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Review Article

New insights in nanocrystal technology chemotherapeutic drugs targeting cancer with a translational research paradigm

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Abstract: Nanocrystal formulation has been increasingly investigated for overcoming the limitations of Biopharmaceutical Classification System (BCS) Class II and IV chemotherapeutic agents. Nanocrystal (NC) formation is widely accepted for increasing the solubility, biological barrier permeability, and cell internalization of poorly-soluble chemotherapeutic drugs. Nanocrystalization improves the bioavailability of anticancer agents, increasing their cytotoxicity and effectiveness for cancer treatment. NCs are nanodrug particles that are coated with a thin polymer or surfactant layer, that enhance their stability, solubility, and internalization in tumor cells. For active targeting, NCs can be decorated with ligands, e.g., proteins and amino acids. NCs also undergo passive targeting by high cellular uptake and retention in the mononuclear phagocyte system (MPS). They are prepared by either top-down, or bottom-up methods or a combination and can be scaled up for industrial manufacturing. NCs are safely administered by oral, parenteral, or transdermal routes. This review highlights the role of morphology and stabilizer, with brief discussions on nanocrystal production, ligand conjugation on drug nanocrystal targeting, and uptake in cancer cells. The benefits of NC formulations over conventional drug delivery are presented by discussing aspects of cytotoxicity studies of anticancer drugs.

Keywords: Nanocrystallization, bioavailability, targeting, ligand, morphology, cytotoxicity

Introduction

Drug targeting, also called smart drug delivery design, is aimed to increase the drug concentration at the diseased site, protecting healthy tissues from unwanted side effects. Site-specific targeting of therapeutic agents is among the major drug delivery-associated difficulties. However, it has become possible in the past few decades to formulate drug nanocarriers targeted for treating tumors with minimum toxic effects on the normal tissues [1, 2]. Passive targeting relies on the properties of nano drug formulations and their interaction with the biological environment, while active drug targeting involves drugs reaching specifically the targeted site by specific recognition with surface ligands. Conventional drug administration presents toxic side effects due to nonspecific biodistribution, whereas targeted drug delivery systems prolong and localize their interaction specifically with diseased tissues. The development of nanocarriers like polymeric nanoparticles, liposomes, and micelles is expected to reduce the drug-related undesirable side effects. They can also target small molecules

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such as genes, peptides, and small interfering RNAs (siRNAs) to the desired site [3-6]. However, despite the desirable characteristics of nanocarriers, they usually have low drug loading capacity. Therefore, formulating pure nano-sized stabilized drug particles is an alternative to drug carriers that can reach directly to the site of action [7, 8].

Pharmaceutical NCs are nano-sized particles composed of the pure drug stabilized by excipients (mainly surfactants and polymers). Their size, ranging from a few nanometers to less than 1 μm using the minimum quantity of excipients, will favor their solubility and bioavailability. They possess appropriate saturation solubility and dissolution rate velocity due to a high surface-to-volume ratio. Nanocrystallization techniques are being adopted for poorly soluble drugs belonging to BCS II and class IV [9, 10]. NC also overcome the limitations of polymeric nanoparticles (NP), where NP sometimes exhibit low bioavailability due to entrapment in the matrix [10]. NC are prepared by either top-down or bottom-up methods. Top-down techniques are wet milling, high-pressure homogenization, micro-fluidization, and spray drying, whereas nanoprecipitation is bottom-up technology [11, 12]. These methods can efficiently produce drug NC of small particle size with higher solubility and tumor cell permeation. In addition to stabilize the drug nanocrystals, stabilizing agents have a remarkable effect in cell internalization. The drug nanocrystals in suspension or lyophilized powder may be formulated and administered as oral, nasal, ocular, parenteral, and other dosage forms [10, 13]. The nanocrystals improve the pharmacokinetics, variation in the crystal structure, and anisotropy. Morphologic characteristics of NCs play a significant role in uptake, distribution, and retention in tumor cells [14]. NCs preparations are studied for safe and efficient drug delivery by targeting cancer cells without affecting viability of normal cells [15]. NC can be loaded in different carrier systems e.g. microspheres, hydrogels, or *in-situ* gels for controlled drug release [16-19]. NC targeting has been studied by using stabilizers, like Pluronics, hydroxy propyl methyl cellulose (HPMC), polyethylene glycols (PEG), D-α-tocopheryl polyethylene glycol succinate (TPGS), surfactants such as Tween 80, and ligands like transferrin, biotin, and folic acids [20-22]. NC coating by avidin-biotin modified RBC membrane has been reported to possess higher targeting ability, drug loading, and sustained release [23, 24]. A brief compilation of the literature on NC formulations of anticancer drugs of Class II and IV, showing formulation methods and dynamic light scattering (DLS) results, is given in **Table 1**.

This review discusses the advantages of NC formulations. We are focused on the perspective of improved drug targeting by NC formation for the treatment of different cancers. We illustrate how the morphological properties, stabilizer role, and surface functionalization by ligand attachment can affect the NC targeting. A brief discussion of NC formulation techniques is also presented. A comprehensive and up to-date research survey of scientists supporting the improvements in cancer treatment by NC targeting is shown. A comparison of cytotoxicity of anticancer drugs with their NC formulations is discussed to evaluate NC's benefits.

Nanocrystallization techniques

Pharmaceutical nanocrystals mainly consist of therapeutic ingredients with the minimum amount of excipients for stabilization. The simple nanocrystal engineering technologies and low ingredient concentration save on production cost. Therefore, nanocrystals are easily scaled up for industrial manufacturing. Furthermore, nanocrystals can conveniently be sterilized by dry heat, steam, filtration, and radiation, depending on the drug stability [8, 25]. Top-down and bottom-up techniques are used for nanocrystal synthesis. Both methods are illustrated in **Figure 1**. In some situations, to obtain further size reduction and uniformity, a combination of the two methods could be adopted.

Top-down method

Top-down methods involve high energy to disintegrate the drug to obtain nano-sized particles, either by wet milling or high-pressure homogenization technologies. These technologies have a high percentage yield that makes them suitable for large-scale manufacturing compared to bottom-up methods. Wet milling, specifically wet bead milling, involves the size reduction of poorly water-soluble drugs suspended in water

Table 1. Nanocrystal formulations of BCS Class II and IV cytotoxic drugs using different stabilizers/ligands, formulation methods, DLS results and evaluation on different cell lines

Drug [BCS class]	Stabilizers	Nano crystallization Technique	Particle size Range [nm]	PDI Range	Zeta potential (mV)	Ref.
Paclitaxel [IV]	Tween-80, Glycol Chitosan (GC), sodium alginate (SA) PS sulfonate	Microfluidizer	194-382	0.134-0.194	-18 to -46	[100]
	Hyaluronic acid Transferrin	Nanoprecipitation	236-339	0.14-0.21	-2.7 to -16.6	[92]
	Poloxamer 188 and PEG-400	High pressure Homogenization	210.6 ± 16.8	0.16 ± 0.06	-30.3 ± 5.3	[120]
Campothecin [IV]	Hyaluronic acid (HA)	Nanoprecipitation by Probe Sonicator	196-400	0.19 to 0.220	-4 to -28	[121]
	Boric acid and PVAL	Nanoprecipitation by Probe Sonicator	204-496	0.172-0.586	-5.82 to -40.1	[122]
Docetaxel [IV]	Tween 80	Nanoprecipitation by Probe Sonicator	526-543	0.18-0.24	-9.6 to 10.1	[123]
	Transferrin	Nanoprecipitation, by Probe Sonicator	405-468	0.18-0.23	-15 to -18	[93]
Etoposide [IV]	Pluronic F-127	Antisolvent precipitation	117 ± 28	0.12-0.13	-16 to -20	[112]
Sorafenib [II]	Polaxamer 407	High pressure homogenization	141-544	0.188-0.797	-7 to -28	[124]
Oridonin [IV]	Polyvinyl pyrrolidone K30 (PVP K30)	Antisolvent Probe sonication	285-295	0.24-0.27	0.035-0.085	[125]
Parthenolide [IV]	Pluronic F68 and lecithin	High-pressure homogenization method [PHPH]	126.9 ± 2.31	0.230 ± 0.024	-11.18 ± 0.59	[32]

