Stabilizers used in nano-crystal based drug delivery systems.

Ganesh Shete[a], Hemant Jain[a], Deeksha Punj[a], Hitesh Prajapat[a], Priya Akotiya[a] and Arvind Kumar Bansal[a*]

[a]Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Punjab 160 062, India

Received: December 1, 2014; *Accepted:* December 15, 2014

Review Article

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 though 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

*Corresponding author:Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, S.A.S. Nagar, Sector-67, Mohali, Punjab (INDIA)-160 062, Tel: +91-172- 2214682 Ext: 2126, Fax: +91-172- 2214692, E-mail: akbansal@niper.ac.in; bansalarvind@yahoo.com

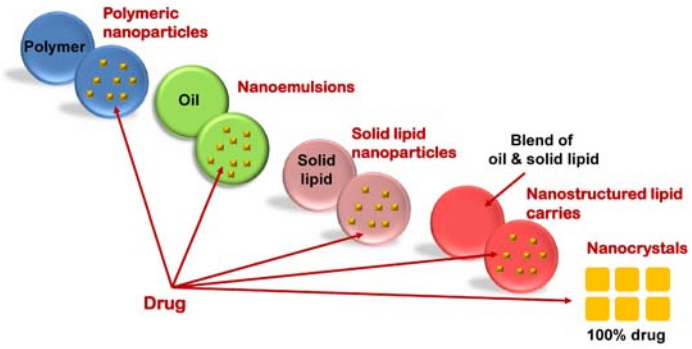

Figure 1 Nanotechnology based drug delivery systems. Reproduced with permission from Reference (16).

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 commerically.

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

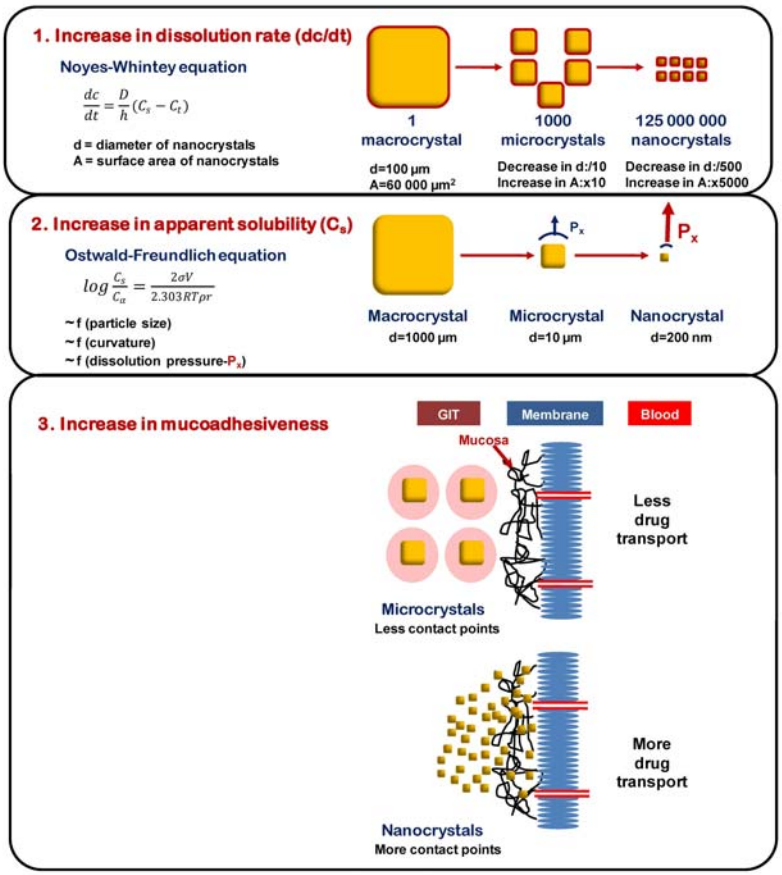

Figure 2 Features of nanocrystals: (1) increased dissolution rate due to increased surface area, (2) increased apparent solubility due to increased dissolution pressure of strongly curved small nanocrystals, and (3) increased mucoadhesiveness of nanocrystals due to increased contact area of small versus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

'top-down' (particle size reduction by mechanical means). Combination methods involving precipitation followed by a size reduction step have also been used. Nano-crystals 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).

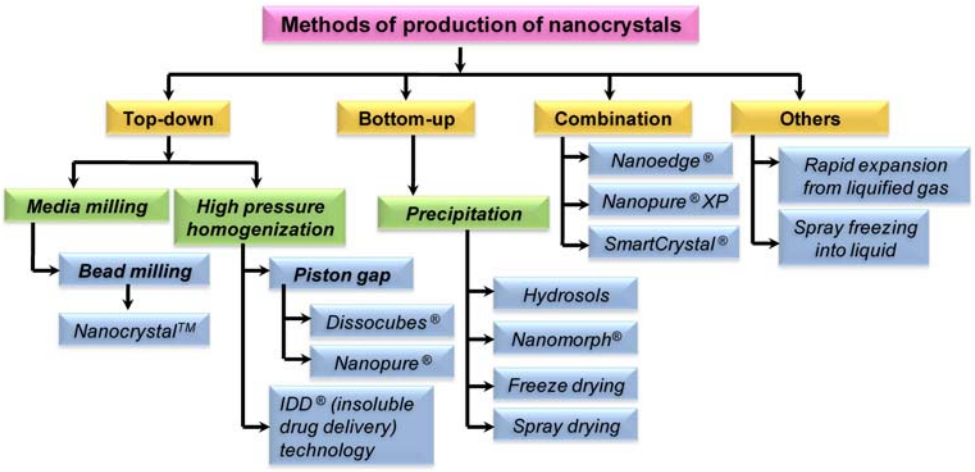

Figure 3 Methods for generating nanocrystals.

