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Nanocrystal Based Therapeutics: Scope and Potential Application in Health Sciences

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ABSTRACT

Nano-crystal (NC) based therapeutic agents are comprised of pure drug crystals with nano-size dimensions. The crystalline nano-crystals (size dimensions, 200 to 500 nm) are generally produced by nano-crystallization technique. NCs are fabricated from the pure core drug alone or with involvement of surfactants or polymeric stabilizing agent based excipients. Owing to their simple structure and compositions, NCs have contemplated an increasing interest in biomedical fields including medicine and pharmacy. They have also been widely exploited in semiconductor, optical and diagnostic field. The NC based formulations can be administered through various administration routes. Moreover, the size of NCs is a certification for their non-toxic tranquil passage through capillary network. In the present review, we have tried to highlight scope and application of drug based nano-crystals in health sciences.

INTRODUCTION

A dosage form, comprising of a poorly soluble core drug, encounters tremendous en-route predicament before reaching to the target site. Solubility, the extent of dissolution of the drug solute in a given solvent to accomplish a homogenous system; on one hand, and its desired concentration in systemic circulation on the other, is obligatory in achieving desirable pharmacological responses. In 1995, Amidon proposed the first bio-pharmaceutics classification system (BCS) [1]. In this system, drugs are divided into four classes, based on their solubility and membrane permeability: BCS Class I (high solubility, high permeability), BCS Class II (low solubility, high permeability), BCS Class III (high solubility, low permeability), and BCS Class IV (low solubility, low permeability). Insoluble drugs are placed in BCS Classes II and IV, with 70% of them in Class II.

At present, about 90% of all new drugs that are in the developmental process or have emerged as a potent pharmaceuticals on the basis of high throughput screening methods (i.e. identification of new chemical entities), exhibit poor solubility in aqueous medium [2]. In general, sparingly soluble drugs confront many biopharmaceutical/clinical issues when used in development of oral Drug Delivery System (DDS). On the same line, low bioavailability, high fasted/fed state variation, retarded onset of action, lack of dose proportionality, high inter-patient variation and local irritation etc; are some other concerns that may have correlation with solubility of

the drug [3]. The low bioavailability of insoluble drugs can be significantly improved by salinification, structural transformation, and other related pharmaceutical methods [4-6]. Some of the conventional approaches used in the solubilization of poorly soluble drugs include pH adjustment, emulsification (such as micro-emulsion, self-emulsifying DDS, or self-micro emulsifying DDS), complexation, micellar systems, surfactants/co-solvents, liposomisation and cyclodextrin mediated solubilization etc [7]. However, the above specified approached employed for solubilization of a drug candidate relies on usage of additives or excipients. Unfortunately, inclusion of excipients to develop a novel formulation, may pose altogether different sets of problems. A variety of smarter nonspecific approaches, employed in development of a given formulation, can be extended for almost any drug molecule. To date, the classical approach for increasing the dissolution rate of poorly soluble drugs is to reduce particle size, which is generally achieved through micronization [8]. It seems that further improvement in drug dissolution rate and also the bioavailability asks for a shift from micronization to nanotization. Thanks to tremendous advancement in process technologies and analytical methods developed in last decades, a considerable number of pharmaceutical nanocrystal products are now available in the market and several others are under development [9].

Among various novel drug delivery systems, nanoparticles, have emerged as a suitable drug vehicle in regulating pharmacokinetics, pharmacodynamics and eventually the bioactivity of the active core compound [10]. Nanotechnology can improve the solubility of insoluble drugs that in turn may ensue in increased bioavailability of the core drug. The nano-drug particles range from 1–1000 nm in size. Depending on the status, nano-drugs can be divided into two general categories: drug nanocrystals (nano-crystallized forms of the drugs) and drug nano-carriers (drug dispersed/nano-crystallized in a suitable carrier). Due to the quantum-size effect, the as-generated nano-particles acquire a large amount surface energy, making them thermodynamically unstable but kinetically steady. Early studies on nano-drugs focused mainly on nano-carrier preparations, such as liposomes, solid lipid nanoparticles, micelles, virosome and nano-capsules etc. Nanoparticles entail en-route shielding of the associated drug molecules and eventually facilitate their distribution to desirable therapeutic target. In spite of their widely acclaimed potential for sustained drug release and potential to accumulate at the desired site, nano-particles do come across with series of barriers that impede attainment of desirable therapeutic outcome.

Table 1: Properties of drug nanocrystals.

1. Particle size; < 1 µm
2. 100% Drug, No excipient
3. Generally needed to be stabilized by surface active agent
4. Crystalline or amorphous structure (Amorphous state offering advantages)
5. Increase in saturation solubility
6. Increase in dissolution velocity

The table is adapted/modified from [9].

WHAT ARE NANO-CRYSTALS?

There is in depth discussion about the definition of a nano-particle, which pertains to the specified size dimension of an entity to be classified as a nanoparticle. Depending on the specified discipline, e.g: in colloid chemistry, particles are classified as nanoparticles when they are in size below 100 nm or even below 20 nm. Based on the size unit, in the pharmaceutical arena, nanoparticles are specified to have a size range between a few nanometers and 1000 nm (=1 µm); while microparticles possess a size range of 1–1000 µm.

Drug nano-crystals are defined as particles with a nanometer size range exhibiting crystalline character. They boast of an additional advantage of entertaining cent per cent drug loading, since these are basically consisting of core drug only and do lack presence of any carrier molecule. Dispersion of drug nanocrystals in liquid media leads to generation of so called “nano-suspension” in contrast to “micro-suspension” or “macro-suspension”. Various widely employed dispersion media include water (for aqueous formulations), or polyethylene glycol, various oil based mediums etc. for non-aqueous formulations [9]. Occasionally, beside core drug, a NC formulation may contain one or more stabilizers dispersed in aqueous or non-aqueous media. The employed stabilizers can be classified as a safe excipient (surfactants, salts, sugars or buffers etc). Depending on the technology employed, processing of drug microcrystals to drug nanoparticles can lead to either crystalline or to an amorphous product, especially when precipitation method is used for synthesis of NCs. In the strictest sense, such an amorphous drug nanoparticle should not

be called nanocrystal. However, we indiscriminately refers "nanocrystals for the amorphous state".