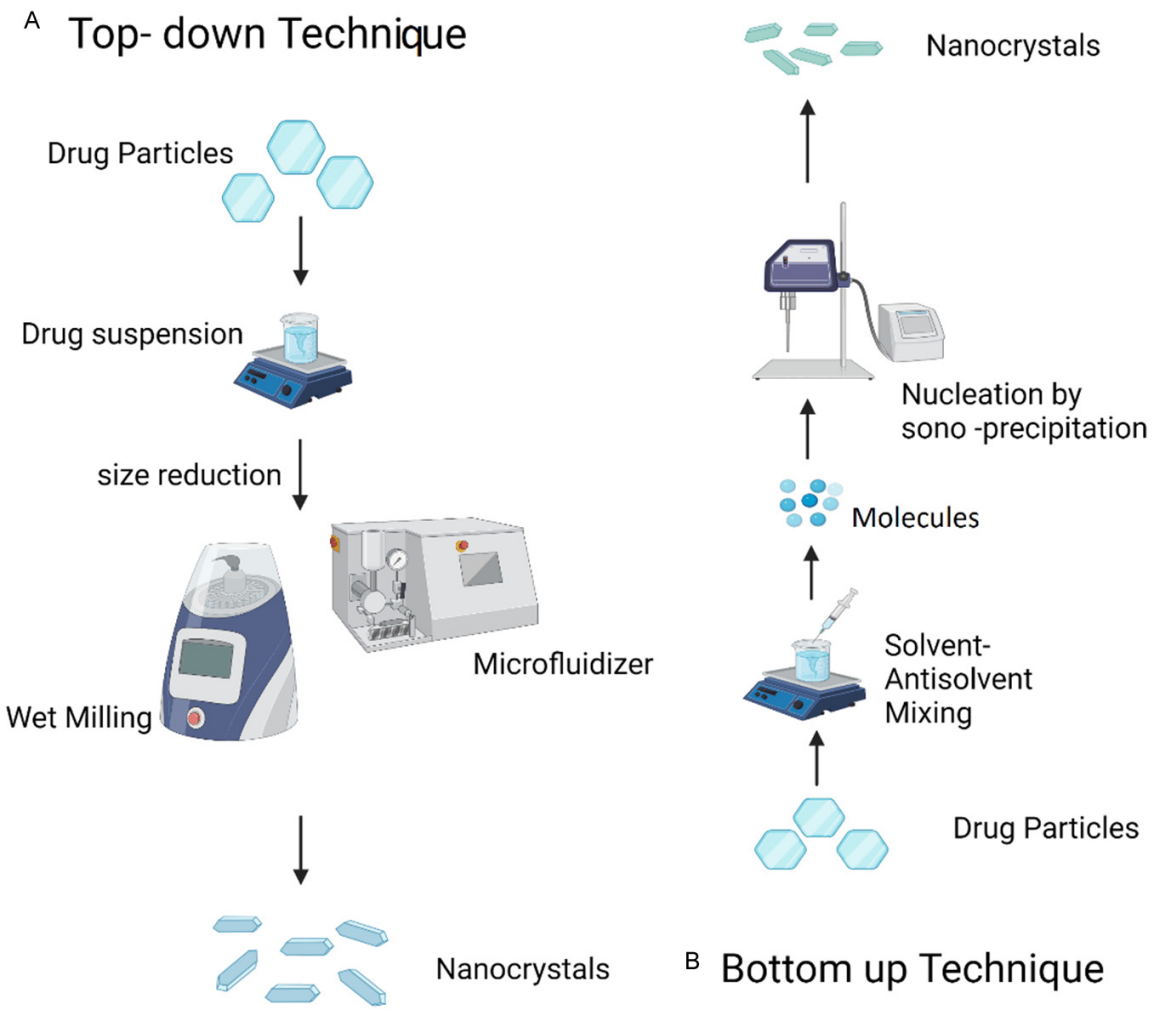

Figure 1. Schematic illustration of nanocrystal production methods, showing general processing of (A) top-down and (B) bottom-up technologies (created with BioRender).

with a nominal quantity of stabilizer by high energy forces in a tube/chamber containing inert grinding media. The grinding materials are either metallic (carbon steel, chrome steel, or stainless steel) or nonmetallic (alumina, zirconium oxide, glass) beads, ranging from 0.05 to 2 mm in size. The particle size reduction mechanism mainly involves impact, high shear forces, and attrition generated by friction among particles. Wet bead milling is usually preferred to high-pressure homogenization due to particle size uniformity, easy scale-up, and convenience for handling a wide range of drug quantities [26-28].

High-pressure homogenization is the second well-adopted top-down technology, using high pressure for particle size reduction in liquid media in the presence of surfactants by particle collision, shear forces, and cavitation forces. Piston-gap homogenization and microfluidization (jet-stream homogenization) are the two main homogenization procedures. The advantages of high-pressure homogenization are aqueous free preparation of nanocrystals and aseptic production for parenteral administration [29-31]. Particle size reduction and poly dispersibility index (PDI) of nanocrystals prepared by high-pressure homogenization depends on homogenization pressure. Parthenolide nanocrystals were successfully prepared by high-pressure homogenization using lecithin and F68 as stabilizers. During homogenization, the stabilizers rapidly cover the nanocrystal surface, providing electrostatic and steric stabilization for efficient particle size reduction.

The particle size reduction was mainly related to homogenization pressure i.e., by increasing from 500 bar to 1000 bar reduced the particle size from 208.2 nm to 126.9 nm and the PDI decreased from 0.333 to 0.230 [32].

Particle size reduction by top-down methods also depends on the physical properties of the active ingredient, as it is affected by initial particle size, polymorphism, morphology, and Young modulus [11, 33-36]. Young modulus determines the strength, elastic, and plastic deformation of solid materials. Some specialized processes like freeze-drying and spray drying can be used to modify drugs with characteristics suitable for nanocrystallization by top-down procedures. Drug particles obtained have porous structures with reduced crystallinity after treatment by freeze-drying or spray drying. The particles have an irregular molecular arrangement compared to unmodified crystalline particles. Due to porosity and brittleness, they have more breakage points and are well adapted for particle size reduction by high impact, shear, and attrition forces. For example, the optimized particle size of glibenclamide was obtained from amorphous freeze-dried powder after treatment by high-pressure homogenization [37]. In another research, resveratrol pre-treated by spray drying resulted in nanosuspensions with significantly smaller particle sizes without reduction in crystallinity [38].

Bottom-up method

With bottom-up technology, also named nanoprecipitation, the drug is solubilized in a solvent and the solution is added to a non-solvent containing a stabilizer [39]. Nanoprecipitation method was first introduced by List and Sucker in 1988 [40]. Crystal growth is generated by nucleation occurring by either solvent-antisolvent mixing or solvent removal. The blending of drug solution and antisolvent could be done by magnetic stirring or impeller blades. This is followed by sonication to promote nucleation. Optimization of the process variables and formulation aspects of nanoprecipitation is required to obtain the desired nanocrystal formulations. It primarily depends on the physical properties and concentration of the drug and, to a lesser extent, the stabilizing agents. Processing parameters influencing the final product are stirring speed, the ratio of solvent-antisolvent, type and depth of the sonicator's probe and temperature [41-43]. Using organic solvent for drug solubilization has led to limited application of bottom-up techniques. This is because of the lower solubility of some new therapeutic agents in easily removable organic solvents like acetone, ethanol, or methanol, but which remain soluble in dimethyl sulfoxide (DMSO). DMSO itself is toxic and removal consumes time that increases the overall production cost [44].

With the advancement in pharmaceutical technology, supercritical antisolvent precipitation using supercritical fluids is used in nanocrystal production. Supercritical fluids behave like liquids and convert to gas above their critical point e.g., carbon dioxide, ammonia, ethane, ethylene [45]. The drug in a solvent is sprayed on supercritical antisolvent in a chamber, resulting in rapid nucleation and nanocrystal formation. This technique yields smaller particle size, and uniformity at ambient temperature suitable for thermosensitive materials. Another technology for nanocrystal production, the spray flash evaporation system, is well adapted for many benefits of short processing time and low temperature. The drug, dissolved in low boiling point solvents like acetone, ethanol or methanol at a concentration of 1 to 10% w/v, under high pressure of 40 bars, is introduced to a vacuum chamber. The pressure drop creates instant evaporation and crystallization [46].

Combined technologies

Both top-down and bottom-up approaches for nanocrystal production have limitations in terms of running time of energy equipment or initial pre-treatment to obtain micronized drug particles [11]. To attain the specific benefits and minimize the disadvantages of these two approaches, combination technologies can be adopted. One commonly used combination technology is NANOEDGETM introduced by Baxter (Baxter Pharmaceutical Solutions, LLC, US). This involves a pre-treatment for microprecipitation of crystals, usually followed by high energy homogenization. The microprecipitation step decreases energy cost for running high shear equipment, whereas subsequent treatment by high-pressure homogenizer increases size uniformity and thermodynamic stability of nanocrystals [47, 48]. Other combination

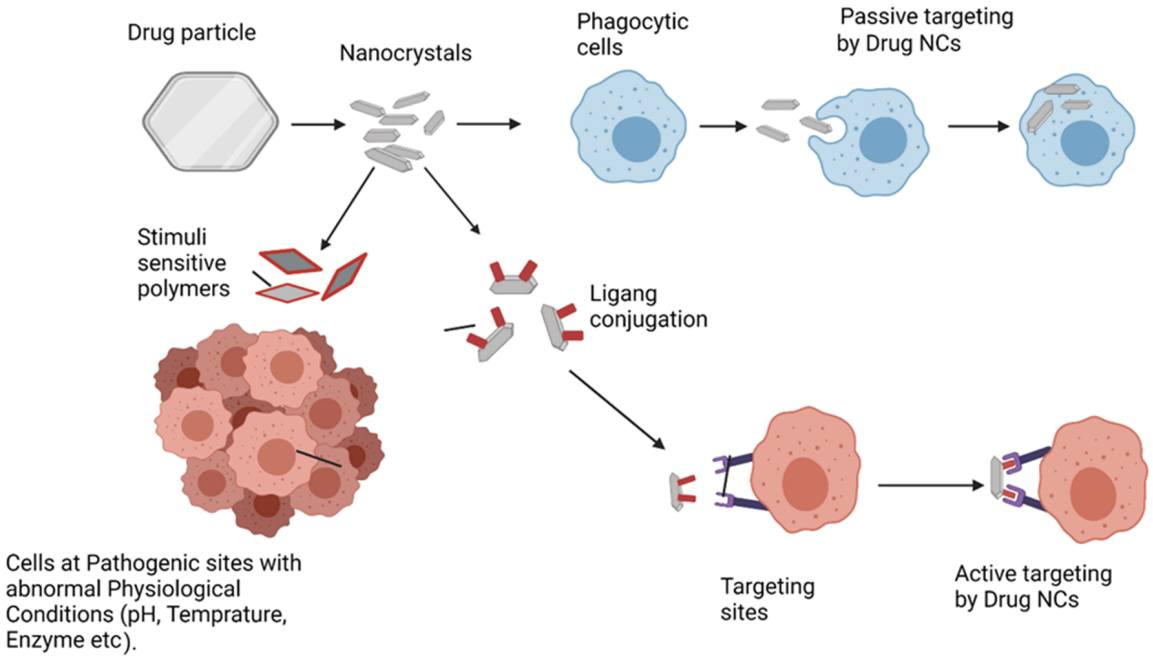

Figure 2. Mechanisms of active and passive targeting to cancer cells by drug nanocrystals (Created by BioRender).

approaches are H42 and H96 technologies involving a pre-treatment by spray drying and lyophilization (freeze-drying) followed by high-pressure homogenization. Both spray drying and lyophilization remove excessive moisture from drug particles, making them more brittle/breakable by high-pressure homogenization. With freeze-drying, H96 technology is more suitable for thermosensitive drugs [49, 50].

