing drug nano-crystals is to 'stabilize' the free energy contributed by the newly created surfaces. Effective stabilization requires fast adsorption of the stabilizers onto these surfaces. Surface free energy determinations have been reported to be useful for the selection of stabilizers. Van Eerdenbrugh *et al.* described the analysis of surface free energy required for the selection of stabilizer for nano-crystalline formulations of several drugs (163). For polymeric stabilizers, the surface free energies of the drug and the stabilizers need to be similar for the effective stabilization of nano-crystals. The addition of a surfactant appears to provide benefits when the difference in the surface free energy between the drug and the polymer is high. The addition of a surfactant modulates the surface free energy encouraging favorable interaction between the drug and the stabilizer (193).

Selection based on the affinity of the stabilizer towards the nano-crystal surface

The affinity of a stabilizer to the particle surface can be studied using several different analytical methods. Atomic force microscopy (AFM) has been reported to be a useful tool for the selection of stabilizers as it provides information on the extent of interaction between the drug and the stabilizer. It also provides information about the characteristics of the adsorbed layer and the strength of the interfacial film. This information is useful for predicting wettability, aggregation, crystal growth and Ostwald ripening of the colloidal particles. AFM has been successfully used for the investigation of the interaction between polymeric stabilizers and ibuprofen to determine their suitability for the preparation

and stabilization of ibuprofen nanosuspensions. It was found that HPMC and HPC interacted strongly with ibuprofen resulting in extensive surface adsorption, confirming their suitability for preparation of an ibuprofen nanosuspension (138).

The stabilizer should be adsorbed onto the particle surfaces in order for suitable stabilization to be achieved. Furthermore, the adsorption should be strong enough to last for a long time. Adsorption of the stabilizer may occur through ionic interaction, hydrogen bonding, van der Waals attraction, ion-dipole interaction or by a hydrophobic effect. The surface of wet-milled drug nano-crystals is hydrophobic. High affinity of the stabilizer to the hydrophobic surfaces is thus important. In light of this, HLB values can help in selecting appropriate stabilizer (110).

Solubility of a drug in the dispersion vehicle

The solubility of a drug molecule in aqueous stabilizer solutions may also play a role in the stabilization of nano-crystals. In microfluidization processes, a higher solubility of ibuprofen in the presence of a stabilizer contributed to Ostwald ripening and an increase in particle size. Ostwald ripening is the change of an inhomogeneous structure over time, i.e., small crystals dissolve, and redeposit onto larger crystals. It is directly correlated to the concentration of the drug in the dispersed phase (194). Aqueous surfactant solutions increase the apparent solubility of small molecular weight materials by forming micelle-like structures and thus they result in Ostwald ripening. These systems may therefore present stability problems on storage.

Crystal growth inhibition

Ochi *et al.* developed nano-crystal formulations of meloxicam by wet-milling followed by lyophilization (195). Hydrophilic polymers were used as aggregation inhibitors. The polymers were selected based on high-throughput

screening of crystal growth inhibition from supersaturated meloxicam solutions. Supersaturation of meloxicam was observed in PVP K30, HPC, and Povacoat Type F (polyvinyl alcohol/acrylic acid/methyl methacrylate copolymer) solutions. The particle size distributions of pulverized meloxicam with PVP K30, HPC and Povacoat Type F were in the nanometer range following lyophilization. Micron-sized aggregates were formed in the formulations after storage at 60°C for 21 days. A correlation was observed between the effectiveness of the crystal growth inhibition of the polymer and the solubility of meloxicam in the polymer solution. This study showed that hydrophilic polymers that inhibit crystal growth in supersaturated meloxicam solutions tend to prevent aggregation. In this study, the stabilizers had been screened using the drug solubilization capacity of a polymer solution. Although this method can help narrow down the candidates for stabilizing a nano-crystal formulation, there are some limitations to this approach. Some combinations of polymers and drug molecules can induce a polymorphic transformation in the drug (196, 197). In addition, surfactants cannot be screened using this method because their ability to prevent precipitation could result in the overestimation of the solubility of the drug (4, 198, 199).

FUTURE TRENDS

The last two decades have witnessed the evolution of drug nano-crystals from an exploratory approach to a successful commercial approach in the field of drug delivery. Most of the research has focused on the discovery of novel technologies for producing nano-crystals. These technologies have been patented both by drug delivery and pharmaceutical companies. As a result, a wide selection of technologies are now available in the market. The research on nano-crystals is currently focused on the mechanistic understanding of drug/stabilizer interactions to provide a scientific framework for the selection of stabilizers. Attempts are being made to evaluate novel combinations of existing

stabilizers, to achieve synergistic effects. Another area of research is the selection of novel excipients for the stabilization of nano-crystals. These advancements will assist in the development of novel approaches for nano-crystal stabilization.

CONCLUSIONS

The use of colloidal systems in the pharmaceutical industry has gained considerable attention. These systems have been investigated primarily to increase the dissolution rate/bioavailability of poorly water soluble drugs. However, the physical stability of sub-micron size particles remains a challenge during pharmaceutical product development. The addition of electrostatic or steric stabilizers, or combinations of both, is a common approach to increase the stability of colloidal systems. Understanding particle-particle interactions within the colloidal system assists in the selection of stabilizer. Various factors related to drug, stabilizer and dispersion medium influence the activity of stabilizer. A scientific understanding of the mechanisms involved in the stabilization of nano-crystals will help in the rational selection of stabilizers. This, together with the discovery of new stabilizers, can broaden the scope of applications of nano-crystals.

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