Table 2: Examples of the available drug NC products. WBM: Wet Ball Milling, HPH: High pressure homogenization; (modified after [9].

Trade name	Active Pharmaceutical ingredients	Company	Effective against	Methods	Formulation
Rapamune	Rapamycin	Wyeth Pharmaceuticals	Immunosuppressant	WBM	Tablet
Emend®	Aprepitant	Merck	Anti-emetic	WBM	Capsule
Gris-Peg®	Griseofulvin	Novartis	Anti-fungal	Precipitation	Tablet
Tricor®	Fenofibrate	Abbott	Hypercholesterolemia	WBM	Tablet
Avinza®	Morphine sulphate	King Pharma	Anti-chronic pain	WBM	Capsule
Verelan PM	Verapamil HCl	Schwarz Pharma	Anti-arrhythmia	WBM	Capsule
Megace ES®	Megestrol acetate	Par Pharma	Contraceptive/ anti-carcinogenic	WBM	Suspension
Triglide®	Fenofibrate	Skye Pharma Inc.	Hypercholesterolemia	WBM, HPH	Tablet
Azopt®	Brinzolamide	Alcon	Glaucoma	WBM	Suspension
Ritalin LA®	Methyl-phenidate hydrochloride	Novartis	Anti-psychotic	WBM	Capsule
FocalinXR®	Dexmethyl-phenidate hydrochloride	Novartis	Anti-psychotic	WBM	Capsule
Herbesser®	Diltiazem hydrochloride	Mitsubishi Tanabe Pharma	Anti-angina	WBM	Tablet
Zanaflex™	*Tizanidine hydrochloride*	Acorda	Muscle relaxant	WBM	Capsule
Naprelan®	naproxen sodium	Wyeth	Anti-inflammation	WBM	Tablet
Theodur®	Theophylline	Mitsubishi Tanabe Pharma	Bronchial dilation	WBM	Tablet, capsule
Cesamet®	*Nabilone*	Lilly	Anti-emetic	Precipitation	Capsule
Paxeed®	Paclitaxel	Angiotech	Anti-inflammatory	-	Injectable micellar (Phase II trial)
Nucryst®	Silver	Nucryst Pharmaceuticals	Anti-bacterial	Reactive magnetron sputtering	Acticoat (anti-microbial barrier dressing)
Entresto	sacubitril/valsartan	Novartis	Chronic heart failure	---	Tablet
Invega Sustenna®	Paliperidone palmitate	Johnson & Johnson	Anti-depressant	WBM, HPH	Tablet

SALIENT FEATURES OF NANOCRYSTALS

The special features of drug nanocrystals (Figure 1) that demarcate them from other drug nanocarriers are as follows:

1. Drug NCs possess increasing adhesiveness to biological mucosa including gastrointestinal (GI) mucosa [11]. The increased adhesiveness can be ascribed to the increased contact area of NCs. The large surface area facilitates better oral absorption of poorly soluble core drug molecules. It also helps in greater saturation solubility and dissolution rate [7,12].

2. Depending on the process of preparation, NCs may induce transformation of crystalline structure, increase in amorphous fraction that may sometimes lead to synthesis of completely amorphous particles. Compared to equally sized drug NCs in the crystalline form, drugs in the amorphous state possess higher solubility and faster dissolution rate due to the higher inner energy [13,14].

3. Drug nano-crystal synthesis is not regulated by encapsulation of core material in shell or other capsular entities of the nanocarriers. Unlike nanocarriers, it facilitates a wide and adjustable dosage range, which generally relates to drug-loading capacity. Clinical requirement can thus be easily met. Even large doses (therapeutic dose > 500 mg) of the nano-preparations can be administered in the host.

4. Nanocrystals can be developed in many usable forms. Employing methods viz. spray drying, freeze drying and fluidized bed drying etc, nanocrystal suspension can be solidified and developed into solid dosage forms such as capsules, tablets, inject able lyophilized powder.

5. The particle size is an important parameter for nano-preparations; it is closely related to solubilizing effects and to bioavailability in orally administered drugs. The size of the nanoparticles can be accurately controlled. As the drug exists in nano-crystallized form, the measured size reflects the actual particle size.

6. The methods, employed for preparing nanocrystals, are versatile, simple to perform, and easily scaled-up for industrial applications and mass production. Conventional equipment, such as high-pressure homogenizers, high-pressure micro jets, and wet-grinding machines, can be used to make drug nanocrystals.

7. Nanocrystals can be administered by intravenous injection (nanosuspensions) and are able to efficiently reach the target tissue or organ with 100% bioavailability [15].

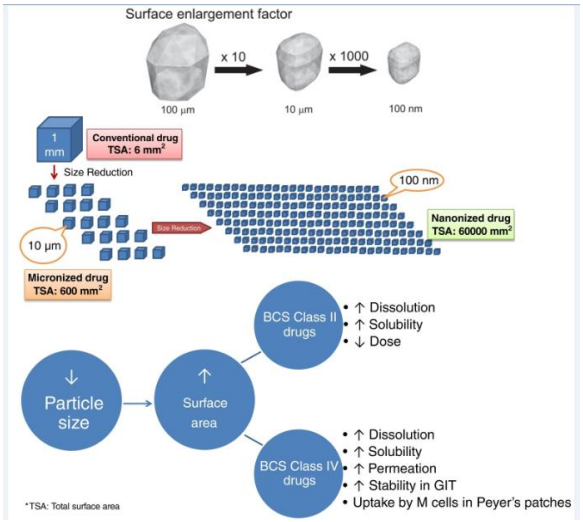

Figure 1: (A) Comparison of micro-sized and nano-sized drug crystal in size and distribution in droplets [80]; (B) Advantageous absorption behavior of drug nanocrystals beyond micronized drug [81].

