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A recent trend of drug-nanoparticles in suspension for the application in drug delivery

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ABSTRACT

Persistent development in nanomedicine has enabled successful nano-sizing of most drug samples, which in turn imparts remarkable properties to the drugs such as enhanced solubility and bioavailability for the applications in drug delivery. In this context, several review articles are available in scientific domain covering inorganic nanoparticles such as Au, Ag, SPIONs, Qdots, carbon nanotubes and graphene however this review covers the development of drug nanoparticles together with their possibilities and limitation from fabrication (bottom up *vs* top down) to application in drug delivery during the last 5 years. In addition, some distinguished studies and novel drug particles are presented in order to contribute significantly towards the understanding of drug nanocrystals and its use in drug delivery.

Keywords: Nano-suspension, Amorphous / Crystalline nanoparticles, Top down / Bottom up, Drug-nanocrystals, Nano-carriers, Drug Delivery

Introduction

Modern material science is no longer restricted to the metallurgical application, as the advancement in the nanotechnology is opening unlocked doors in various fields. For over a decade, extensive research is being undertaken using nanoparticles for its application in medicines, environmental projects, electrical and electronics, aerospace and lifestyle industries. These nanoparticles of nanometer in size ranging from 1 to 1000 nm possess great advantages as compared to the microsized particles.

Development of a large number of drugs and their delivery without any side effects to the patients are the real challenges faced by scientific community. Most of the drug molecules are large organic molecules and have poor solubility in water. Hence a huge effort has been made for nanosizing the drug particles in either an amorphous or crystalline nanosuspension for applications in passive targeting due to enhanced membrane diffusion. Similarly an effort has been made for the development of several carriers for delivering drugs and functionalisation of carriers to enhance the active delivery at a target organ. Furthermore, the nanosize of drug particles imparts remarkable properties to the drugs such as enhanced solubility and bioavailability. Therefore this review is focussed on critically highlighting the nanomedical use of various drug nanocrystals, especially for drug delivery applications.

Recent trends

Nanoparticles' use has increased exponentially for the drug formulations and drug delivery in the last decade. Increased efforts are applied to monitor the structure – function relationship of the nanoparticles for targeted drug delivery applications. There has been some novel nanodrugs produced recently such as the Copazone, Welchol, VivaGel and RenaGel. Copazone is a random co-polymer of the four main amino acids which is employed to treat muscular sclerosis [1]. Welchol is a drug which helps to lower the low density lipoprotein (LDL) cholesterol and blood sugar level by binding the cholesterol [2]. VivaGel is a topically administered vaginal virucide which is a multivalent lysine based dendrimer product [3]. RenaGel is an oral polymeric sequestrant that binds to phosphate and is used to treat chronic kidney disease [4]. In addition, a timeline is reviewed in Figure 1 to show the FDA approved amorphous and crystalline drugs since 1984 [5].

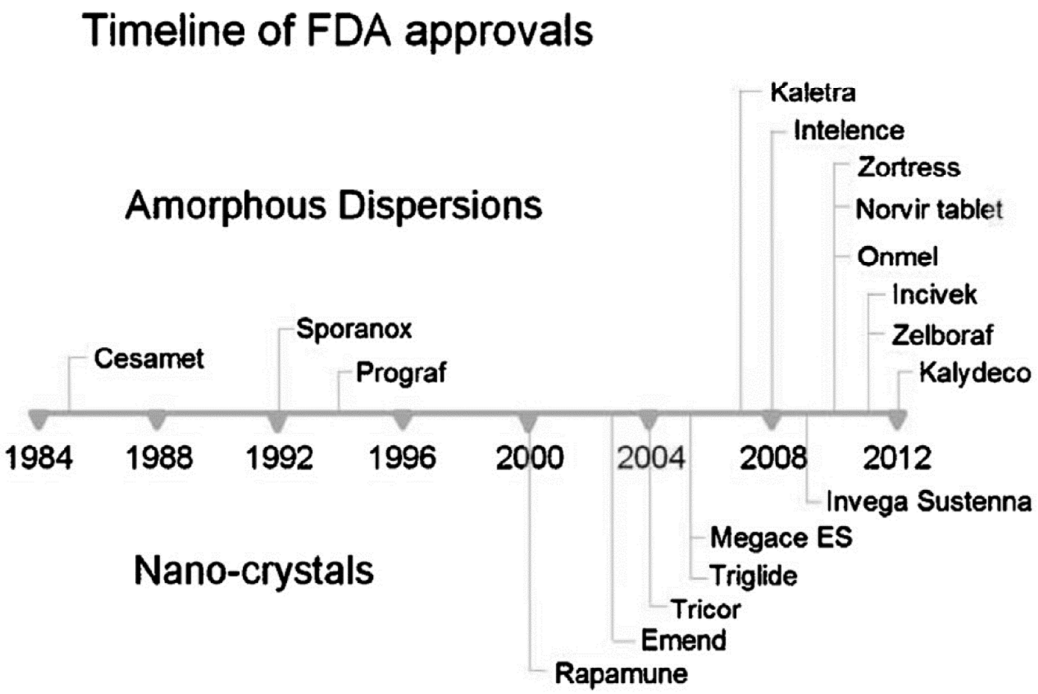

Figure 1: Timeline reviewing FDA approved crystalline & amorphous drugs
(Reproduced with permission from ref [5])

Most of the current cancer therapies and drug formulations are based on the nanoparticle approach to improve the bioavailability and solubility of the encapsulated drugs at the target site. There is an increased use of nanosuspensions for drug delivery application, especially for topically administered drugs since nanosuspensions increases the bioavailability of drugs due to increased permeation. For instance, as shown in Figure 2, nanosuspensions exhibit higher permeability and high concentration gradient for both topical and subcutaneously administered drugs compared to microparticles [6]. A multi-disciplinary principle is employed nowadays to tailor the nanoparticles in order to incorporate specific properties, such as DNA functionalisation using molecular biology and genetics, ion conductivity using the semiconductor physics, solubility and stability using the organic chemistry, and so on. For example, β-Lapachone or simply β-Lap is a novel anticancer drug demonstrated to be delivered by 30 nm PEG-PLA polymer micelles to the lungs of mice. The drug was injected intravenously and was bioactivated by the enzyme NAD(P)H:quinon oxidoreductase-1 (NQO1) that is found in excess levels at the non-small-cell lung cancer sites [7].

