Review Article

Engineered Nanocrystals for Poorly Soluble Drug Delivery: A Review

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Biopharmaceutical classification system class II drugs contribute to 60 %-70 % of the drugs with low solubility and high permeability, which are problematic for the formulation of dosage forms. Now-a-days, attempts have been made very extensively to overcome the problems related to the solubility of drugs in order to maximize drug bioavailability at targeted sites in the body. The oral route for pharmaceutical delivery is the most widely used and accepted of all drug delivery routes. In spite of having better therapeutic effects, many biological entities are rarely used for oral drug delivery due to the problems of poor solubility, poor permeability and poor stability in the gastrointestinal environment, as well as poor oral bioavailability. In this context, nanonization of such drugs has emerged as an important tool. The industry and academics are investing a lot of effort and money in creating the nanocrystal products because drug nanocrystals are unique drug delivery platforms that play a substantial and distinctive role in drug delivery. In the present review, we discuss the advantages and limitations of nanocrystallized drugs, stabilization techniques, special properties of nanocrystals, methods of preparation of nanocrystals, mechanism of action of nanocrystals in the human body, *in vivo* fate of nanocrystals, the products offered on the market using nanocrystal-based formulations which are being developed by the various pharmaceutical companies for drug delivery, several routes of applications of the nanocrystallized drugs, role of nanocrystals in targeted drug delivery, hybrid nanocrystals for *in vivo* imaging, future prospectives which may be useful to conduct further nanocrystal-based scale-up research projects.

Key words: Poorly water soluble drugs, nanonization, nanocrystals, drug delivery

Poorly soluble drugs possess limited solubility and dissolution velocity, due to which they display many biopharmaceutical issues in oral drug delivery such as low bioavailability, delayed onset of action, high fasted or fed state variation, a lack of dose proportionality, high inter-patient variation, and local irritation[1]. These poorly soluble drugs have been sub classified into two types of molecules *viz*., grease ball and brick dust compounds[2]. Grease ball molecules are extremely lipophilic compounds (log p>4, melting temperatures <200°) with low solubility due to the solvation process because they are unable to establish bonds with water molecules. Whereas, brick dust molecules show lower log p<2 values and melting points >200°, whose water solubility is constrained due to the tight intermolecular bonds within the crystal structure.

The rapidly developing discipline of nanoscience and more specifically, nanocrystal technology can enhance the bioperformance of poorly soluble medicinal molecules[3,4]. The particle size diminution of the drug to the nanometer scale contributes to an increased particle surface area and curvature and thus enhanced saturation solubility, dissolution velocity, increased adhesiveness to surface/cell membranes and further acceptable bioavailability upon oral administration[5]. Hence, nanocrystals have exceptional qualities that allow them to solve the solubility issues with poorly soluble pharmaceuticals. Enhancing bioavailability, a high drug load, a low incidence of side effects from excipients, a rapid onset of action,

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flexibility in the choice of administration routes (including oral, parenteral, ocular, pulmonary and dermal), no fasted/fed state variation and an overall improvement in effectiveness and safety are all the features of nanocrystal formulations.

Presently, drug nanocrystals are receiving a lot of attention at the moment as a viable strategy since the number of medications that are poorly soluble is rising in the drug development process, their pharmacoeconomic value, straightforward production and safe composition, which consists of 100 % drug. The pharma companies can benefit from the nanocrystal approach due to the possibility of product line extension offered by the regulatory authorities (Food and Drug Administration (FDA)) for already existing drug formulations[6,7]. In recent times, nanotechnology has been encountered all around our daily lives, where nanonized agents can provide a whole range of benefits, may it be in the computer field, biotechnological field, cosmetic industry, food industry, nutraceutical field and medical applications, thus helping to achieve progress all around us.

Drug nanocrystals are crystals with a size in the nanometer range, which means they are nanoparticles with a crystalline character. There are debates over the definition of a nanoparticle, which stipulates that depending on the discipline, a particle's size must be below 100 nm or even below 20 nm. For example, in colloid chemistry, particles are only considered nanoparticles when their size is below 100 nm or even below 20 nm. According to the size unit, nanoparticles in the pharmaceutical industry should be categorized as being between 1 nm and 1000 nm in size. One distinguishing feature is that, unlike polymeric nanoparticles drug nanocrystals are made entirely of the drug and there is no carrier substance[8]. Different actives for solubility chart based on Biopharmaceutical Classification System (BCS) of drugs[9] are given in Table 1.