STABILITY OF NANOCRYSTALS

The particle size diminution to the nanometer size range, in the as-synthesized NCs, contributes to an augmented particle surface area and curvature. This in turn contributes to enhance dissolution, saturation solubility, and increased bio-adhesiveness to surface or cell membranes. An increase in the particle surface area contributes to elevated surface interactions that may ensue in high tendency to aggregate. As colloidal system, NCs divulge thermodynamically unstable condition due to increased Gibbs free energy in the system. It results in the expansion of additional interfaces and eventually a tendency to reduce the total free energy [16]. Consequently, the stability of NCs based formulations is majorly affected by the particle aggregation. Still, the long-term stability is considered a special feature of drug nanocrystals [11].

Nanoparticles dispersed in a given medium have a tendency to aggregate. Kinetically, the aggregation process depends on activation energy of the system, which can be increased by including stabilizers to the system [16]. The appropriate stabilizer thus provides a barrier to aggregation (Figure 2).

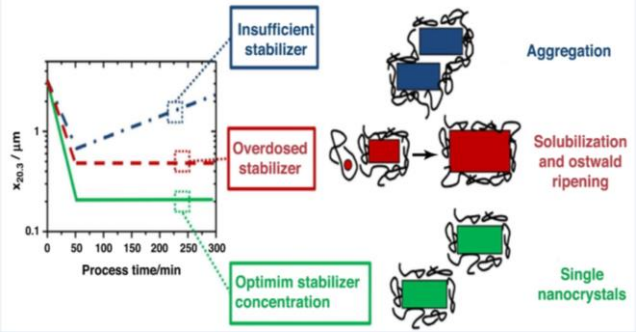

Figure 2: A suitable concentration of stabilizer should be present in the system to produce nanosuspensions with small particle size and to assure colloidal stability. Excess stabilizer should be avoided to prevent solubilization and increase of particle size due to Ostwald ripening. Adapted, with permission, from [82].

Theoretically, the principle of an energetic barrier can be explained by the DLVO theory, according to Russians Deryagin and Landau and the Dutch scientists Verwey and Overbeek. The theory basically describes the interaction of solid particles in a liquid medium in terms of attractive and repulsive interactions between the electric double layers surrounding the particles in solution [17,18]. The balance between the attractive and repulsive forces is adjusted by the stabilizers in the nano-crystalline systems. Hence, the stability of NCs mostly hinge on the stabilizer, its type and strength etc [19]. The NC suspension can show good physical stability by an absence of aggregation and Ostwald ripening phenomenon [20]. Physical stability might be attained by means of either of the multiple types of stabilizers such as the ionic surfactants, non-ionic surfactants, and amphiphilic copolymers, which can provide an electrostatic and steric repulsion between the nanocrystals, thus, preventing the aggregation of NCs. It was reported that a combination of electrostatic and steric stabilizer usually had a better effectiveness for stabilizing drug nanocrystals. The stabilizer actually diffuses quickly and shelters the surface of the crystals, thus, soothing the system by safeguarding an

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electrostatic and static barrier between the crystals. Various desirable characteristic ought to be present in an ideal stabilizer, include its adequate affinity for the drug particle surface, high diffusion velocity to encompass the surface and must be safe to the body and should be available in appropriate quantity for complete coverage of the particle surface. A good stabilizer delivers sufficient electronic or steric repulsion between the particles.

Ostwald ripening phenomenon [20] ensures that the solute concentration in the vicinity of smaller particles is higher than the large particles due to the higher saturation solubility of small particles. Therefore, driven by the concentration gradient, the molecules surrounding the small particles will diffuse towards the large particles. Subsequently, the re-crystallization on the surface of the larger particles occurs and leads to the formation of microparticles. In general, to rule out Ostwald ripening phenomenon, the narrow size distribution of drug nanocrystals should be taken into consideration. Besides the physical stability, drug nanocrystals can be used for the stabilization of a chemically labile drug. The increased stability of drug nanocrystals can be explained by a shield effect of surfactants and a monolayer of degraded drug molecules which acts as the surface of drug nanocrystals for protecting the drug underneath from degradation. Altogether, the selection of an accurate type and amount of stabilizer is thus, very crucial for the as-synthesized NC product quality.

In one of our pioneering studies, we recently reported that *Aloe vera* leaf extract, which has the potential to induce nanoparticle synthesis of various metals, can facilitate biomimetic synthesis of drug nanoparticles such as 5-FU [21] and Amphotericin B [22] as well. It seems that *Aloe vera* leaf extract mediated nanocrystal synthesis progresses via a self-nucleation process initially that eventually ensues in formation of primary particles [23]. The formed primary nuclei function as a seed to facilitate particle growth accompanied by an increase in the thermodynamic stability. In general, synthesis is facilitated by heterogeneous nucleation and growth; a process referred to as Ostwald ripening [20]. In this process, higher oligomers trap the monomers in their initial orientation and inhibit structural rearrangements. It can, therefore, be stipulated that the drug dimers/tetramers can act as seeding point (nucleus) for the condensation of monomers and eventually lead to the formation of supra-aggregated nanocrystals. The contents of Aloe vera function both as a driving force as well as stabilizing agent in the whole process [22]. It has been suggested that microenvironment of chromophoric groups of a chemical compound affects the Sπ life-span mainly by tuning the relative energy of the Sπ state and the closely lying Sn dark state. The stability of π-π* transition (in the case of 5-FU; it is the transfer of an electron from the lone pair of oxygen or nitrogen atom towards the more diffuse π* orbital) increases both with the polarity and hydrogen bonding ability of 5-FU with the external milieu. Further, it implies that with such microenvironment, the dynamics of Sπ is not influenced by Sn. Moreover, since the nano-particle framework is providing relatively less polar environment to the entangled drug molecules when compared to that of free form of the drug, it eventually results in broadening of the absorption spectrum [21].