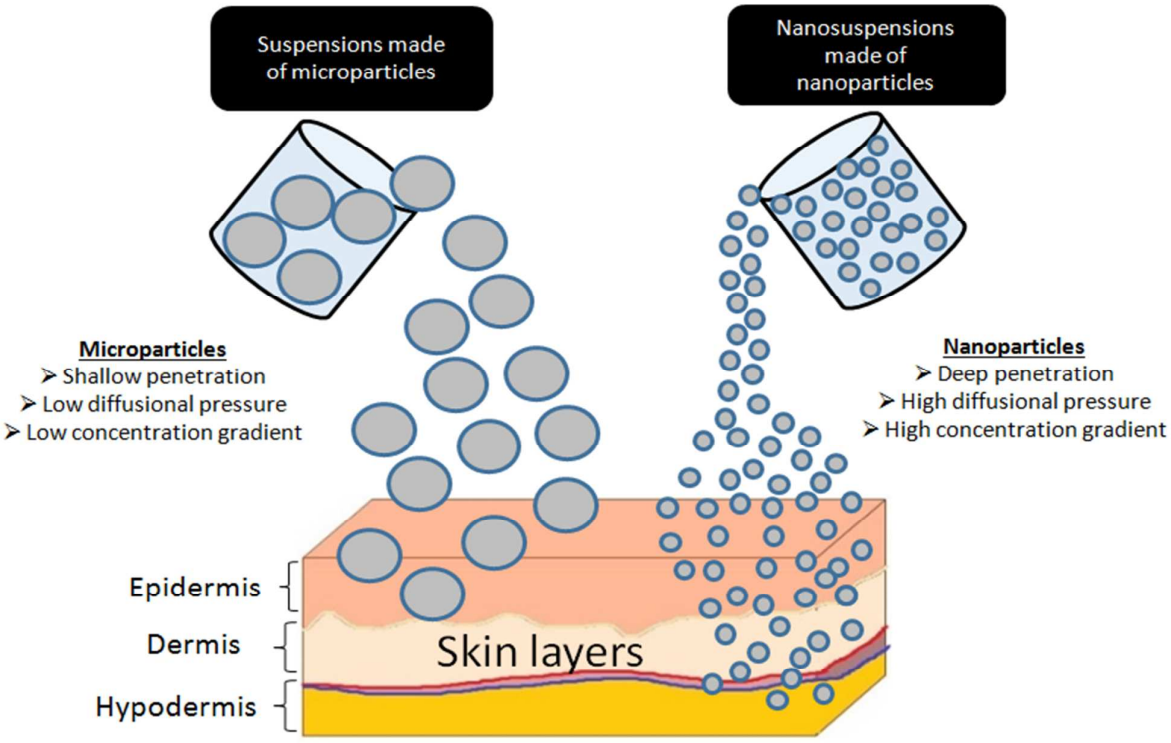

Figure 2: Scheme showing enhanced permeability of drug nanosuspension

Drug particles in Nanosuspension

In simple terms, a submicron colloidal dispersion of the nanoparticles (in either amorphous or crystalline form) is called as a nanosuspension. A drug specific nanosuspension consists of dispersed, finely colloidal and mostly biphasic drug particles with an overall size of less than 500 nm [8]. These drug-nanoparticles conjugates can be stabilised using polymers and surfactants before administration through various routes such as topical, oral, pulmonary, ocular or parenteral [6]. Different ingredients are used for formulation of nanosuspensions such as organic solvents, cosurfactants, stabilisers and other additives such as salts, polyols, buffers, cryoprotectants and osmogents [9].

Solubility and bioavailability of drugs are the key issues that are mitigated using the nanosuspensions. In addition, the nanosuspension also affects the drug's pharmacokinetics that can improve its efficacy and safety. Both chemical and physical modifications are applied to improve the solubility and bioavailability of drugs [10]. Chemical modifications include synthesising the soluble salts and prodrugs, whereas the physical modifications include reducing particle size, use of

surfactants for solublisation/complexation, forming pseudo-polymorphs/polymorphs and preparing drug dispersions [11-15]. Different types of composite structure results from nanoparticles in suspension, as shown in Figure 3. Various factors and techniques are responsible to form composite structures such as the core shell, internal dispersion, agglomeration, coating/surface modification, hollow, porous, nano-dense body, nano-porous body and nano-thin film [16].