DIFFERENT TYPES OF NANOPARTICLES

Polymeric nanoparticles:

Biodegradable nanoparticles, as effective drug delivery system are being applied extensively over the past few decades. Nanoparticles formulated from various natural and synthetic polymers have gained importance. This drug delivery system provides targeted drug delivery, increased bio-availability and sustained release of drugs and protects drugs from enzymatic degradation[10,11].

Fullerenes:

A fullerene is a molecule made up of carbon in different shapes such as tubes, hollow sphere, and ellipsoid. Fullerene is similar to Graphite in structure.

Nanotubes (NTs):

NTs are cylindrical fullerenes. NTs have a closed end as well as open end. Fullerenes show various therapeutic properties such as targeting cancerous cells, binding specific antibiotic to the specific structure of bacteria etc.

TABLE 1: DIFFERENT ACTIVES FOR SOLUBILITY CHART BASED ON BCS CLASSIFICATION OF DRUGS[9]

BCS class	Solubility	Permeability	Examples of drugs
I	High	High	Amlodipine, bisoprolol, cetirizine, citalopram/escitalopram, donepezil, doxazosin, enalapril, loratadine, mirtazapine, ondansetron, pravastatin, quinapril, ramipril, sertraline, sildenafil, terbinafin, tramadol, venlafaxine, zolpidem
II	Low	High	Aceclofenac, carbamazepine, carvedilol, clopidogrel, ebastine, ibuprofen, irbesartan, lamotrigine, lorazepam, lovastatin, mycophenolate mofetil, quetiapine, risperidone, simvastatin
III	High	Low	Alendronic acid, anastrazole, cefaclor, codeine, fluconazole, gabapentin, isoniazid, lamivudine, letrozole, levetiracetam, levofloxacin, lisinopril, losartan, pyrazinamide, ranitidine, risedronic acid, terazosin, topiramate
IV	Low	Low	Acetaminophen, amoxicillin, cefixime, cefuroxime auxetil, famotidine, hydrochlorothiazide, oxcarbazepine

Solid Lipid Nanoparticles (SLNs):

SLNs are lipids in nature which remain in solid phase at normal room temperature. SLNs are composed of solid hydrophobic core and a single coating layer of phospholipids. SLNs are stabilized by different surfactants for emulsification and also show many properties such as increased bio-degradability, increased bio-availability and drug targeting in the brain. SLNs have vast applications in cancer. SLNs have ability to accumulate tumor and also allow anticancer drugs delivery to the brain.

Super paramagnetic nanoparticles:

These are attracted towards a specific magnetic field. When the magnetic field is removed, these cannot retain their residual magnetism. Particles range in the size of 5-100 nm and used for selective magnetic bio-separations and can be visualized in Magnetic Resonance Imaging (MRI). These work on the principle of magnetic field and heated to trigger the drug release. These have also shown major role in cancer therapy and diagnosis.

Nanostructure Lipid Carriers (NLC):

NLC are prepared by using blend of solid lipids and liquid lipids. The particles remain in solid state at normal room temperature. NLC and the Lipid Drug Conjugate (LDC) nanoparticles are prepared in the form of matrices. These matrices increase drug loading capacity and bio-availability. These also have applications in the fields of cosmetics, food, agricultural and used in the delivery of anti-inflammatory drugs.

Nanoshells:

Nanoshells also known as core-shells are spherical cores of concentric particles which are surrounded by an outer coating of thin layer of another material. Nanoshells have biomedical imaging and therapeutic applications.

Gold nanorods:

Gold nanorods were first prepared in mid-1990. These exhibit distinct optical and electronic properties and depend on shape, size and aspect ratios. These can be easily stabilized and conjugated to antibodies.

Quantum Dots (QD):

QD are known as semiconductor nano-crystals and core-shell. These are 2-10 nm in size. These are used as drug delivery system for various hydrophilic drugs such as small interfering RNA and anti-sense oligodeoxy-nucleotide as well as targeting antibodies, peptides etc. QD have extensive applications in imaging contrast.

Nanofibers:

Nanofibers are produced by electro spinning technique in which fabrication of polymers in a fine and dense mesh works directly from solution and requires an electric field. These have dimension less than 100 nm as mentioned. Polymeric nanofibers are effective carriers for drug delivery and show advantages such as specific surface with small pore size, porosities, reduced toxicity and increased therapeutic level and bio-compatibility.

Ceramic nanoparticles:

Ceramic nanoparticles are porous in nature and particle size is less than 50 nm. These possess distinct properties such as sol-gel process, work in ambient temperature condition and product produced of desired size, shape and porosity as well as effective in hiding the uptake by reticulo-endothelial system.