NANO-CRYSTALS ASSOCIATED TOXICITY

There is a great deal of concern regarding toxicity issues associated with NCs. The NCs have potential to enter the cell and invoke damage to various cell components. In general, the nanoparticles (in size range of 100-1000 nm) can be taken up by quite limited number of cells with phagocytic activity. However, the nanoparticles with size below 100 nm can be taken up by various types of cells (via endocytosis) which lead to the high risk of toxicity. Additionally, the persistency of nanoparticles in the body after administration also affects the risk of toxicity. It should be considered that the nanoparticles can be degraded in the body or at least be eliminated; otherwise they are bio-persistent. The non-biodegradable nano-particles cannot be easily eliminated because they are too large for renal clearance. Normally, they stay within the cells, cannot exocytose, and remain as a waste. Therefore, the non-biodegradable nano-particles are not welcome in pharmaceutical products.

A Nanotoxicological Classification System (NCS) is applied to arrange the toxicity risk of nanoparticles [24]. The size and persistency related risks are combined to classify the NCS of nano-particles as follows. Class I is classified for the nanoparticles with size above 100 nm and biodegradable, Class II is for the nanoparticles with size above 100 nm and non-biodegradable, Class III is for the nanoparticles with size

below 100 nm and biodegradable, and Class IV is for the nanoparticles with size below 100 nm and non-biodegradable. Normally, the nanocrystals belong to the low risk class of nanoparticles, because their particle size can be made to be higher than 100 nm and they are also biodegradable (the dissolution occurs when the water is sufficient). The NCs are a priori low risk or non-risk nanoparticles, due to their particle size (generally > 100 nm) and biodegradable nature (dissolution occurs in sufficient water amount). Consequently, the toxic risk of NCs is limited. However, they can cause undesired systemic effects in the body. When the nanoparticles are taken up by the cells of the immune system, they can trigger an immune response and irritate the immune system. Hence, the development of nanocrystals requires the careful investigation to acieve the potential effects and less toxicity.

BIO-DISTRIBUTION OF NANO-CRYSTALS

NCs with the size range of about 100-1000 nm can only be taken up by a limited number of cells with phagocytic activity, e.g. the macrophages of the Mononuclear Phagocytic System (MPS), which is not easy to access [25]. In contrast, particles; with a size range below 100 nm; can be taken up by endocytosis. Taking into consideration the frame work of sequential barriers that a nanoparticle has to come across inside the host body, it is imperative to appreciate putative obstacles that may hinder site specific delivery of drug loaded nanoparticles. Overall, attributes such as size, shape, surface charge deformability and degradability of nano-particles regulate their bio distribution and plasma half-lives. Nanoparticles have been reported to preferentially accumulate at site of injury, infection and inflammation, mostly because of endothelial dysfunction and blood vessel fenestration at such sites [26]. In fact, surface properties of nanoparticles play important role in their sequestration that generally begins with opsonization that involves adsorption of plasma proteins including serum albumin, apo-lipoproteins, complement components and immunoglobulins etc. This eventually leads to avid uptake of opsonised nanoparticles by components of reticuloendothelial system as well as residential macrophages present in various vital organs.

As specified above, such development may play important role in the control of the disease, where host's immune system play important role. Various proteins, cytokines, and other biological factors present in plasma may categorically get adsorbed on the surface of nanoparticles forming 'Corona' [27,28]. The stability and composition of the corona depends on nanoparticle size and surface chemistry. Moreover, the corona is not a static entity; it exchanges over time to adapt its composition to the surrounding biological environment [29]. Some particles with a hard core comprising of a biological macromolecule interact strongly with the nanoparticle surface, characterized by high affinity and often also low off-rates. The hard corona may be surrounded by an outer layer of weakly associated biological macromolecules characterized by lower affinity and higher off rates. Other kinds of nanoparticles may have a "soft" corona only, i.e. most of the biological macromolecules will be in rapid exchange with the surrounding environment [30]. Regardless of the fact that whether the corona is "hard" or "soft", the biological macromolecules that surround a nanoparticle, is crucial to regulate its fate since this corona is what cells "see" and interact with [31]. Such adsorption, not only facilitate/inhibit interaction of doped nanoparticles with biological barrier but also decide their biological half-life. Among various biological barriers, components of the reticulo-endothelial system (RES) play important role in regulation of passive targeting. The above specified fate of administered nanoparticlesshould be considered as one aspect, where nanosized particles are avidly taken up by components of reticulo-endothelial system based biological barrier of the host (as pointed out by reviewer also). In fact, this has already been established for various lipid based nanoparticles [32,33].

It has been reported that the nano-particle bearing macrophages may act as 'Trojan horse' [34] that can carry the active drug to the inflicted site of the body. In general, residential and peripheral monocytes/macrophages have tendency to accumulate at site of infection/cancer. In fact, in doing so, macrophages with accumulated nanoparticles now act as secondary depot [35], or 'cellular drug reservoirs', gradually releasing the drug into the surrounding milieu [36], They may also provide an indirect boost for disease clearance as the drug can be passively targeted to the site of infection/cancer [37] as stated above and thus, can justify the superior efficacy of nano-particulate form of the drug over its free form. The grafting of specific ligands on the surface of

nanoparticles may further form the basis of the selective and specific targeting of the core drug to the active site.

It is well known that the nanocarriers accumulate preferentially at tumor or inflammatory sites, by virtue of the Enhanced Permeability and Retention (EPR) effect of the site specific vasculature [38,39]. The disturbed microvasculature may also cause localized acidosis [40]. Additionally, lymphatic vessels are either absent or ineffective causing inefficient drainage from the tumour tissue site leading to effective retention or accumulation of the nano drug carriers (EPR effect). In fact, the EPR effect/passive targeting provides selective localization thus prevents undue toxicity of the drugs [41] (Figure 3).