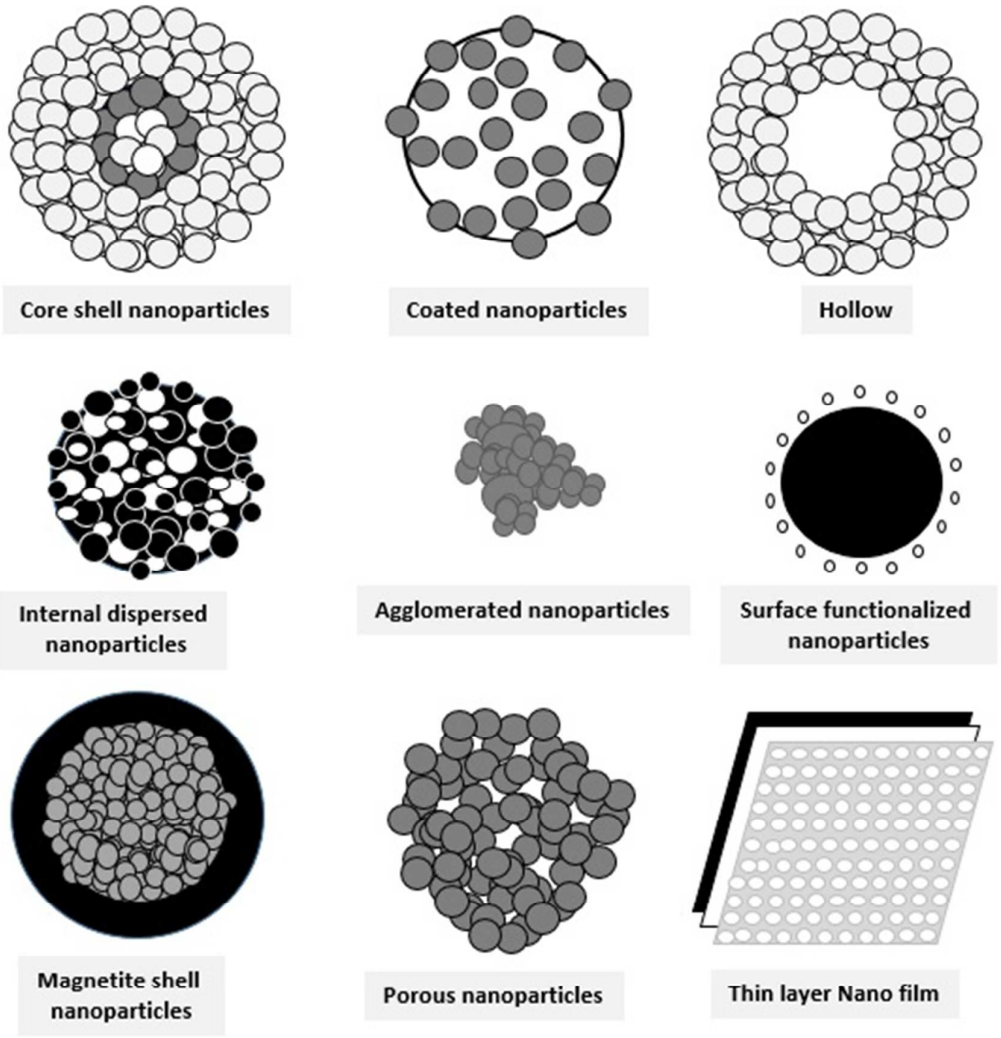

Figure 3: Types of composite structures formed from nanoparticles in suspension

Functionalisation of nanocrystals

A schematic review of the various ligands surface functionalised on a nanocrystals core is shown in Figure 4 [17]. It can be seen that a drug nanocrystal core can be surface modified for specific applications by tailoring the type of ligands attached to it

with the help of a polymer coating. For instance, magnetic ligands can be used for magnetically targeting to tumor sites followed by hyperthermia applications, where the drug could be released systematically due to localised hearing. The use of image contrast ligand facilitates the diagnostic monitoring of drug release and provide real time data about the pharmacokinetics of the drug. Furthermore, the surface of nanocrystal core could be functionalised with uptake, targeting or adhesion ligands to provide an active transport or targeting to tumor sites in *in vivo*. For instance, nanocrystal implants are exploiting these surface functionalised drug nanocrystals as therapeutic vehicles to identify, transfect and rebuild damaged regions in the body [18].

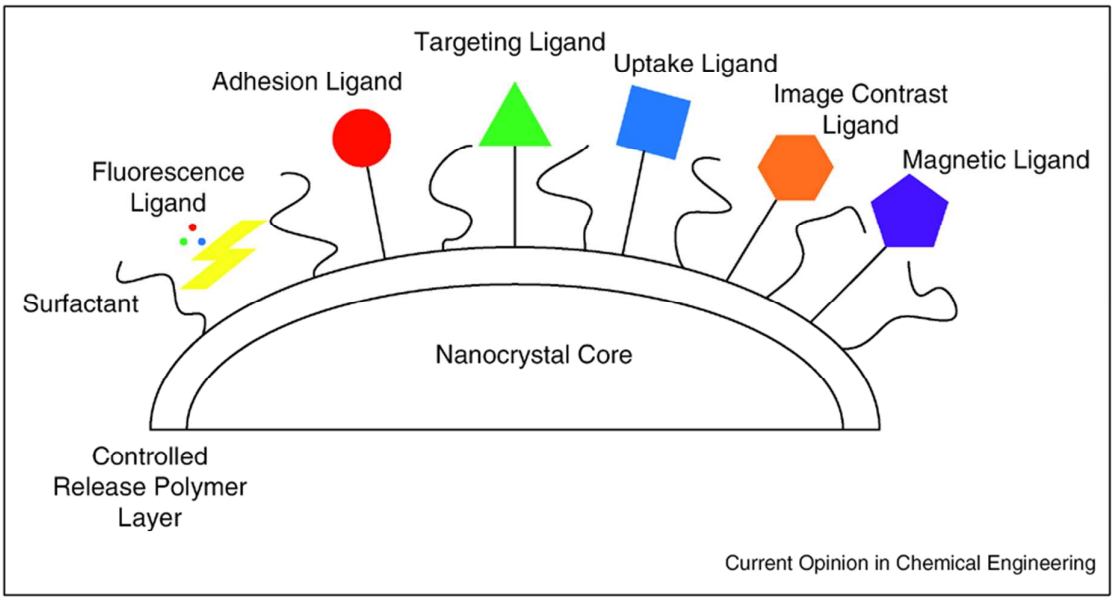

Figure 4: Surface functionalization of nanocrystals (Reproduced with permission from ref [17])

Methods of preparation of nanosuspension

Nanosuspensions can be prepared using both bottom-up and top-down approaches. These methodologies are widely applied in nanomedicine due to its consistency to produce nanoparticles of controlled aspect ratio [19]. The bottom-up methods are called as conventional precipitation methods whereas the top-down methods are referred to as disintegration methods. The top-down methods are further classified as media milling and high pressure homogenisation (HPH) methods, where the latter

is sub-classed into DissoCubes (HPH in water), Nanopure (HPH in nonaqueous media) and Nanoedge (HPH and precipitation) [20]. Figure 5 present a schematic diagram of two different approaches for the fabrication of drug nanoparticles.