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 >1μm. 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 nm	Particles invisible by electron microscopy; Pass through semi-permeable membrane	Oxygen molecules dissolved in water
Colloidal dispersion	1 nm-1000 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 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

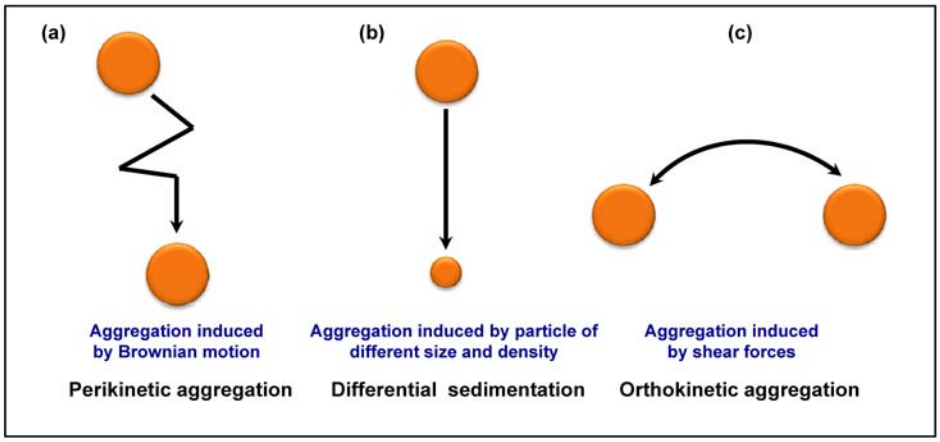

Figure 4 Schematic representation of (a) perikinetic aggregation (b) differential sedimentation and (c) orthokinetic aggregation.

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 NANO-CRYSTALLINE 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

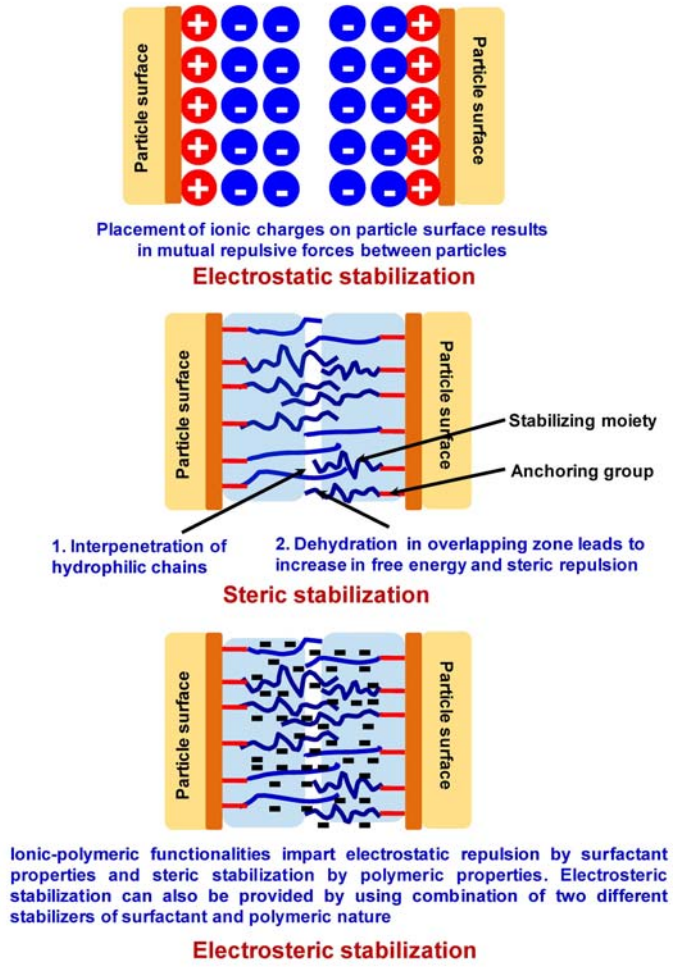

Figure 5 Schematic illustration of electrostatic, steric and electrosteric stabilization.

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-

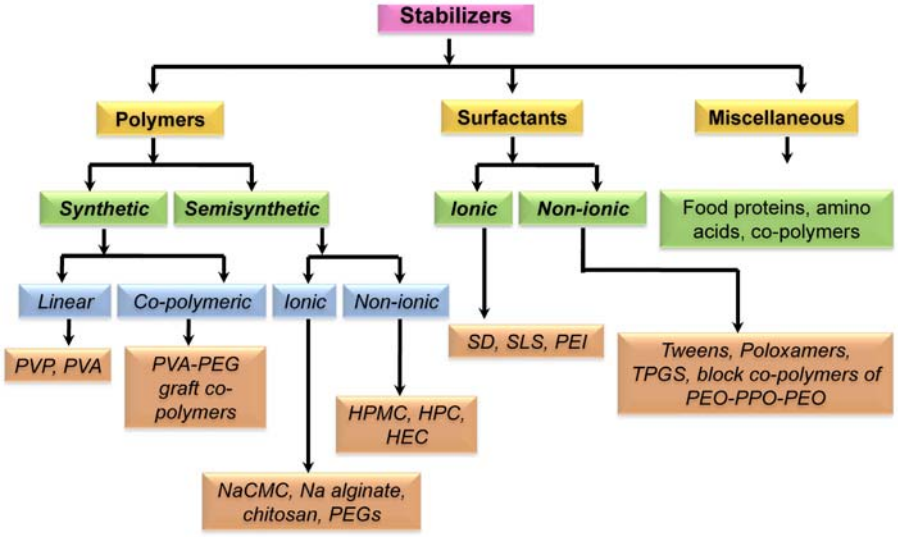

Figure 6 Various types of the stabilizers used for stabilization of nanoformulations. PVP (Povidone), PVA (Polyvinyl alcohol), PEG (Polyethylene glycol), HPMC (Hypromellose), HPC (Hydroxypropyl cellulose), HEC (Hydroxyethyl cellulose), NaCMC (Carboxymethylcellulose sodium), SD (Docusate sodium), SLS (Sodium lauryl sulfate), PEI (Polyehtylene 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 nano-crystalline 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,