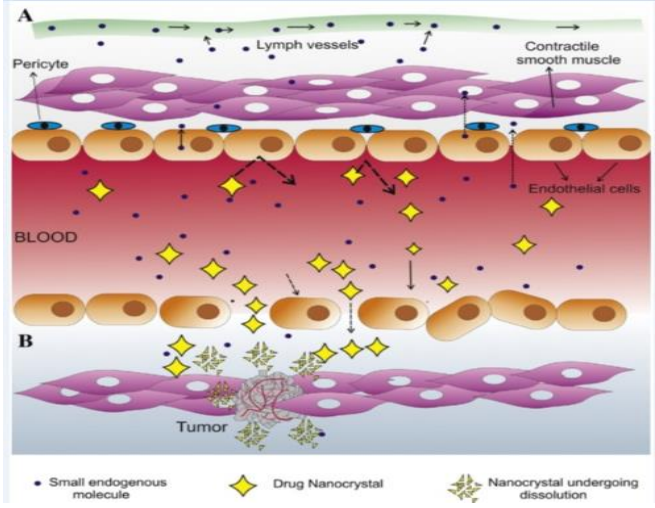

Figure 3: EPR effect; (A) Normal vessel: the narrow gap junctions present in between endothelial cells allow only small molecules to penetrate, screening out colloidal sized particles. Notice the ordered structure of cells in the presence of functional lymphatic drainage. Lymph flow regularly filters out accumulated material. (B) Tumor microenvironment: The vascular endothelium in and around tumor gets disjointed, irregular and leaky allowing effective penetration of nanocrystals. Absence of dysfunctional lymphatic vessels further delay clearance of these particles leading to their enhanced accumulation at tumor site (Reprinted with permission from [83].

Nanocrystals with their unique size attain an innovative bio-distribution profile which is not possible with simple micronization of the drug molecules. Zhang et. al., whilst investigating the in-vivo anti-tumor effect of camptothecin nanocrystals in MCF-7 xenografted Balb/C mice observed significant tumor suppression due to their five-fold higher tumor accumulation as compared to camptothecin solution. They attributed enhanced efficiency of nanocrystals to EPR effect [42]. Moreover, the resistance offered by bilayer plasma membrane will be more or less of same degree for free versus ensembled aggregated forms of the drug because they share common intrinsic properties. However, nanoparticles have advantage in the sense that single particle is harboring tens of thousands of molecules. The internalization of one ensembled particle will enable simultaneous release of several thousand parent molecules due to the change in its Critical Micelle Concentration (CMC) as the drug will leave the compact nanostructure in its monomeric (free) from; thus will be available for its action.

CO-CRYSTALS

Crystal engineering offers an alternative approach to enhance the drug solubility, dissolution and bioavailability of co-crystals. Te co-crystal is a crystalline solid with multiple components, usually two, present in a definite stoichiometric ratio in the crystal lattice. Non-covalent forces govern the interactions among cocrystal components. The cocrystals genesis make use of hydrogen bonds including the strong O••••H•••••O hydrogen bonds, van der Waals forces, halogen bonding and π-π interactions etc. [43]. Key hydrogen bonding types, 'synthons', involved in the synthesis of cocrystals are depicted in figure 4.

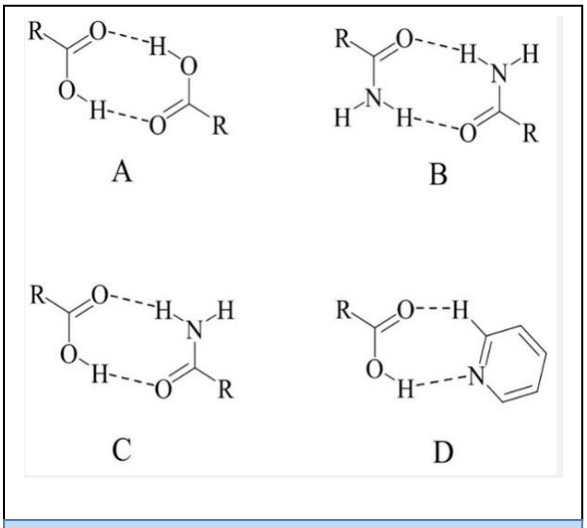

Figure 4: Various hydrogen bonding motifs involved in formation of co-crystals (A - carboxylic acid-carboxylic acid, B -amine-amine, C - carboxylic acid-amine, D – carboxylic acid-pyridine).

Among many different types of cocrystals, the pharmaceutically active cocrystals are consisting of Active Pharmaceutical Ingredient (API) and a coformer. The coformer should be safe for human consumption and should be classified under a GRAS ('Generally Regarded as Safe' declared by the US Food and Drug Administration) umbrella. A few examples of such pharmaceutical cocrystals are carbamazepine-saccharin, indomethacin-saccharin, itraconazole-L-malic acid and adefovirdipivoxil-dicarboxylic acids (suberic acid or succinic acid) [44-47]. Pharmacologically relevant physico-chemical properties such as dissolution rate, solubility, stability, and bioavailability of the active pharmaceutical ingredients may be enhanced via cocrystallization. For instance, enhanced solubility of carbamazepine was reported via its cocrystallization with numerous coformers including nicotinamide and saccharin. Further, increased dissolution rates and physical stability of theophilline were reported via its cocrystallization [48-50]. Also, carbamazepine upon its cocrystallization with saccharin showed enhanced dissolution, solubility, oral absorption and stability [51].

The key advantage of formulating the cocrystals is that without altering the pharmacological properties, the Active Pharmaceutical Ingredient (API) will benefit of their physicochemical properties enhancements because of the presence of coformer in crystal structure which is a property modifying component. The effect on the physicochemical properties of the API is dependent on the available coformer [52-55]. Another unique advantage of cocrystals over the more common salts is that cocrystals can be made for non-ionisable APIs as well as for those complex drugs which have sensitive functional groups that may not survive the harsh reaction conditions of strong acids or bases [56,57]. There are several other main advantages behind the formulating the cocrystals. Cocrystals have the potential to shorten the drug development timeline of APIs. Shortened development times equate to less cost, which is appealing to pharmaceutical companies. Solid state synthesis techniques of cocrystals can be classified as green chemistry as they offer high yield, no solvent use and there are few by-products. Pharmaceutical cocrystals are structurally different to their bulk forms; it is possible to patent cocrystals of existing APIs as a new crystal form. Pharmaceutical cocrystals can enhance the physicochemical properties of drugs such as melting point, tabletability, solubility, stability, bioavailability, permeability.