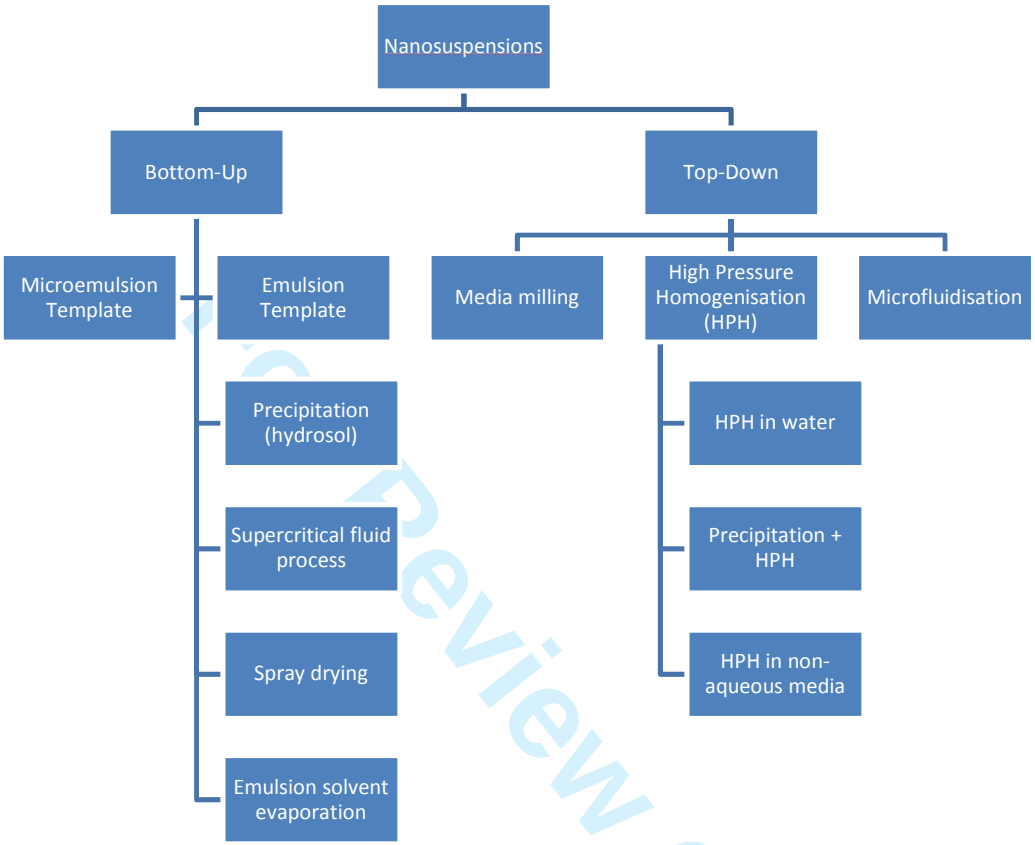

Figure 5: Various methods of nanosuspension preparation

Top down method of nanosuspension preparation aims to reduce particle size into nanoparticles using wet milling techniques such as high pressure homogenisation, microfluidisation and media milling. There are no harmful solvents used in these techniques but media milling method requires high energy inputs [21]. In general, considerable amounts of heat is generated in the top down methods leading to destruction of heat sensitive drug components. For example, milling is reported to cause mechanical activation at surfaces of drug particles [22]. High surface energetics lead to generation of amorphous regions and crystal defects. Further crystallisation of the drug particles reduces the physical and chemical stability of particles on storage [23].

Bottom up method of nanosuspension preparation aims to produce nanoparticles from precipitate by first dissolving the drug in an organic solvent followed by adding a precipitating agent in the presence of a stabiliser [24]. Some forms of this method include supercritical fluid processes, solvent anti-solvent method, emulsion solvent evaporation and spray drying. Some of the limitations of bottom up method include generation of unstable polymorphs, solvates and hydrates of the nanosuspension constituents [25, 26]. Furthermore, the difficulty of completing removing the solvents from the final product is persistent in bottom up method. This reduces the physical and chemical stability of the formulation, as it is often seen that bottom up method results in needle shaped particles due to rapid unidirectional growth [24].

A study of some of the recent nanosuspensions prepared through various routes for drug delivery is shown in table 1 [27 - 42]. The table highlights both amorphous and crystalline nanosuspensions along with the particle size, method of preparation and main conclusions from the studies.

Table 1: A study of the recent (5 years) drug nanoparticles in both crystalline and amorphous forms [27 – 42]

Type of nanoparticle	Particle size (nm)	Drug/Polymer used	Method of preparation	Focus of article	Conclusion	*Ref.*
Amorphous nanoparticle suspension	350	Cylosporin A (CyA)	Bead milling	Enhance applications in psoriasis or dermal penetration	Higher penetration using amorphous CyA nanoparticles	*27*
Amorphous nanosuspension	50-500	Isradipine	Sonoprecipitation	Enhance drug delivery for increased dissolution rates	Improved drug release rate was observed	*28*
Nanosuspension (crystalline to amorphous)	300 -500	Myricetin	Nanosizing process	Effect of stabilizers on bioavailability/ dissolution of poorly water-soluble drugs	Stabilizers enhanced dissolution, amorphisation enhanced solubility	*29*
Nanosuspension (crystalline to amorphous)	208 -246	Cur-TPGS NSs (Curcumin-D-α-Tocopherol PEG 1000 succinate)	Sonoprecipitation, ultrasonic homogenization	To enhance the solubility of formed nanosuspension	Cur-TPGS NSs showed higher solubility & oral adsorption	*30*
Nanosuspension (crystalline)	< 150	Anticancer compound, SN 30191	Wet milling	Evaluation of crystalline nanosuspension as a cancer drug	Crystalline nanosuspension showed high tolerance & drug delivery	*31*
Nanocrystals to Amorphous nanosuspension	150-175	Curcumin	Antisolvent precipitation method	Enhance bioavailability of curcumin by nanosizing and amorphisation	Nanosized & amorphous nanosuspension shows the maximum stability	*32*
Crystalline nanosuspension	200-350	BCS class II/IV compounds	Spray drying	Enhance dissolution using nanocrystalline suspensions	Faster dissolution was obtained subject to drug-to-excipient ratio	*33*
Amorphous &	NA	Nanosuspensions	Freeze drying	Effect of critical formulation	Freeze drying does not	*34*

crystalline nanosuspension		with steric stabilizers		temperature (CFT) and freeze drying on drug's stability	affect drug's stability	
Amorphous & crystalline nanosuspension	300	Hydrocortisone (HC)	Microfluidic nanoprecipitation (bottom-up) & wet milling (top-down)	Bottom up and top down methods for ophthalmic drug	Top-down showed high stability as compared to the bottom-down	*35*
Crystalline nanosuspension	88, 122, 362	Compounds (celecoxib, griseo-fulvin, fenofibrate, and compound-X)	Ultracentrifugation & filtration	Dissolution rate dependence on the Noyes-Whitney equation	Low dependence	*36*
Amorphous and crystalline nanosuspensions	274	Ziprasidone	Lyophilisation and milling	Improving drug absorption in the fasted state (in-vitro studies)	Amorphous nanosuspension showed high absorption	*37*
Crystalline & amorphous nanosuspension	323-734	Itraconazole (ITZ)	HME and wet milling	Effect of methods on Itraconazole (ITZ) for both *in vitro* & *in vivo*	HME method showed higher dissolution & bioavailability	*38*
Crystalline & amorphous nanosuspension	300	Drug/polymeric complex	Wet-milling	Increase solubility & dissolution rate by nanosizing	Higher solubility & dissolution rate upon nanosizing	*39*
Amorphous nanosuspension	>600	Ezetimibe nanosuspension	Precipitation	Enhance oral bioavailability of ezetimibe	Amorphous precipitation increases oral bioavailability	*40*
Crystalline nanosuspension	699	Fenofibrate	Bead-milling method Spray drying	Effect of methods on bioavailability & dissolution	The dissolution was found faster in the spray dried powder	*41*
Crystalline nanosuspension	397	Indomethacin	Spray drying	Effect of spray drying on powder yields	High yields with lower moisture content	*42*