Some combination technologies combine the two top-down techniques, such as wet ball milling and high-pressure homogenization. First, the drug particle suspension is subjected to low energy ball milling, with conditions adjusted to yield a particle size in sub-micron range from 600 nm to 1 micron, then treated by high-pressure homogenization. The pretreatment with ball milling reduces the overall cost and saves time and energy by a high-pressure homogenizer. Moreover, this method ultimately yields particles with better stability and uniformity but relatively larger particle size compared to the above-mentioned combination technologies [51].

Cellular uptake and targeting of nanocrystals

The presence of cell receptors has a significant role in cell permeation of few drugs and their cellular uptake. However, numerous drugs have remarkable therapeutic effects but poor permeation characteristics and cellular uptake. These limitations may be overcome by drug nanocrystal modification and formulation techniques to enhance bioavailability and increase cell internalization. Surface modification by ligands and stabilizers increases active drug targeting for site-specific cytotoxic effects. Furthermore, the internalization of nanocrystals is influenced by morphologic characteristics, the type of stabilizer used to coat the nanocrystal surface, and ligand attachment specific to infectious cells, as illustrated in **Figure 2**.

Role of particle size and morphology

The particle size can affect the retention of drug nanocrystals in cells since the drug nanocrystals smaller than 150 nm showed higher solubility and better accumulation in the liver, lungs, and kidneys than drug solution [14]. However, sometimes nanoparticles ranging from 100 to 150 nm may possess pharmacokinetic behavior like a solution. Due to their immediate dissolution in blood, they are usually not recognized by the MPS [8]. In comparison, larger nanocrystals (300-400 nm or more) exhibit different biodistribution characteristics

into solution since they are recognized as foreign agents by MPS, readily phagocytized, and distributed to the liver, spleen, and lungs [26, 51]. The MPS clearance after intravenous injection is also influenced by the stabilizers and method of preparation i.e., bottom-up or top-down techniques [12]. From phagocytic cells of MPS, hydrophobic agents cross phagolysosomal membranes, enter the cytoplasm, and diffuse out due to the drug concentration gradient.

Among morphologic characteristics, the shape of nanocrystals has a significant effect on their cellular uptake. Rod-shaped drug nanocrystals have better retention and invasiveness into pathogenic sites than spherical ones. Rod-like nanocrystals of immunomodulatory drugs are efficient to passively target the phagocytic cells as demonstrated with doxorubicin. An anthracycline was formulated since Cis-aconityl-doxorubicin labeled cellulose nanocrystal rods were prepared with enhanced cellular uptake. This shape-dependent cytotoxicity was also observed in rod-shaped 10-hydroxycamptothecin and needle-like camptothecin nanocrystals. Differently shaped nanocrystals have been evaluated for the cellular internalization in human nasopharyngeal epidermal carcinoma KB cells. Confocal microscopy and fluorescence detection also showed higher retention of rod-like nanocrystals in comparison to spherical ones. This higher retention and enhanced permeability of chemotherapeutic drug nanocrystals are favorable in passive targeting of tumor cells. The disrupted vasculature, lymphatic drainage system, and defective endothelium of tumor cells enhance drug nanocrystals' uptake [52, 53].

Role of the nature of the stabilizer

Stabilizers have a role in the stability of drug NC. Addition of polymers and surfactants is required to stabilize NCs, to facilitate their production at manufacturing scale [54]. Particle size reduction in the absence of stabilizer results an increase in surface area, and free surface energy may lead to a higher magnitude of attractive molecular forces. As a result of these unsatisfied bonding forces at the particle surface, they can cohere and result in aggregates of larger size. Further, after being lyophilized, the drug particles may develop electrostatic forces due to internal friction. The intensity of charges depends on the nature of compounds and the agitation produced. To maintain nanocrystals' integrity, steric stabilizers such as nonionic surfactants, e.g. polysorbates, vitamin E TPGS 1000, or sorbitan esters, are added to form a thin layer around the drug particles. This provides a mechanical barrier preventing their aggregation and size increment. The presence of stabilizers on nanocrystals may be confirmed by various analytical techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier Transform Infrared(FT-IR) spectroscopy and Raman spectroscopy [55].

The nanocrystal surface modification by stabilizers is essential for internalizing tumor cells and avoiding rapid clearance by MPS. There are various stabilizers used for NCs to prevent their aggregation, and maintain their particle nanosize required forimproved bioavailability and other pharmacokinetic parameters. Ionic surfactants e.g. sodium dodecyl sulfate (SDS) or cetyltrimethylammonium bromide (CTAB) increase the electrostatic charge of the particles, thereby inducing interparticle repulsion and improving their stability. Moreover, the PEG coatings on NC shield them from undesirable interaction with the MPS, aggregation, and opsonization, prolonging circulation time. This ultimately increases the half-life of the drug in the body [56]. Polymer stabilizers like Poloxamers F-68, F-127, F-188, and, among celluloses, hydroxypropyl methylcellulose HPMC, are widely used in nano crystallization. By absorbing on the surface of drug nanocrystals, Poloxamers stabilize the nanodrug particles by steric hindrance, preventing aggregation. HPMC inhibits crystal growth by hydrogen bonding, due to a high degree of substitution of methoxy and hydroxypropoxy groups [57].

The nature of the stabilizer has a significant effect on drug transport and targeting by increasing the permeability of the drug. It may enhance the influx of drug nanocrystals by opening the tight junctions in cells, increasing the paracellular transport of the particles across biologic barriers. Therefore, selecting an appropriate stabilizing agent is critical before choosing other parameters for the formulation of drug nanocrystals [28, 57]. The stabilizer must have an affinity for the crystal surface for

adsorption, providing a strong interfacial film to avoid unnecessary shedding. The stabilizer selection should be based on the interaction between stabilizer and drug NCs, which can be confirmed by atomic force microscopy [58]. However, an optimum strength of interfacial film between stabilizer and NCs should be critically evaluated in stimuli sensitive nanodrug crystals. Stabilizer shedding in response to cellular enzymes or other stimuli is beneficial for the therapeutic effects. For example, TPGS induces P-glycoprotein (P-gp) inhibition, therefore increasing the uptake of nanocrystals in tumor cells [59]. The efficacy of TPGS was evaluated by observing higher cytotoxicity of paclitaxel nanocrystals (PTX-NC) on KB and H460 cell lines compared to paclitaxel (PTX) and other nanocrystal formulations without TPGS [60]. Similarly, Tween 80 facilitates cell membrane permeation by P-gp inhibition and drug retention in the cytosol and minimum efflux to extracellular fluid [15]. The toxicity to MCF-7 and MDA-MB cells of the drug solution and paclitaxel nanocrystals containing Tween 80 were compared. The IC_{50} of pure drug solution was 12.17 (±1.36) nM and 7.02 (±1.27) nM on MCF-7 and MDA-MB cells, respectively. In comparison, PTX-NC with Tween 80 (PTX/NC-T80) had higher toxicity on MCF-7 with an IC_{50} of 7.41 (±0.96) nM and MDA-MB cells with an IC_{50} of 4.82 (±0.32) nM.

In addition to the stabilizer layer, the presence of ligands facilitates site-specific targeting of the drugs to tumor cells. Stimuli-sensitive polymers responding to physiochemical changes (such as temperature, pH, ionic concentration, enzymes, and magnetic field) have been used successfully in controlled release drug delivery systems. Therefore, they are effectively used as stabilizers in nanocrystal preparation for drug targeting. For example, the pH of the tumor cells or tissues is usually lower than in normal cells, so pH-responsive polymers may be used for drug delivery [61]. Hyaluronic acid (HA)-coated camptothecin nanocrystals showed a pH-triggered release behavior, where camptothecin was released more rapidly, more than 55% in a mild acidic condition [pH 5.5] within three hours, while it was only 25% at pH 7.4 PBS [62]. $CaCO_3$/doxorubicin nanocrystals were prepared by high-pressure homogenization. They exhibited selective toxicity at pH 4.8 i.e., 96%, without any nonspecific cytotoxicity since nanocrystals had minimum toxic effects at neutral pH 7.4 [59]. Reactive oxygen species (ROS)-sensitive paclitaxel nanocrystals were prepared by wet milling, using a library of 10 redox-sensitive amphiphilic block copolymers. ROS-responsive biodegradable polymeric stabilizers were prepared by post-polymerization modification via the thiol-yne reaction [63]. The hydrophobic thiols have an affinity for the hydrophobic surface of oxidation-sensitive paclitaxel nanocrystals. This study concluded that site-specific shedding of stabilizers is used in drug targeting by cellular uptake. Therefore, other stimuli-sensitive polymers can target the drug to specific infectious regions for localized effects without affecting the healthy cells e.g. poloxamers including Pluronic F68 and Pluronic F127, Poly[ethylene glycol]-poly[3-caprolactone]-poly[ethylene glycol] (PCEC).

Ligand attachment

In addition to the stabilizer layer, the presence of ligands facilitates site-specific targeting of the drugs to tumor cells. The ligand molecules can bind specific sites specifically expressed or overexpressed by the malignant cells, without significantly affecting healthy sites. Numerous receptors present on cells may serve as docking sites for anticancer drug targeting including folate receptors, transferrin receptors, sigma receptors [64], bombesin receptors [65], fibroblast growth factor receptors, and follicle-stimulating hormone receptors. Understanding the appropriate interactions between nanocrystals and cell membrane receptors is significant for therapeutic application [66, 67]. Several factors like ligand molecule length and density affect the equilibrium in receptor-mediated endocytosis. Higher density and rigidity increase the uniform distribution of ligand molecules on the drug particle surface. The ligand length is proportional to binding affinity on cell membranes, but it is usually harder for phagocytic cells to engulf the drug nanocrystals with more extended ligands [68-72].