Nanoerythrosomes:

Nanoerythrosomes are derived from a red blood cell membrane by the process of haemo-dialysis through filter. Nanovesicles are defined pore size and composed of proteins, phospholipids and cholesterol. These can load a variety of biologically active agents such proteins. Nanoerthroysomes composed of a natural membrane allows the insertion of recombinant ligands along with better stability.

Reasons describing why nanocrystals are better than microcrystals:

Enhanced saturation solubility and dissolution velocity than microcrystals, leading to increased bioavailability; high adhesiveness especially in comparison to microcrystals, also serves as a crucial component for enhanced absorption of poorly soluble drugs; high stability due to the lack of aggregation and Ostwald ripening (crystal growth), especially when compared to microsuspensions.

Improved biological performance of the nanocrystal drug in any delivery forms irrespective of the route of administration[12].

Advantages of nanocrystals[13]:

Nanocrystals offer a multitude of advantages, including applicability to all routes of administration in any dosage form, enhanced saturation solubility, increased absorption rate leading to improved bioavailability, rapid, simple and cost-effective formulation development, reduced required drug dose, minimized fed/fasted variability and intersubject variability, high drug loading capacity, enhanced reliability, improved biological performance of drugs, high stability, rapid onset of therapeutic effect and high adhesiveness.

Limitations:

They can be applied only to BCS II class drugs. Drug nanocrystal manufacture requires expensive equipment, which drives up the price of dosage form manufacturing. The nanocrystals formation and their stability depend on the molecular structure of the drug, so only certain classes of compounds will qualify.

Properties of nanocrystals[8]:

The following factors are the primary causes of the elevated dissolution velocity and subsequent elevation in bioavailability:

Increase of dissolution velocity by surface area enlargement: An increase in surface area results from the size reduction, as given by the Noyes-Whitney equation (1897), an increased dissolution velocity.

$dc/dt=DA(C_s-C_b)/h$

Where, dc/dt=dissolution velocity; D is diffusion coefficient; A is surface area; C_s is saturation solubility and C_b is bulk concentration and h is diffusional distance over which the concentration gradient occurs. A low dissolution velocity almost always coincides with low saturation solubility.

Increase in saturation solubility: The saturation solubility C_s is a foundation on the compound, the dissolution medium and also the temperature. Nonetheless, C_s is also a function of the particle size below a crucial size of 1-2 μm. It increases as the particle size decreases below 1000 nm. As a result, nanocrystals of pharmaceuticals have higher saturation solubility. Ostwald's Freundlich equation directly describes the relationship between the saturation solubility of the drug and the particle size.

$\log C_s/C_x=2\sigma V/2.303\ RT\ pr$

Where, C_s is saturation solubility; C_x is solubility of the solid consisting of large particles; σ is interfacial tension of substance; V is molar volume of the particle material; R is gas constant; T is absolute temperature and r is radius of particle

An increased adhesiveness to surface/cell membranes: In most cases, the higher adhesiveness of nanomaterials can be attributed to an increased contact area of small particles as compared to large particles (at identical total particle mass).

Stabilization of drug nanocrystals: Nanocrystals of drug molecules are nothing but nanosized, solid drug particles encompassed by a stabilizer layer. Producing nanocrystals is often a very simple process; however, maintaining their stability and choosing the appropriate stabilizers are typically the most difficult and time-consuming aspects of the process. The selection of the stabilizer is made only on the basis of the necessity of physical stability; for example, keeping the particle size at a nanometer level for as long as feasible following the fabrication of drug nanocrystals[14].

The enormous surface area of drug nanocrystals causes them to have a sufficiently high surface charge or free energy, either of which may result in the particles being attracted to one another or being agglomerated. It is possible for nanocrystals of a small size to boost the solubility of drugs beyond the saturation threshold, which then encourages the recrystallization of the drug into larger particles, also called as Ostwald ripening. At the end, these procedures will result in an integrity breach of the formulation that will not be recoverable[14,15].

CLASSIFICATION OF STABILIZERS[13]

Synthetic stabilizers:

Linear stabilizers include Polyvinyl Pyrrolidone (PVP), Polyvinyl Alcohol (PVA) and co-polymeric stabilizers includes PVA-Polyethylene Glycol (PEG) graft co-polymers.

Semi synthetic stabilizers:

Ionic stabilizers include sodium Carboxymethyl Cellulose (NaCMC), sodium alginate, chitosan, PEGs and non-ionic stabilizers include Hydroxypropyl Methylcellulose (HPMC), hydroxypropyl cellulose, hydroxyethyl cellulose.