Size measurement of nanoparticles in suspension

The size and number of atoms within a single nanoparticle controls the morphological aspects of these particles, in addition to other chemical and physical properties. For instance, the smallest hydrogen atom has a diameter of about 0.074 nm whereas a lead atom is about 0.32 nm. Therefore in a single nanoparticle, there can be ten to hundreds of atoms present based on the bonding types and composition [16]. One of the most important properties attained on transforming micro-sized particles into nanoparticles is the massive increase in the surface area. This increased surface area imparts enhanced solubility and reaction rates for the particles, which is a key desire in modern science. Other properties such as dielectric constant, melting point and even crystal lattice are affected due to the "size effect".

The size measurement for nanoparticles can be done using a range of characterisation techniques such as imaging using electron microscope from SEM and TEM, peak widths at half maximum from X-ray diffraction, surface area analysis from BET and Brownian motion calculation using Dynamic Light Scattering in Zetasizer instrument. The imaging techniques are considered to be relatively easy and quick as compared to others since it can provide visual inspection of the particles as well. However, the sample preparation is a critical step that determines the quality of image an accuracy of size measurements.

The X-ray method is on the other hand considered complex but relatively precise in estimating the average particle size from peak properties. But on a nanoscale, it is also subjected to limitations of sample preparation and orientation to the electron beam. Surface area analysis from BET is relatively simpler to estimate particle size but the assumption of spherical shape for calculation is not applicable to all types of nanoparticles. Furthermore, the inner structure and surface state of particles can affect the calculations and thereby the size of nanoparticles. The application of Zetasizer is widely recognised in literatures for determining the size of particles in nanosuspensions [16, 43 - 45].

A summary of the most common analytical techniques for characterisation of nanoparticles is shown in the Figure 6. These techniques are helpful in studying various aspects of the nanoparticles such as X-ray diffraction for amorphous or

crystallinity study, SEM/EDX and AFM for surface morphology study, TEM for internal structure study, AGM, VSM and SQUID for magnetic property study and so on.

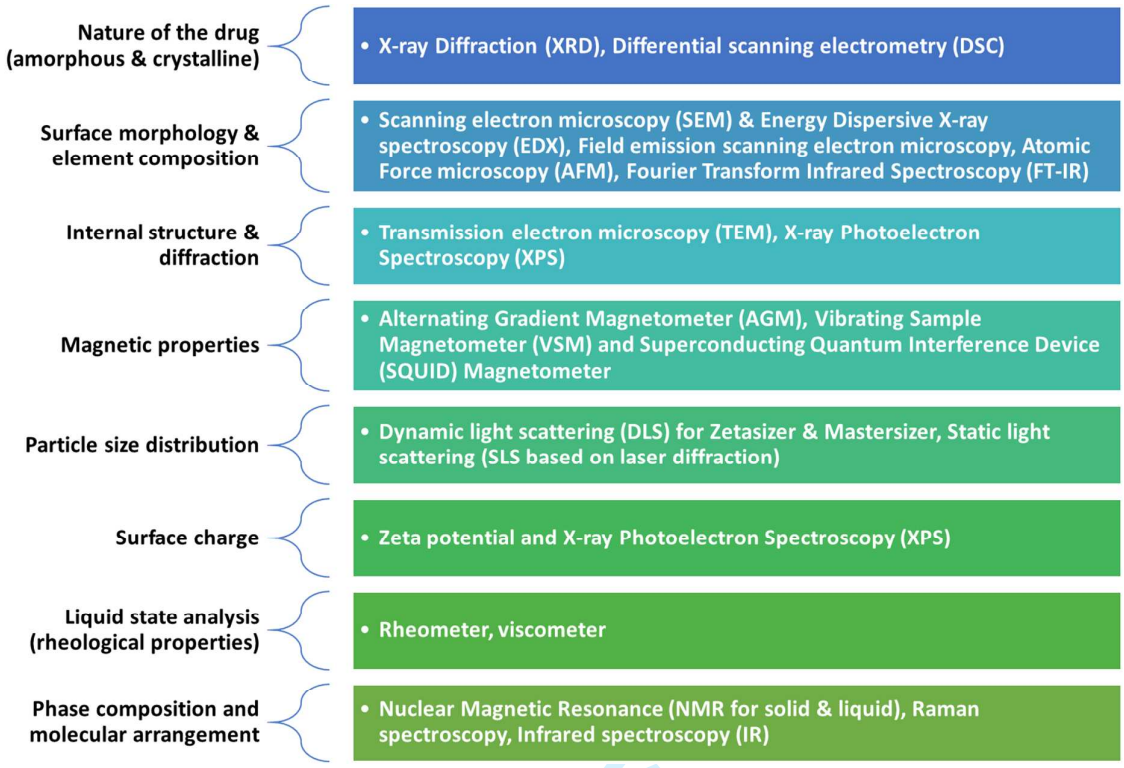

Figure 6: Useful analytical techniques to study various properties of nanoparticles

Amorphous vs Crystalline Nanoparticles in Nanomedicine

It is possible to synthesise various drug nanoparticles used in nanomedicine as either amorphous or crystalline forms. On one hand, there is a well-defined crystal structure with some crystal defects located on the surface and bulk of the crystalline nanoparticles. On the other hand, structural disorderedness is evident throughout the nanoparticles in amorphous state providing a more porous framework with high structural defects [46 - 48]. These structural differences arise due to different methods of nanoparticles preparation. Both bottom up and top down approaches can produce particles with varying crystallinity and other physio-chemical properties.