Some of the commonly used ligands, ligand-conjugated chemotherapeutic agents, and their enhanced permeation in cancer cell models and cell lines are presented in **Table 2**. The surface decoration of nanoformulations by ligands is an attractive therapeutic tool for cancer treatment by active targeting. The folate receptors are commonly overexpressed on the

Table 2. Examples of ligand-conjugated nanocrystals for the targeting of drugs to tumor cells

Chemotherapeutic agent	Ligands	Tumor cells/cell lines	References
Calcium fluorapatite	Folic acid	HepG2 and MCF-7	[126]
Campothecin and paclitaxel	Folic acid	Human lung cancer and murine breast cancers	[87]
Paclitaxel	Folic acid	4T1 breast cancer cells	[108]
Cadmium selenide/Cadmium Sulphide (CdSe/CdS)	Folic acid and Biotin	HeLa, KB cells	[88]
Paclitaxel	Transferrin, Hyaluronic acid	MCF-7	[127]
Doxetaxel	Transferrin	A549	[93]
Cadmium chalcogenide	Transferrin	H460	[94]

plasma membrane of most cancer cells, including lung, endometrial, breast, ovarian, and kidney cancers [73-78]. Folate receptors mediate the cellular uptake of folic acid, also known as vitamin B9, an essential vitamin for cell proliferation. Therefore, folate receptors are most expressed in rapidly dividing cancerous cells. However, the folic acid is also taken by healthy cells through proton-coupled folate transporter and reduced folate carrier system [79-82]. The effect of folic acid conjugated cellulose nanocrystals was determined on folate receptor-positive cells, KB cells, and human breast cells (MDA-MB-468A). This formula showed selective targeting and higher internalization in the cell lines. This finding suggest its diagnostic use as an imaging agent for tumor detection at the early stages [83]. Elongated folic acid-modified cellulose nanocrystals and unmodified cellulose crystals were evaluated for cellular uptake to human brain tumor cells DBTRG-05MG, H4, and C6 rat brain tumor cells. Folate receptor expression of the cells was verified by immunofluorescence staining. The modified nanocrystals exhibited higher cellular binding in DBTRG-05MG, H4, and C6 cells. They were 1452, 975, and 46 times higher, respectively, than nanocrystals without folic acid. The cellular internalization was by caveolae-mediated endocytosis in DBTRG-05MG and C6 cells and in H4 cells by clathrin-mediated endocytosis [82, 84].

Folic acid-conjugated bioluminescent calcium fluorapatite nanocrystals for cancer cell fluorescence imaging were tested in folate receptor positive cells. The nanocrystals were biocompatible and readily dispersible in water [85]. Furthermore, by grafting the nanocrystal's surface with folic acid (Quantum dots conjugated to folic acid), a highly sensitive recognition of the targeted sites was demonstrated by confocal laser scanning microscopy in HeLa, T47D, and MCF-7 cells. HeLa and T47D cells overexpressed folate and showed high internalization up to 95% and 90%, respectively. In comparison, MCF-7 cell line had a lower growth rate, with negligible expression of folate receptors had low internalization (3% of labeling) [86].

A nanocrystal formulation of paclitaxel and campothecin was developed using Pluronic F127 polymer as a stabilizer by three-phase nanoparticle engineering technology, including amorphous precipitation, amorphous aggregation, and nanocrystal stabilization [87]. The nanocrystals were made of over 99% of the drug with a high ratio of drug to excipient. Antitumor activity was evaluated *in vivo* in two tumor models in mice, human lung, and murine breast cancers, resulting in significant tumor growth inhibition after intravenous and oral administration. The nanocrystals were further modified for targeted delivery of paclitaxel by conjugating a folate ligand to the outer stabilizer layer of F127. *In vitro*, the folic acid conjugated nanocrystals showed high cell toxicity. The paclitaxel nanocrystals induced maximum toxicity at 10% concentration of F127-folate.

Biotin and folic acid functionalized Cadmium selenide/Cadmium sulphide (CdSe/CdS) nanocrystals were formulated by new seeded-type growth [88]. Biotin-conjugated nanorods were evaluated in HeLa cells and exploited as an alternative class of fluorescent molecular probes for cell and tissue imaging. Other nanocrystals conjugated with folic acid were studied for selective targeting on human nasopharyngeal epidermal carcinoma (KB) cells, showing the use of these materials for targeting and imaging specific tumor cells [88].

In addition to folic acid, the surface of nanodrug delivery systems may be modified by different agents including proteins, polysaccharides, peptides, aspartame, and small mo-

lecules that enhance the antitumor effect [89]. Among these, transferrin has broad utility as a surface-modifier of nanocrystals for targeting tumor cells due to higher iron demand of cancer cells and overexpression of transferrin-receptor. Therefore, transferrin-receptor-mediated endocytosis is an efficiently involved cellular uptake pathway for delivering anticancer agents [90, 91]. Transferrin conjugated nanocrystals have enhanced internalization into various cell lines such as A549, MCF-7, MDA-MB-231 cells, HeLa cells, Caco-2 cells, and human H460 cells. For example, transferrin-modified paclitaxel nanocrystals with TPGS as the stabilizer were prepared successfully for oral administration with improved intestinal absorption and resulted in a higher antitumor effect [60]. HA can specifically react with the overexpressed CD44 receptors on MDA-MB-231 cells. Therefore, HA-modified paclitaxel nanocrystals were prepared and evaluated for cytotoxicity and cellular uptake in MDA-MB-231 cells. Nanocrystals exhibited 30.8% cellular uptake, whereas drug solution exhibited 15% [62]. In another study, different types of paclitaxel nanocrystals were prepared by the nano-precipitation method. The surface of nanocrystals was modified with transferrin and hyaluronic acid and compared with unmodified paclitaxel nanocrystals. All nanocrystals, modified and unmodified, had a mean particle size of 230-340 nm. The modified nanocrystals had higher cellular uptake in MCF-7 cells. The cell growth inhibition in MCF-7 cells was significantly higher by modified nanocrystals than unmodified nanocrystals and pure drug. On the other hand, transferrin and hyaluronic acid-modified nanocrystals were safer in normal cells. HaCaT cells had a cell inhibition of 11 to 12%, while in unmodified paclitaxel nanocrystals and pure drug, the cell inhibition was 17% and 23%, respectively [92]. Transferrin modified docetaxel nanocrystals after 24 hours of incubation in A549 cells showed higher cytotoxicity (66.9% ± 3.8%) than unmodified docetaxel nanocrystals (55.5% ± 6.1%) and pure drug (15.5% ± 5.7%) at a docetaxel concentration of 100 μg/ml [93].

A stronger binding approach of ligands/biomolecules to drug crystal surface is needed to improve cellular targeting and nanocrystal uptake. This need of nanocrystal modification is critical for biomedical application. A facet engineering approach was adopted for significantly enhancing transferrin binding to cadmium chalcogenide nanocrystals. Their cellular uptake was evaluated by confocal microscopy on HeLa cells [94]. The stronger binding between transferrin and nanocrystals resulted in greater uptake of these protein - nanocrystal conjugates into HeLa cells. Transferrin binding was obtained by inner-sphere thiol complexation, evaluated by competitive adsorption experiments and theoretical calculations. High thiol content contributed to the higher binding of transferrin to nanocrystals possessing soft metals by metal-thiol complexation. Further, molecular dynamics simulation revealed that facet-dependent transferrin modification occurred by the differential affinity of crystal facets to the monomolecular layer of water molecules, which hinders access to exposed facets [95]. Chemical complexation is widely adopted for adsorption of ligand macromolecules onto metal-containing nanomaterials, and may be extended to different biocompatible nanocrystals preferably containing soft metals (e.g., Au, Ag, Pt, Pd, and Zn) having a strong affinity to form coordination bonds with thiol-containing ligands [96, 97].

Transferrin conjugated to alloyed quaternary nanocrystals was found to have high recognition of tumor sites possessing higher cellular uptake in tumor cells and low toxicity to healthy cells. Stable, biocompatible nanoconjugate of transferrin were anchored to Ag-In-Zn-S quantum dots on the surface of doxorubicin nanocrystals. The drug nanoconjugate exhibited a concentration-dependent cytotoxic effect on the H460 cell line (human non-small cell lung carcinoma) [98].

Drug targeting in in-vivo studies

The nanocrystals have been found safe and efficient by oral, parenteral, pulmonary, and ophthalmic routes. Particle size and surface properties determine their *in vivo* fate by influencing the absorption, dissolution, distribution and cellular uptake that further. After oral administration, drug dissolution from formulation to luminal fluids is the rate-limiting step. Particle size reduction leading to higher specific surface area of nanosuspensions increases their dissolution rate. In addition to particle size reduction, a stabilizer layer enhances the stability, solubility, and bioavailability [8, 99].

The oral route has been found efficient for administering nanocrystals for cancer treatment. The improvement in bioavailability and other beneficial effects of chemotherapeutic drugs has been investigated in many studies. The oral *in-vivo* pharmacokinetic studies demonstrated that paclitaxel nanocrystals exhibit significant increase in area under curve (AUC 0-t), maximum concentration (C_{max}), mean residence time, MRT, and decrease in time to reach maximum concentration (T_{max}), compared to plain paclitaxel crystals. The increase in AUC of nanocrystals was almost 9-10 fold compared to plain paclitaxel crystals [100].

Finding an *in vitro-in vivo* correlation (IVIVC) is important for evaluating oral formulations. IVIVC was established for a drug formulation Foscan® comprising temoporfin for palliative photodynamic therapy of head and neck squamous cell carcinoma. This was done based on *in vitro* drug release, particle characterization and *in vitro* tests in HL 60 cells, a prediction of drug accumulation in the human liver and lungs. In that research study, the significance of particle size and release rate was determined [101]. Owing to poor solubility of many chemotherapeutic agents and other drugs, oral administration route has limitations. In many cases, a carrier system is needed for an effective drug targeting, as porous, positively charged N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) NP were found as efficient carrier for PTX-NC. HTCC nanoparticles loaded with PTX-NC were showing more drug distribution. *In vivo* imaging studies confirmed their greater accumulation in subcutaneous tumor tissue than simple drug nanocrystals [102].

