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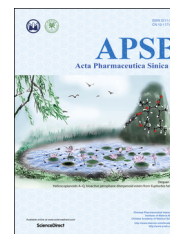
REVIEW

Parenteral nanosuspensions: a brief review from solubility enhancement to more novel and specific applications

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Abstract Advancements in *in silico* techniques of lead molecule selection have resulted in the failure of around 70% of new chemical entities (NCEs). Some of these molecules are getting rejected at final developmental stage resulting in wastage of money and resources. Unfavourable physicochemical properties affect ADME profile of any efficacious and potent molecule, which may ultimately lead to killing of NCE at final stage. Numerous techniques are being explored including nanocrystals for solubility enhancement purposes. Nanocrystals are the most successful and the ones which had a shorter gap between invention and subsequent commercialization of the first marketed product. Several nanocrystal-based products are commercially available and there is a paradigm shift in using approach from simply being solubility enhancement technique to more novel and specific applications. Some other aspects in relation to parenteral nanosuspensions are concentrations of surfactant to be used, scalability and *in vivo* fate. At present, there exists a wide gap due to poor understanding of these critical factors, which we have tried to address in this review. This review will focus on parenteral nanosuspensions, covering varied aspects especially stabilizers used, GRAS (Generally Recognized as Safe) status of

stabilizers, scalability challenges, issues of physical and chemical stability, solidification techniques to combat stability problems and *in vivo* fate.

1. Introduction

Lead molecule selection through *in silico* methods, including high throughput screening; have led to increase in the propensity of poorly water-soluble candidates being selected, and at the same time rejection of around 70% of these drug candidates due to their poor water solubility. Many of these new chemical entities (NCEs, around 40%)[1] get processed and are killed at the final development stage resulting in wastage of money and resources. Because of their unacceptable physicochemical profile, such compounds are often considered as time and resources consuming only and are discarded either at initial or final stages, without any significant contribution in the synthesis pipeline[2]. Solubility and permeability are the two important factors governing oral bioavailability of compounds after oral absorption[3]. Deviation in solubility and permeability directs to unpredictable absorption profile and low oral bioavailability[4]. In the past few years, lead molecule optimization processes have governed the selection of molecules having potency and selectivity but these compounds lack suitable physicochemical properties because of their bulky structure and higher molecular mass. There is a dire need to incorporate appropriate physicochemical properties along with selectivity and potency[5]. Gardener et al.[6] have nicely reported incorporation of physicochemical parameters in drug development process. Fig. 1[6] provides a glimpse of parameters to be taken care while designing a drug development strategy. To obtain an adequate amount of bioavailability, researchers have explored numerous techniques *viz.* use of co-solvents, micelle formation, cyclodextrin complexation, lipid-based nanoformulations, polymeric nanoparticulate systems, micronization, and nanonization. All aforementioned techniques have their own advantages and limitations[7]. Among these techniques, nanonization is one of the widely used technique for improvement in solubility and bioavailability, as evidenced by huge literature and approved marketed products such as Rapamune®, Emend®, Triglide®, Tricor®, Megace ES® already available in market[8]. Recent reports on the current market scenario of nanocrystals claim that it will occupy around 60% of a $136 billion nanotechnology-enabled drug delivery market by 2021[9]. There are statistical predictions that the total market in 2021 will be $136 billion, with nanocrystals and other nanocarriers, of which $81 billion will be due to nanocrystal formulations alone. This itself is an indicative of vast market potential of nanocrystals[10].

These advancements are attributed to benefits offered by this technology such as ease of formulation, scale-up compatibilities, fewer controversies towards nanotoxicology and regulatory aspects[11]. Besides aforementioned advantages, nanocrystals offer easy conversion to solid dosage forms (tablets/capsules/powder for redispersion and many more). Nowadays, researchers are expanding horizons in nutraceuticals by using nanocrystals, as they offer an increase in saturation solubility, dissolution velocity and at the same time provide all the advantages of nanoformulation, with a slight addition of the surfactant[12]. The advent of nanotechnology has created a new boom in all fields, including chemical, physical and life sciences, which has given a new path for drug delivery in pharmaceuticals[13,14]. Various routes for administration of nanocrystals[7] are proposed as well, including oral[15], parenteral[16], dermal[17,18], systemic[19], pulmonary[20] and ocular[21–23].

By definition, nanocrystals are crystals of the drug in nano-size range stabilized with only minute amounts of surfactants, therefore possessing higher theoretical drug loading as compared to any other nanoparticulate system. This high loading capacity makes these systems as better drug carriers for transporting the higher load of a drug to the targeted site for therapeutic activity[24]. Marketed formulations based on nanocrystals technologies are mostly for oral administration. The first nanocrystal-based formulation was approved in 1990s (Ambisome®) and since then almost forty commercial nanocrystal-based formulations are available in the market. Recently *in vivo* studies done on animal models have suggested the adaptability of nanocrystals formulation as suitable enough to be tuned to change improve targeting, pharmacodynamic and pharmacokinetics of less soluble drug candidates[25]. Parenteral administration, by all means, is critical and requires strict control over many parameters, like for parenteral nanosuspension, size becomes the main constraint as it decides the *in vivo* fate of administered nanocrystals. Researchers have reported that nanocrystals of size 200 nm and more dissolve slowly and are taken up by the macrophages in liver whereas nanocrystals of 100 nm size and below, dissolve rapidly[26]. All the basic information of solubility enhancement mechanism and preparation methods is already reviewed by many authors[7,27]. The present review will focus on parenteral nanosuspension covering all aspects including surfactants used till date, preparation, stability issues and strategies to combat stability problems, *in vivo* fate after parenteral administration, marketed formulations and strategies to play with pharmacokinetics. In-general methods of preparation along with some advances and specific features of nanocrystals are discussed in brief.
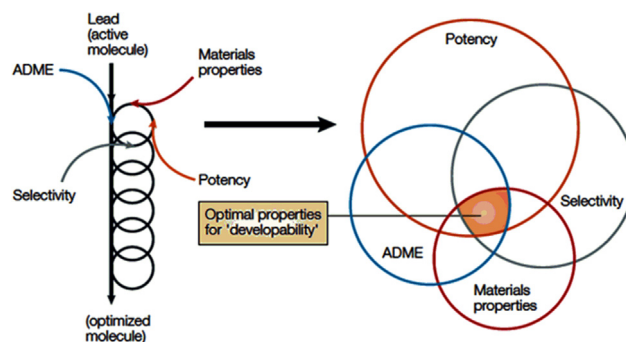
Figure 1 Parameters to be critically monitored during initial screening of lead optimization. Reprinted (adapted) with permission from Ref. 6. Copyright © 2004 Nature Publishing Group.
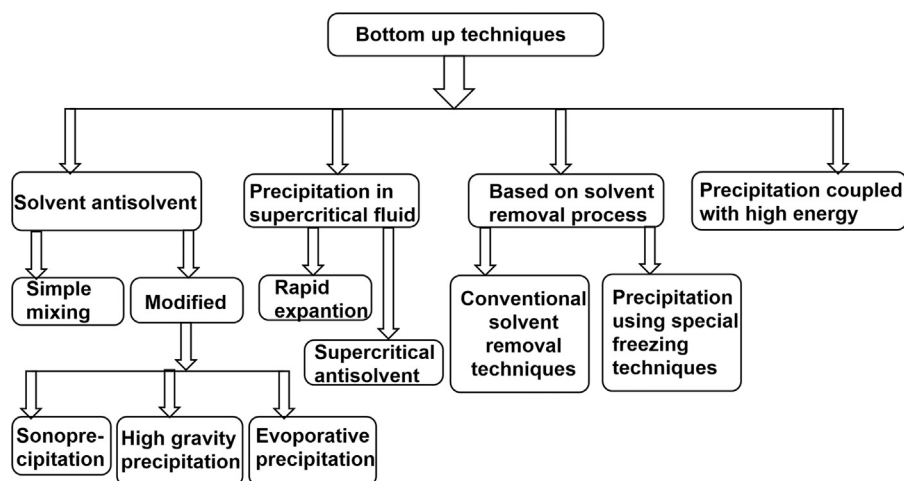
Figure 2 Advances in bottom-up techniques for preparation of nanocrystals.

2. Specific features and production

Parenteral aqueous nanosuspensions are prepared to deal with two major issues *viz.* to reduce the side effects of existing parenteral product or to target drug of interest to specific part of the body. Production techniques are classified either as bottom-up or top-down techniques[7]. In general top-down techniques start from larger size particles and produces crystals of a drug in nano-size range by using mechanical forces (attrition, cavitation, and others). In contrast to this, bottom-up technique starts from molecular level of drug and precipitation of drug crystals in nano range takes place in conditions, which influence nucleation process[28]. Advancements in technology include the use of supercritical fluid and solvent evaporation processes. Scalability issues at industrial level have made these advancements less preferable over already established ones[2].

The present classification system of nanocrystals technologies broadly categorizes them into two generations, from which the first generation includes basic bottom-up and top-down technologies (nanoprecipitation, media milling, high-pressure homogenization (HPH) and others). Second generation production techniques have improved properties and are based on combinatory approaches (smartCrystal technology). Second generation techniques provide formulation scientists with benefits of faster production, better size reduction, improved physical stability and controlled pharmacokinetic profile of prepared nanocrystals. SamrtCrystal is "tool box" for various combination processes *e.g.* H42 (combination of spray drying and HPH) and H96 (combination of lyophilization and HPH). Nanocrystals of approximately 50 nm can be easily achieved with smartCrystal technologies[26]. Proceeding section will give a brief introduction about aforementioned production techniques.

2.1. Bottom-up method

The bottom-up nanoprecipitation technique has been utilized since decades for preparation of micronized particles. Use of this technique for nanocrystal production started in the late 1980s[28]. Bottom-up technique initiates from molecular level of drug (drug dissolved in organic solvent) and subsequently, nucleation is governed by various means, including solvent evaporation, ultrasonic waves, supercritical fluid and others. Solvents used in nanoprecipitation technique are the ones which have some amount of miscibility with antisolvent used[29]. Commonly used solvents are ethanol, methanol, acetone, dimethylsulfoxide, dichloromethane and many more. Most common choice of antisolvent is water; buffers are rarely used but their use has been reported as well. This production process is very tedious and has critical process parameters to be taken care of such as rate of precipitation and prevention of crystal growth. Sometimes there is a risk of residual organic solvents and complexity in process control which creates a barrier for scale-up[7].

The BASF company have patented industrially useful nanosuspension precipitation method developed by Auweter, Horn and co-workers[24] basically for colorants in colloidal dispersion form. This technology has been used by various industries to generate nanocrystals with the desired size range. Other bottom-up technologies such as high gravity controlled precipitation technology, liquid jet precipitation, sonocrystallization and multi-inlet vortex mixing are being explored in recent years[30]. Fig. 2 provides a glimpse of conventional and novel bottom-up techniques, utilized for effective production of nanocrystals. De Waard et al.[31] developed a novel bottom-up process for fenofibrate to produce drug nanocrystals by controlled crystallization during freeze-drying. Numerous novel bottom-up methods are well-reported in existing literature. However; the acceptability of this technique is still limited to lab-scale production only, owing to poor understanding of critical process parameters, especially those affecting industrial scale production. This is one of the major reasons why most of the commercially available products are based on top-down technique only[32].