Most of the drug nanoparticles are prepared using top down approaches, among which high pressure homogenisation (HPH) is widely used. HPH process consists of 3 main steps [49 - 54]:

- Dispersion of powdered drug in a solution of water and stabiliser
- Comminution of coarse particles of drug under low pressure homogenisation to prevent blockage of homogenisation chamber
- High pressure homogenisation using repetitive cycles to attain the desired particle size and distribution

The number of cycles and pressure of homogenisation are the critical factors which control the particle size and distribution. For example, the polydispersity index and particle size of amoitone B nanocrystals is reported to gradually reduce with an increase in number of cycles and homogenisation pressure in a stepwise manner [52]. However, once the particles become highly uniform non-aggregated then a continuous increase in the pressure or number of cycles has little effect on particle size and polydispersity index [52].

HPH is also often combined with precipitation process to prepare nanocrystals. In this process, first the precipitation of drug is done in a liquid medium that produces comminuted drug crystals. Then high pressure homogenisation using repetitive cycles is applied to achieve the desired particle size [55, 56]. The main requirement of this process is that the drug should dissolve in at least one solvent and get precipitated using a miscible non-solvent. This poses challenges of potential toxicity from solvent media and its complete removal from the final drug preparation [57].

Wet ball milling technique uses a milling chamber to comminute a drug material that is dispersed in water and agitated along with surfactants or stabilisers. In this technique, mechanical shear and attrition is used due to collision of the drug particles with each other, milling media and walls of the milling chamber [58]. Small pearls, stainless steel, cross linked polystyrene, glass or ceramic beads of size 0.3 mm or higher are used as milling media. However, the final size of the drug nanoparticles depends on other factors such as viscosity of the dispersion media, concentration of the surfactant, temperature conditions, duration of milling process and properties of drug dispersed [59].

The bottom up process offer smaller particle sizes than most top down approaches but its production scale is also much smaller. The fundamental of this process is precipitation of drug nanocrystals in two steps procedure: nucleation followed by crystal growth [60]. Hence it is important to control the nucleation step since if the

number of nuclei formed is higher, it will limit the growth of every nuclei. The use of ultrasound in combination to precipitation process results in cavitation effects which can speed up the nucleation process and minimize agglomeration [60, 61].

Another bottom up process is called as evaporative precipitation in aqueous solution, where the drugs are dissolved in ethanol at 60°C. The solution is then added dropwise with constant stirring into water at 0°C. This temperature difference promotes supersaturation and nucleation which leads to a smaller size as compared to HPH technique. For example, the anticancer drug riccardin D was prepared as nanocrystals using evaporative precipitation and HPH technique with a size of 184 nm and 815 nm respectively [51]. This study shows that a smaller particle size can be prepared using evaporative precipitation as a bottom up process which provides a higher control on particle size.

Precipitation techniques of bottom up processes usually produces nanodrugs of lower crystallinity as various factors affect the polymorphic form of the drug nanoparticles, such as degree of supersaturation, type of solvent-antisolvent, etc. Solid state characterisation techniques such as XRD, NMR, DSC and FTIR can be used to study the crystallinity and other properties. However, the amorphous state of the nanoparticles is thermodynamically favourable during precipitation since it exhibits a metastable state with high energy that imparts quicker solubility of the drug. But, the property of the drug is also a significant factor to determine the resulting state of nanoparticles, as some drugs like Ibuprofen mostly precipitates in crystalline state, whereas Cefuroxime axetil precipitates as amorphous particles [62, 63].

The structural differences between the two (crystalline and amorphous) states of nanoparticles impart unique properties to the drugs which is exhibited either as advantages or disadvantages in terms of properties and specific applications. For instance, Table 2 critically studies both amorphous and crystalline drug nanoparticle along with their respective potential merits and demerits [46 - 48, 64 - 69].

Table 2. Comparison of amorphous and crystalline nanoparticles

Types of Drug Nanoparticles	Advantages	Disadvantages
Amorphous NPs	Disorderedness improves thermodynamic driving force	Wide size range (nm to μm)
	High water solubility	Abrupt surface areas
	Enhanced Dissolution rate	Low impurity of drug
	High oral absorption	Physical and chemical instability in GI tract
	High saturation solubility (Ex-Chloramphenicol palmitate)	High pharmacokinetic variability
	Ease of production	Low suitability for intravenous routes
Crystalline NPs	Low pharmacokinetic variability in patients	Lattice energy barrier for dissolution limits
	Narrow size range of crystals (< 1 μm)	Need stabilisation by surfactants
	Physical and Chemical Stability from enzymatic degradation	Aggregation due to high surface area and surface energy
	High Purity and drug loading (100%)	Requires extensive crystallisation steps
	Suitable for intravenous administration	Low oral absorption

Nanosuspensions for drug delivery

Nanosuspensions are powerful for drug delivery applications and several latest review articles have provided an in depth discussion on the various routes of administration. In brief, it is important to focus on key developments in this area by reviewing strategic articles and routes of administration. For instance, oral drug delivery is one of the most preferred routes of drug administration but is limited due to low oral absorption and bioavailability. Drug nanoparticles in suspension can tackle this problem by increasing the area of absorption under the curve as shown by naproxen nanoparticles with a 218% increase. Danazol – an inhibitor of gonadotropin when used in suspension showed an increased bioavailability of 82.3 as opposed to conventional dispersion of 5.2% [6]. In addition, lyophilised

nanosuspensions offer faster dissolution rate with an increased permeability, as shown in case of oleanolic acid [70]. A schematic diagram shown in Figure 7 highlights the enhanced permeability and retention (EPR) effect of drug nanocrystals, effective for tumour targeted drug delivery [71].