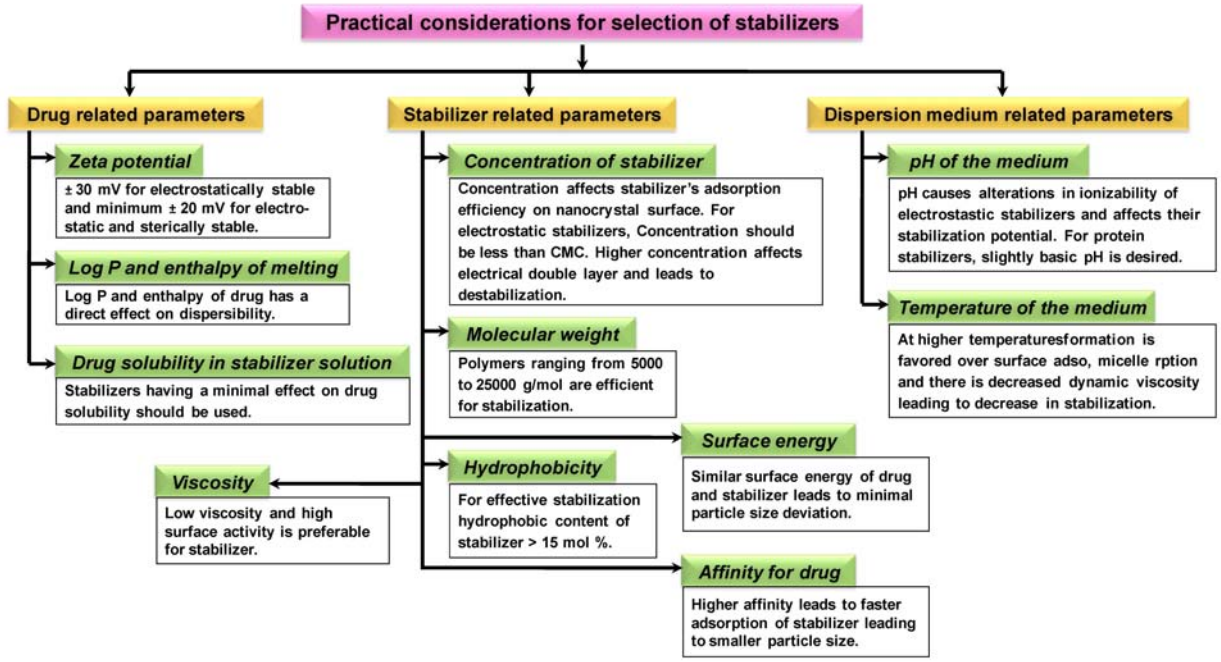

Figure 7 Practical considerations for the selection of stabilizers.

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	Nanosupension	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	Nanosupension	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 hydro-phobicity. 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 nanos-uspensions, 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:

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

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 nanosuspen-sions 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 an 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. Super-saturation of meloxicam was observed in PVP K30, HPC, and Povacoat Type F (polyvinyl alcohol/acrylic acid/methyl metha-crylate co-polymer) solutions. The particle size distri-butions 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.

REFERENCES

1 Gupta S, Kesarla R, Omri A. Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. ISRN Pharm, 2013: 16, 2013.

2 Che E, Zheng X, Sun C, Chang D, Jiang T, Wang S. Drug nanocrystals: a state of the art formulation strategy for preparing the poorly water-soluble drugs. Asian J Pharm Sci, 7(1): 85-95, 2012.

3 Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods, 44(1): 235-249, 2000.

4 Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery

and development settings. Adv Drug Deliver Rev, 46(1-3): 3-26, 1997.

5 Stella VJ, Nti-Addae KW. Prodrug strategies to overcome poor water solubility. Adv Drug Deliver Rev, 59(7): 677-694, 2007.

6 Elder DP, Holm R, Diego HL. Use of pharmaceutical salts and cocrystals to address the issue of poor solubility. Int J Pharm, 453(1): 88-100, 2013.

7 Sanghvi R, Evans D, Yalkowsky SH. Stacking complexation by nicotinamide: A useful way of enhancing drug solubility. Int J Pharm, 336(1): 35-41, 2007.

8 Jouyban-Gharamaleki A, Valaee L, Barzegar-Jalali M, Clark BJ, Acree Jr WE. Comparison of various cosolvency models for calculating solute solubility in water-cosolvent mixtures. Int J Pharm, 177(1): 93-101, 1999.

9 Kiss N, Brenn G, Pucher H, Wieser J, Scheler S, Jennewein H, Suzzi D, Khinast J. Formation of O/W emulsions by static mixers for pharmaceutical applications. Chem Eng Sci, 66(21): 5084-5094, 2011.

10 Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm, 50(1): 47-60, 2000.

11 Kaushal AM, Gupta P, Bansal AK. Amorphous drug delivery systems: molecular aspects, design, and performance. Crit Rev Ther Drug, 21(3): 133-193, 2004.

12 Sarker DK. Engineering of nanoemulsions for drug delivery. Cur Drug Deliv, 2(4): 297-310, 2005.

13 Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Rel, 70(1): 1-20, 2001.

14 Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliver Rev, 47(2): 165-196, 2001.

15 Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliver Rev, 59(6): 522-530, 2007.

16 Müller RH, Gohla S, Keck CM. State of the art of nanocrystals- special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm, 78(1): 1-9, 2011.

17 Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: a review. Crit Rev Ther Drug, 19(2): 99-134, 2002.

18 Müller RH, Heinemann S. Emulsions for intravenous administration. I: Emulsions for nutrition and drug delivery. Pharm Industrie, 55(9): 853-856, 1993.

19 Storm G, Wilms HP, Crommelin DJ. Liposomes and biotherapeutics. Biotherapy, 3(1): 25-42, 1991.

20 Crommelin DJ, Storm G. Liposomes: from the bench to the bed. J Lipos Res, 13(1): 33-36, 2003.

21 Müller R, Shegokar R, M Keck C. 20 Years of Lipid Nanoparticles (SLN NLC): Present State of Development Industrial Applications. Cur Drug Discov Tech, 8(3): 207-227, 2011.

22 Li D, Kaner RB. Shape and aggregation control of nanoparticles: not shaken, not stirred. J Am Chem Soc, 128(3): 968-975, 2006.

23 Gao L, Zhang D, Chen M. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanopart Res, 10(5): 845-862, 2008.

24 Hecq J, Deleers M, Fanara D, Vranckx H, Amighi K. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. Int J Pharm, 299(1): 167-177, 2005.

25 Keck CM, Müller RH. Nanotoxicological classification system (NCS)- A guide for the risk-benefit assessment of nanoparticulate drug delivery systems. Eur J Pharm Biopharm, 84(3): 445-448, 2013.

26 Möschwitzer J, Müller RH. Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. Eur J Pharm Biopharm, 62(3): 282-287, 2006.

27 Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov, 3(9): 785-796, 2004.

28 Gao L, Liu G, Ma J, Wang X, Zhou L, Li X, Wang F. Application of drug nanocrystal technologies on oral drug delivery of poorly soluble drugs. Pharm Res, 30(2): 307-324, 2013.