2.2. Top-down method

Two basic size reduction techniques classified under top-down methods are wet milling and HPH[7]. The top-down techniques start with larger crystals of a drug, (μm in range) and size reduction takes place by virtue of applied mechanical forces as described in preceding sections. Wet milling technique is preferred mostly over dry (jet) milling because it gives particle size in the nm range. Most of the marketed products are reported to be based on pearl mill (bead mill) technology[24]. Table 1 provides brief information about various marketed products based on nanocrystal technology. Construction wise pearl mills consist of milling chamber, milling

Table 1 Nanosuspension based marketed products.

Product name	Drug used	Indication	Marketed by (company)	Production technique	Administration route	Solved problem	Refs.
Tricor[®]	Fenofibrate	Hypercholesterolemia	Abbot pharma	Elan nanocrystals	Oral	Solubility enhancement	39,40
Sporanox[®]	Itraconazole	Antifungal	Janssen pharma	Sporanox	Oral	Oral bioavailability	41
Invega sustenna[®]	Paliperidone palmitate	Antipsychotic	Janssen pharma	Elan nanocrystals	Intravenous	Modified treatment	42
Rapamune[®]	Sirolimus	Immunosuppressant	Wyeth	Elan nanocrystals	Oral	Dissolution and erratic absorption	43,44
Triglide[®]	Fenofibrate	Hypercholesterolemia	First horizon pharma	Skye pharma	Oral	Oral bioavailability	45
Emend[®]	Aprepitant	Antiemetic	Merck	Elan nanocrystals	Oral	Absorption	8,46,47

motor, and the recirculation chamber. Micronization by media milling is conducted by loading drug suspension in surfactant solution to the milling chamber. Milling media is added in optimized amount which is responsible for generation of forces utilized in size reduction. Process parameters and material attribute like the amount of the drug, amount of milling pearls used, milling speed, milling time and temperature must be controlled to obtain particles in the desired size range[28]. Another alternative top-down method was developed by Muller and co-workers, called piston-gap homogenizer, wherein the suspension is passed at high speed through a small orifice and during passage through the orifice collision of crystals results in the particle size reduction[24,33–35]. Erosion from the milling pearl, during the milling process, is the major limitation of the method, but in recent years, yttrium stabilization of milling media has ameliorated this problem. Another technique is microfluidizer technology, which can generate smaller size crystallites with a collision of two fluid streams at very high pressure[2]. Microfluidizer technology is one of the top-down processes which is based on the principle of jet-stream homogenization. Either Z-type or Y-type of collision chamber is used. The cavitation forces generated during the process, particle collision and shear forces are responsible for particle size reduction up to desired range[36]. Apart from aforementioned limitations, top-down techniques make the drug substance more prone towards phase transition, which can ultimately affect the *in vivo* performance of the drug product[7,37].

2.3. Combination technologies

Combination techniques simply combine bottom-up and top-down technologies. It is a two-step process which involves nanoprecipitation in the first step followed by HPH[2,7].

In the context of parenteral nanosuspension, it is very difficult to control various process parameters and Baxter is one of the industries focusing on intravenous suspensions of drug molecule presently. The smartCrystal technology is one amongst the best-suited techniques, combining benefits of fast production and at the same time production of very small nanocrystals, as already mentioned in preceding sections[24]. Recent advances in the field of combination techniques are triple combination *i.e.* precipitation-lyophilization-homogenization technique, supercritical fluid methods, spray freezing into liquids (SFL), solution-enhanced dispersion by the supercritical fluids (SEDS), aerosol solvent extraction (ASES) and evaporative precipitation into an aqueous solution (EPAS)[30].

Salazar et al.[38] have reported a comparative evaluation of a combination approach (freeze drying followed by HPH/wet ball milling) with conventional top-down technique and found combination approach to be better regarding size control. Glibenclamide was taken as a model drug and two subsequent steps were reported to be performed including freeze drying followed by wet ball milling. Authors have shown the effect of solvents (dimethyl sulfoxide: *tert*-butanol) on the porosity of powder obtained after the first step of freeze drying. Scanning electron microscopic (SEM) images obtained for different ratios of dimethyl sulfoxide and *tert*-butanol showed that the material obtained was porous and fragile with different morphologies. Fig. 3 gives a glimpse of the process as well as SEM images obtained for the different combination of solvents[38]. At the industrial scale, the major challenge is to obtain less than 100 nm particle size using conventional approach only, and in such situation, combinatory approaches are very useful[36].
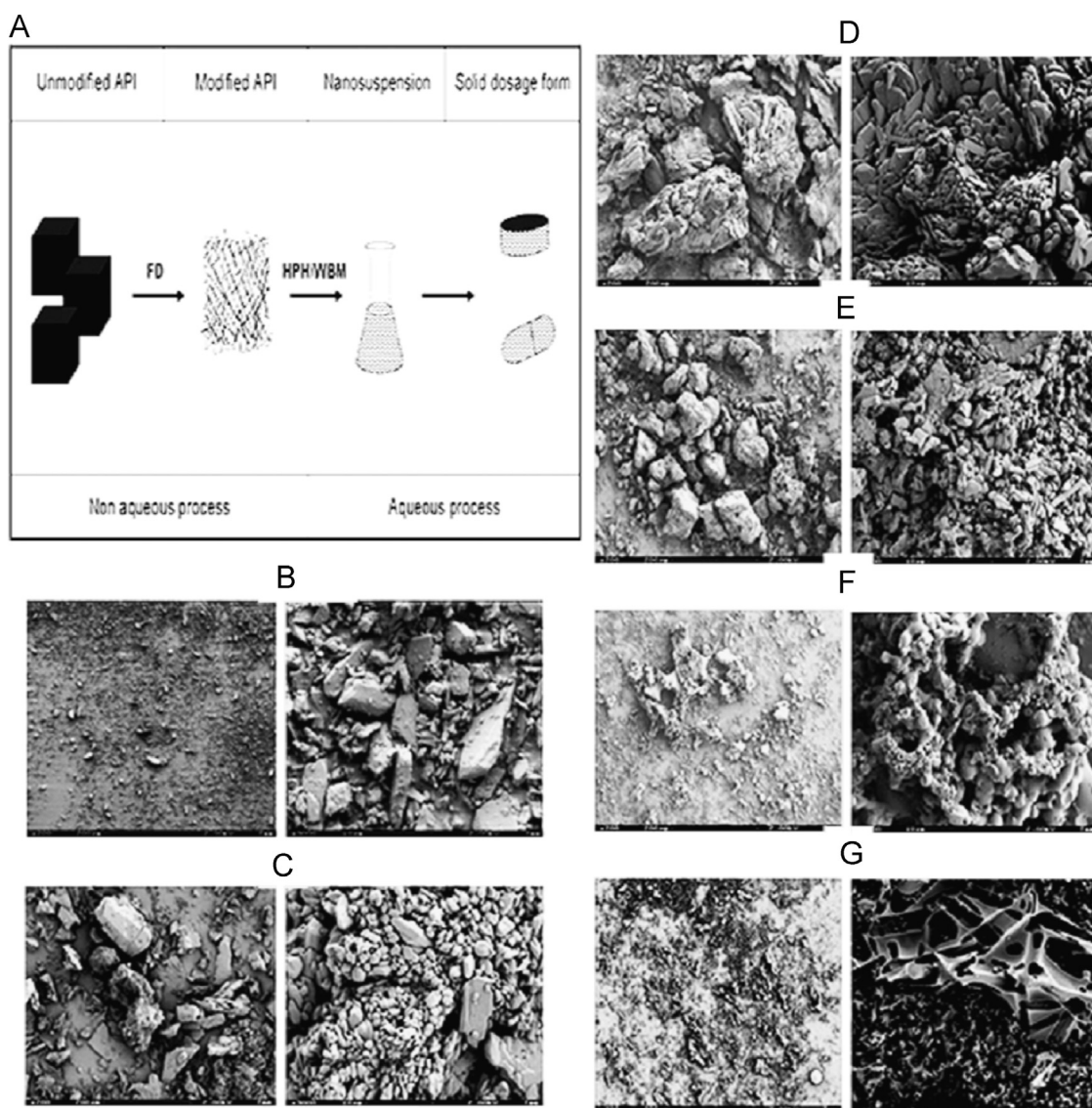
Figure 3 SEM pictures of the freeze-dried powders: (B) unmodified API; (C) dimethyl sulfoxide:*tert*-butanol (DMSO:TBA), 90:10 (low TBA); (D) 75:25, (E) 50:50 (medium); (F) 25:75; and (G) 10:90 (high TBA). Left: 200 μm scale bar/right: 10 μm scale bar (3000 × magnification). Reprinted (adapted) with permission from Ref. 38. Copyright © 2012 Elsevier.

3. Parenteral nanosuspension

Currently, nanosuspension technology has been broadly applied to resolve the problem of poor water solubility for drug candidates administered by either route, oral or parenteral. Nanocrystals are delivered parenterally to address two main issues *viz.* to reduce toxicity issues of non-aqueous existing formulation and to provide targeted effect[48]. A Parenterally administered nanosuspension formulation offers reduced toxicity as compared to conventional drug deliveries[16]. Size range between 100 and 300 nm is ideal for the enhanced permeability and retention effect (EPR effect) to achieve drug concentration in solid tumors which are reported to have very dense vasculature[7]. Proceeding section will deal with stabilizers used till date, recent advances, and applications of parenteral nanosuspension.

3.1. Stabilizers used in parental nanocrystals

Being very versatile technique, nanocrystals are preferred over numerous other techniques of solubility enhancement and have emerged as leading technique in last decade. Nanocrystals are solid drug particles surrounded by the layer of a stabilizing agent. Preparation of nanocrystals is quite easy, but the stability and selection of stabilizer in optimum concentration are critical in formulation development. Not all the stabilizers are suitable for parenteral use, due to associated toxicity and microbial load issues. Polymers and surfactants are used as stabilizers for pharmaceutical nanocrystals[49] and the amount used for stabilization is very critical. Sinha et al.[32] have nicely mentioned the effect of concentration of surfactants as well as polymers regarding critical micelle concentration (CMC) and critical flocculation

concentration (CFC) respectively. In general, it is assumed that higher the concentration, better the chances of adsorption and stabilization of nanocrystals, but beyond a concentration level (CMC/CFC) micellar solubilization phenomenon is prominent leading to Ostwald ripening and system destabilization[32]. By this means, selection of surfactant type and concentration to be used is important. Reports have shown lecithin to be the stabilizer of choice for parenteral and autoclavable nanosuspension[50]. Proceeding section deals with various stabilizers reported in the production of parenteral nanosuspension.