Although nanocrystal formation has considerably attracted pharmaceutical scientists, improper absorption or insufficient retention in the gastrointestinal tract (GIT) remains a problem. Retention time in the GIT can be increased by loading the nanocrystals in mucoadhesive drug delivery systems. Nanocrystals, due to their size and almost pure drug content, are uniformly distributed in a suitable mucoadhesive carrier and can be conveniently released at the targeted site. Silybin nanocrystal loaded in mucoadhesive microspheres with drug had a loading capacity of up to 35.41 ± 0.31%. An *ex-vivo* study was performed on rat intestine to determine the mucoadhesiveness. It was found that the nanocrystal loaded microspheres were retained on intestine for more than one hour. Pharmacokinetic study showed a three-fold increase in bioavailability compared to free drug. Mucoadhesive microspheres, by releasing the drug nanocrystals constantly, can provide a uniform therapeutic effect for a longer time [18]. Pluronic F127-based thermosensitive *in situ* hydrogel was prepared and loaded with imatinib nanocrystals for locally treating cervicovaginal cancer. The hydrogel was found efficient for mucoadhesivesness, prolonging *in vivo* intravaginal residence time and significant sustained effects in comparison to hydrogel containing imatinib. The enhanced inhibition of tumor growth in mice was observed with negligible mucosal toxicity [103]. Similarly mucoadhesive hydrogel was prepared for local delivery of cisplatin to colorectal cancer [104].

For treating lung cancer, particle size of nanocrystals determines retention in the pulmonary tract. By applying aerosols of drug nanocrystals, droplets are efficiently spread on the lungs surface due to suitable stabilizers, and dissolve rapidly in fluid linings the lungs, with rapid onset of local and systemic effect [54]. In addition to particle size, the nanocrystal shape influences cellular uptake and retention. For example, rod-shaped camptothecin nanocrystals of size 250 nm had a better accumulation in the lungs than the spherical ones. This was primarily because the rod-shape hinders escape from lung tissues [26]. Moreover, brain tissues can take up nanocrystal coated with tween 80, poloxamers or sodium dodecyl sulfate. Specifically, polysorbate 80-coated nanocrystals can cross the blood-brain barrier; hence, they can treat different types of brain tumor.

Regarding injectable nanosuspensions, particle size affects the pharmacokinetic behavior. For example, after injecting a nanosuspension of particle size about 100 nm, the pharmacokinetics was found to be similar to the solution, while larger-sized particles (800 to 900 nm) were accumulated in the liver. Nanocrystals, due to their smaller size, had a greater dissolution rate with extended elimination [105].

In chemotherapy, temoporfin is used in palliative care of squamous cell carcinoma of the head and neck. Two advanced *in silico* models were developed to determine the pharmacoki-

netics of temoporfin nanocrystals. Model A was a dissolution based *in silico* model, assuming that the drug rapidly diluted after administration had high plasma protein binding. The composition used for *in vitro* release profile in phosphate buffer saline 7.4 was 0.01% Methyl β Cyclodextrin (Me-β-CD) and 10% Fetal bovine serum (FBS), whichreflects the dissolution pressure applied to the formulation more realistically. Model B was based on dissolution and distribution that provide better correlation of *in vivo* and *in vitro* data. In this model, circulating blood is considered as the central compartment. After injection, some drug remains at the injection site in a precipitated state while the nanocrystal fraction circulates abruptly and increases the initial plasma concentration. Dissolution of drug crystals and circulating fraction of nanocrystals are responsible for the plasma concentration time profile in humans. The release rate and particle size play a major role for biodistribution [101].

Cannabidiol (CBD) is a poorly water-soluble drug and is subjected to massive first-pass metabolism resulting in low oral bioavailability. CBD nanocrystals were prepared through antisolvent precipitation method for intramuscular injection to improve bioavailability. They displayed a particle size of 141.7 ± 1.5 nm. *In vitro* drug dissolution rates were 42.91% and 91.57% of CBD physical mixture and CBD nanocrystal freeze-dried powder, respectively, within 15 min. A higher dissolution rate of nanocrystals was attributed to increased surface area. C_{max} after CBD nanocrystal freeze-dried powder IM injection was 239.41 ± 16.92, which was much higher than orally administered CBD nanocrystal freeze-dried powder at 151.40 ± 35.78. Similarly, AUC 0-24 h value of the IM administered formulation was 2 to 2.2 fold greater than oral administration, while bioavailability also enhanced up to 7.8 fold [114]. Tenofovir was converted into long-acting prodrug nanocrystals, which increased its cellular uptake and retention. Two nano formulations, NM1TFV and NM2TFV, were prepared, which after a single IM administration demonstrated an effective dose for 2 months [106].

Curcumin (Cur) is a potential antineoplastic drug bearing low systemic toxicity and high therapeutic efficiency. However, when loaded in nanocrystals, uncontrollable drug release and high systemic metabolism hamper its further application in chemotherapy. The surface of curcumin nanocrystals (Cur-NC) was made hydrophilic by modifying with HA, which reduced its cellular uptake and prolonged its biodistribution. Flow cytometry confirmed apoptotic effects indicating its cytotoxicity. The half-life for Cur, Cur-NC and HA-Cur-NC were 11.14 ± 1.63 h, 14.66 ± 6.58 h and 53.06 ± 18.21 h, respectively, indicating that half-life was increased many fold in hyaluronic acid coated Cur-NC. Moreover, its AUC was 6.3-fold higher than simple Cur-NC. Coating of NC improved bioavailability and stability, but could inhibit leakage of drug *in vivo* [107].

Polyethylene glycol (PEG) and folic acid (FA) were coated on PTX-NCs (PTX NCs-PEG-FA). The NCs were prepared by thin-film hydration method which is a bottom-up technique. FA and PEG decorated Paclitaxel nanocrystals demonstrated higher stability and were studied for tissue distribution in breast cancer treatment in mice. The size of modified PTX-NC was 201.90 ± 2.92 nm and was unchanged for 168 h in the plasma, while nonmodified PT-NC exceeded 600 nm in similar conditions. It was revealed by cellular uptake experiments and cellular growth inhibition studies, that FA and PEG modification of PTX NC prolonged the circulation in the bloodstream compared to unmodified PTX NC and taxol. Surface functionalization by FA increased the cytotoxic effect on 4T1 breast cancer. They were also studied in an *in-vivo* cancer model, where they showed significantly higher PTX accumulation and effectively inhibited tumor growth. Modified PTX NCs displayed longer persistence in the blood indicated by a high mean residence time (MRT), area under the plasma concentration time curve (AUC), and lower clearance [108].

In another study, HA anchored paclitaxel nanocrystals HA-PTX/NC were formulated by homogenization, a top-down method. The presence of HA improved the chemotherapeutic efficacy by effective inhibition of lung metastasis in an LA-7 tumor-bearing rat model. HA coated Paclitaxel NCs with particle size around 250 nm imparted both longer circulation and tumor targeting. *In vivo* studies revealed prolonged blood circulation time, with an 8.4-fold increase of AUC 0-∞, active targeting, and reduced lung metastasis and toxicity in the LA-7 tumor bear-

ing rat model compared to Taxol™. Hyaluronic acid on PTX nanocrystals is a promising approach for anticancer therapy since the study showed a significant increase in the blood circulation time of PTX [109].

Zhang et al., also studied the advantage of preparing PEGylated paclitaxel nanocrystals by testing them on breast cancer and lung metastasis. The nanocrystals were prepared by probe sonication using antisolvent precipitation. In this work, an *in vivo* study was performed on breast cancer xenografted mice model and a model of lung tumor metastasis was quantified by luciferase activity. In both breast cancer xenografted mice and lung tumor metastasis, PEG modified NCs showing highly significant ($P<0.001$) and significant ($P<0.05$) tumor inhibition compared to saline PTX and PTX-NC groups, respectively, after intravenous administration. These studies suggest the advantages of PEGylated PTX nanocrystals as alternative drug delivery systems for anticancer therapy [110]. Similarly, effective tumor inhibition was observed by transferrin-modified PTX NCs. The study was performed on KB-bearing mice, where surface modification of NCs by transferrin showed a 45% inhibition compared to an unmodified PTX nanosuspension showing 28% inhibition. These results suggest the benefit of using a serum protein in a non-covalent manner in conjunction with paclitaxel nanocrystals as a promising drug delivery model for anticancer therapy [111].

The concentration profile of another anticancer drug, etoposide ETO, was assessed after administration of its NC formulation and Toposar (etoposide injection USP). Injection of NC formulations induced a significantly higher ETO plasma concentration than the Toposar with an AUC 0-120 min almost twofold greater and a higher mean residence time ($P<0.05$) [112, 113]. Ganta et al. intravenously injected asulacrine, ASL NCs and had a 2.7-fold lifespan enhancement in the plasma compared to the asulacrine solution. The pharmacokinetics and tissue distribution of ASL, administered either as a nanosuspension or as a solution were compared after i.v. administration to mice. By evaluating the pharmacokinetic parameters in plasma, ASL nanosuspension exhibited a significantly ($P<0.01$) reduced C_{max} and AUC (0-infinity) and a significantly ($P<0.010$) greater volume of distribution, clearance, and elimination half-life compared to the ASL solution. In contrast, the ASL nanosuspension resulted in a significantly greater AUC (0-infinity) in liver, lung, and kidney (all $P<0.01$), but not in heart [14]. Besides the solid form of NCs increasing the plasma lifetime, the use of stabilizer comprising PEG is known to reduce protein binding and therefore extend the particle's plasma concentration [114]. Therefore, drug nanocrystal formations have been found therapeutically efficient by systemic administration. Particle size reduction and stabilization modify the pharmacokinetics of nanocrystals compared to conventional drug formulations through increased absorption, distribution, and AUC. Nanocrystals possess increased surface area, increasing the area of contact between the dissolving fluid and solid, hence drug molecules are distributed in higher concentration to the infected or targeted tissues [115]. However, certain other factors may affect distribution. Usually, an equilibrium is established between drug concentration in systemic circulation and diseased targeted cells, so drug concentration may also depend on blood circulation in infected tissues.