29 De Waard H, Hinrichs WLJ, Frijlink HW. A novel bottom-up process to produce drug nanocrystals:

controlled crystallization during freeze-drying. J Control Rel, 128(2): 179-183, 2008.

30 Bansal AK, Dantuluri AKR, Shete G, Pawar YB. Nanocrystalline solid dispersion compositions and process of preparation thereof. WO 2013132457 A2. Patent Co-operation Treaty Office, Geneva, Switzerland., 2013.

31 Sinha B, Müller RH, Möschwitzer JP. Bottom-up approaches for preparing drug nanocrystals: Formulations and factors affecting particle size. Int J Pharm, 453(1): 126-141, 2013.

32 Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm, 364(1): 64-75, 2008.

33 Gao L, Liu G, Ma J, Wang X, Zhou L, Li X. Drug nanocrystals: In vivo performances. J Control Rel, 160(3): 418-430, 2012.

34 Shegokar R, Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm, 399(1): 129-139, 2010.

35 Hogg R, Healy TW, Fuerstenau DW. Mutual coagulation of colloidal dispersions. Tras Farad Soc, 62: 1638-1651, 1966.

36 Gregory J. Adsorption and flocculation by polymers and polymer mixtures. Adv Colloid Inter Sci, 169(1): 1-12, 1987.

37 Gregory J. The density of particle aggregates. Water Sci Tech, 36(4): 1-13, 1997.

38 Gregory J. Monitoring particle aggregation processes. Adv Colloid Inter Sci, 147-148: 109-123, 2009.

39 Heinson WR, Sorensen CM, Chakrabarti A. Shear History Independence in Colloidal Aggregation. Langmuir, 28(31): 11337-11342, 2012.

40 Haines BA, Martin AN. Interfacial properties of powdered material; Caking in liquid dispersions I. Caking and flocculation studies. J Pharm Sci, 50(3): 228-232, 1961.

41 Berre FL, Chauveteau G, Pefferkorn E. Perikinetic and orthokinetic aggregation of hydrated colloids. J Colloid Interf Sci, 199(1): 1-12, 1998.

42 Hansen PH, Bergström L. Perikinetic aggregation of alkoxylated silica particles in two dimensions. J Colloid Interf Sci, 218(1): 77-87, 1999.

43 O'Melia CR, Tiller CL. Physicochemical aggregation and deposition in aquatic environments. Environ Part, 2: 353-386, 1993.

44 Westbrook C, Ball R, Field P, Heymsfield AJ. Theory of growth by differential sedimentation, with application to snowflake formation. Phys Rev E, 70(2): 021403, 2004.

45 Hansen PH, Malmsten M, Bergenståhl B, Bergström L. Orthokinetic aggregation in two dimensions of monodisperse and bidisperse colloidal systems. J Colloid Interf Sci, 220(2): 269-280, 1999.

46 Mumtaz H, Hounslow M, Seaton N, Paterson W. Orthokinetic aggregation during precipitation: A computational model for calcium oxalate monohydrate. Chem Eng Res Des, 75(2): 152-159, 1997.

47 Ain-Ai A, Gupta PK. Effect of arginine hydrochloride and hydroxypropyl cellulose as stabilizers on the physical stability of high drug loading nanosuspensions of a poorly soluble compound. Int J Pharm, 351(1): 282-288, 2008.

48 Yu W, Xie H. A review on nanofluids: preparation, stability mechanisms, and applications. J Nanomat, 2012: 1-17, 2012.

49 Bhakay A, Merwade M, Bilgili E, Dave RN. Novel aspects of wet milling for the production of microsuspensions and nanosuspensions of poorly water-soluble drugs. Drug Dev Ind Pharm, 37(8): 963-976, 2011.

50 Srinarong P, de Waard H, Frijlink HW, Hinrichs WLJ. Improved dissolution behavior of lipophilic drugs by solid dispersions: the production process as starting point for formulation considerations. Expert Opin Drug Deliver, 8(9): 1121-1140, 2011.

51 Kesisoglou F, Panmai S, Wu Y. Nanosizing- oral formulation development and biopharmaceutical evaluation. Adv Drug Deliver Rev, 59(7): 631-644, 2007.

52 Einarson MB, Berg JC. Electrosteric stabilization of colloidal latex dispersions. J Colloid Interf Sci, 155(1): 165-172, 1993.

53 Fritz G, Schädler V, Willenbacher N, Wagner NJ. Electrosteric stabilization of colloidal dispersions. Langmuir, 18(16): 6381-6390, 2002.

54 Romero-Cano MS, Martin-Rodriguez A, De Las Nieves FJ. Electrosteric stabilization of polymer colloids with different functionality. Langmuir, 17(11): 3505-3511, 2001.

55 Peltonen L, Hirvonen J. Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods. J Pharm Pharmacol, 62(11): 1569-1579, 2010.

56 Van der Hoeven PC, Lyklema J. Electrostatic stabilization in non-aqueous media. Ad Colloid Interfac, 42(12): 205-277, 1992.

57 Hang J, Shi L, Feng X, Xiao L. Electrostatic and electrosteric stabilization of aqueous suspensions of barite nanoparticles. Powder Technol, 192(2): 166-170, 2009.

58 Manciu M, Ruckenstein E. Role of the hydration force in the stability of colloids at high ionic strengths. Langmuir, 17(22): 7061-7070, 2001.

59 Ducker WA, Senden TJ, Pashley RM. Direct measurement of colloidal forces using an atomic force microscope. Nature, 353(6341): 239-241, 1991.

60 Moorthi C, Kathiresan K. Fabrication of highly stable sonication assisted curcumin nanocrystals by nanoprecipitation method. Drug Inv Today, 5(1): 66-69, 2013.

61 Yoon R-H, Yordan JL. Zeta-potential measurements on microbubbles generated using various surfactants. J Colloid Interf Sci, 113(2): 430-438, 1986.

62 Childress AE, Elimelech M. Effect of solution chemistry on the surface charge of polymeric reverse osmosis and nanofiltration membranes. Journal Membrane Sci, 119(2): 253-268, 1996.

63 Vergote G, Vervaet C, Van Driessche I, Hoste S, De Smedt S, Demeester J, Jain R, Ruddy S, Remon JP. An oral controlled release matrix pellet formulation containing nanocrystalline ketoprofen. Int J Pharm, 219(1): 81-87, 2001.