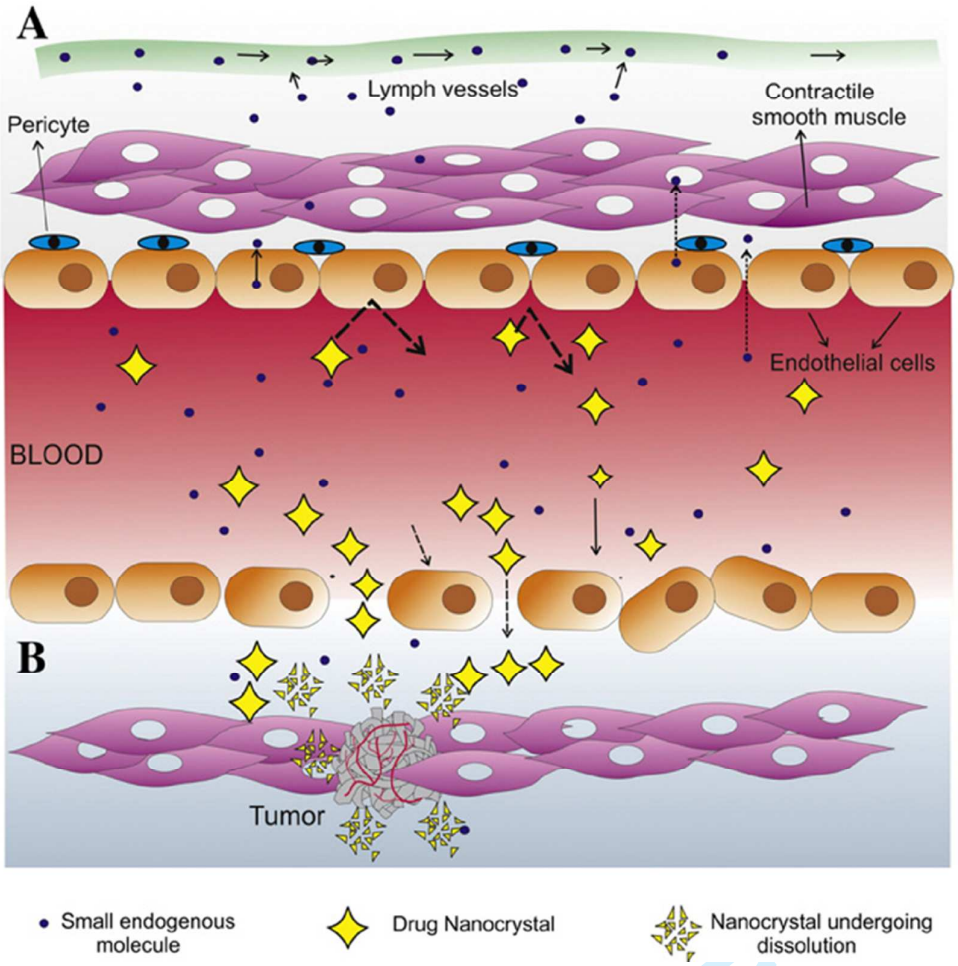

Figure 7: EPR effect; (A) Normal vessel: the narrow gap junctions present in between endothelial cells allow only small molecules to penetrate, screening out colloidal sized particles. Notice the ordered structure of cells in the presence of functional lymphatic drainage. Lymph flow regularly filters out accumulated material. (B) Tumour microenvironment: The vascular endothelium in and around tumour is disjointed, irregular and leaky allowing effective penetration of nanocrystals. Absent or dysfunctional lymphatic vessels further delay clearance of these particles leading to their enhanced accumulation at tumour site (Reproduced with permission from ref [71]).

Nanosuspensions are also compatible for parenteral routes of drug administration like intravenous, interperitoneal and intra-articular. One of the key requirements for drug molecules administered through parenteral route is to highly soluble or have a particle size of less than 5 μm to prevent blockage of capillaries. It has been reported that nanosuspensions have increased the efficiency of parenterally administered drugs like paclitaxel [72], aphidicolin [73], clofazimine [74] and busulfan [75].

Pulmonary drug delivery is dependent on the solubility of the drugs in pulmonary secretions. It is possible to nebulise the aqueous nanosuspensions using ultrasonic or mechanical nebulisers for delivery to lungs. This can lead to a uniform distribution of the drug nanoparticle in the aerosol droplet allowing for rapid dissolution and diffusion in the lungs. Furthermore, the adhesiveness of the drug to the mucosal membrane will be increased and therefore prolonging the drug's residence time at the site of absorption. One such example is seen from budesonide drug that was nebulised successfully using an ultrasonic nebuliser [6].

Other key applications include use of nanosuspensions for ocular drug delivery as shown by dexamethasone study for anti-inflammation activity in rabbit [76], targeted drug delivery as shown by aphidicolin study against Leishmania-infected macrophages [73], mucoadhesion of drug for reducing infection as shown by mucoadhesive Buparvaquone [6] and topical drug delivery [8].

In vitro stability of drug nanocrystals

Some of the areas of concern for *in vitro* stability are related to the pharmaceutical application of drug nanocrystals such as agglomeration, crystal growth and sedimentation. Drug nanocrystals can undergo amorphisation, especially upon application of nanosuspension methodologies such as top down and bottom up approaches [77]. Stabilisers are normally used to prevent the agglomeration of drug nanocrystals but it was found that drug-polymer interactions can substantially reduce the crystallinity. For instance, a study provided concrete evidence of Naproxen drug undergoing amorphisation due to the generation of amorphous layer on nanocrystals surface when subjected to media milling in the presence of hydroxypropyl methylcellulose [78].

Cell culture assays are generally the standardised protocol for evaluating the performance of drug nanocrystals. However some of the key limitations of these

assays are linked to their working conditions and parameters. For instance, a fundamental limitation of these assays is due to its finite volume and closed system features. This can greatly affect the therapeutic efficacy and dissolution rate of the drug depending on the administered dose, especially if it is above the drug's saturation solubility. This in turn affects the cytotoxicity based on the incubation time, since the low dissolution rate drugs naturally exhibit lower cytotoxicity effects [79]. A recent study has established these results by showing that longer incubation times such as 72 hours, for 240 nm Camptothecin's drug nanocrystals showed similar cytotoxicity results as that to the solution [80].

Furthermore, a recent study also established the fact that drug nanocrystals can aggregate within the cells to form rough edges with time. This can greatly affect the overall size and shape of the drug nanocrystals and therefore degrade the drug's pharmacokinetics and its release. For instance, in the study, antiretroviral drug Ritonavir was loaded as large drug nanocrystals of about 300 to 900 nm into the macrophages. It was found that 24 hour post uptake, 68% of the drug nanocrystals were intact due to aggregation and acted as rough edged reservoirs to sustainably release the drug over a period of 2 weeks and longer [81]. As an extension to this work, it was also found that surfactant coating, shape and type of drug nanocrystals were influential parameters which affected its release, uptake and antiretroviral activity. It was found in the study that drug nanocrystals with irregular and round edges showed low cell uptake, while rod shaped drug nanocrystals showing regular and smooth edges showed a rapid uptake and release from macrophages [82].