CRYSTALLIZATION APPROACHES

Drug NCs are commonly prepared in a liquid dispersion medium NPS. The nanocrystallization techniques are mainly bifurcated into bottom-up and top-down approaches. Although, combination of both the approaches also exists. The versatility of the procedure and the attainable particle sizes are the most significantfacets for the accomplishment of this technology.The top-down nanocrystallizationmethods are high energy processes where micronsized drug crystals are reduced to nano-dimension under mechanical grinding or high pressure, via wet media milling and High-Pressure Homogenization (HPH), respectively.

Milling Process

Nanomilling is the most utilized technique for production of drug nanocrystals, and most commercial products are produced by some kind of milling technique. A schematic presentation of the nanomilling process is presented in (Figure 2). The wide utilization of milling is based on considerably easy scale-up and good repeatability of the technique. In nanomilling of pharmaceuticals, size reduction is performed in the liquid suspension form. The coarse aqueous suspension of the drug and stabilizer(s) is fed into the mill together with hard beads as milling media. The mechanical attrition of drug particles with the milling media decreases particle size of the drug material. There are two basic milling principles: either the milling medium is moved by an agitator, or the complete container is moved in a complex movement leading consequently to a movement of the milling media. In the wet media milling method the drug particles are dispersed in a surfactant/stabilizer solution and the obtained micro suspension is then subjected to milling energy. The classical Nano Crystals® technology uses a bead or a pearl mill to achieve particle size diminution. The particle size is reduced by the shear forces generated by the movement of the milling media. The crystals are ground between the moving pearls, moved by an agitator, resulting in a NPS. The milling time depends on many factors such as the surfactant content, hardness of the drug, viscosity, temperature, energy input, size of the milling media. The milling time can last from about 30 minutes to hours or several days [58].

Generally, after wet media milling process crystalline structures have been reported [59].

High pressure homogenization

The second most vital technique to produce drug NCs is High Pressure Homogenization (HPH). First, the jet stream principle where high energy fluid streams of the suspension collide. Second, the piston-gap homogenization in water or third, the piston-gap homogenization in water-reduced/non-aqueous media in which a drug/surfactant microsuspension is forced with a high velocity by a piston under pressure, e.g. Microfluidizer, IDD-PTM (insoluble drug delivery microparticle technology), Dissocubes® technology (piston gap homogenization in water) and Nanopure® technology(water mixtures or in nonaqueous media) respectively. HPH techniques usually promote the formation of amorphous state, which may be prone for recrystallization.

The Microfluidizer technology can generate small particles by a frontal collision of two fluid streams under pressures up to 1700 bar [60]. This leads to particle collision, shear forces and also cavitation forces [61]. It can be achieved with jet stream homogenizers such as the microfluidizer (Microfluidizer®, Microfluidics Inc.). The collision chamber can be designed in two shapes, being either Y-type or Z-type. Surfactants are required to stabilize the desired particle size. Unfortunately, a relatively high number of cycles (50 to 100 passes) are necessary for a sufficient particle size reduction. SkyePharma Canada Inc. (formerly RTP Inc.) uses this principle for their Insoluble Drug Delivery – Particles (IDD-P™) technology to achieve the production of submicron particles of poorly soluble drugs.

In contrast, the Dissocubes® technology employs piston-gap homogenizers. The technology was developed by Müller and colleagues [62,63] and later acquired by SkyePharma PLC. It is the production of nanoparticle suspensions in water at room temperature. A drug powder is dispersed in an aqueous surfactant solution and subsequently forced by a piston through the tiny homogenization gap with pressures ranging up to 4000 bar, typically 1500 to 2000 bar (Figure 5). The width of the homogenization gap, depending on the viscosity of the suspension and the applied pressure, ranges from approximately. 5 to 20 µm. The resulting high streaming velocity of the suspension causes an increase in the dynamic pressure which is compensated by a reduction in the static pressure below the vapor pressure of the aqueous phase (according to Bernoulli's law [62]). Formation of gas bubbles occurs because the water starts boiling at room temperature. These gas bubbles collapse immediately when the liquid leaves the homogenization gap being again under normal air pressure of 1 bar.

Another approach using the piston-gap homogenizer is the Nanopure® technology, owned and developed by PharmaSol GmbH in Berlin. The technology uses dispersion media with a low vapor pressure and optionally homogenization at low temperatures. The cavitation in the homogenization gap is very little or nonexistent. Even without cavitation, the size diminution was sufficient [64]. The remaining shear forces, particle collisions and turbulences are sufficient to achieve nanoparticles. The optional low temperatures while homogenizing allow the processing of temperature labile drugs.

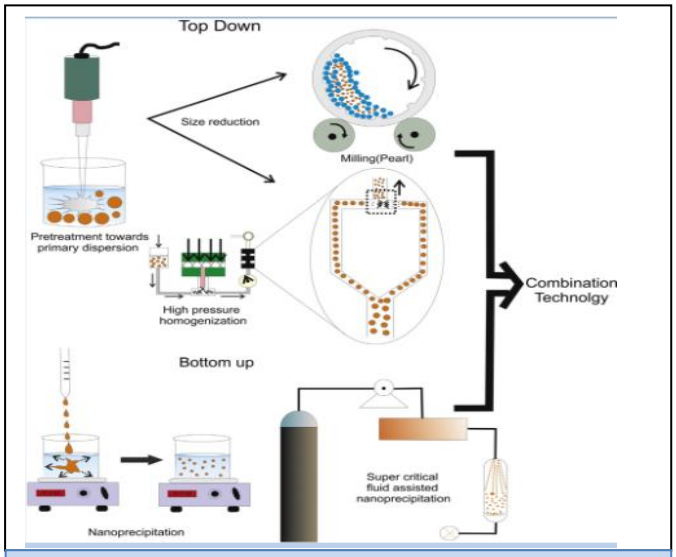

Figure 5: Manufacturing techniques employed towards fabrication of nanocrystals. Top-down methods involves primary drug dispersion and downsizing of drug particles by milling, cavitation, grinding, impaction, shearing, and attrition. Bottom-up techniques work on the principle of nanoprecipitation in which drug solution is introduced to a non-solvent system to initiate nucleation process followed by size growth. Stabilizers are often incorporated in formulation mixture to prevent size fluctuation. A combination of more than one conventional method can also be employed in fabrication of nanocrystals [83].