Surfactants stabilizers:

Ionic stabilizers include docusate sodium, Sodium Lauryl Sulfate (SLS), polyethylene imine, non-ionic stabilizers include tweens, poloxamers, D-α-Tocopheryl Polyethylene Glycol Succinate (TPGS), block polymer of Polyethylene Oxide (PEO)-Polypropylene Oxide (PPO)-PEO.

Others:

Food-proteins, amino acids, co-polymers are other polymers. The mechanism of stabilization of nanocrystals and the stabilizing agents used is given in Table 2. Characterization parameters for nanocrystals are given in Table 3.

PRODUCTION TECHNOLOGIES FOR NANOCRYSTALS

Nanocrystals are made up of the drug itself with nominal utilization of an excipient, i.e., a surface stabilizing agent. The reduced overall cost of production can be attributed to simplistic manufacturing technology and low ingredient requirements that make manufacturing easy to scale up. Conventional methods, including heat/steam/radiation sterilization can be easily used for the sterilization of nanocrystals[16].

The two basic approaches for the preparation of nanocrystals are bottom-up and top-down technologies and a sequence of these two technologies is now-a-days most widely used, few of which are also patented, often sufficient to induce size reduction.

Bottom-up technologies:

They are also termed as nanoprecipitation. This method is formerly used in the preparation of drug nanoparticles in the decade of the 1980. The basic rule is that a solvent is used to dissolve the drug, the solution is then added to a non-solvent or anti-solvent and as a consequence, the dissolved drug precipitates. By controlling the structure of the particles (amorphous *vs.* crystalline), the crystals growth into the μm size range can be avoided[16]. However, most of the modern therapeutic entities are not easily soluble in common organic solvents, which themselves are toxic and difficult to completely remove, which limits the applicability of the method[17]. The development of new technologies throughout the course of time has made it possible to create nanocrystals through the application of a number of different supercritical fluid technologies, which involve Gas Anti-Solvent (GAS) recrystallization, an Aerosol Solvent Extraction System (ASES)[18], Atomized Rapid Injection for Solvent Extraction (ARISE), Rapid Expansion of Supercritical Solution (RESS)[19] and Depressurization of an Expanded Liquid Organic Solution (DELOS)[20]. The most commonly utilized supercritical fluid for pharmaceutical applications is CO_2.

TABLE 2: MECHANISM OF STABILIZATION OF NANOCRYSTALS AND THE STABILIZING AGENTS USED[13]

Mechanism of stabilization	Stabilizing agent	Techniques
Electrostatic methods	SLS	Nanoprecipitation and bead milling
Steric methods	HPMC,PVA K15, poloxamer 188, poloxamer 407	Antisolvent precipitation, ball milling, high pressure homogenization
Electrosteric methods	PVP K30, SLS, poloxamer 188, lecithin	High pressure homogenization, emulsion diffusion

TABLE 3: CHARACTERIZATION PARAMETERS FOR NANOCRYSTALS[13]

S.no	Characterization parameters	Methods
1	Mean particle size and size distribution	Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM),
2	Structure and morphology	Light microscopy, SEM, TEM, field emission scanning electron microscopy, atomic force microscopy
3	Surface charge	Zeta potential analysis
4	Solid state analysis/physical state characterization	Crystalline powder X-ray diffraction, differential scanning calorimetry, FT-IR studies
5	Solubility	UV spectroscopy
6	Drug content determination	UV spectroscopy, HPLC

Top-down technologies:

In top-down technologies, one starts with large crystals in the µm range and goes down to the nanodimension by diminuting the crystals, i.e., performing a milling process. Dry milling (eg., jet milling) is not efficient to obtain a particle size in the nanometer range; therefore, wet milling is applied. The drug particles are first dispersed in a solution containing a surfactant and a stabilizer, forming a macrosuspension, which is then subjected to milling energy. This process is referred to as wet milling, which can be either high or low depending upon the nature of the particles, leading to the formation of nanosuspension. Pearl milling or bead milling, also called Nanocrystal™ technology, is a low-energy milling process that has produced many nano-sized commercial products. Various commercial nanocrystal based products have already been prepared through this technique because of its widespread and reliable nature[15,16,21].

High Pressure Homogenization (HPH/ Dissocubes®) technique, which was invented by Muller *et al.*[16], in the 1990s, is the extensively used technique, which may involve lyophilization, spray drying, piston cap or micro-fluidization for the manufacture of nanocrystals[13]. Production of nanocrystals using homogenization methods may involve one of three important technologies *viz*., Microfluidizer technology (IDD-PTM technology), piston gap homogenization in water (Dissocubes® technology) or water mixtures or non-aqueous media (Nanopure® technology).