In vivo stability of drug nanocrystals

A wide range of factors affect the stability and performance of drug nanocrystals in *in vivo*, such as the dissolution rate, half-life, charge, shape, particle distribution profile and toxicity effects. Both biological and physicochemical parameters affect the biodistribution and pharmacokinetics of the drug nanocrystals in *in vivo*. For instance, a study highlighted the effect of agglomeration on the cell's uptake efficiency, showing that drug nanocrystals of size 50-100 nm were not internalised by the cells [83]. Furthermore, the un-dissolved drug nanocrystals injected intravenously remained in blood and interacted with plasma proteins that lead to surface opsonisation and formation of protein-drug agglomerates. This facilitates the recognition and clearance of the protein-drug agglomerates from the blood by the

tissue macrophages and circulating phagocytes that are directly in contact with blood [84].

Dissolution of the drug nanocrystals and its accumulation in liver and spleen are the key limitations which is inherent of the nanocrystals [85]. There are several studies which demonstrates the dissolution profile of low cytotoxic drug nanocrystals in *in vivo* models such as rats, dogs, rabbits, etc and also pinpoint their accumulation in tissues, especially the macrophages of spleen and Kupffer cells of the liver [86]. Figure 8 presents a recent study showed the *in vivo* application of 450 nm Veirapine nanocrystals in mice [87]. The study indicated an EPR effect in the liver of mice for the drug nanocrystals as compared to drug in solution. However, the size of drug nanocrystals significantly affects their dissolution and accumulation profiles. For instance, a study showed the use of nanocrystals of Oridonin drug with two sizes ranges; 100 nm and 900 nm. It was found that when these drug nanocrystals were IV injected in rabbits, the 100 nm nanocrystals showed complete dissolution within 10 minutes whereas the 900 nm nanocrystals underwent complete dissolution in 2 hours [88].

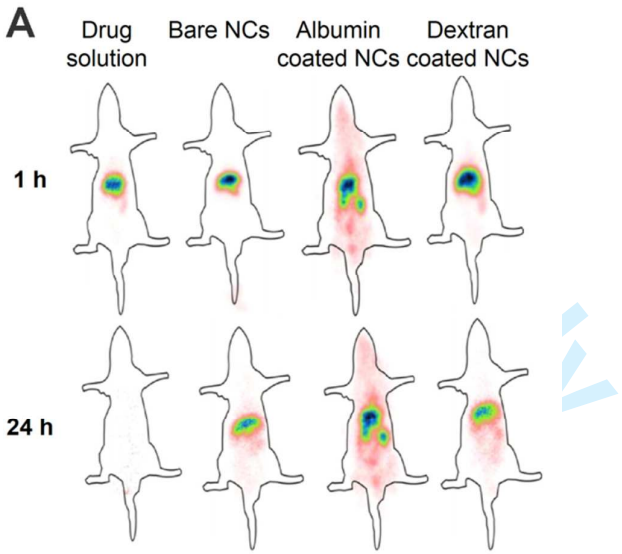

Figure 8: Particle-like behavior of NCs *in vivo*. (A) Gamma scintigrams depicting biodistribution of bare radiolabelled nevirapine 450-nm NCs and NCs surface-coated with albumin and dextran at 1 h and 24 h in rat compared to the drug in solution. (Reproduced with permission from reference [87])

Benefits of nanosuspensions in drug delivery

There are several benefits of nanosuspensions for use in drug delivery applications, such as [89 - 91]:

- improves solubility of drugs insoluble in organic media and water, as alternative to lipid systems
- helps to decrease the dosage requirements by increasing absorption and bioavailability of drugs
- helps in formulations of high melting point and high log P value drugs
- reduces systemic toxicity of drugs
- increased physical stability of drugs
- offers high resistance to oxidation and hydrolysis
- offers passive targeting

Conclusion

To this date, various types of nanoparticles are employed in the field of nanomedicine, for drug delivery, imaging, diagnostics and several other applications. Even though a large number of studies are dedicated towards the carrier molecules such as organic and inorganic nanoparticles, it is important to understand the physiochemical properties of the effective drug itself for both *in vivo* and *in vitro* studies. There are several different processes that are used for synthesising the drug nanoparticles in both crystalline and amorphous forms, but these processes are broadly classified as top down and bottom up approaches. It is therefore important to choose the right methods of nanosizing the drug to control the degree of crystallinity and properties like particle size, morphology and surface areas.

Nanosuspensions are an attractive solution for drug delivery, especially for oral and dermal routes of administration and are in turn favourable towards both amorphous and crystalline drug nanoparticles. It is highly anticipated that nanosuspension can dramatically improve the drug efficacy and bioavailability for all major drug classes, provided suitable solvents and stabilising agents are discovered. Therefore further studies should be directed towards finding a surfactant free route of drug delivery such as the food-derived or blood-derived proteins which poses no toxicity problems *in vivo*.

Future Perspective

Targeted nanodrugs have already showed higher performance as compared to conventional drugs through almost all routes of administration. However the number of nanomedicines commercialising every year is far less than the total articles published. There seems to be a gap that is created between the laboratory tests and actual clinical trials due to the various differences that arise during scale up process and clinical trial stage. Therefore, adequate characterisation should be carried out for the synthesised drug nanoparticles and performance should be evaluated based on specific parameters such as surface area, surface charge and dispensability in nanosuspension. Foremost, crystallisation methods should be standardised for certain drug classes and appropriate control should be established on the aspect ratio of the drug nanocrystals for potential drug delivery both via passive and active targeting routes.

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Executive summary

Fabrication of drug nanoparticles in suspension

- Drug particles in nanosuspension mitigate solubility and bioavailability issues of drugs.
- Two main approaches for the synthesis of nanosuspension such as top-down and bottom up.
- Crystalline and amorphous nanoparticle with variety of shapes, sizes and morphology can be designed
- A range of characterisation techniques can be used for tuning the properties of drug nanoparticles

Nanosuspensions in Drug Delivery

- Poor solubility of drug molecules triggered the development of drug nanoparticulates for drug delivery
- Nanosuspensions are highly desirable for oral route of drug administration
- It is also compatible for parenteral routes of drug administration like intravenous, interperitoneal and intra-articular.
- Nebulising of nanosuspensions aids the drugs to be used for pulmonary routes
- Drug nanocrystals have higher EPR (enhanced permeability and retention) effect
- *In vitro* studies of drug nanocrystals are limited by agglomeration, crystal growth and sedimentation
- *In vivo* studies of drug nanocrystals face challenges due to bio-distribution and dissolution barriers along with accumulation tendencies in liver and spleen.

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The author have used the following keys to highlight the articles which are of "interest" and of "considerable interest"

** = of interest, ** = of considerable interest*

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