The bioavailability characteristics of nanocrystals may be modified by coating with certain polymers. Therefore, such polymeric substances function as surface modifiers, which influence the biodistribution of nanocrystals. They are physically absorbed on a nanocrystal surface and modify the permeation and retention. For example, nevirapine nanocrystals were coated by polyethylene glycol (PEG) 1000. This reduced the phagocytosis of drug nanocrystals by macrophages [116]. PEG resists interactions with components of the blood stream. PEG coating on nanodrug carriers protects them from aggregation, opsonization, and phagocytosis, thereby prolonging systemic circulation time. For this, it is the widely used "stealth" polymer in drug delivery, due to its long history of safety in humans and classification as Generally Regarded as Safe (GRAS) by the FDA [57]. Many diseases causing deformed neovascularization like diabetes, some ocular disorders, asthma, and multiple sclerosis can be targeted by PEGylated nano carriers [117]. Furthermore, focused ultrasound nano carriers are efficient targeting agents for *in vivo* delivery to the blood brain barrier. This is a non-invasive

treatment for brain disorders, and injuries [118, 119]. This technique is attracting many pharmaceutical scientists and medical researchers for the treatment of tumors without affecting normal healthy cells.

Conclusion

Targeted drug delivery by nanocrystal preparations has remarkable potential in cancer treatment. Nano crystallization technology, by particle size reduction, increases the solubility and overcomes or minimizes bioavailability problems of poorly soluble chemotherapeutic drugs. Drug nanocrystals are targeted to tumor cells, and possess higher cellular uptake and accumulate in high concentrations in the cancer tissue. The drug targeting is due to their morphologic characteristics that facilitates passive targeting. The active targeting is possible by ligand attachment on nanocrystals, which targets nanocrystals by interaction of ligands to the receptors on cancer cells. Nanocrystals may be used for -specific targeted drug delivery with higher cell internalization, preserving normal body tissues, and minimizing drug-related side effects.

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Disclosure of conflict of interest

None.

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References

[1] Muller RH and Keck CM. Challenges and solutions for the delivery of biotech drugs–a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 2004; 113: 151-170.

[2] Bertrand N and Leroux JC. The journey of a drug-carrier in the body: an anatomo-physiological perspective. J Control Release 2012; 161: 152-163.

[3] Zhang L, Hou S, Mao S, Wei D, Song X and Lu Y. Uptake of folate-conjugated albumin nanoparticles to the SKOV3 cells. Int J Pharm 2004; 287: 155-162.

[4] Sudimack J and Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev 2000; 41: 147-162.

[5] Vader P, van der Aa LJ, Storm G, Schiffelers RM and Engbersen JF. Polymeric carrier systems for siRNA delivery. Curr Top Med Chem 2012; 12: 108-119.

[6] Veiseh O, Kievit FM, Ellenbogen RG and Zhang M. Cancer cell invasion: treatment and monitoring opportunities in nanomedicine. Adv Drug Deliv Rev 2011; 63: 582-596.

[7] Kreuter J, Petrov V, Kharkevich D and Alyautdin R. Influence of the type of surfactant on the analgesic effects induced by the peptide dalargin after its delivery across the blood-brain barrier using surfactant-coated nanoparticles. J Control Release 1997; 49: 81-87.

[8] Gao L, Liu G, Ma J, Wang X, Zhou L and Li X. Drug nanocrystals: in vivo performances. J Control Release 2012; 160: 418-430.

[9] Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov 2004; 3: 785-796.

[10] Joshi K, Chandra A, Jain K and Talegaonkar S. Nanocrystalization: an emerging technology to enhance the bioavailability of poorly soluble drugs. Pharm Nanotechnol 2019; 7: 259-278.

[11] Chang TL, Zhan H, Liang D and Liang JF. Nanocrystal technology for drug formulation and delivery. Front Chem Sci Eng 2015; 9: 1-14.

[12] Liu T, Yu X, Yin H and Möschwitzer JP. Advanced modification of drug nanocrystals by using novel fabrication and downstream approaches for tailor-made drug delivery. Drug Deliv 2019; 26: 1092-1103.

[13] Zhao J, Liu Y, Wang L, Zhou Y, Du J and Wang Y. Functional and modified nanocrystals technology for target drug delivery. J Nanosci Nanotechnol 2018; 18: 5207-5221.

[14] Ganta S, Paxton JW, Baguley BC and Garg S. Formulation and pharmacokinetic evaluation of an asulacrine nanocrystalline suspension for intravenous delivery. Int J Pharm 2009; 367: 179-186.

[15] Yusefi M, Soon ML, Teow SY, Monchouguy EI, Neerooa BNHM, Izadiyan Z, Jahangirian H, Rafiee-Moghaddam R, Webster TJ and Shameli K. Fabrication of cellulose nanocrystals as potential anticancer drug delivery systems for colorectal cancer treatment. Int J Biol Macromol 2022; 199: 372-385.

[16] Yang M, Abdalkarim SYH, Yu HY, Asad RAM, Ge D and Zhou Y. Thermo-sensitive composite microspheres incorporating cellulose nanocrys-

tals for regulated drug release kinetics. Carbohydr Polym 2023; 301: 120350.
[17] Lin Z, Mei D, Chen M, Wang Y, Chen X, Wang Z, He B, Zhang H, Wang X, Dai W, Yin Y and Zhang Q. A comparative study of thermo-sensitive hydrogels with water-insoluble paclitaxel in molecule, nanocrystal and microcrystal dispersions. Nanoscale 2015; 7: 14838-14847.
[18] Wang Y, Li H, Wang L, Han J, Yang Y, Fu T, Qiao H, Wang Z and Li J. Mucoadhesive nanocrystal-in-microspheres with high drug loading capacity for bioavailability enhancement of silybin. Colloids Surf B Biointerfaces 2021; 198: 111461.
[19] Kumar A, Valamla B, Thakor P, Chary PS, Rajana N and Mehra NK. Development and evaluation of nanocrystals loaded hydrogel for topical application. J Drug Deliv Sci Technol 2022; 74: 103503.
[20] Sarwar AR, Iqbal FM, Jamil MA and Abbas K. Nanocrystals of mangiferin using design expert: preparation, characterization, and pharmacokinetic evaluation. Molecules 2023; 28: 5918.
[21] Salgado C, Guénée L, Černý R, Allémann E and Jordan O. Nano wet milled celecoxib extended release microparticles for local management of chronic inflammation. Int J Pharm 2020; 589: 119783.
[22] Rahim S, Ghamsari MS and Radiman S. Surface modification of titanium oxide nanocrystals with PEG. Scientia Iranica 2012; 19: 948-953.
[23] Wang C, Wang M, Zhang Y, Jia H and Chen B. Cyclic arginine-glycine-aspartic acid-modified red blood cells for drug delivery: synthesis and in vitro evaluation. J Pharm Anal 2022; 12: 324-331.
[24] Chai Z, Ran D, Lu L, Zhan C, Ruan H, Hu X, Xie C, Jiang K, Li J, Zhou J, Wang J, Zhang Y, Fang RH, Zhang L and Lu W. Ligand-modified cell membrane enables the targeted delivery of drug nanocrystals to glioma. ACS Nano 2019; 13: 5591-5601.
[25] Lu Y, Li Y and Wu W. Injected nanocrystals for targeted drug delivery. Acta Pharm Sin B 2016; 6: 106-113.
[26] Gao L, Zhang D, Chen M, Duan C, Dai W, Jia L and Zhao W. Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions. Int J Pharm 2008; 355: 321-327.
[27] Malamatari M, Taylor KMG, Malamataris S, Douroumis D and Kachrimanis K. Pharmaceutical nanocrystals: production by wet milling and applications. Drug Discov Today 2018; 23: 534-547.
[28] Peltonen L and Hirvonen J. Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods. J Pharm Pharmacol 2010; 62: 1569-1579.
[29] Merisko-Liversidge E, Liversidge GG and Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci 2003; 18: 113-120.
[30] Chin WW, Parmentier J, Widzinski M, Tan EH and Gokhale R. A brief literature and patent review of nanosuspensions to a final drug product. J Pharm Sci 2014; 103: 2980-2999.
[31] Keck CM and Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm 2006; 62: 3-16.
[32] Liang P, Wu H, Zhang Z, Jiang S and Lv H. Preparation and characterization of parthenolide nanocrystals for enhancing therapeutic effects of sorafenib against advanced hepatocellular carcinoma. Int J Pharm 2020; 583: 119375.
[33] Müller RH, Gohla S and Keck CM. State of the art of nanocrystals--special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm 2011; 78: 1-9.
[34] Sharma P, Zujovic ZD, Bowmaker GA, Marshall AJ, Denny WA and Garg S. Evaluation of a crystalline nanosuspension: polymorphism, process induced transformation and in vivo studies. Int J Pharm 2011; 408: 138-151.
[35] Cerdeira AM, Gander B and Mazzotti M. Role of milling parameters and particle stabilization on nanogrinding of drug substances of similar mechanical properties. Chem Eng Technol 2011; 34: 1427-1438.
[36] Liu T, Müller RH and Möschwitzer JP. Production of drug nanosuspensions: effect of drug physical properties on nanosizing efficiency. Drug Dev Ind Pharm 2018; 44: 233-242.
[37] Salazar J, Müller RH and Möschwitzer JP. Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets. Eur J Pharm Sci 2013; 49: 565-577.
[38] Liu T, Müller RH and Möschwitzer JP. Systematical investigation of a combinative particle size reduction technology for production of resveratrol nanosuspensions. AAPS PharmSciTech 2017; 18: 1683-1691.
[39] Moorthi C and Kathiresan K. Fabrication of highly stable sonication assisted curcumin nanocrystals by nanoprecipitation method. Drug Invention Today 2013; 5: 66-69.
[40] List M and Sucker H. Pharmaceutical colloidal hydrosols for injection. GB Patent 1988; 2200048.
[41] Soliman KA, Ibrahim HK and Ghorab MM. Effects of different combinations of nanocrystallization technologies on avanafil nanoparticles: in vitro, in vivo and stability evaluation. Int J Pharm 2017; 517: 148-156.