Combination technologies:

The combination technologies generally combine a pre-treatment step followed by a high energy process. Baxter developed the NANOEDGE™ technology. In the first step of the process, crystals are precipitated and after that the resulting suspension is put through a high-energy process, which is commonly high-pressure homogenization[22].

Smartcrystals® is another patented combination technology developed by Abbott, USA, with the intention of accelerating the size reduction process[23]. Different solvents used, mixing time, temperature for the formulation of nanocrystals are given in Table 4[24-33]. Examples of nanocrystalline drugs on the market approved by FDA are given in the Table 5[34-38].

MECHANISM OF ACTION OF THE VARIOUS NANO DRUG DELIVERY SYSTEMS

While being fabricated to avoid the body's immune mechanisms, the nanoparticles can be used to increase the efficacy, safety and tolerability of incorporated drugs. Nanoparticle-based formulations are known to show high solubility, controlled release and improved pharmacokinetic and pharmacodynamic properties when compared to the raw drug[39,40].

TABLE 4: DIFFERENT SOLVENTS USED, MIXING TIME, TEMPERATURE FOR THE FORMULATION OF NANOCRYSTALS

Drug used	Solvent	Mixing time	Temperature	References
Amisulpride	Methanol	2 h (1000 rpm)	27°	[24]
Baicalein	Ethanol	5 min (1600 rpm)	27°	[25]
Candesartan cilexetil	Acetone	4 h (600 rpm)	25°	[26]
Clarithromycin	Acetone	30 min (10 000 rpm)	27°	[27]
Glyburide	Dimethyl Sulfoxide (DMSO)	2 min (10 000 rpm)	27°	[28]
Lornoxicam	DMSO	2 h (1400 rpm)	27°	[29]
Luliconazole	Methanol	2 h (2000 rpm)	2°	[30]
Nitrendipine	DMSO, acetone, acetonitrile	2 h (1000 rpm)	5°	[31]
Paclitaxel	Methanol, ethanol, methylene chloride, ethyl acetate, DMSO	20 min (1200 rpm)	4°	[32]
Repaglinide	Acetone	15 min (10 000 rpm)	27°	[33]

TABLE 5: EXAMPLES OF NANOCRYSTALLINE DRUGS ON THE MARKET APPROVED BY FDANANOCRYSTALS

Drug (trade name)	Indication	Making company	Year of approval	Dosage form	Reference
Methylphenidate HCl (Ritalin LA®)	Antipsychotic (Attention deficit hyperactivity disorder)	Novartis	1955	Capsule	[13,35,36]
Theophylline (Elixophyllin®)	Bronchial dilation	Nostrum labs Inc	1979	Elixir,oral liquid	[36, 38]
Griseofulvin (Gris-Peg®)	Anti-fungal	Novartis	1982	Tablet	[13, 36,38]
Nabilone (Cesamet®)	Anti-emetic	Lilly Pharma	1985	Capsule	[13, 36,38]
Olanzapine (Zyprexa®)	Schizoprenia, Bipolar disorder	Lilly Pharma	1996	Tablet	[36]
Naproxen sodium (Naprelan®)	Anti-inflammatory	Almatica	1996	Tablet	[13, 36,38]
Brinzolamide (Azopt®)	Glaucoma	Novartis	1998	Suspension	[13, 36,38]
Rapamycin (Rapamune®)	Immunosuppressant	Wyeth Pharmaceuticals	2000	Suspension, tablet	[13, 36]
Tizanidine HCl (Zanaflex® capsules)	Muscle relaxant	Accorda	2002	Capsule	[13, 36]
Aprepitant (Emend®)	Anti-emetic	Merck	2003	Capsule	[13,35,36]
ß-tricalcium	Synthetic bone graft substitute	Orthovita, Inc.	2003	Bone graft substitute	[34]
phosphate (Vitoss®)					
Fenofibrate (Tricor®)	Hypercholesterolemia	Abbott Labs	2004	Tablet	[13, 35,36]
Calcium hydroxyapatite (Ostim®)	Osteoconduction (ability of a bone to grow on a suface)	Osartis GmbH & Co.	2004	Nanocrystalline paste	[34]
Fenofibrate (triglide®)	Hypercholesterolemia	Skye Pharma marketed by Sciele Pharma Inc.	2005	Tablet	[13, 34]
Megestrol acetate (megace ES®)	Antianorexic/appetite stimulant	Par Pharmaceutical Companies, Inc.	2005	Suspension	[13, 36]
Morphine sulphate (Avinza®)	Psychostimulant	King Pharmaceuticals	2002	Capsule	[13]
Dexmethylphenidate	Antipsychotic	Novartis	2005	Capsule	[13, 36]
HCl (Focalin XR®)					
Paliperidone palmitate (Invega Sustenna®)	Antidepressant	Johnson and Johnson	2009	Suspension in prefilled syringe	[13, 36]
Diltiazem (Herbesser®)	Antiangina	Mitsubishi Tanabe Pharma	2002	Tablet	[13]
Verapamil HCl (Verelan®)	Hypertension	Schwarz Pharma	1998	Capsule	[13]
Theophylline (Theodur®)	Asthma, Chronic Obstructive Pulmonary Disease	Mitsubishi Tanabe Pharma	2008	Tablet, capsule	[13]
Dantrolene sodium (Ryanodex®)	Malignant benign hypothermia	Eagle Pharmaceutical	2014	Lyophilized powder for reconstitution	[37]