3.1.1. Poloxamers

Poloxamers are synthetic polymers consisting of a chain made up of closely packed block copolymers of ethylene oxide and propylene oxide. Commonly used concentrations for nanosuspension preparation is 0.2% to 0.6% by intramuscular and intravenous route[51,52]. They are widely used in the preparation of nanoparticles[53], micelle carriers[54], thermo-sensitive hydrogels[55] and as tissue scaffold[56] in biomedicine applications following their physical and chemical properties. They are generally considered as safe for topical, oral and parenteral applications and are approved by FDA as a pharmaceutical ingredient and food additives. Nowadays, their use as a stabilizing agent is very common for nanocrystal formulations. Fabrication of poloxamers involves the addition of ethylene oxide to polyoxypropylene glycol, which again is a product of the reaction between polyethylene glycol (PEG) and propylene oxide. These types of block polymers are reported to be more advantageous as stabilizers compare to traditional homopolymers[57]. Commonly used poloxamer in nanocrystals formulations is poloxamer 188 and (Pluronic® F68) poloxamer 407 (Pluronic® F127). Both of these polymeric stabilizers are the non-ionic linear triblock copolymer consisting of the hydrophobic central segment of polypropylene oxide and two hydrophilic side segments of polyethylene oxide. The adsorption on crystal surface is governed by hydrophobic polypropylene oxide chains. However, the reverse structured poloxamers are more advantageous, due to inter-particle bridging and aggregation after nanocrystals formation as compared to Pluronics® [58]. During selection of stabilizers several factors affect stability and particle size of the nanosuspension formulation viz. morphology of polymers, functional groups, hydrophilic or hydrophobic ratio in stabilizer molecule and molecular weight[59,60]. Polymer composition has the direct effect on the stability of nanosuspension. Polymers and other stabilizers used should have optimum hydrophilic to lipophilic balance in controlling the crystal growth and enhancing the wettability of poorly soluble drug[61]. Stabilizers work by two mechanisms of stabilization viz. steric stabilization as well as electrostatic stabilization[62]. Adsorption plays a key role in providing steric stabilization by means of surface adsorbed bulky groups, while the formation of electrical double layer results in electrostatic stabilization. The surfactants having small molecular weights are generally reported to facilitate the dissolution of hydrophobic drugs[61]. Functional group present on stabilizer and drug surface also plays an important role in particle size and stability[63].

Several authors have reported nanocrystals of drug (nimodipine, paclitaxel, indomethacin, budesonide) stabilized with the help of single or the combinations of poloxamers[64]. Hydrophilic polymers are considered less competent stabilizers for nanocrystals formulation as compared to amphiphilic polymers and surfactants, due to the absence of thermodynamic driving forces required to adsorb on hydrophobic surfaces of nanocrystals drug particles[62]. Additionally, amphiphilic polymers are known to increase wettability and decrease the interfacial tension of nanocrystals thus providing better stabilization. The selection of stabilizer totally depends on the drug compound of interest in the formulation, but due to versatile and advantageous nature of the poloxamer, they are effective for most of the compounds from biopharmaceutical classification system-II (BCS class-II)[49]. Viitala et al. have reported better stabilization of itraconazole nanosuspension prepared with the aid of poloxamers whereas other surfactants screened, failed to do so[58,65,66]. A wide range of Pluronics® with different grades is reported for the preparation of parenteral nanosuspensions. Danhier et al.[67] have reported parenteral (intravenous) nanosuspension of a novel poorly water-soluble anti-cancer multi-targeted kinase inhibitor, MTKi-327 with the aid of Pluronic® F108. Authors have investigated the role of four different formulation approaches to tackle the problem of poor water solubility viz. polymeric nanoparticles, parenteral nanosuspension, polymeric micelles and solution prepared with the aid of Captisol®. Results of comparative analysis of aforementioned approaches suggested nanosuspension to be effective formulation approach due to higher tolerated dose and 2.4-fold increase in aqueous solubility as compared to all other investigational approaches[67]. Table 2 provides inactive ingredient guide (IIG) and GRAS status of commonly used stabilizers in parenteral nanosuspension.

3.1.2. Amino acid derived co-polymers

The amino acid derivative polypeptides are commonly used in the parenteral nanocrystal formulation as a stabilizer. Albumin (intravenous concentration allowed is 2%), lysine, leucine (intravenous concentration allowed is 52.6%) and transferrin are amongst the most common among copolymers which are used to achieve dual application of both surface stabilization and targeting. Albumin is one of the anciently used moieties having single polypeptide chain of 585 amino acids, containing protein and enzyme. In aqueous nanocrystal, formulation leucine acts as a lubricant as well as anti-adherent due to its hydrophobic nature. These co-polymers are reported to show enhanced stabilization effect when used in combination with each other. On similar lines, Frank and Boeck[66] have introduced two amino acid derivatives for parenteral nanocrystals formulation viz. proline (intravenous concentration allowed is 35.6%) and arginine (intravenous and intramuscular concentration allowed is 78% and 39.5%, respectively)[52,68]. Authors proposed the use of these stabilizers to formulate BI XX drug in the form of nanocrystals. Use of aforementioned amino acid-based stabilizers has been reported to achieve desired particle size and physical stability of the formulation.

3.1.3. D-α-Tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS)

Vitamin E TPGS is a waxy material obtained from esterification of vitamin E carboxylic group with PEG 1000 which is water soluble in nature. It is a non-ionic multi-role surfactant used widely in the pharmaceutical, nutraceutical and cosmetic formulations[76]. Commonly used concentrations are 200–300 mg/L by intravenous route[52,77]. It is used mainly in the formulation of self-emulsifying drug delivery system owing to its emulsifying properties. As a water-soluble compound vitamin E TPGS is also used as an effective source of natural vitamin E for both therapeutic and

Table 2 Commonly used surfactants in parenteral nanosuspensions with their daily uptake limits.
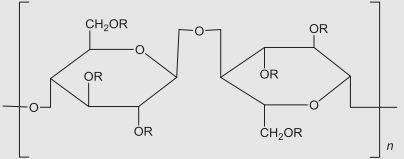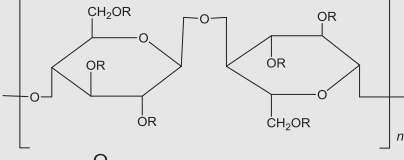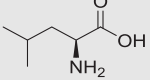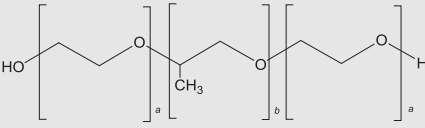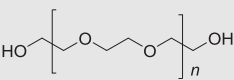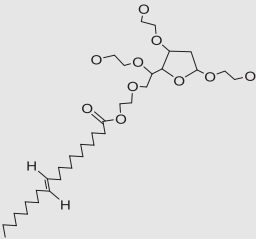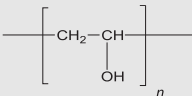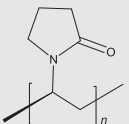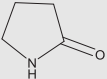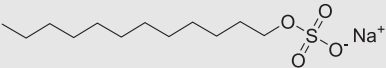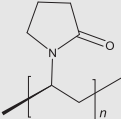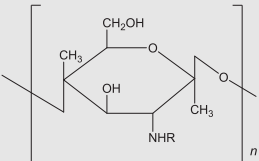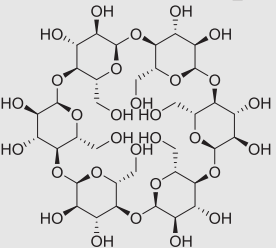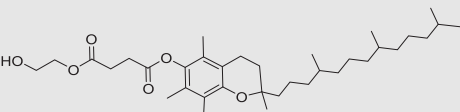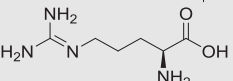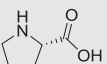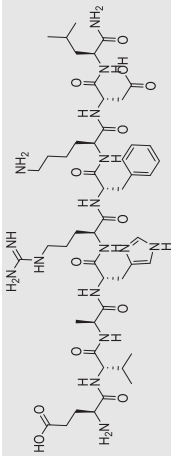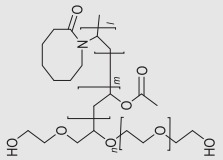
Nos.	Ingredient	Chemical structure (Monomeric units)$_n$	GRAS status and daily uptake limits with a particular route of administration	IIG limit (maximum potency/unit dose)	Refs.
1	Hydroxypropyl cellulose (HPC)		• Not specified by WHO for human use • Lethal dose, 50% (LD$_{50}$) (rat intravenous administration: 0.25 g/kg)	*	52,68
2	Hypromellose (HPMC)		• Estimated daily intake (EDI) of 5.50 g for Human • LD$_{50}$ (mouse, IP): 5 g/kg • LD$_{50}$ (rat, IP): 5.2 g/kg	*	52,68
3	Leucine		• It is generally regarded as a nontoxic and non-irritant material • Only moderately toxic by the subcutaneous route. • LD$_{50}$ (rat, IP): 5.379 g/kg	IV-52.6%	52,69,70
4	Poloxamer F68, F127		• No adverse effects at IV administration up to 0.5 g/kg/day in dogs • 0.5 g/kg/day to rabbits • LD$_{50}$ (mouse, IP): 1 g/kg, • LD$_{50}$ (rat, IV): 7.5 g/kg	Poloxamer F68: IM-0.2%, IV-0.6%,	52,68
5	Polyethyleneglycol (PEG) 400		• The WHO has set an estimated acceptable daily intake of polyethylene glycols at up to 10 mg/kg body-weight • LD$_{50}$ (mouse, IV): 8.6 g/kg • LD$_{50}$ (rat, IV): 7.3 g/kg	IM-20%, IV-75.58%	52,68
6	Polysorbate 80		• The WHO has set an estimated acceptable daily intake for polysorbates 25 mg/kg body-weight • LD$_{50}$ (mouse, IV): 4.5 g/kg • LD$_{50}$ (rat, IV): 1.8 g/kg	IM-12%, IV infusion-58.5%	52,68
7	Polyvinylalcohol (PVA)		• PVA is generally considered a nontoxic material • LD$_{50}$ (mouse, oral): 14.7 g/kg • LD$_{50}$ (rat, oral): > 20 g/kg	Intravitreal-0.12 mg	52,68
8	Povidon		• A temporary acceptable daily intake for povidone has been set by the WHO at up to 25 mg/kg body-weight. • LD$_{50}$ (mouse, IP): 12 g/kg	IV-0.2%, IM-0.9%	52,68

Table 2 (*continued*)