64 Aoki T, Decker EA, McClements DJ. Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. Food Hydrocolloid, 19(2): 209-220, 2005.

65 Rao YM, Kumar MP, Apte S. Formulation of nanosuspensions of albendazole for oral administration. Current Nanosci, 4(1): 53-58, 2008.

66 Jódar-Reyes AB, Martín-Rodríguez A, Ortega-Vinuesa JL. Effect of the ionic surfactant concentration on the stabilization/destabilization of polystyrene colloidal particles. J Colloid Interf Sci, 298(1): 248-257, 2006.

67 Badawy AME, Luxton TP, Silva RG, Scheckel KG, Suidan MT, Tolaymat TM. Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticles suspensions. Env Sci Tech, 44(4): 1260-1266.

68 Dong XM, Kimura T, Revol J-Fo, Gray DG. Effects of ionic strength on the isotropic-chiral nematic phase transition of suspensions of cellulose crystallites. Langmuir, 12(8): 2076-2082, 1996.

69 Pelton R. Temperature-sensitive aqueous microgels. Adv Colloid Interfac, 85(1): 1-33, 2000.

70 Chaubal MV, Popescu C. Conversion of nanosuspensions into dry powders by spray drying: a case study. Pharm Res, 25(10): 2302-2308, 2008.

71 Basa S, Muniyappan T, Karatgi P, Prabhu R, Pillai R. Production and in vitro characterization of solid dosage form incorporating drug nanoparticles. Drug Dev Ind Pharm, 34(11): 1209-1218, 2008.

72 Schmidt C, Bodmeier R. Incorporation of polymeric nanoparticles into solid dosage forms. J Control Rel, 57(2): 115-125, 1999.

73 Washington C. Stability of lipid emulsions for drug delivery. Adv Drug Deliver Rev, 20(2): 131-145, 1996.

74 Napper DH. Flocculation studies of sterically stabilized dispersions. J Colloid Interf Sci, 32(1): 106-114, 1970.

75 Lasic DD. Sterically stabilized vesicles. Angew Chem Int Edit, 33(17): 1685-1698, 1994.

76 Kato K, Uchida E, Kang E-T, Uyama Y, Ikada Y. Polymer surface with graft chains. Prog Polym Sci, 28(2): 209-259, 2003.

77 Discher DE, Eisenberg A. Polymer vesicles. Science, 297(5583): 967-973, 2002.

78 Battaglia L, Gallarate M. Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery. Exp Opin on Drug Deliv, 9(5): 497-508, 2012.

79 Shenoy DB, Sukhorukov GB. Engineered microcrystals for direct surface modification with layer-by-layer technique for optimized dissolution. Eur J Pharm Biopharm, 58(3): 521-527, 2004.

80 Giermanska-Kahn J, Schmitt V, Binks BP, Leal-Calderon F. A new method to prepare monodisperse Pickering emulsions. Langmuir, 18(7): 2515-2518, 2002.

81 Kesisoglou F, Panmai S, Wu Y. Nanosizing- oral formulation development and biopharmaceutical evaluation. Adv Drug Deliver Rev, 59(7): 631-644, 2007.

82 Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm, 62(1): 3-16, 2006.

83 Yu WW, Wang YA, Peng X. Formation and stability of size-, shape-, and structure-controlled CdTe nanocrystals: ligand effects on monomers and nanocrystals. Chem Mater, 15(22): 4300-4308, 2003.

84 Pincus P. Colloid stabilization with grafted polyelectrolytes. Macromolecules, 24(10): 2912-2919, 1991.

85 Napper DH. Colloid stability. Ind Eng Chem Prod Rd, 9(4): 467-477, 1970.

86 Zimmermann E, Müller RH. Electrolyte-and pH-stabilities of aqueous solid lipid nanoparticle (SLN) dispersions in artificial gastrointestinal media. Eur J Pharm Biopharm, 52(2): 203-210, 2001.

87 Wu L, Zhang J, Watanabe W. Physical and chemical stability of drug nanoparticles. Adv Drug Deliver Rev, 63(6): 456-469.

88 Singh SK, Srinivasan KK, Gowthamarajan K, Singare DS, Prakash D, Gaikwad NB. Investigation of preparation parameters of nanosuspension by top-down media milling to improve the dissolution of poorly water-soluble glyburide. Eur J Pharm Biopharm, 78(3): 441-446.

89 Kong HJ, Bike SG, Li VC. Electrosteric stabilization of concentrated cement suspensions imparted by a strong anionic polyelectrolyte and a non-ionic polymer. Cement Concrete Res, 36(5): 842-850, 2006.

90 Kong H-J, Bike SG, Li VC. Development of a self-consolidating engineered cementitious composite employing electrosteric dispersion/stabilization. Cement Concrete Comp, 25(3): 301-309, 2003.

91 Ghosh I, Bose S, Vippagunta R, Harmon F. Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibit crystal growth. Int J Pharm, 409(1): 260-268.

92 Napper DH. Steric stabilization. J Colloid Intef Sci, 58(2): 390-407, 1977.

93 Napper DH. Steric stabilization and the Hofmeister series. J Colloid Intef Sci, 33(3): 384-392, 1970.

94 Liz-Marzan LM, Giersig M, Mulvaney P. Synthesis of nanosized gold-silica core-shell particles. Langmuir, 12(18): 4329-4335, 1996.

95 Antonietti M, Förster S, Hartmann J, Oestreich S. Novel amphiphilic block copolymers by polymer reactions and their use for solubilization of metal salts and metal colloids. Macromolecules, 29(11): 3800-3806, 1996.

96 Devissaguet JP, Fessi C, Puisieux F, Thies C. Process for the preparation of dispersible colloidal systems of a substance in the form of nanoparticles, US 5118528 A.

97 Dickinson E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloid, 17(1): 25-39, 2003.

98 Dickinson E. Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocolloid, 23(6): 1473-1482, 2009.

99 Oupicky D, Howard KA, Konak Cr, Dash PR, Ulbrich K, Seymour LW. Steric stabilization of poly-L-Lysine/DNA complexes by the covalent attachment of semitelechelic poly [N-(2-hydroxypropyl) methacrylamide]. Bioconjugate Chem, 11(4): 492-501, 2000.

100 Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliver Rev, 47(1): 113-131, 2001.

101 Iijima M, Kamiya H. Surface modification for improving the stability of nanoparticles in liquid media. KONA Powder Particle J, 27: 119-129, 2009.