Particle size:

Nanomaterials are advantageous over microscale particles and their small size and high mobility make them capable of higher cellular uptake and suitable for a wider range of cellular and intracellular targets[41,42].

Surface charge:

Zeta potentials having a value of ±30 mv have been reported to be stable in suspension, preventing the aggregation of particles[43]. The surface charge of nanomaterials is crucial for drug loading.

Drug loading:

The incorporation of a drug on or in nanomaterials is referred to as drug loading. An ideal nanoparticle drug delivery system should have a high drug-loading capacity without aggregation. High drug loading capacity can minimize administration or the number of doses[44].

Drug targeting:

Targeting of tumors leads to improved chemotherapy and nanomaterials provide a highly specific and versatile platform for cancer treatment. This is due to enhanced permeability and subsequent drug retention[45].

Binding to the receptor sites:

The level of blood components (eg., opsonins) is determined by the size of the nanoparticles, the hydrophobicity of their surfaces and the functions of their surface coatings that bind to their surface influencing the *in vivo* fate of nanoparticles[46].

Release of drug:

Manipulation of particle size gives a trigger for tuning drug release rates[47]. Most of the marketed nanocrystal formulations are formulated using top-down, wet media milling techniques. The first nanocrystal technology based product approved by the FDA and introduced in the market was rapamune tablets, containing the immunosuppressant drug rapamycin or sirolimus, by Wyeth Pharmaceuticals in 2000. This formulation was known to show 21 % higher bioavailability than the commercially available sirolimus oral solution.

TARGETED DRUG DELIVERY OF NANOCRYSTALS[48]:

Targeted drug delivery is now becoming vital because by targeting drugs directly to the cells or tissues where the therapeutic action is needed, the drug's efficacy can be increased while the possible side effects are decreased. This is highly exploited in cancer treatments due to the fact that most of the novel, potent anticancer drugs are poorly water-soluble[49]. Nanosuspensions are useful for targeted delivery because their surface characteristics and behavior *in vivo* may be easily adjusted by choosing the most appropriate stabilizers. There is growing evidence that nanocrystals of drugs can accumulate in tumors through a mechanism known as Extended Producer Responsibility (EPR), which stands for increased permeability and retention and the example that can be quoted here is Kangius RF therapy. As a result, drug nanocrystals present a fascinating opportunity for the treatment of cancer, replacing radiotherapy and chemotherapy. Kayser also created the formulation of aphidicolin as a nanosuspension in order to boost the drug's targeted impact against macrophages that were infected with *Leishmania*[50,51]. Another example is the successful targeting of amphotericin to pulmonary aspergillosis in the form of suspension instead of stealth liposomes[52].

Bupravaquone nanocrystals suspended in a mucoadhesive drug delivery system showed better targeting of the gastrointestinal parasite *Cryptosporidium parvum* and were considered highly stable than the pure drug[53]. It was reported that surface modification of atovaquone nanocrystals with surfactants like sodium dodecyl sulfate enhanced its brain uptake and efficacy to decrease parasite load (*Toxoplasma gondii*) in comparison to commercial micronized atovaquone suspension (Wellvone®) oral administration of 100 mg of drug/kg[54]. In a recent study, poorly soluble anti-retroviral nevirapine was converted into nanocrystals using cold HPH followed by surface modification with serum albumin, dextran and PEG. The macrophage absorption of the drug was at least four times higher in the formulation that was coated with serum albumin than that in the free drug. Studies on the biodistribution of nanocrystals in rats found an enhanced concentration of the drug in the brain, liver and spleen in compared to a free drug that was given Intravenous (IV)[55].