[42] Bosselmann S, Nagao M, Chow KT and Williams RO 3rd. Influence of formulation and processing variables on properties of itraconazole nanoparticles made by advanced evaporative precipitation into aqueous solution. AAPS PharmSciTech 2012; 13: 949-960.
[43] de Waard H, Frijlink HW and Hinrichs WL. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharm Res 2011; 28: 1220-1223.
[44] Montes A, Gordillo M, Pereyra C and de la Ossa EM. Particles formation using supercritical fluids. In: Mass Transfer-Advanced Aspects. Citeseer; 2011.
[45] Spitzer D, Pichot V, Pessina F, Schnell F, Klaumünzer M and Blas L. Continuous and reactive nanocrystallization: new concepts and processes for dual-use advances. Comptes Rendus Chimie 2017; 20: 339-345.
[46] Möschwitzer J and Müller RH. New method for the effective production of ultrafine drug nanocrystals. J Nanosci Nanotechnol 2006; 6: 3145-3153.
[47] Salazar J, Müller RH and Möschwitzer JP. Combinative particle size reduction technologies for the production of drug nanocrystals. J Pharm (Cairo) 2014; 2014: 265754.
[48] Shegokar R and Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm 2010; 399: 129-139.
[49] Verma S, Gokhale R and Burgess DJ. A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions. Int J Pharm 2009; 380: 216-222.
[50] Müller RH and Keck CM. Second generation of drug nanocrystals for delivery of poorly soluble drugs: smartCrystal technology. Eur J Pharm Sci 2008; 1: S20-S21.
[51] Liu G, Zhang D, Jiao Y, Guo H, Zheng D, Jia L, Duan C, Liu Y, Tian X, Shen J, Li C, Zhang Q and Lou H. In vitro and in vivo evaluation of riccardin D nanosuspensions with different particle size. Colloids Surf B Biointerfaces 2013; 102: 620-626.
[52] Li N, Lu W, Yu J, Xiao Y, Liu S, Gan L and Huang J. Rod-like cellulose nanocrystal/cis-aconityldoxorubicin prodrug: a fluorescence-visible drug delivery system with enhanced cellular uptake and intracellular drug controlled release. Mater Sci Eng C Mater Biol Appl 2018; 91: 179-189.
[53] Zhang H, Hollis CP, Zhang Q and Li T. Preparation and antitumor study of camptothecin nanocrystals. Int J Pharm 2011; 415: 293-300.
[54] Li J, Wang Z, Zhang H, Gao J and Zheng A. Progress in the development of stabilization strategies for nanocrystal preparations. Drug Deliv 2021; 28: 19-36.
[55] Jarvis M, Krishnan V and Mitragotri S. Nanocrystals: a perspective on translational research and clinical studies. Bioeng Transl Med 2018; 4: 5-16.
[56] Suk JS, Xu Q, Kim N, Hanes J and Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev 2016; 99: 28-51.
[57] Tuomela A, Hirvonen J and Peltonen L. Stabilizing agents for drug nanocrystals: effect on bioavailability. Pharmaceutics 2016; 8: 16.
[58] Shete G, Jain H, Punj D, Prajapat H, Akotiya P and Bansal AK. Stabilizers used in nano-crystal based drug delivery systems. Journal of Excipients and Food Chemicals 2014; 5: 184-209.
[59] Dintaman JM and Silverman JA. Inhibition of P-glycoprotein by D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS). Pharm Res 1999; 16: 1550-1556.
[60] Liu Y, Huang L and Liu F. Paclitaxel nanocrystals for overcoming multidrug resistance in cancer. Mol Pharm 2010; 7: 863-869.
[61] Tang H, Zhao W, Yu J, Li Y and Zhao C. Recent development of pH-responsive polymers for cancer nanomedicine. Molecules 2018; 24: 4.
[62] Wang J, Muhammad N, Li T, Wang H, Liu Y, Liu B and Zhan H. Hyaluronic acid-coated camptothecin nanocrystals for targeted drug delivery to enhance anticancer efficacy. Mol Pharm 2020; 17: 2411-2425.
[63] Fuhrmann K, Połomska A, Aeberli C, Castagner B, Gauthier MA and Leroux JC. Modular design of redox-responsive stabilizers for nanocrystals. ACS Nano 2013; 7: 8243-8250.
[64] Georgiadis MO, Karoutzou O, Foscolos AS and Papanastasiou I. Sigma receptor (σR) ligands with antiproliferative and anticancer activity. Molecules 2017; 22: 1408.
[65] Pooja D, Gunukula A, Gupta N, Adams DJ and Kulhari H. Bombesin receptors as potential targets for anticancer drug delivery and imaging. Int J Biochem Cell Biol 2019; 114: 105567.
[66] Verma A and Stellacci F. Effect of surface properties on nanoparticle-cell interactions. Small 2010; 6: 12-21.
[67] Leroueil PR, Hong S, Mecke A, Baker JR Jr, Orr BG and Banaszak Holl MM. Nanoparticle interaction with biological membranes: does nanotechnology present a Janus face? Acc Chem Res 2007; 40: 335-342.
[68] Ding HM and Ma YQ. Role of physicochemical properties of coating ligands in receptor-mediated endocytosis of nanoparticles. Biomaterials 2012; 33: 5798-5802.
[69] Verma A, Uzun O, Hu Y, Hu Y, Han HS, Watson N, Chen S, Irvine DJ and Stellacci F. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. Nat Mater 2008; 7: 588-595.

[70] Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME and DeSimone JM. The effect of particle design on cellular internalization pathways. Proc Natl Acad Sci U S A 2008; 105: 11613-11618.
[71] Chithrani BD, Ghazani AA and Chan WC. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 2006; 6: 662-668.
[72] Gao H, Shi W and Freund LB. Mechanics of receptor-mediated endocytosis. Proc Natl Acad Sci U S A 2005; 102: 9469-9474.
[73] Martín-Sabroso C, Torres-Suárez AI, Alonso-González M, Fernández-Carballido A and Fraguas-Sánchez AI. Active targeted nanoformulations via folate receptors: state of the art and future perspectives. Pharmaceutics 2021; 14: 14.
[74] Dainty LA, Risinger JI, Morrison C, Chandramouli GV, Bidus MA, Zahn C, Rose GS, Fowler J, Berchuck A and Maxwell GL. Overexpression of folate binding protein and mesothelin are associated with uterine serous carcinoma. Gynecol Oncol 2007; 105: 563-570.
[75] Hartmann LC, Keeney GL, Lingle WL, Christianson TJ, Varghese B, Hillman D, Oberg AL and Low PS. Folate receptor overexpression is associated with poor outcome in breast cancer. Int J Cancer 2007; 121: 938-942.
[76] Toffoli G, Cernigoi C, Russo A, Gallo A, Bagnoli M and Boiocchi M. Overexpression of folate binding protein in ovarian cancers. Int J Cancer 1997; 74: 193-198.
[77] Weitman SD, Frazier KM and Kamen BA. The folate receptor in central nervous system malignancies of childhood. J Neurooncol 1994; 21: 107-112.
[78] Weitman SD, Weinberg AG, Coney LR, Zurawski VR, Jennings DS and Kamen BA. Cellular localization of the folate receptor: potential role in drug toxicity and folate homeostasis. Cancer Res 1992; 52: 6708-6711.
[79] Weitman SD, Lark RH, Coney LR, Fort DW, Frasca V, Zurawski VR Jr and Kamen BA. Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues. Cancer Res 1992; 52: 3396-3401.
[80] Hutson JR, Stade B, Lehotay DC, Collier CP and Kapur BM. Folic acid transport to the human fetus is decreased in pregnancies with chronic alcohol exposure. PLoS One 2012; 7: e38057.
[81] Salazar MD and Ratnam M. The folate receptor: what does it promise in tissue-targeted therapeutics? Cancer Metastasis Rev 2007; 26: 141-152.
[82] Dong S, Cho HJ, Lee YW and Roman M. Synthesis and cellular uptake of folic acid-conjugated cellulose nanocrystals for cancer targeting. Biomacromolecules 2014; 15: 1560-1567.
[83] Bittleman KR, Dong S, Roman M and Lee YW. Folic acid-conjugated cellulose nanocrystals show high folate-receptor binding affinity and uptake by KB and breast cancer cells. ACS Omega 2018; 3: 13952-13959.
[84] Talekar M, Ganta S, Amiji M, Jamieson S, Kendall J, Denny WA and Garg S. Development of PIK-75 nanosuspension formulation with enhanced delivery efficiency and cytotoxicity for targeted anti-cancer therapy. Int J Pharm 2013; 450: 278-289.
[85] Xu J, Shen X, Jia L, Xu Z, Zhou T, Li X, Ma T, Li H, Wang Y and Zhu T. Facile synthesis of folic acid-conjugated fluorapatite nanocrystals for targeted cancer cell fluorescence imaging. Materials Letters 2017; 203: 37-41.
[86] Monteiro CAP, Oliveira ADPR, Silva RC, Lima RRM, Souto FO, Baratti MO, Carvalho HF, Santos BS, Cabral Filho PE and Fontes A. Evaluating internalization and recycling of folate receptors in breast cancer cells using quantum dots. J Photochem Photobiol B 2020; 209: 111918.
[87] Liu F, Park JY, Zhang Y, Conwell C, Liu Y, Bathula SR and Huang L. Targeted cancer therapy with novel high drug-loading nanocrystals. J Pharm Sci 2010; 99: 3542-3551.
[88] Gidwani B, Sahu V, Shukla SS, Pandey R, Joshi V, Jain VK and Vyas A. Quantum dots: prospectives, toxicity, advances and applications. J Drug Deliv Sci Technol 2021; 61: 102308.
[89] Almalik A, Donno R, Cadman CJ, Cellesi F, Day PJ and Tirelli N. Hyaluronic acid-coated chitosan nanoparticles: molecular weight-dependent effects on morphology and hyaluronic acid presentation. J Control Release 2013; 172: 1142-1150.
[90] Pang Z, Gao H, Yu Y, Chen J, Guo L, Ren J, Wen Z, Su J and Jiang X. Brain delivery and cellular internalization mechanisms for transferrin conjugated biodegradable polymersomes. Int J Pharm 2011; 415: 284-292.
[91] Han S, Li X, Zhou C, Hu X, Zhou Y, Jin Y, Liu Q, Wang L, Li X and Liu Y. Further enhancement in intestinal absorption of paclitaxel by using transferrin-modified paclitaxel nanocrystals. ACS Appl Bio Mater 2020; 3: 4684-4695.
[92] Sohn JS, Yoon DS, Sohn JY, Park JS and Choi JS. Development and evaluation of targeting ligands surface modified paclitaxel nanocrystals. Mater Sci Eng C Mater Biol Appl 2017; 72: 228-237.
[93] Choi JS and Park JS. Development of docetaxel nanocrystals surface modified with transferrin for tumor targeting. Drug Des Devel Ther 2017; 11: 17-26.
[94] Qi Y, Zhang T, Jing C, Liu S, Zhang C, Alvarez PJJ and Chen W. Nanocrystal facet modulation to enhance transferrin binding and cellular delivery. Nat Commun 2020; 11: 1262.