Nos.	Ingredient	Chemical structure (Monomeric units)$_n$	GRAS status and daily uptake limits with a particular route of administration	IIG limit (maximum potency/unit dose)	Refs.
9	2-Pyrrolidone		• Poisonous by IV route. Toxic by ingestion, skin contact, and IP routes. It is an experimental teratogenic, mutagenic additive • LD_{50} (mouse, IV): 0.155 g/kg • LD_{50} (mouse, oral): 5.13 g/kg • LD_{50} (rabbit, SC): 8.0 g/kg • LD_{50} (rat, IP): 2.472 g/kg • LD_{50} (rat, IV): 0.0805 g/kg	SC-25.85%	52,68
10	Sodium lauryl sulfate (SLS)		• Should not be used by IV route • LD_{50} 0.5–5.0 g/kg	*	52,68
11	Cross povidone		• Acceptable daily intake in humans has not been specified by the WHO. • LD_{50} (mouse, IP): 12 g/kg	IM-0.02%	52,68
12	Chitosan		• Chitosan has been shown to be biodegradable. • LD_{50} (mouse, oral): >16 g/kg	*	52
13	Cyclodextrin		• Cyclodextrin-α: LD_{50} (rat, IP): 1.0 g/kg • LD_{50} (rat, IV): 0.79 g/kg • Cyclodextrin-β: LD_{50} (mouse, IP): 0.33 g/kg • LD_{50} (mouse, SC): 0.41 g/kg • LD_{50} (rat, IP): 0.36 g/kg • LD_{50} (rat, IV): 1.0 g/kg • LD_{50} (rat, oral): 18.8 g/kg • LD_{50} (rat, SC): 3.7 g/kg • Cyclodextrin-γ: LD_{50} (rat, IP): 4.6 g/kg • LD_{50} (rat, IV): 4.0 g/kg • LD_{50} (rat, oral): 8.0 g/kg	IV-5%	52,68
14	D-α-Tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS)		• The WHO has set an acceptable daily intake of tocopherol used as an antioxidant at 0.15–2.0 mg/kg body-weight	IV-225 mg/L	52,68
15	Arginine		• Oral supplementation with L-arginine at doses up to 20 g daily are generally well tolerated	IM-78%, IV-39.5%	52,68,71,72
16	Proline		• Generally regarded as safe	IV infusion-35.6%	73,51,68

17	Serum albumin		• Essentially nontoxic and non-irritant • LD$_{50}$ (monkey, IV): >12.5 g/kg • LD$_{50}$ (rat, IV): >12.5 g/kg	52,68
18	Soluplus®		IV-2% *	74,75

*Parameter not found in the literature. SC, Subcutaneous; IM, Intramuscular; IV, Intravenous; IP, Intraperitoneal

nutritional purposes. Physical properties of vitamin E TPGS makes it a good plasticizer for several newer applications. Since so many years, it was used in oral formulations, but nowadays it is also explored for its use in parenteral and topical delivery routes. Due to its antioxidant properties, vitamin E TPGS is attractive for industries dealing in cosmetics. Very high physical stability of vitamin E TPGS makes it less vulnerable towards effects of light, heat, oxygen and oxidizing agents. It is stable up to 4 years in closely packed container at room temperature and presents stability issues only when in contact with basic medium[49]. Vitamin E TPGS in combination with the PEG is reported to give extended release, especially of drugs having a short half-life in plasma and enhancing the bioavailability of drug[78]. It is having amphiphilic structure, owing to the presence of alkyl tail and hydrophilic polar head, with comparatively low CMC of 0.02% (w/w), and hydrophilic-lipophilic balance value of 13.2. Due to these aforementioned properties of vitamin E TPGS, it is used in various drug delivery systems such as nano formulations, liposomes, prodrug, nutraceuticals, cosmeceuticals and micellar formulations.

Apart from all other benefits, vitamin E TPGS has permeation glycoprotein (P-gp) inhibitory activities and is used to overcome the multidrug resistance (MDR) effect in case of anticancer drugs by ameliorating cellular uptake. Many researchers are working in this direction and paclitaxel/docetaxel nanocrystals are successfully developed with vitamin E TPGS showing improved anticancer profile[79]. Liu et al.[80] have investigated the effect of TPGS stabilized paclitaxel nanocrystals on KB, H460, and NCI/ADR-RES cells and found the aforementioned formulation to be more effective as compared to nanocrystals stabilized with Pluronic® F127 and marketed formulation Taxol®. Fig. 4[80] provides an overview of % cell viability in various cell lines using vitamin E TPGS as a stabilizer.

Vitamin E TPGS is well reported for its stabilization as well as solubilization effects in various oral and parenteral formulations[76]. Baert et al.[81] have reported rilpivirine nanosuspension stabilized with vitamin E TPGS, where it was used for long-acting parenteral formulations providing prophylactic treatment in HIV.

Ge et al.[82] have reported preparation of ursolic acid nanocrystals utilizing vitamin E TPGS as a stabilizer and found many folds enhancement in bioavailability as compared to other techniques, due to P-gp inhibition effect on intestinal epithelium cells. Several such examples are reported in the literature showing P-gp[83] inhibitory effect of this compound, however, covering them all is beyond the scope of this article[84]. All these properties of vitamin E TPGS coupled with low toxicity profile makes it a suitable candidate for parenteral applications.

3.1.4. Soluplus®

It is an innovative excipient that gives new levels of solubility and bioavailability of poorly water-soluble drugs. Chemically, it is a polyvinyl caprolactam-polyvinyl acetate-PEG graft copolymer. The allowed concentration of Soluplus® by intravenous and intramuscular routes is 75% and 20% respectively[52,72]. Soluplus® is an effective excipient and has been used in formulation development of poorly water-soluble drugs utilizing spray drying, hot melt extrusion, electrospinning, solvent casting, high shear dispersions, solvent evaporation, thermal heating, co-milling and ball milling. Soluplus® was developed by BASF industries to be used in the preparation of the solid solution, but it is used for various other applications including, as a binder in wet granulation
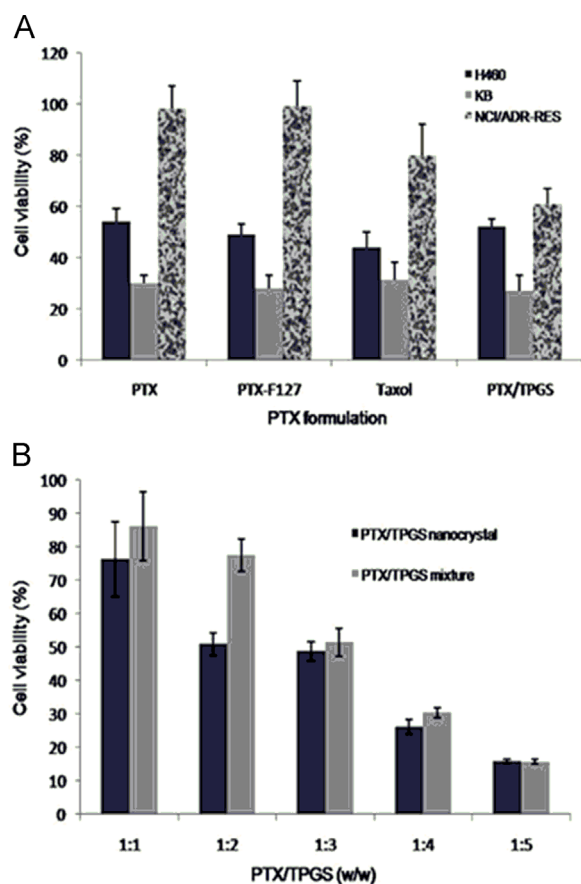
Figure 4 Cell viability after being treated with different formulations for 48 h. A, Effects of paclitaxel/TPGS nanocrystals, paclitaxel/F127 nanocrystals, Taxol and paclitaxel at the same 5 µmol/L paclitaxel concentration on NCI/ADR-RES, KB and H460 cells. B, Effects of paclitaxel/TPGS nanocrystals (10 µmol/L) with different amount of TPGS and paclitaxel/TPGS mixture (10 µmol/L) with different amount of TPGS. Reprinted (adapted) with permission from Ref. 80. Copyright © 2010 American chemical society.

or dry binder in direct compression for tableting, owing to its versatile nature. Soluplus® exhibits high flowability and controllable extrudability features which makes it suitable for use as stabilizers in pharmaceuticals[49].

Yang et al.[85] have reported fenofibrate nanocrystals stabilized with the combination of hydroxypropylmethylcellulose (HPMC) and Soluplus® prepared with media milling technique. Authors have reported better physical stability of prepared nanocrystals due to weaker Ostwald's ripening effect of Soluplus®. Additionally, this excipient was found to improve bioavailability by altering drug permeability in intestine. The permeation studies performed *in vitro* in Caco-2 cell line and *in vivo* transport studies in Beagle dogs show safety and permeability enhancer property of the Soluplus® in nanocrystals formulation as compared to pure drug[86]. All these studies are performed for oral/intragastric administration and use of Soluplus® *via* parenteral route still needs to be explored concerning its toxicity.

3.1.5. Cellulose-based polymers
HPMC is a cellulose-based polymer, used in pharmaceutical development and is considered as a non-toxic and non-irritant

ingredient. It is semi-synthetic non-ionic polymer widely used in nanocrystals formulations to provide physical stabilization using steric stabilization mechanisms. HPMC is suitable for various routes of administration including ocular, oral and parenteral routes. Characteristics of prolonged contact with mucosa, low toxicity, high swelling ability, and lubrication effect, makes it most appropriate excipient for ophthalmic nanosuspensions[87]. The concentration of 5.50 g/day is commonly used for HPMC *via* intravenous route[52].

Tuomela et al.[88] have recently reported the use of HPMC in ocular drug delivery as a stabilizer in intraocular pressure releasing nanosuspension formulation. The formulation was developed using HPMC as a stabilizer by wet milling method at different PH conditions. Optimization of nanocrystals formulation with various concentrations revealed 25% (w/w) HPMC to be giving better stabilization. Due to the presence of hydroxypropoxy and methoxy groups on the HPMC surface, it provides good attachment of brinzolamide with hydrogen bonding. High molecular weight also plays an important role for steric stabilization in the above-mentioned drug formulation[88].

Apart from HPMC other cellulose-based non-ionic polymers are also used as stabilizers in preparation of nanocrystals *viz.* hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and others. The hydrogen bonding between polymer and drug surface inhibits the crystal growth by virtue of which all these acts as good stabilizer[49].

Ghosh et al.[89] have studied the effect of different polymeric stabilizers (HPMC, polyvinyl pyrrolidone, and HPC) on process parameter associated variabilities in size and polydispersity of nanosuspension. They used the quality by design (QbD) approach and found that the type of polymeric stabilizers plays an important role in the production of the nano range particles. Among all other polymeric stabilizers compared, authors found HPMC stabilized nanosuspension was exhibiting least variations in final size and dispersity upon exposure to the different processing parameters. Overall results revealed that the choice of stabilizer to be used is a key factor along with the concentration to be used in the successful development of stable nanosuspension. Authors reported steric stabilization and hydrogen bond making tendency of polymer, responsible for better stabilization effect[89].