Precipitation Methods

The bottom-up approaches are generally based on the drug precipitation from a supersaturated solution of the drug. These precipitation approaches can be kept in four different groups: precipitation by liquid solvent-antisolvent addition, precipitation in the presence of supercritical fluid, by the removal of solvent and in the presence of high energy processes [19]. The precipitation based method has potential to produce amorphous material. However, the amorphous form may be transformed to crystalline state in the presence of water. Another precipitation method is the preparation of amorphous drug nanoparticles, for example, as carotene nanoparticles in the food industry ([65], Contribution of lymphatically transported testosterone undecanoate to the systemic exposure of testosterone after oral administration of two andriol formulations in conscious lymph duct-cannulated dogs [65], eg, Lucarotin® or Lucantin® (BASF). A solution of the carotenoid, together with a surfactant in a digestible oil, are mixed with an appropriate solvent at a specific temperature. To obtain the solution a protective colloid is added. This leads to an O/W two phase system. The carotenoid stabilized by the colloid localizes in the oily phase. After lyophilization X-ray analyzes shows that approximately 90% of the carotenoid is in an amorphous state. This technology is used for pharmaceuticals by Soliqs (Ludwigshafen, Germany) and advertised under the trade name NanoMorph®.

Supercritical Fluid (SCF) has been expected to have a high solubility against insoluble organic compounds, since it shows high solubility under high temperatures and high pressure. On the basis of this idea, the use of SCF has been examined for the purpose of improving there precipitation method. The new method is called Supercritical Fluid Crystallization (SCFC) method [66]. Applying the additional outsources energy like SCF to the reprecipitationmethod is quite useful for extending the preparation possibility of organic nanocrystals.

Combination strategies

Combination of the bottom-up and top-down approaches provides an efficient method to produce smaller particle sizes. Basically the combination methods consist of a pre-treatment step followed by a high energy top-down process, for e.g. Nanoedge™ technology (Baxter) [9]. Combination technology also overcome the deficiencies like congestionin equipment and relatively long process times. The company Baxter uses for its NanoEdge™ technology a precipitation step with subsequent annealing step by applying high energy, eg, high shear and/or thermal energy [66]. PharmaSol uses in its Nanopure XP technology a pre-treatment step with subsequent homogenization to produce particles well below 100 nm. Drug nanocrystals with a size of about 50 nm and below are distinctly smaller than the wavelength of the visible light, and so the nanosuspensions are translucent [9].

Preparation of co-crystals

As far as methods of preparation of cocrystals are concerned, solution methods and grinding methods are the most commonly used ones. Other less frequently used methods of cocrystal preparation include supercritical fluid involved methods, ultrasound assisted methods and hot stage microscopy used especially for screening of cocrystals [56]. The method or conditions used for the preparation of cocrystals must be carefully chosen depending on whether cocrystals of equimolar mixtures or nonequimolar mixtures are to be prepared. For yielding cocrystals of equimolar composition of the components, the solvent or the solvent mixture should enable equal solubility of the components to facilitate their congruent saturation. In contrast, for yielding cocrystals of nonequimolar composition of the components, the solvent or the solvent mixture should enable differential solubility of the components to facilitate their noncongruent saturation which will lead to cocrystal formation [56]. Solution cocrystallization is carried out to yield cocrystals of either equimolar composition or nonequimolar composition of the components. The solution crystallization methods discussed under this section include: evaporation cocrystallization, reaction crystallization and cooling crystallization. Evaporation cocrystalization is carried out to obtain cocrystals of the stoichiometric ratio of cocrystal components. Accordingly, equal solubility of the components in the solvent is an essential requirement for the successful formation of cocrystals. Basavoju and others prepared indomethacin-sachcharincocrystals using the solution crystallization method [56,68]. Reaction cocrystallization is the method of choice for the formation of cocrystals of components with nonequalsolubilities. In this method, one component is gradually added to a saturated or almost saturated solution of

the other component. Cocrystals may form as the solution becomes saturated with the gradually added component. Successful preparation of cocrystals using reaction crystallization was reported by Childs and coauthors who investigated 18 coformers for the preparation of carbamazepine cocrystals [69]. Cooling crystallization involves the heating of a solution of the components to a high temperature to allow their complete dissolution and subsequent cooling down of the solution to facilitate the precipitation of cocrystals. This method has gained much interest as a technique facilitating large scale formation of cocrystals [70]. Caffeine – p-hydroxybenzoic acid and carbamazepine-nicotinamide are two cocrystal systems successfully formed using cooling crystallization [71,72].

Grinding methods for the preparation of cocrystals have gained increased interest lately. The two grinding methods used are neat grinding and liquid-assisted grinding. Neat grinding or dry grinding, which is a solvent free method, is used for the preparation of cocrystals of components of stoichiometric ratio, and carried out using mortar and pestle or ball mill or vibratory mill. High solid state vapor pressure of one or both components of the cocrystals is an essential requirement for the formation of cocrystals via this method [73]. The other grinding method, solvent-assisted grinding or kneading or wet co-grinding or solvent drop, uses a minute amount of solvents to increase the kinetic energy of the active ingredient and the coformer. The two grinding methods – neat grinding and liquid-assisted grinding – are lower in cost and more environmentally friendly than solvent based methods due to the use of little or no solvents [56].