Both passive and active ways of tumor targeting can be accomplished through the utilization of nanotechnology. These methods are respectively referred to as "passive targeting" and "active targeting". In the case of active tumor targeting, a ligand or antibody that is specific to membrane-bound receptors of the tumor is attached to nanosized delivery systems, which results in better tumor targeting than passive tumor targeting. Passive tumor targeting is based on the unique property of nanosized delivery systems to accumulate in tumor tissue because of the EPR effect. Active tumor targeting is superior to passive tumor targeting[56].

It is possible to administer diagnostic probes along with anticancer medications through the use of systems that are based on nanotechnology. These tumor theranostic systems successfully cure cancer by recognizing the tumor, determining its stage and tracking the progression of the tumor all at the same time[57,58].

Some of the existing nanocrystal technologies for target delivery make use of a variety of modification technologies. These technologies include magnetic nanocrystals, PEGylated chitosan modified nanocrystals, cationic nanocrystals, and pluronic modified nanocrystals[59].

APPLICATIONS OF NANOCRYSTALS

Oral drug delivery:

It is the widely used and convenient route of administration for nanocrystal based products. They have been developed either in liquid oral dosage forms (i.e., suspensions) or in solid oral dosage forms (i.e., tablets and capsules). After the manufacture of nanosuspensions, the next stage is solidification, which results in the formation of solid oral dosage forms.

Rapamune® (Wyeth) is the first nanocrystalline-based formulation of the macrocyclic immunosuppressive medicine sirolimus (rapamycin) and it is accessible both as an oral suspension and as tablets. It inhibits the production of macrocyclic immunosuppressant proteins[60].

Parenteral drug delivery:

Nanotherapeutics hold great potential for selective and controlled drug delivery to target cells and organs[5,15]. Additional benefits of nanocrystals for parenteral drug delivery include a high drug loading and an ease of sterilisation of these formulations through the use of conventional methods such as gamma irradiation, filtration and thermal sterilisation. These are just some of the advantages of nanocrystals[34]. When nanocrystals are injected directly into the blood stream, they are subjected to an immediate sink condition and may completely and rapidly dissolve. In this case, the pharmacokinetic profile is similar to that of a drug solution[61,62].

Regarding IV administration, a few studies have reported the development of nanocrystals as a tumor-targeting drug delivery approach. The improved permeability and retention properties that permit passive accumulation of particles in tumor tissues have been the primary motivations for formulating pharmaceuticals as nanocrystals for IV administration[63].

Pulmonary delivery:

Nanocrystal aerosols constitute ideal carriers used for poorly soluble drugs, which are used for the treatment of pulmonary diseases[64] because the targeting of the deep lung requires an aerodynamic particle size in the range 1-5 μm[65]. Another important feature of nanocrystals with regard to pulmonary application is their increased adhesiveness in comparison to larger particles. Nanocrystals would hence better and longer adhere to the mucosal surfaces of the lungs, thereby enhancing drug absorption[66]. However, no studies have yet been done about the fate of aerosolized nanocrystals and many mechanisms seem to be involved[67].

Ocular applications:

Ocular drug delivery is the preferred route of administration for pathologies of the eye such as infections, inflammation, dry eye syndrome, glaucoma and retinopathies. Nanocrystals can represent a promising technology for ocular administration because the increased saturation solubility and dissolution rate would lead to a high concentration gradient, which would facilitate the permeation of the dissolved drug molecules across the corneal and conjunctival epithelium, in addition to which nanocrystals can be used to prolong the residence time in the cul-de-sac due to their increased adhesiveness[64].

Dermal applications:

Dermal nanocrystals are available on the market in cosmetic products, more precise than submicron

crystals because their size is greater than 100 nm. Rutin submicron crystals in the line Juvedical® by Juvena Switzerland and Hesperidin crystals in the product Platinum Rare® by the company La Prairie® are two of the most expensive cosmetic products in the world. Rutin nanocrystals showed about 1000 fold higher activity than the water soluble synthetic rutin-glycoside derivative[68]. The anti-oxidant impact of the nanocrystal formulations was demonstrated in an experiment that was conducted *in vivo*[69].

Therefore, the flux is increased and the passage of the dissolved molecules across the membrane is accompanied by the immediate dissolution of other crystals. The increased saturation solubility of nanocrystals results in a higher concentration gradient between the external and internal sides of the stratum corneum. Moreover, the adhesive properties of nanocrystals result in better and longer stickiness[70].

Others:

PX-18 nanocrystals showed neuroprotective effects in cerebral ischemia/reperfusion in rodents[71].