3.1.6. Hydrophobins
These are protein-based surfactants obtained from filaments fungi which stabilize nanocrystals by forming the self-assembling monolayer with hydrophilic-hydrophobic interfaces. Hydrophobin forms a protein shell around hydrophobic particles which gives dispersion in water and restricts the aggregation of particles. These are classified into two classes based on of their hydropathy and nature of amino acid sequence and have a significant change in their structural assembly[90]. Hydrophobins are nontoxic in nature and have the activity to prevent immune response by making a layer around nanoparticles. Adsorption of these between hydrophilic–hydrophobic interfaces without losing their folded structure is possible due to its amphiphilic nature. Nowadays, different classes of hydrophobins are used to improve solubility and bioavailability. Apart from it, these can be used in targeted and controlled drug delivery systems. Because of its good stability, versatile nature and good adsorption properties, it is used to produce various nanoparticles successfully. Valo et al.[90] have reported nanocrystals of drug beclomethasone dipropionate stabilized with hydrophobins and found it to be physically stable
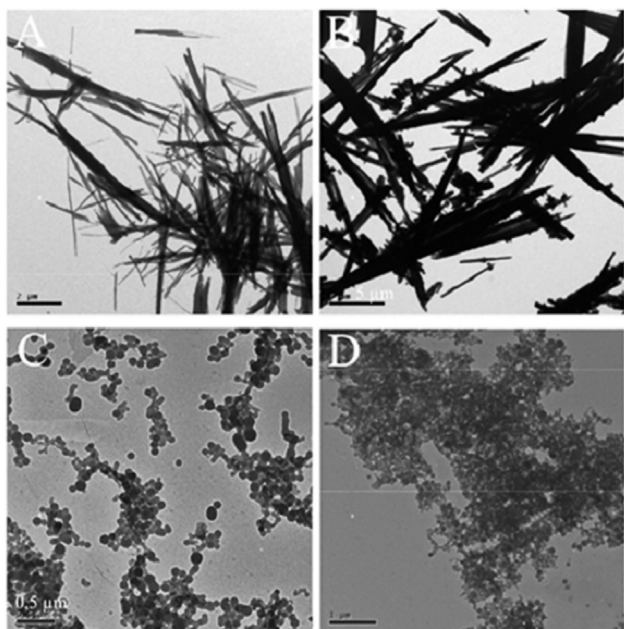
Figure 5 TEM images showing beclomethasone dipropionate (BDP) precipitation in deionized water (A) without class II hydrophobin (HFBII), (B) with 0.005% HFBII, (C) with 0.05% HFBII, and (D) with 0.1% HFBII (Scale bar: 0.5 μm). Reprinted (adapted) with permission from Ref. 90. Copyright © 2010 American chemical society.

system depicted by transmission electron microscopy (TEM) images of stabilized nanocrystals (Fig. 5) prepared with nanoprecipitation method. Till date hydrophobin's use as a stabilizing agent is reported only for the topical and oral nanosuspensions, that leaves a lot of room for expanding its horizons for parenteral administration, provided suitable toxicity are generated first[90,91].

3.1.7. Miscellaneous

Above mentioned stabilizers are commonly used for the production of nanosuspensions. Apart from these some non-conventional stabilizers are also used these days[92]. Polyvinyl alcohol (PVA) is water soluble synthetic polymer used as an effective stabilizer in nanocrystals formulations. Xia et al.[93] have reported PVA stabilized nitrendipine nanocrystals to enhance the dissolution rate and oral bioavailability. Prepared nanosuspension (combination method of precipitation-ultrasonication) showed particle size and zeta potential of 209 ± 9 nm and -13.9 ± 1.9 mV respectively. The in vitro dissolution of nitrendipine was found to be increased by nanonization of drug particles[93]. Intravenously allowed level of PVA in general is 0.12 mg[52].

Recently a research group have reported the use of chitosan as a cationic stabilizer for preparation of itraconazole nanocrystals using the conventional method of HPH method and investigated its stabilization effect. Physical stability of prepared nanocrystals was found to be significantly improved without any changes or new modification observed in crystal structure[94]. Another research group working on chitosan-based atorvastatin nanocrysta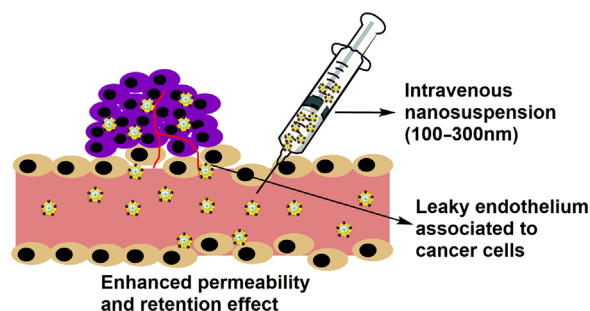ls investigated the effect of the cationic charge of stabilizer on the nanocrystals particle size and stability. Nanocrystals of the drug were prepared with desired specifications and effect of chitosan as potential stabilizer has been evaluated[94].

Figure 6 EPR effect shown by smaller size nanocrystals.

3.2. Recent advances

Application of nanocrystals in parenteral drug delivery is explored very rapidly nowadays due to its important role in solubility and bioavailability enhancement[7]. As already described in preceding sections aqueous parenteral nanosuspensions are prepared for two reasons viz. either to overcome side effects of existing parenteral formulation or to provide targeting effects. Passive targeting can be achieved by the interplay of particle size as nanocrystals in the range of 100–300 nm are reported to be better candidates for EPR effect as shown in Fig. 6. Most of the times anticancer drugs including paclitaxel, docetaxel, curcumin, camptothecin and others are formulated using nanocrystals technology for the intravenous administration[80] keeping in view the passive targeting effect. For active targeting effect surface, modified nanocrystals are being explored in recent years. Recently, Liu et al.[95] have reported Paclitaxel nanocrystal formulation coated with vitamin E TPGS, where surface coating served the dual purpose of stabilization as well as P-gp inhibition. As already described in preceding sections, vitamin E TPGS works as virtuous P-gp inhibitor as well as a stabilizer; authors have combined these properties to overcome multidrug resistance of various anticancer drugs. This changing trend is itself indicative of a paradigm shift from simply solubility enhancement to more novel and specific applications of nanocrystals. Table 3 enlist recently reported nanosuspensions used for parenteral applications.

Apart from surface modifications for active targeting, effects of nanocrystal size on passive targeting is explored as well. Use of novel stabilizing agents to enhance stability and achieve desired size range is reported by authors. Strict control over size range indirectly exerts an effect on passive targeting and lymphatic clearance. Newer stabilizers which are more biocompatible are being explored these days to address toxicity issues as well[8,25].

Frank and co-authors[66] developed nanosuspension formulation utilizing novel amino acid stabilizers (proline and arginine) utilizing media milling process and found it physically stable showing enhanced dissolution and bioavailability of drug BIXX.

On similar lines, Zakir et al.[134] recently reported amphotericin B nanocrystal-based formulation for parenteral administration. They have used Tween 80, Pluronic® F68 and Soya lecithin as the stabilizers to achieve stability and desired particle size range. Nanocrystal formulation for the parenteral administration with smaller particle size was successfully produced with the NanoEdge™ technology[134]. The technology utilizes basic precipitation process followed by annealing step applying high energy either in the form of shear or thermal energies[33]. The combinational step of imparting high shear or thermal energy provides an important role in nano-sizing.

Table 3 Recently reported nanosuspension based parenteral formulations.

Nos.	Active ingredient	Category	Method of nanosuspension production	Surfactant used	Concentration of surfactant used	Purpose of the study	Refs.
1	*p*-Terphynyl derivative	Anticancer	Precipitation-microfluidization method	Pluronic F-68	0.08%, *w/v*	Solubility enhancement	16
2	Etoposide	Anticancer	High pressure homogenization –solvent precipitation	Bovin serum albumin	3%, *w/v*	Targeted delivery	96
3	Azithromycin	Antibiotic	Reactive precipitation method	Soybean Lecithin	1%	Targeted delivery	97
4	Fenofibrate	Reduces cholesterol level	Stirred ball mill	HPMC	5%, 7.5%, 10%	Controlled delivery	98
5	Silybin	Hepatoprotective	High pressure homogenization	Lecithin Poloxamer 188	0.2% and 0.1, respectively	Solubility enhancement	99
6	Simvastatin	Lipid lowering agent	Nanoprecipitation technique	PVP K-30 SLS	4% and 0.02%, respectively	Solubility enhancement	100
7	Itraconazole	Anti-fungal	–	Poloxamer 188	–	Solubility enhancement	19
8	Clofazimine	Antimycobacterial	High pressure homogenizer	Pluronic F-68	0.5%	Targeted delivery, Stability	101
9	Paclitaxel	Anticancer	High-pressure homogenizer	Human serum albumin PEG	4.5% and 10%. respectively	Targeted delivery	102
10	Hyaluronic acid and paclitaxel	Anticancer	Electrostatic attraction method	Pluronic F-127 Chitosan	–	Targeted delivery	103
11	Paclitaxel	Anticancer	Antisolvent precipitation method (probe sonication)	PVP K-30	0.01%	Targeted delivery	68
12	Bexarotene	Anticancer	High pressure homogenizer	Pluronic F-68	0.02%	Solubility enhancement	104
13	Paclitaxel	Anticancer	High pressure homogenizer	Sodium poly styrene sulphonate Glycon Chitosan Tween 80	0.1%, 0.1%, 0.5% and 0.5%, respectively	Solubility enhancement	105
14	Bexarotene	Anticancer	Precipitation + microfluidization	Pluronic F-68	0.5%	Targeted delivery	106
15	Paclitaxel 1-methyltryptophan	Anticancer	Nano-precipitation method	PVA Pluronic F-68	2% of both	Targeted delivery	107
16	Paclitaxel	Anticancer	Wet media milling method	Cremophor EL	0.1%	Targeted delivery	108
17	Paclitaxel	Anticancer	Nano-precipitation method	TPGS	0.4%	Toxicity and PK study	109
18	Paclitaxel	Anticancer	Nano-precipitation method	TPGS	0.4%	Drug resistance study	110
19	Paclitaxel	Anticancer	High pressure homogenization	Poloxamer 188 PEG-400 Tween-80 CMC-Na Tyloxapol	0.08%	PK evaluation study	64
20	Paclitaxel	Anticancer	Emulsification+high speed homogenization	Poloxamer-188	0.5%	Solubility enhancement	111
21	Quercetin	Antioxidant	HPS Bead milling Cave precipitation	Tween-80	2%	Solubility enhancement	37
22	Baicalein	Anticancer	Antisolvent recrystalization + high pressure homogenization	Polysorbate -80 SLS Poloxamer-188	0.1%–0.5%	Solubility enhancement	20