PRODUCTS IN THE MARKET

NCs have been developed for a wide variety of therapeutic applications including oral, dermal, pulmonary, systemic administration, as well as targeted drug delivery and intra-peritoneal chemotherapy. The availability of large scale production approaches at effectively low cost and coherently meeting the regulatory necessities are the essential prerequisite for introduction of NC based therapeutic to the pharmaceutical market. There are various existing commercial NC products available in the market. The first marketed product introduced in 2000 by Wyeth Pharmaceuticals (Madison, NJ) was Rapamune, an immunosuppressant that contains sirolimus (rapamycin) derived from an actinomycetes, Streptomyces hygroscopicus. It was manufactured utilizing wet ball milling top-down nanocrystallization method. Rapamune is available in oral suspensions as well as in tablet formulations; overcoming the unpalatable taste and restricted cold storage conditions of the earlier formulation. The bioavailability of NCs tablet is 21% higher compared to the oral solution. Emend® (Merck) is the second NC based marketed product launched in 2001. It was developed using nanosuspension of a precipitant, having anti-emetic property and is a high-affinity antagonist of human substance P/neurokinin 1 receptors. It was formulated as a spray coated solid capsule dosage form which exhibited enhanced bioavailability due to reduced fast and fed state variations. Avinza® (King Pharma) is morphine sulphate extended release capsule taken orally to treat chronic to severe pain, developed into NCs utilizing wet ball milling top-down nanocrystallization method. TriCor® and Triglide® are tablet formulations of fenofibrate designed to improve the bioavailability and to overcome the fast and fed state dependent absorption variations associated with other formulations of this drug. Megace ES® is a liquid dispersion dosage form designed to improve dissolution, and bioavailability of megestrol acetate and thereby provides reduced dosing volume compared to other dosage forms of the drug. InvegaSustenna® was developed as a once monthly extended release sterile injectable liquid dispersion dosage form of paliperidone palmitate (intramuscular suspension) available in prefilled syringes and it stands unique for being available at variable dose strengths with a two year shelf life period.

Other product synthesized using wet ball milling are Verelan PM® (Schwarz Pharma, Anti-arrhythmia), Azopt® (Alcon, Glaucoma), FocalinXR®/Ritalin LA® (Novartis, anti-psychotic), Herbesser® (Mitsubishi Tanabe Pharma, anti-angina), Zanaflex™ (Acorda, muscle relaxant), Naprelan® (Wyeth, anti-inflammation) andTheodur® (Mitsubishi Tanabe Pharma, bronchial dilation). Cesamet®/Lilly is an anti-emetic formulation prepared by precipitation method. At clinical phases II-III, NCs based products in the queue are Paxeed®/Angiotech (paclitaxel, anti-inflammatory), Semapimod®/Cytokine Pharmasciences (guanylhydrazone, TNF-α inhibitor), Theralux®/Celmed (thymectacin, anti-

cancer),Semapimod® (guanylhydrazone), and Nucryst®/Nucryst Pharmaceuticals (silver, anti-bacterial) [9]. Each of the commercialized NC products represents a rational formulation design.

Different formulations of pharmaceutical cocrystals are available in the market such as Viagra (Pfizer) to treat erectile dysfunction and pulmonary arterial hypertension, Entresto (Novartis) for treatment of chronic heart failure and some others under clinical developments [52,53,56,57,74].Curcumin, being a natural product with numerous important bioactivities, is much researched as an active ingredient in cocrystal formation with a variety of coformers. Yet, only a handful of curcumin cocrystals have been formed to-date while most attempts have led to the formation of other solid forms such as eutectics and solid dispersions possessing a number of properties significant especially pharmaceutically [75]. Curcumin-resorcinol and curcumin-pyrogallol are two examples of co-crystals synthesized via altering the reactivity of the keto-enol group of curcumin. Curcumin: resorcinol – 1:1 (mol/mol) cocrystal was obtained via the solution crystallization method by Sanphui and coworkers. They showed, using single crystal X-ray diffraction, that two resorcinol molecules form O•••••H•••••O hydrogen bonds with the keto-enol group of curcumin, in the crystal habit of the cocrystal. Further, they observed an auxiliary role played by π-interactions between resorcinol and curcumin in stabilizing the structure. Curcumin: pyrogallol – 1:1 (mol/mol) cocrystal, also, consisted of hydrogen bonding between the keto-enol group of curcumin and pyrogallol. Interestingly, each pyrogallol molecule formed hydrogen bonds with the phenol hydroxyl groups of two other curcumin molecules exhibiting trimer synthons. Sanphui and coauthors utilized liquid-assisted grinding, a method involving low solvent amounts, to prepare the cocrystals in bulk. The intrinsic dissolution rate and apparent solubility of the cocrystals were much higher than those of curcumin, and the curcumin-pyrogallolcocrystal exhibited highest values. Apart from the fact that curcumin-resorcinol and curcumin-pyrogallolcocrystals show promise in enhancing the bioavailability of curcumin, the fact that both resorcinol and pyrogallol possess bioactivities may further buttress their use as pharmaceutical cocrystals [76]. Other pharmaceutical cocrystals of curcumin include curcumin-4,4′-bipyridine-N,N′-dioxide [77], curcumin-salicylic acid, curcumin- hydroxyquinol [78] and curcumin-dextrose [79]. Curcumin-salicylic acid and curcumin-hydroxyquinolcocrystals exhibited higher dissolution rates and curcumin-dextrose cocrystal showed higher solubility than curcumin, that may enhance the bioavailability of curcumin, the active pharmaceutical ingredient under consideration.

CONCLUSION

Nanocrystals as well as cocrystals have been widely used to achieve pharmacological objectives similar to those for which polymeric nano-particles are employed. New technologies are in expansion to develop a dosage form with higher drug loadings, better re-dissolution at the site of action that eventually ensues in improved drug targeting. Another benefit associated with drug nanocrystals based formulations, is their potential to provide smaller dose administration to achieve moderate blood plasma level and thus lower down the side effects that may arise from larger dosage employed in conventional formulations. Furthermore, drug nanocrystals can be administered to the host body via various routes such as oral, parenteral, ocular, pulmonary and dermal delivery etc. The particle size and persistency of nanocrystals are important parameters that determine the interaction between nanocrystals and the cells. and their risk of toxicity. Additionally, the nanoparticles can lead to an irritation of the immune systems. Therefore, the nanotoxicity should be concerned when the nanocrystals are prepared. In the future, the development of stealth nano-crystals and active targeting nanocrystals modified with functionalized surface will be the next important part of work for drug nanocrystals.

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