In nutrition:

Nanocrystals are also a well suitable formulation for poorly soluble nutraceuticals like Coenzyme Q_{10} (CoQ_{10}), rutin, hesperidin, apigenin etc., which showed superior performance against the marketed products when tested for *in vitro* release pattern[72].

Hybrid drug nanocrystals for *in vivo* drug imaging:

The continual breakdown of drug nanocrystals in living organisms makes it difficult to follow their movement *in vivo* and there are only a few methods that may be used for detection. Active Pharmaceutical Ingredient (API) and fluorescent colors come together to form hybrid nanocrystals[64,8,73], which were invented to track nanocrystals after adiminstration. It can be achieved through the bottom-up method.

Following the lead of autofluorescent crystals, fluorophores were introduced into the lattice of nanocrystals in the form of defects, resulting in the formation of hybrid nanocrystals. This allowed for the simultaneous achievement of therapeutic and bioimaging functions[74,75,76]. At first, water-soluble Near-Infrared (NIR) fluorophores were employed to make hybrid nanocrystals. These fluorophores included fluorescein, rhodamine B and FPR-749. From these hybrid nanocrystals, the free dye molecules could be easily separated[74]. The crystalline nature of the nanocrystal is well preserved when only 1 % of dye is used for preparing the hybrid NCs[8].

This method was developed for imaging diseases in living organisms, such as the distribution of drugs during the treatment of cancer[76]. Water bound macadam has the ability to directly nanosize water-insoluble dyes like nile red, which can then be used to investigate nanocrystal penetration and accumulation in hair follicles with the assistance of fluorescence imaging as well as microscope technologies[77].

Future perspectives:

The unique benefits that nanocrystals bring to the pharmaceutical industry are attributable to the diminution of particle size of the drug and the corresponding increase in its surface, curvature, saturation solubility as well as the increased dissolution velocity. This results in the problems of low solubility and low bioavailability of poorly water soluble drugs being solved, which is a huge step forward for the pharmaceutical industry. The nanoparticulate applications become significant *via* the various administrative routes. In depth *in vivo* and *in vitro* studies of the biological effects, optimization of synthesis technology, processing methods for drug nanocrystals and improved preparation techniques are required[78].

In certain circumstances, not only do drug nanocrystals have a higher bioavailability, but they also exhibit sustained release qualities and the capacity to target particular organs or tissues. In addition, when the need calls for it, nanocrystals can be converted into traditional dosage forms such as tablets, capsules, injections, aerosols, suspensions, gels and so on. Using this high-pressure homogenization process which allows for the manufacture of nanocrystals on massive scale. It has come to people's attention as a potentially fruitful strategy that can be employed to obtain commercial advantages as a result of the increased market value of nanocrystals and the growing number of items that are promoted using them[79]. As a result of the studies that were conducted in order to find new technologies for the fabrication

of nanocrystals, there is now a vast array of methods that may be utilized for the production of nanocrystals on both the laboratory scale and the industrial scale. In the pharmaceutical industry, the success of nanocrystals for the administration of pharmaceuticals that are poorly water soluble can be attributed to the ease with which they can be produced including the availability of diverse formulation processes. Due to the rapid disintegration of the nanoparticles, nanocrystals also make it possible to achieve a rapid beginning of action. This is because the drug may be absorbed more rapidly. This is an advantage, particularly for medicines that require a quicker start to their effect on the body. It is possible to modify the surface of a nanocrystal in order to induce either a prolonged or targeted release[80]. Nanocrystals can be efficiently used in industrial fields other than the pharma field to provide effective, bioavailable nutraceuticals and cosmetic products in the future.

CONCLUSION

Owing to their ease of manufacture and simple limited number of requirements for the formulation, nanocrystals make the most desirable nanoparticles. When compared to the specific solubilization technologies, which are only applicable to a limited number of different types of drug molecules, nanocrystals have the potential to be a superior formulation technology for poorly soluble drugs. This is because the technology is applicable to almost all drugs. A huge number of nanocrystal products are in the clinical trial phase which, upon therapeutic acceptance can be successfully released into the market in the upcoming years. As of now, the development is focused mainly on the oral and IV routes. Experimentation on the various other routes of drug nanocrystal administration, they can be successfully exploited as a better choice of poorly soluble drugs formulations. The dried nanocrystals can be suitable in the pharmaceutical processing such as granulation which can be further tableted or filled in hard gelatin capsules or also incorporated into other pharmaceutical dosage forms upon slight modifications.

Conflict of interests:

The authors declared no conflict of interests.

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