23	Puerarin	Cardiovascular system	High pressure homogenization	Sodium dodecyl sulphate	3.75%	Solubility enhancement	112
24	Docetaxel	Anticancer	High shear homogenization	Pluronic F-127 Pluronic F-68 Tween-80	1%–5%	Targeted delivery	113
25	Curcumin	Anticancer	Media milling	Tween-80	0.2%	Solubility enhancement	114
26	Benzimidazole	Anticancer	Nanoprecipitation, media milling	Pluronic F-108 Tween-80	10%	Bioavailability enhancement	115
27	Itraconazole	Antimycotic agent	Pearl milling technique	Poloxamer-407	3%	Solubility enhancement	51
28	Nimodipine	Calcium-channel blocker	High pressure homogenization	Poloxamer-188 Tween-80	0.6% 0.5%	Solubility and bioavailability enhancement	116
29	Asulacrine	Anticancer	High pressure homogenization	Poloxamer	1%	Bioavailability enhancement	117
30	Nevirapine	Antiretroviral	High pressure homogenization	Tween-80 Poloxamer-188 PVP	1.0% 0.5%0.3%	Targeted delivery	118
31	Oridonin	Anticancer	High pressure homogenization	Pluronic f-68 Lecithin	0.5%	PK and tissue distribution study	119
32	1,3-Dicyclohexylurea	Antihypertensive	Wet media milling	PVP	20%	PK evaluation	120
33	Ibuprofen	Anti-inflammatory	Nano-precipitation	Tween-80	0.02%	Bioavailability enhancement	121
34	Acyclovir	Antiviral	Nano-precipitation	Tween-80	0.02%	Bioavailability enhancement	122
35	Hydrocortisone, prednisolone	Glucocorticoid	High-pressure homogenization	Pluronic F-68	0.1%	Targeted and delayed drug delivery	123
36	Fluticasone	Anti-inflammatory	Wet media milling technique	Tween-80	0.5%	Targeted drug delivery	124
37	Sulfacetamide	Antibiotic	Nano-precipitation	Pluronic F-68	1%	Targeted drug delivery	125
38	Cloricromene	Antithrombotic and antiplatelet	Nano-precipitation	Tween-80	0.02%	Bioavailability and stability enhancement	126
39	Camptothecin	Antitumor	Anti-solvent precipitation with sonication	Pluronic F-127 PVP-K30 HPMC PEG 8000	Not reported	Targeted delivery	127
40	Paclitaxel	Antitumor	Wet media milling	Pluronic F-127	1%	Targeted delivery	128
41	Budesonide	Glucocorticoid	High pressure homogenization	Span 85 Lecithin	0.5% 0.5%	Targeted delivery	129
42	Deacety mycoepoxydiene	Anticancer	High pressure homogenization	Poloxamer 188 Lecithin HPMC PVP	0.5% 0.5% 0.1% 0.1%	Solubility enhancement	130
43	Atovaquone	Antimicrobial	High pressure homogenization	Tween 80 Poloxamer 184	0.1% 0.1%	Targeted delivery	131
44	Bupravaquonone	Antibiotic	High pressure homogenization	Poloxamer 188 Lecithin	1.0% 0.5%	Targeted delivery	132
45	Omeprazol	Proton pump inhibitor	High pressure homogenization	Poloxamer 188	1%	Stability enhancement	133

'–' Data not available.
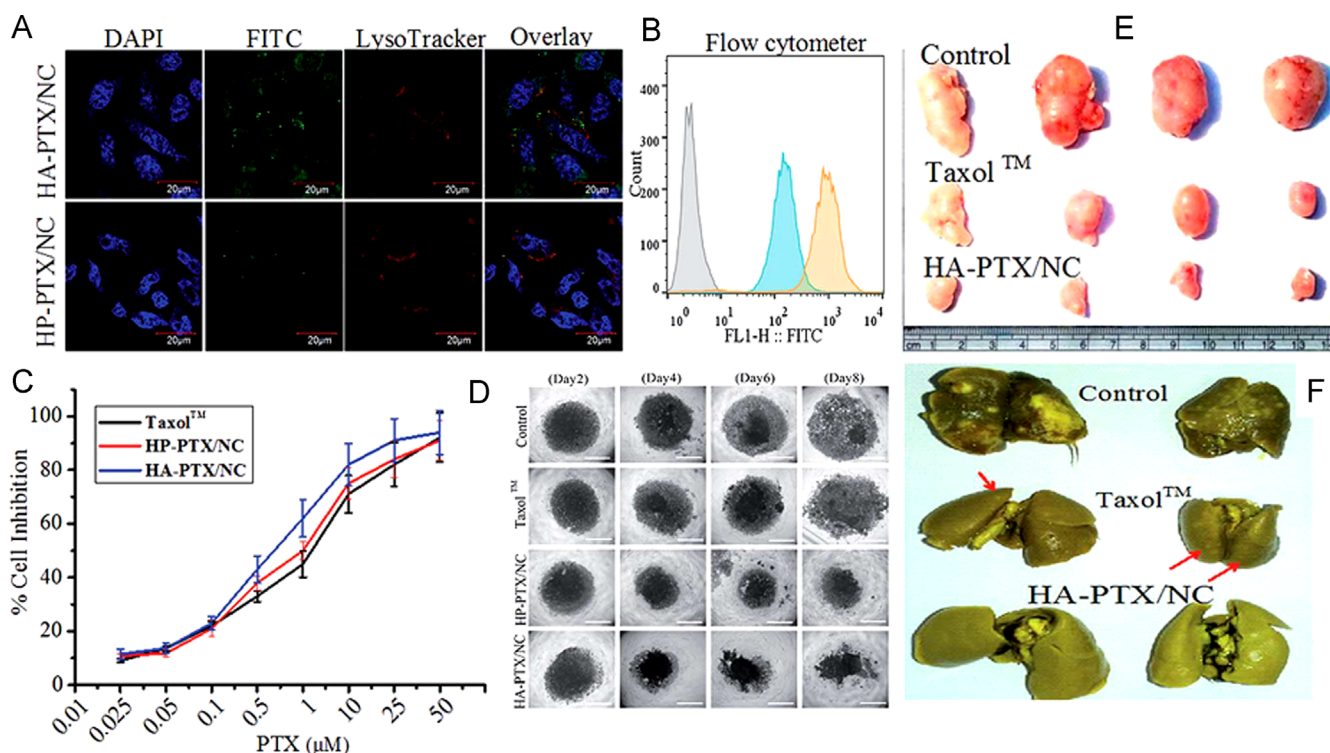
Figure 7 Fluorescein isothiocyanate (FITC) tagged nanocrystals uptake by (A) Confocal laser scanning microscopy (CLSM), (B) Flow cytometry (blue: hyaluronic acid-paclitaxel/nanocrystal (HP-PTX/NC); orange: HP-PTX/NC); (C) Cytotoxicity of paclitaxel formulation on MDA-MB 231 cells after 48 h, M, mol/L; (D) Microscopic images of MDA-MB-231 spheroids on 2–8 days after incubation of different formulations at alternate days (paclitaxel equivalent dose 0.5 μmol/L). Scale bar 500 μm. (E) and (F), Antitumor efficacy of control, Taxol™ and HA-PTX/NCs against LA-7 mammary gland rat cancer model at a dose equivalent to 10 mg/kg paclitaxel. (E) Morphology of the harvested tumors at the end of the study; (F) Lungs isolated from animals of different groups at the end of tumor regression study to demonstrate metastasis of tumor cells to lungs. Reprinted (adapted) with permission from Ref. 103. Copyright © 2016 Royal Society of Chemistry.

Nanotechnology gives a lot of new opportunities in the field of drug delivery as well as challenges while focusing on targeting aspects. Targeting to the specific site is one of the major challenges and offers several avenues of research. Nanocrystals surface can be suitably modified with the specific targeting ligand to avoid cellular uptake of the nanocrystals, as well as targeting to specific site[135]. Nowadays researchers are focusing on means surface modifications which are more complex instead of the simple step of coating.

Recently Sharma et al.[103] have reported hyaluronic acid anchored paclitaxel nanocrystals for the enhanced anticancer effect of the drug. Drug nanocrystals of <250 nm were prepared and cell based *in vitro* evaluation of HA-paclitaxel/NC on 2D monolayer and 3D spheroids demonstrated that hyaluronic acid coating was able to significantly improve the therapeutic efficacy (Fig. 7)[103] as compared to free drug and marketed formulation. Authors reported a significant increase in circulation lifetime of these surface modified nanocrystals as evidenced by 8.4-fold increase in area under curve the (AUC). At the same time, the enhanced anticancer effect was contributed to the interaction of hyaluronic acid with CD44 receptor which significantly enhanced the cellular uptake of these nanocrystals. Reduced lung metastasis and reduced toxicity in LA-7 tumor-bearing rat model was observed in comparison to marketed formulation Taxol™[103].

Apart from targeting to cancer cells, use of apolipoprotein E, Tween 80 and poloxamer 188 are well reported for effective transport against the blood–brain barrier (BBB) and can be effectively used for decoration of drug nanocrystal surface, requiring higher transport from BBB. Table 4 reports some of the patented nanosuspension drug products.

In on similar lines, Zhan et al.[60] have reported camptothecin nanocrystals decorated with silver nanoparticles. Authors have reported excellent dispersion properties along with enhanced dissolution rate and cellular uptake. Silver nanoparticles are reported to inhibit P-gp and authors have tried to combine advantages obtained from the broad-spectrum anticancer agent and P-gp inhibition in single nanocrystal formulation. Prepared nanocrystals were tested for *in vitro* cytotoxicity in both drug sensitive and resistant cell lines and found them to be showing extreme and indiscriminate cytotoxicity in both the cells.

Recently, authors are working in the field of chemically modified drug nanocrystals and exploring the field to incorporate properties like sustained release as well as enhanced circulation time. Zhang et al.[139] have reported PEGylated paclitaxel nanocrystals with prolonged circulation time. For this purpose pretreatment of drug paclitaxel was done with PEG 5000 and then nanocrystals were prepared by simple nanoprecipitation method augmented by probe sonication. An average particle size of paclitaxel nanocrystals and PEGylated paclitaxel nanocrystals was found to be 240 and 330 nm, respectively. PEG molecules were found to cover drug nanocrystals with 11.54 nm fixed aqueous layer thickness. Surface modified nanocrystals showed higher stability in both the storage conditions as well as physiological conditions. In breast cancer xenograft mice model and lung

Table 4 Patents related to parenteral nanosuspension.

No.	Title	Area of invention/final product description	Advantages/applications/claims	Refs.
1	An aqueous suspension of submicron 9-hydroxyrisperidone fatty acid esters	A pharmaceutical composition suitable as a depot formulation for administration via IM or SC route	Administration by IM or SC injection provides therapeutically effective formulation by improving bioavailability	42
2	Pharmaceutical nanosuspension for medicament administration as systems with increased saturation solubility and rate of solution	Preparation of nanosuspension formulation	Nanosuspension formulation provides good bioavailability by increasing the saturation solubility and dissolution rate compared with powders of active compounds	136
3	Nano-particulate formulation of fenofibrate	Nanosuspension prepared using vitamin E TPGS as a stabilizer	Stable fenofibrate nano-particulate (nanosuspension) with vitamin E-TPGS without the addition of any stabilizer	137
4	Parenteral and oral formulations of benzimidazole	Parenteral and oral nanosuspension	Benzimidazole nanosuspension formulation for oral and parenteral delivery, which provides a better effect on oral bioavailability and good therapeutic efficacy	115
5	Novel compositions	The final product as nanosuspension for parenteral administration	Spironolactone nanosuspension preparation for solubility enhancement and improving bioavailability by increasing saturation solubility	137

cancer model, the modified nanocrystals revealed higher anticancer effect as compared to simple drug nanocrystals. The results were quite promising and the prepared formulation was giving better activity when compared with the marketed formulation, Taxol®.