102 de las Heras Alarcon C, Pennadam S, Alexander C. Stimuli responsive polymers for biomedical applications. Chem Soc Rev, 34(3): 276-285, 2005.

103 Kipp JE, Wong JCT, Doty MJ, Rebbeck CL. Microprecipitation method for preparing submicron suspensions, US 7037528 B2.

104 Weers JG, Schutt EG, Dellamary LA, Tarara TE, Kabalnov A. Stabilized preparations for use in metered dose inhalers, WO 1999016422 A1.

105 Gilbert P, Jones MV, Allison DG, Heys S, Maira T, Wood P. The use of poloxamer hydrogels for the assessment of biofilm susceptibility towards biocide treatments. J Appl Microbiol, 85(6): 985-990, 1998.

106 Stratton LP, Dong A, Manning MC, Carpenter JF. Drug delivery matrix containing native protein precipitates suspended in a poloxamer gel. J Pharm Sci, 86(9): 1006-1010, 1997.

107 Jiang L, Gao L, Sun J. Production of aqueous colloidal dispersions of carbon nanotubes. J Colloid Interf Sci, 260(1): 89-94, 2003.

108 Liversidge GG, Cundy KC. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int J Pharm, 125(1): 91-97, 1995.

109 Xia D, Quan P, Piao H, Piao H, Sun S, Yin Y, Cui F. Preparation of stable nitrendipine nanosuspensions using the precipitation–ultrasonication method for enhancement of dissolution and oral bioavailability. Eur J Pharm Sci, 40(4): 325-334, 2010.

110 Verma S, Gokhale R, Burgess DJ. A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions. Int J Pharm, 380(1): 216-222, 2009.

111 Dong Y, Ng WK, Shen S, Kim S, Tan RB. Preparation and characterization of spironolactone nanoparticles by antisolvent precipitation. Int J Pharm, 375(1): 84-88, 2009.

112 Kayaert P, Van den Mooter G. An investigation of the adsorption of hydroxypropylmethyl cellulose 2910 5 mPa s and polyvinylpyrrolidone K90 around Naproxen nanocrystals. J Pharm Sci, 101(10): 3916-3923, 2012.

113 Mitri K, Shegokar R, Gohla S, Anselmi C, Müller RH. Lutein nanocrystals as antioxidant formulation for oral and dermal delivery. Int J Pharm, 420(1): 141-146, 2011.

114 Bernard VE, Sofie V, Jan V, Ludo F, Jan VH, Guy VdM, Patrick A. Microcrystalline cellulose, a useful alternative for sucrose as a matrix former during freeze-drying of drug nanosuspensions - a case study with itraconazole. Eur J Pharm Biopharm, 70(2): 590-596, 2008.

115 Limayem Blouza I, Charcosset C, Sfar S, Fessi H. Preparation and characterization of spironolactone-loaded nanocapsules for paediatric use. Int J Pharm, 325(1): 124-131, 2006.

116 Zhang J, Lv H, Jiang K, Gao Y. Enhanced bioavailability after oral and pulmonary administration of baicalein nanocrystal. Int J Pharm, 420(1): 180-188, 2011.

117 Kakran M, Shegokar R, Sahoo NG, Al Shaal L, Li L, Müller RH. Fabrication of quercetin nanocrystals: comparison of different methods. Eur J Pharm Biopharm, 80(1): 113-121, 2012.

118 Jiang T, Han N, Zhao B, Xie Y, Wang S. Enhanced dissolution rate and oral bioavailability of simvastatin nanocrystal prepared by sonoprecipitation. Drug Dev Ind Pharm, 38(10): 1230-1239, 2012.

119 Lai F, Pini E, Angioni G, Manca M, Perricci J, Sinico C, Fadda A. Nanocrystals as tool to improve piroxicam dissolution rate in novel orally disintegrating tablets. Eur J Pharm Biopharm, 79(3): 552-558, 2011.

120 Lai F, Sinico C, Ennas G, Marongiu F, Marongiu G, Fadda AM. Diclofenac nanosuspensions: influence of preparation procedure and crystal form on drug dissolution behaviour. Int J Pharm, 373(1): 124-132, 2009.

121 Liversidge GG, Conzentino P. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int J Pharm, 125(2): 309-313, 1995.

122 Zhang D, Tan T, Gao L, Zhao W, Wang P. Preparation of azithromycin nanosuspensions by high pressure homogenization and its physicochemical characteristics studies. Drug Dev Ind Pharm, 33(5): 569-575, 2007.

123 Deng J, Huang L, Liu F. Understanding the structure and stability of paclitaxel nanocrystals. Int J Pharm, 390(2): 242-249, 2010.

124 Nakarani M, Patel P, Patel J, Patel P, Murthy RS, Vaghani SS. Cyclosporine a-nanosuspension: formulation, characterization and in vivo comparison with a marketed formulation. Sci Pharm, 78(2): 345, 2010.

125 Borhade V, Pathak S, Sharma S, Patravale V. Formulation and characterization of atovaquone nanosuspension for improved oral delivery in the treatment of malaria. Nanomedicine, 9(5): 649-666, 2014.

126 Dolenc A, Kristl J, Baumgartner S, Planinšek O. Advantages of celecoxib nanosuspension formulation and transformation into tablets. Int J Pharm, 376(1): 204-212, 2009.

127 Sun W, Mao S, Shi Y, Li LC, Fang L. Nanonization of itraconazole by high pressure homogenization: stabilizer optimization and effect of particle size on oral absorption. J Pharm Sci, 100(8): 3365-3373, 2011.

128 Cerdeira AM, Mazzotti M, Gander B. Miconazole nanosuspensions: influence of formulation variables on particle size reduction and physical stability. Int J Pharm, 396(1): 210-218, 2010.

129 Kayser O, Olbrich C, Yardley V, Kiderlen A, Croft S. Formulation of amphotericin B as nanosuspension for oral administration. Int J Pharm, 254(1): 73-75, 2003.

130 Jacobs C, Müller RH. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Res, 19(2): 189-194, 2002.

131 Gao L, Zhang D, Chen M, Duan C, Dai W, Jia L, Zhao W. Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions. Int J Pharm, 355(1): 321-327, 2008.

132 Peters K, Leitzke S, Diederichs J, Borner K, Hahn H, Müller R, Ehlers S. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. J Antimicrob Chemoth, 45(1): 77-83, 2000.