[95] Penna MJ, Mijajlovic M and Biggs MJ. Molecular-level understanding of protein adsorption at the interface between water and a strongly interacting uncharged solid surface. J Am Chem Soc 2014; 136: 5323-5331.
[96] Lau BL and Hsu-Kim H. Precipitation and growth of zinc sulfide nanoparticles in the presence of thiol-containing natural organic ligands. Environ Sci Technol 2008; 42: 7236-7241.
[97] Wang L, Li J, Pan J, Jiang X, Ji Y, Li Y, Qu Y, Zhao Y, Wu X and Chen C. Revealing the binding structure of the protein corona on gold nanorods using synchrotron radiation-based techniques: understanding the reduced damage in cell membranes. J Am Chem Soc 2013; 135: 17359-17368.
[98] Kowalik P, Bujak P, Penkala M and Pron A. Organic-to-aqueous phase transfer of alloyed AgInS(2)-ZnS nanocrystals using simple hydrophilic ligands: comparison of 11-mercaptoundecanoic acid, dihydrolipoic acid and cysteine. Nanomaterials (Basel) 2021; 11: 843.
[99] Rossier B, Jordan O, Allémann E and Rodríguez-Nogales C. Nanocrystals and nanosuspensions: an exploration from classic formulations to advanced drug delivery systems. Drug Deliv Transl Res 2024; [Epub ahead of print].
[100] Sharma S, Verma A, Teja BV, Shukla P and Mishra PR. Development of stabilized paclitaxel nanocrystals: in-vitro and in-vivo efficacy studies. Eur J Pharm Sci 2015; 69: 51-60.
[101] Jablonka L, Ashtikar M, Gao G, Jung F, Thurn M, Preuß A, Scheglmann D, Albrecht V, Röder B and Wacker MG. Advanced in silico modeling explains pharmacokinetics and biodistribution of temoporfin nanocrystals in humans. J Control Release 2019; 308: 57-70.
[102] Lv PP, Wei W, Yue H, Yang TY, Wang LY and Ma GH. Porous quaternized chitosan nanoparticles containing paclitaxel nanocrystals improved therapeutic efficacy in non-small-cell lung cancer after oral administration. Biomacromolecules 2011; 12: 4230-4239.
[103] Ci LQ, Huang ZG, Lv FM, Wang J, Feng LL, Sun F, Cao SJ, Liu ZP, Liu Y, Wei G and Lu WY. Enhanced delivery of imatinib into vaginal mucosa via a new positively charged nanocrystal-loaded in situ hydrogel formulation for treatment of cervical cancer. Pharmaceutics 2019; 11: 15.
[104] Vakili MR, Mohammed-Saeid W, Aljasser A, Hopwood-Raja J, Ahvazi B, Hrynets Y, Betti M and Lavasanifar A. Development of mucoadhesive hydrogels based on polyacrylic acid grafted cellulose nanocrystals for local cisplatin delivery. Carbohydr Polym 2021; 255: 117332.
[105] Sigfridsson K, Xue A, Goodwin K, Fretland AJ and Arvidsson T. Sustained release and improved bioavailability in mice after subcutaneous administration of griseofulvin as nano- and microcrystals. Int J Pharm 2019; 566: 565-572.
[106] Cobb DA, Smith N, Deodhar S, Bade AN, Gautam N, Shetty BLD, McMillan J, Alnouti Y, Cohen SM, Gendelman HE and Edagwa B. Transformation of tenofovir into stable ProTide nanocrystals with long-acting pharmacokinetic profiles. Nat Commun 2021; 12: 5458.
[107] Ji P, Wang L, Chen Y, Wang S, Wu Z and Qi X. Hyaluronic acid hydrophilic surface rehabilitating curcumin nanocrystals for targeted breast cancer treatment with prolonged biodistribution. Biomater Sci 2020; 8: 462-472.
[108] Zhao J, Du J, Wang J, An N, Zhou K, Hu X, Dong Z and Liu Y. Folic acid and poly(ethylene glycol) decorated paclitaxel nanocrystals exhibit enhanced stability and breast cancer-targeting capability. ACS Appl Mater Interfaces 2021; 13: 14577-14586.
[109] Sharma S, Singh J, Verma A, Teja BV, Shukla RP, Singh SK, Sharma V, Konwar R and Mishra P. Hyaluronic acid anchored paclitaxel nanocrystals improves chemotherapeutic efficacy and inhibits lung metastasis in tumor-bearing rat model. RSC advances 2016; 6: 73083-73095.
[110] Zhang H, Hu H, Zhang H, Dai W, Wang X, Wang X and Zhang Q. Effects of PEGylated paclitaxel nanocrystals on breast cancer and its lung metastasis. Nanoscale 2015; 7: 10790-10800.
[111] Lu Y, Wang ZH, Li T, McNally H, Park K and Sturek M. Development and evaluation of transferrin-stabilized paclitaxel nanocrystal formulation. J Control Release 2014; 176: 76-85.
[112] Martin B, Seguin J, Annereau M, Fleury T, Lai-Kuen R, Neri G, Lam A, Bally M, Mignet N and Corvis Y. Preparation of parenteral nanocrystal suspensions of etoposide from the excipient free dry state of the drug to enhance in vivo antitumoral properties. Sci Rep 2020; 10: 18059.
[113] Ige PP, Baria RK and Gattani SG. Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability. Colloids Surf B Biointerfaces 2013; 108: 366-373.
[114] Peracchia MT, Harnisch S, Pinto-Alphandary H, Gulik A, Dedieu JC, Desmaële D, d'Angelo J, Müller RH and Couvreur P. Visualization of in vitro protein-rejecting properties of PEGylated stealth polycyanoacrylate nanoparticles. Biomaterials 1999; 20: 1269-1275.
[115] Andes D. In vivo pharmacodynamics of antifungal drugs in treatment of candidiasis. Antimicrob Agents Chemother 2003; 47: 1179-1186.
[116] Grande F, Ioele G, Occhiuzzi MA, De Luca M, Mazzotta E, Ragno G, Garofalo A and Muzza-

lupo R. Reverse transcriptase inhibitors nanosystems designed for drug stability and controlled delivery. Pharmaceutics 2019; 11: 197.
[117] Alexis F, Pridgen E, Molnar LK and Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 2008; 5: 505-515.
[118] Nance E, Timbie K, Miller GW, Song J, Louttit C, Klibanov AL, Shih TY, Swaminathan G, Tamargo RJ, Woodworth GF, Hanes J and Price RJ. Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood-brain barrier using MRI-guided focused ultrasound. J Control Release 2014; 189: 123-132.
[119] Kennedy JE, Wu F, ter Haar GR, Gleeson FV, Phillips RR, Middleton MR and Cranston D. High-intensity focused ultrasound for the treatment of liver tumours. Ultrasonics 2004; 42: 931-935.
[120] Wang Y, Li X, Wang L, Xu Y, Cheng X and Wei P. Formulation and pharmacokinetic evaluation of a paclitaxel nanosuspension for intravenous delivery. Int J Nanomedicine 2011; 6: 1497-1507.
[121] Swierniak M, Wojcicka A, Czetwertynska M, Dlugosinska J, Stachlewska E, Gierlikowski W, Kot A, Gornicka B, Koperski L, Bogdanska M, Wiechno W and Jazdzewski K. Association between GWAS-derived rs966423 genetic variant and overall mortality in patients with differentiated thyroid cancer. Clin Cancer Res 2016; 22: 1111-1119.
[122] Zhan H and Liang JF. Extreme activity of drug nanocrystals coated with a layer of non-covalent polymers from self-assembled boric acid. Sci Rep 2016; 6: 38668.
[123] Choi JS and Park JS. Surface modification of docetaxel nanocrystals with HER2 antibody to enhance cell growth inhibition in breast cancer cells. Colloids Surf B Biointerfaces 2017; 159: 139-150.
[124] Kong FH, Ye QF, Miao XY, Liu X, Huang SQ, Xiong L, Wen Y and Zhang ZJ. Current status of sorafenib nanoparticle delivery systems in the treatment of hepatocellular carcinoma. Theranostics 2021; 11: 5464-5490.
[125] Sheng H, Zhang Y, Nai J, Wang S, Dai M, Lin G, Zhu L and Zhang Q. Preparation of oridonin nanocrystals and study of their endocytosis and transcytosis behaviours on MDCK polarized epithelial cells. Pharm Biol 2020; 58: 518-527.
[126] Saljoughi H, Khakbaz F and Mahani M. Synthesis of folic acid conjugated photoluminescent carbon quantum dots with ultrahigh quantum yield for targeted cancer cell fluorescence imaging. Photodiagnosis Photodyn Ther 2020; 30: 101687.
[127] Haddad R, Alrabadi N, Altaani B and Li T. Paclitaxel drug delivery systems: focus on nanocrystals' surface modifications. Polymers (Basel) 2022; 14: 658.