4. *In-vivo* fate of nanocrystals after parenteral delivery

As mentioned in preceding sections, nanocrystals have emerged as potential drug delivery platform for solubility enhancement of poorly water-soluble drug. Apart from the solubility advantages, nanocrystals can be surface-tailored for more advanced applications including targeting *via* the parenteral route. Parenteral administration has advantages of immediate onset of action, reduced dose, and efficacious targeting. Due to these advantages offered by this route of administration, drugs having problems of first-pass metabolism, faster degradation in the gastrointestinal tract and erratic absorption are suitable candidates for parenteral administration. When talking about parenteral administration toxicity-related issues of excipients are to be taken care. A well-known example of the fact is Cremophor EL® which is an integral component of marketed formulation Taxol®. The aforementioned surfactant was found to initiate histamine release eliciting hypersensitivity reactions[140]. Nanocrystals formulation contains very few excipients (stabilizer in minute quantities) and[141,142], toxicity issues related to it are very less. When nanocrystal formulation is prepared, there are many factors to be taken care as they affect the *in vivo* behavior of nanocrystals.

4.1. In vivo fate of nanocrystals administered intravenously

4.1.1. Nanocrystals with smaller size
Particle size is one of the important factors affecting the *in vivo* fate of drug nanocrystals. Nanocrystals with a smaller particle size (less than 100 nm) behave like solution due to very fast dissolution and in contrary, larger crystals show higher cellular uptake by the reticuloendothelial system upon intravenous administration. In practice, the *in vivo* and *in vitro* correlation (IVIVC) is difficult to establish after intravenous administration of nanosuspension owing to either short residence time or fast precipitation. Hence it is extremely important for a formulation scientist to establish meaningful correlation in IVIVC studies[140].

The *in vivo* stability of nanocrystals is a big issue especially when it comes to their fate inside the body. Dissolution rate and half-life *in vivo* are dependent on the size of nanocrystals, the hydrophobicity of drug and presence of a stabilizer. After intravenous administration biodistribution can be mostly affected by the state of agglomeration and surface characteristics of particles.

4.1.2. Larger nanocrystals
Nanocrystals greater than 500 nm are not taken up by cells because of their big size and slowly undergo dissolution or bind to the plasma proteins or are taken up by macrophages. Surface deposition of plasma proteins on nanocrystal surface can lead to aggregation or opsonization by macrophages. Nanocrystals of this size are phagocytosed by macrophages (considered as foreign particles) and may cause toxicity[143]. This mechanism of phagocytosis and clearance is very fast and takes around 5 min to phagocytose almost 90% of administered dose. Macrophages associated with Kupffer cells play an important role and are responsible for the accumulation of most of the dose in the liver. After liver, spleen is the main component responsible for the same
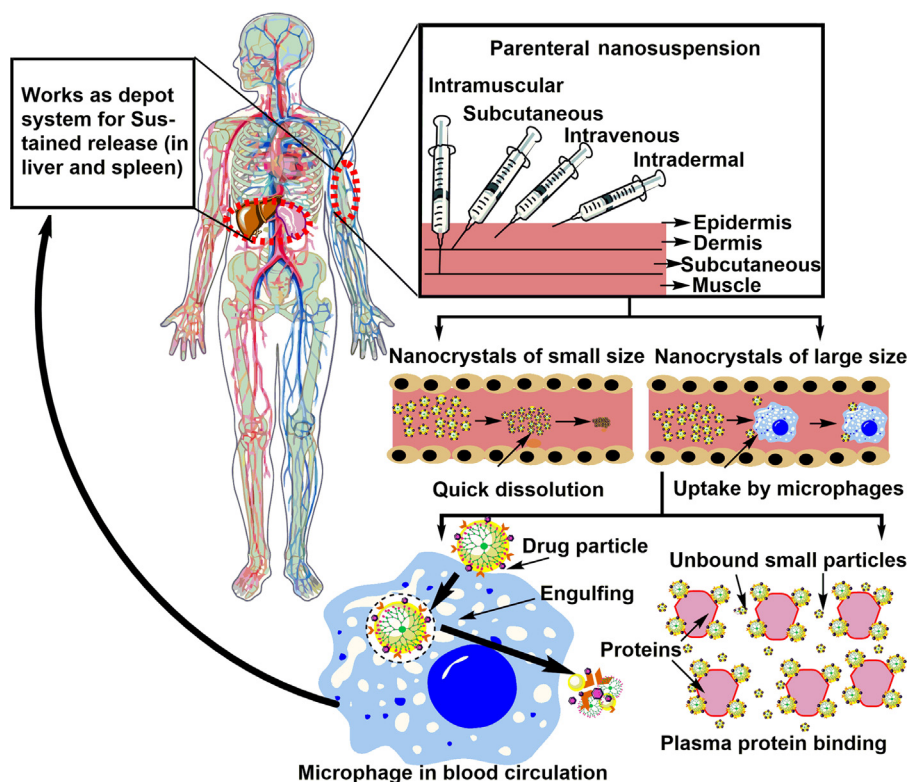
Figure 8 The *in vivo* fate of drug nanocrystals following intravenous administration.

phenomenon. This internalization changes the whole pharmacokinetics of nanosuspension and inversely affects higher dissolution rates. After being taken up by liver macrophages, these cells work as a depot of drug and slowly release drug of interest, which is in absolute contrast to the mechanism of drug release exhibited by nanocrystals under normal conditions[144]. Fig. 8 provides an overview of the *in vivo* fate of drug nanocrystals after parenteral administration.

Route of administration decides which path of nanoparticle will follow after administration and how body cells will consider that nanoparticles. In case of nanocrystals, cellular uptake mechanisms can be of different type after entering to the bloodstream. The paracellular transport mechanism is proposed by some authors, which can be enhanced by the use of surfactants like sodium deoxycholate and others. Particles of 100 nm can be easily transported through this mechanism[144]. However, there is little data available to validate this proof of concept. Another mechanism of cellular uptake is clathrin-mediated endocytosis, non-clathrin mediated endocytosis, phagocytosis, and pinocytosis. Following the intravenous route of administration, nanocrystals remain as such or are present in the form of a molecular dispersion. As mentioned in earlier sections their fate will be eventually decided by the size and surface properties of nanocrystals depending upon which they will be either solubilized or phagocytized[25].

Shegokar et al.[2] have reported the impact of nanocrystals size on body distribution. Nevirapine nanosuspension of particle size 480 nm was administered intravenously to observe its *in vivo* fate. Findings of the study were in accordance with theoretical perception. Around 40% of the administered dose was found in liver and 37% reached to the spleen. The reasons behind this were postulated as nanocrystals size, which was big enough to be taken up by macrophages.

4.2. *In vivo fate of nanocrystals administered by an intramuscular/subcutaneous route*

Contrary to intravenous administration, drug nanosuspensions administered intra-muscularly or subcutaneously (with smaller size) are reported to show sustained drug release irrespective of nanocrystal size. Wei et al.[145] have reported intramuscular administration of curcumin didecanoate nanosuspension and found drug release to be sustained. Similarly, rilpivirine nanosuspension of size 200 nm was reported to show sustained plasma release profile up to 3 months, when administered as single intramuscular or subcutaneous injection[135]. The *in vivo* fate of nanocrystals can be suitably modified upon surface modification. Opsonisation is the most prominent phenomenon that can occur after intravenous administration and can be avoided by surface modification with PEG, which also enhances circulation time of these nanoparticles. Similarly, surface modification using vitamin E TPGS can lead to P-gp efflux inhibition. However, as mentioned earlier; IVIVC correlation is highly needed, to evaluate drug efficacy and avoid probable toxicity.

5. Challenges associated with the development of parenteral nanocrystals

Currently, there are very few marketed formulations based on parenteral nanosuspension technology. United States Food and Drug Administration (US FDA) has approved Invega Sustenna®, the first monthly antipsychotic injection, for use with Elan Drug Technologies (NanoCrystal technology) developed by Janssen Pharmaceuticals in 2012. Invega Sustenna® is the fifth licensed product approved by the US FDA using Elan's NanoCrystal

technology for various formulations[42]. Paliperidone palmitate is an antipsychotic agent belonging to the category of benzisoxazole derivatives. Nanocrystal formulation of a mentioned drug for parenteral use is prepared with the aid of polysorbate 20, PEG 4000, citric acid monohydrate, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate monohydrate, sodium hydroxide, and water for injection as inactive ingredients.

Sporanox IV® is another nanocrystal-based parenteral formulation of antifungal drug itraconazole. Contrary to oral route (Emend®, Rapamune®, Tricor®, Triglide® and others) as mentioned above, there are very less parenteral products based on nanocrystal technologies[2]. The reason behind this could be various challenges associated with development and effectiveness of parenteral nanosuspensions. Physical stability, stability during application and shedding of targeting ligand are some of the bottlenecks hindering the development of parenteral nanocrystal-based formulations. Proceeding sections will deal with challenges associated with parenteral nanocrystal in details.

5.1. Challenges of physical stability

Nanosuspension system is colloidal dispersion which is stabilized by stabilizers (surfactants, polymers or permutation of both). It is a thermodynamically unstable system having a tendency to agglomerate or enhances crystal growth. Despite the fact that the nanosuspension technology has been broadly investigated, stability is still a major concern and creates trouble for industrial scale up. Moreover, the knowledge on the practical correlation between the stability and stabilizer efficacy of nanosuspension formulation is quite insufficient. Stabilizer plays a vital role to put off aggregation of nanosuspension formulation. However, different drug requires different stabilizers in varying concentration and it is unlikely to arrive at a rule of thumb, applicable to all. Unfortunately, there is no experiential or theoretical guideline for the stabilizer selection and its optimization. There is still very little practical knowledge about the stability and stabilizer effectiveness of the formulation. Therefore, to select an appropriate stabilizer for a given nanosuspension formulation is often an important task for formulation scientist. Moreover, the amount and type of stabilizers affect the physical stability and *in vivo* performance of formulation. Some common stability problems are frequently observed in nanosuspension formulation[146].