133 Langevin D. Electric Field Induced Capillary Attraction between Like Charged Particles at Liquid Interfaces. Chem Phys Chem, 4(10): 1057-1058, 2003.

134 Nikolaides MG, Bausch AR, Hsu MF, Dinsmore AD, Brenner MP, Gay C, Weitz DA. Electric-field-induced capillary attraction between like-charged particles at liquid interfaces. Nature, 420(6913): 299-301, 2002.

135 Winslow FH, Matreyek W. Particle size in suspension polymerization. Ind Eng Chem, 43(5): 1108-1112, 1951.

136 Grier DG. When like charges attract: interactions and dynamics in charge-stabilized colloidal suspensions. J Phys-Condens Mat, 12(8A): A85, 2000.

137 Phenrat T, Saleh N, Sirk K, Kim HJ, Tilton RD, Lowry GV. Stabilization of aqueous nanoscale zerovalent iron dispersions by anionic polyelectrolytes: adsorbed anionic polyelectrolyte layer properties and their effect on aggregation and sedimentation. J Nanopart Res, 10(5): 795-814, 2008.

138 Verma S, Huey BD, Burgess DJ. Scanning probe microscopy method for nanosuspension stabilizer selection. Langmuir, 25(21): 12481-12487, 2009.

139 Lee J, Choi JY, Park CH. Characteristics of polymers enabling nano-comminution of water-insoluble drugs. Int J Pharm, 355(1): 328-336, 2008.

140 Sennett P, Olivier JP. Colloidal dispersions, electrokinetic effects, and the concept of zeta potential. Ind Eng Chem, 57(8): 32-50, 1965.

141 Riddick TM. Control of colloid stability through zeta potential. Creative Press, New York , 1968.

142 Kirby BJ, Hasselbrink EF. Zeta potential of microfluidic substrates: 1. Theory, experimental techniques, and effects on separations. Electrophoresis, 25(2): 187-202, 2004.

143 Fu LM, Lin JY, Yang RJ. Analysis of electroosmotic flow with step change in zeta potential. J Colloid Interf Sci, 258(2): 266-275, 2003.

144 Patil S, Sandberg A, Heckert E, Self W, Seal S. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. Biomaterials, 28(31): 4600-4607, 2007.

145 Pretorius V, Hopkins BJ, Schieke JD. Electro-osmosis: A new concept for high-speed liquid chromatography. J Chromatogr A, 99:23-30, 1974.

146 Barrat J, Hansen J. On the stability of polydisperse colloidal crystals. J Phys, 47(9): 1547-1553, 1986.

147 Kirby BJ, Hasselbrink EF. Zeta potential of microfluidic substrates: 2. Data for polymers. Electrophoresis, 25(2): 203-213, 2004.

148 George M, Ghosh I. Identifying the correlation between drug/stabilizer properties and critical quality attributes (CQAs) of nanosuspension formulation prepared by wet media milling technology. Eur J Pharm Sci, 48(1): 142-152.

149 Chow AHL, Chow SF, Zhang XR, Wan KY, Cheng KK, Baum LW. Engineering of polymer-stabilized nanoparticles for drugs with log p values below 6 by controlled antisolvent precipitation, US 20130122058 A1.

150 Brinck T, Murray JS, Politzer P. Octanol/water partition coefficients expressed in terms of solute molecular surface areas and electrostatic potentials. J Org Chem, 58(25): 7070-7073, 1993.

151 Van Eerdenbrugh B, Froyen L, Van Humbeeck J, Martens JA, Augustijns P, Van den Mooter G. Drying of crystalline drug nanosuspensions-the importance of surface hydrophobicity on dissolution behavior upon redispersion. Eur J Pharm Sci, 35(1): 127-135, 2008.

152 Pronk P, Infante Ferreira CA, Witkamp GJ. A dynamic model of Ostwald ripening in ice suspensions. J Cryst Growth, 275(1): e1355-e1361, 2005.

153 Davis SS, Round HP, Purewal TS. Ostwald ripening and the stability of emulsion systems: an explanation for the effect of an added third component. J Colloid Interf Sci, 80(2): 508-511, 1981.

154 Verma S, Kumar S, Gokhale R, Burgess DJ. Physical stability of nanosuspensions: investigation of the role of stabilizers on Ostwald ripening. Int J Pharm, 406(1): 145-152.

155 Wu L, Zhang J, Watanabe W. Physical and chemical stability of drug nanoparticles. Adv Drug Deliver Rev, 63(6): 456-469, 2011.

156 Ghosh I, Schenck D, Bose S, Ruegger C. Optimization of formulation and process parameters for the production of nanosuspension by wet media milling technique: Effect of Vitamin E TPGS and nanocrystal particle size on oral absorption. Eur J Pharm Sci, 47(4): 718-728, 2012.

157 Murau P, Singer B. The understanding and elimination of some suspension instabilities in an electrophoretic display. K Appl Phys, 49(9): 4820-4829, 1978.

158 Koelmans H, Overbeek JTG. Stability and electrophoretic deposition of suspensions in non-aqueous media. Discuss Faraday Soc, 18: 52-63, 1954.

159 Cesarano J, Aksay IA, Bleier A. Stability of aqueous Al_2O_3 suspensions with poly (methacrylic acid) polyelectrolyte. J Am Ceram Soc, 71(4): 250-255, 1988.

160 Rangel-Yagui CO, Pessoa Jr A, Tavares LC. Micellar solubilization of drugs. J Pharm Sci, 8(2): 147-163, 2005.

161 Brode Iii PF. Adsorption of ultra-long-chain zwitterionic surfactants on a polar solid. Langmuir, 4(1): 176-180, 1988.

162 Zhu Y-P, Masuyama A, Kobata Y, Nakatsuji Y, Okahara M, Rose MJ. Double-chain surfactants with two carboxylate groups and their relation to similar double-chain compounds. J Colloid Interf Sci, 158(1): 40-45, 1993.

163 Van Eerdenbrugh B, Vermant J, Martens JA, Froyen L, Van Humbeeck J, Augustijns P, Van den Mooter G. A screening study of surface stabilization during the production of drug nanocrystals. J Pharm Sci, 98(6): 2091-2103, 2009.

164 Wang H, Pan Q, Rempel GL. Micellar nucleation differential microemulsion polymerization. Eur Polym J, 47(5): 973-980, 2011.