5.1.1. Aggregation

Selection of stabilizer type plays an important role in the stabilization and often improper selection can induce aggregation/other stability issues during prolonged storage. Ostwald ripening is a most common phenomenon occurring in nanosuspensions of heterogeneous nature which is most likely the case with most of the developed nanosuspensions[146]. In fact, aggregation is an intrinsic property of nanosuspension formulation, which is due to the propensity of a nanonized system to decrease the Gibbs free energy and initiation of Ostwald ripening. Proper stabilizer selection offering an excellent interaction potential between stabilizer and drug is necessary for the proper stabilization of preparation. When nanosuspension is produced by the top-down approach the surface area largely increases and due to that aggregation of nanosized particles happens. Therefore, the use of stabilizers is extremely important to cover up the surface of nanoparticles during preparation process[147].

5.1.2. Crystalline transformation

To improve physical stability, solidification is considered a good method and various techniques like spray drying, freeze drying, electrospraying and pelletization are used. Formulation of the amorphous form requires very less energy than the crystal form formation and exhibits higher saturation solubility and higher dissolution velocity than the crystalline state. As a result, the crystalline drug is more stable and mostly best choice for the nanosuspension preparation. Once the amorphous material is produced, there is a risk of crystalline transformation during release or storage and thus crystalline transformation is an unavoidable thing in storage stage of the nanosuspension. Due to crystalline transformation, solubility may vary and may result in the alteration in plasma drug concentrations[148].

5.1.3. Sedimentation/creaming

Nanosuspension formulations are mostly colloidal dispersion and are neither like true solutions nor a coarse dispersion rather an intermediate between them. Sedimentation is an incident of appearance for unstable nanosuspension formulation[147]. The primary step of instability is aggregation and Ostwald ripening. Nevertheless, when the gravity of drug nanoparticles becomes larger than their buoyancy force provided by dispersion system, the sedimentation takes place. Flocculation is also one of the types of sedimentation and it may occur by neutralization charge, particle-polymer complex, depletion flocculation or due to polymer bridging mechanism. The flocculation depends upon the two things polymer and its property of interaction with nanocrystals surface[149].

5.1.4. Crystal growth

Crystal growth is the common phenomenon in the colloidal suspension and it is known as Ostwald ripening which is responsible for the change in size distribution and particle size[150]. According to Ostwald–Freundlich equation, smaller particles have more saturation solubility than bigger particles and this creates concentration gradient between small and large particles. The molecules in formulation disseminate the small particles (higher concentration surrounding areas) from larger particles (surrounded with lower drug concentration) and create an unsaturated solution in the vicinity of large particles which leads to a process of the Ostwald ripening and crystal growth. In other terms, the process of the diffusion leaves an unsaturated solution surrounding the small particles, giving rise to drug crystallization on large particles. The stabilizers play an essential role here *i.e.* they reduce the interfacial tension between the solid particles and a liquid medium and thereby avoid the Ostwald ripening. Verma et al.[151] have reported the preparation of indomethacin nanosuspension stabilized using various stabilizers, the effect of the stabilizer concentration and its type on Ostwald ripening.

5.2. Challenges of chemical instability

Commonly, the nanosuspension formulations are prepared using water as a dispersion medium. Though non-aqueous solvents are also used to the same extent, the major proportion is still aqueous. Consequently, the chemical stability, like oxidation and hydrolysis, are the major concerns in the nanosuspension formulation. Appropriate packaging is a potential approach ensuring the chemical stability of formulation by protecting the drug from chemical hazards, light sensitivity, oxidation and hydrolysis[152].

Conversion of final product into the solid and concentrated state protects the drug from chemical hazards, but hydrolyzable compounds are still very difficult to protect[146]. It was reported that a significant enhancement of chemical stability of quercetin nanocrystal formulation occurred by using evaporative precipitation method and HPH considerably improved chemical stability and dissolution rate[153].

5.3. Challenges due to the shedding of targeting ligand

Targeting ligands, in general, are not covalently attached to the surface of nanocrystals and physical adsorption is the prime most concepts utilized for surface modification. Physical adsorption provides weak bonding which could be unstable in biological environments and is a major challenge when the foremost objective is to provide targeting to a specific site. In some cases, this ligand shedding is desirable, as in case of P-gp substrate stabilized with vitamin E TPGS. Shedding of this ligand is involved in P-gp inhibition and provides better efficacy[59].

6. Strategies to combat stability issues

Various solidification techniques are used to overcome issues related to physical as well as chemical stability. Solidification results in physical stability of nanosuspensions, as particle motion is hindered and chances of collision become less. Removal of aqueous part reduces/ends most of the chemical reactions resulting in chemical stability. Researchers have explored various solidification techniques *viz.* freeze drying, spray drying, the formation of hydrogels, pelletization, printing and electrospraying which are suitable for removal of aqueous content and enhancing physical as well as chemical stability. Among them freeze drying[154], spray freeze drying[155], printing[155], coating[155] are the techniques already reported for solidification of parenteral nanosuspension. Other possible solidification techniques, which can be explored in the near future based on the properties of drug incorporated and excipients used, are also discussed briefly. Proceeding section will deal with some of the solidifications techniques in brief.

6.1. Freeze drying

Freeze drying technique (well-known as lyophilization) is predominantly used for solidification of pharmaceuticals. Freeze drying technique is considered a safe technique for solidification of heat sensitive products like vaccines and various protein-containing formulations including nanosuspension. Freezing, primary drying, and secondary drying are three main steps in the process of lyophilization. Freezing step converts most of the aqueous component to the ice while primary drying involves sublimation of ice crystals. Secondary drying step removes an unfrozen aqueous component from the system[59]. Cryoprotectants are added to avoid the particle aggregation in formulation during the lyophilization process. Finally, the product is obtained as highly porous powder cake with very less water content. Selection of cryoprotectants and freezing rate both are critical parameters in this technique.

6.2. Spray drying

Spray drying technique is very simple and convenient technique for the conversion of liquid formulation into the solid powder[156].

This technique is working on the principle of spraying liquid in the form of small droplets to a preheated chamber by using nozzle and dried at a predefined temperature. Inlet temperature and spraying rate are the critical process parameters for this technique[157]. Nowadays, an electro-spraying technique is widely used for the solidification of liquid formulation which utilizes electrical current for atomization in place of pressurized air[59].

6.3. Spray-freeze drying

Spray-freeze drying technique is a fusion of both the aforementioned techniques. This technique is particularly used for the thermo-sensitive products (vaccines, proteins, *etc.*). In this technique, nanosuspension is passed through a nozzle to obtain droplets and then these are immediately frozen in liquid nitrogen. The remaining process is similar to freeze drying. Spray freeze-drying produces injectable as well as inhalable delivery products[156]. The technique provides a good alternative especially for thermo-sensitive drug compounds and provides less particle size in comparison to the simple spray dried product.

6.4. Coating

Deposition of nanosuspension on the exterior of pellets, granules, sugar beads with the help of coater is another technique for the solidification of nanosuspension. This method requires an adequate quantity of excipients to stabilize the system and also acts as coating agents[156]. He et al.[158] have reported preparation of indomethacin nanosuspension by precipitation– ultrasonication method using various proteins obtained from food as stabilizers. In this study, solidification was carried out using coating onto the pellets and results obtained suggested faster dissolution profile than the unprocessed crystals[158].

6.5. Incorporation in granules and pellets

Incorporation of nanosuspension in granules and pellets is another approach used for solidification of the nanosuspension formulation[156], In this technique, nanosuspension is incorporated in the granules or pellets utilizing various instruments such as fluidized-bed granulator, spheronizer etc. Mitri et al.[159] have reported preparation of the lutein nanosuspension by HPH method and further solidification here was done with the formation of pellets by incorporation of nanosuspension using the spheronization technique. Finally, these prepared pellets were filled into capsules. This prepared formulation has shown higher saturation solubility and increased release rate[159]. However, this technique is reportedly not used for parenteral nanosuspension so far.

6.6. Printing

Printing technique is based on the principle of deposition of liquid or dispersed drug for manufacturing solid oral or pulmonary dosage forms. These techniques play an important role for narrow therapeutic index drugs and administering an amalgamation of drugs by printing more than one layer *via* barrier coating. A significant application of printing technology is a preparation of personalized medicines[156]. Nowadays, Inkjet printing, flexographic printing, and 3D printing are three mainly applied printing technologies used, which appears to be promising for use in parenteral nanocrystal-based formulations.

6.7. Using aerosol flow reactor

The aerosol flow reactor is one of patented technology for solidification of nanosuspension formulation for better stability. It is a single step continuous method with atomization of liquid flow *via* nitrogen (carrier gas) for solidification[156]. The liquid feed is atomized by nebulizer and the droplets produced are suspended in a carrier gas (N_2) which are then fused in the heated tubular laminar flow reactor with constant temperature, where solidification occurs. Laaksonen et al.[160] prepared the nanoparticulate indomethacin by aerosol flow reactor and wet milling technique. Particles obtained from this technique were showing good release and dissolution profile. Detailed investigation of nanocrystals produced using this technique is however still lacking for it to be used safely for parenteral administration.

6.8. Electrospraying

Electrohydrodynamic atomization (EHDA) is a technique based on electromechanical and hydrodynamic forces working in combination with each other. Thakkar et al.[161] have recently reported preparation of erlotinib nanosuspension utilizing nanoprecipitation technique. A comparative evaluation of electrospraying and lyophilization techniques was done utilizing electrospraying as novel solidification technique. Flow rate, applied voltage, and tip to collector distance are critical parameters to be taken care with the use of this technique. While this technique was explored for obtaining a nano sprayed powder for orally dispersible tablets, it would be equally interesting to investigate the feasibility of using this technique for parenteral application. Electrospraying offers the advantage of using a different polymer which could also be used to obtain customized release rate of parenteral formulation.

7. Conclusion and future prospects

In comparison to other nanocarriers, nanocrystals are most successful as evidenced by the lesser time between inventions to market stage. A total number of products in the market (Tricor® a blockbuster product with sales of > 1 billion US dollar per year) and ongoing clinical trials further support this fact. Toxicity issues associated with nanocrystals are negligible as these are made majorly of the drug itself, thus get easily dissolved without leaving any residual carrier and can be of size > 100 nm. Again uptake of drug nanocrystals by macrophages is majorly governed by the interplay of size/surface properties and can affect drug release (sustained release by working as depot system). Higher drug concentrations are associated with toxicity issues, especially in case of anticancer molecules which are very potent. However, very few articles talks about the *in vivo* fate of nanocrystals administered through various routes. The prime bottleneck is IVIVC of nanocrystal-based formulation which is needed to have a better understanding of its *in vivo* fate. A surface modification which provides stable interactions even at physiological conditions is one more thrust area of research; however, undesirable targeting ligand shedding still pose a major challenge. Some researchers have focused on pre-treatment of drug (PEGylation of drug followed by nanocrystals preparation) with the ligand to provide stable binding, which requires the involvement of chemical processes and needs further systematic investigation. Most of the nanocrystal-based studies lack stability under physiological conditions, a critical parameter which can affect most desirable properties *i.e.* solubility

enhancement of nanocrystals. An investigation in directions above is needed for better understanding of nanocrystal technology and for providing new insights in this field before it could be safely extrapolated to formulations delivered parenterally.

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