165 Singh SK, Srinivasan K, Gowthamarajan K, Singare DS, Prakash D, Gaikwad NB. Investigation of preparation parameters of nanosuspension by top-down media milling to improve the dissolution of poorly water-soluble glyburide. Eur J Pharm Biopharm, 78(3): 441-446, 2011.

166 Canelas DA, Betts DE, DeSimone JM. Dispersion polymerization of styrene in supercritical carbon dioxide: importance of effective surfactants. Macromolecules, 29(8): 2818-2821, 1996.

167 Baines FL, Dionisio S, Billingham NC, Armes SP. Use of block copolymer stabilizers for the dispersion polymerization of styrene in alcoholic media. Macromolecules, 29(9): 3096-3102, 1996.

168 Napper DH. Flocculation studies of non-aqueous sterically stabilized dispersions of polymer. Trans Faraday Soc, 64: 1701-1711, 1968.

169 Lee J, Lee S-J, Choi J-Y, Yoo JY, Ahn C-H. Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion. Eur J Pharm Sci, 24(5): 441-449, 2005.

170 Black SD, Mould DR. Development of hydrophobicity parameters to analyze proteins which bear post-or cotranslational modifications. Anal Biochem, 193(1): 72-82, 1991.

171 Pasquali RC, Taurozzi MP, Bregni C. Some considerations about the hydrophilic-lipophilic balance system. Int J Pharm, 356(1): 44-51, 2008.

172 Pouton CW, Porter CJH. Formulation of lipid-based delivery systems for oral administration: materials,

methods and strategies. Adv Drug Deliver Rev, 60(6): 625-637, 2008.

173 Gershanik T, Benita S. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. Eur J Pharm Biopharm, 50(1): 179-188, 2000.

174 Binks BP. Particles as surfactants-similarities and differences. Curr Opin Colloid In, 7(1): 21-41, 2002.

175 Shinoda K. The correlation between the dissolution state of nonionic surfactant and the type of dispersion stabilized with the surfactant. J Colloid Interf Sci, 24(1): 4-9, 1967.

176 Zajic JE, Panchal CJ, Westlake D. Bio-emulsifiers. Crc Cr Re Microbiol, 5(1): 39-66, 1976.

177 Landfester K, Willert M, Antonietti M. Preparation of polymer particles in nonaqueous direct and inverse miniemulsions. Macromolecules, 33(7): 2370-2376, 2000.

178 Binks BP, Lumsdon SO. Influence of particle wettability on the type and stability of surfactant-free emulsions. Langmuir, 16(23): 8622-8631, 2000.

179 Binks BP, Lumsdon SO. Catastrophic phase inversion of water-in-oil emulsions stabilized by hydrophobic silica. Langmuir, 16(6): 2539-2547, 2000.

180 Kholodenko AL, Douglas JF. Generalized Stokes-Einstein equation for spherical particle suspensions. Phys Rev E, 51(2): 1081, 1995.

181 Harris KR. The fractional Stokes-Einstein equation: Application to Lennard-Jones, molecular, and ionic liquids. J Chem Phys, 131(5): 054503, 2009.

182 Zwanzig R, Harrison AK. Modifications of the Stokes-Einstein formula. J Chem Phys, 83(11): 5861-5862, 1985.

183 Edward JT. Molecular volumes and the Stokes-Einstein equation. J Chem Educ, 47(4): 261, 1970.

184 Rachmawati H, Shaal LA, Müller RH, Keck CM. Development of curcumin nanocrystal: physical aspects. J Pharm Sci, 102(1): 204-214, 2013.

185 Caruso F. Nanoengineering of particle surfaces. Adv Mater, 13(1): 11-22, 2001.

186 Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid St M, 6(4): 319-327, 2002.

187 De Cuyper M, Joniau M. Mechanistic aspects of the adsorption of phospholipids onto lauric acid stabilized magnetite nanocolloids. Langmuir, 7(4): 647-652, 1991.

188 Lin D, Xing B. Tannic acid adsorption and its role for stabilizing carbon nanotube suspensions. Environ Sci Technol, 42(16): 5917-5923, 2008.

189 Obeidat WM, Schwabe K, Müller RH, Keck CM. Preservation of nanostructured lipid carriers (NLC). Eur J Pharm Biopharm, 76(1): 56-67, 2010.

190 Sun B, Yeo Y. Nanocrystals for the parenteral delivery of poorly water-soluble drugs. Curr Opin Solid St M, 16(6): 295-301.

191 He W, Lu Y, Qi J, Chen L, Hu F, Wu W. Food proteins as novel nanosuspension stabilizers for poorly water-soluble drugs. Int J Pharm, 441(1): 269-278, 2013.

192 Kakran M, Shegokar R, Sahoo NG, Gohla S, Li L, Müller RH. Long term stability of quercetin nanocrystals prepared by different methods. J Pharm Pharmacol, 64(10): 1394-1402, 2012.

193 Gupta AK, Wells S. Surface-modified superparamagnetic nanoparticles for drug delivery: preparation, characterization, and cytotoxicity studies. IEEE Trans Nanobioscience, 3(1): 66-73, 2004.

194 Verhiest K, Mullens S, De Wispelaere N, Claessens S, DeBremaecker A, Verbeken K. Nano-yttria dispersed stainless steel composites composed by the 3 dimensional fiber deposition technique. J Nucl Mater, 428(1): 54-64.

195 Ochi M, Kawachi T, Toita E, Hashimoto I, Yuminoki K, Onoue S, Hashimoto N. Development of nanocrystal formulation of meloxicam with improved dissolution and pharmacokinetic behaviors. Int J Pharm, 474(1): 151-156.

196 Lin N, Huang J, Chang PR, Feng J, Yu J. Surface acetylation of cellulose nanocrystal and its reinforcing function in poly (lactic acid). Carbohyd Polym, 83(4): 1834-1842.

197 Hilton JE, Summers MP. The effect of wetting agents on the dissolution of indomethacin solid dispersion systems. Int J Pharm, 31(1): 157-164, 1986.

198 Dai W-G. In vitro methods to assess drug precipitation. Int J Pharm, 393(1-2): 1-16, 2010.

199 D'Souza SS, DeLuca PP. Methods to assess in vitro drug release from injectable polymeric particulate systems. Pharm Res, 23(3): 460-474, 2006.