Formulation and Evaluation of Nanocrystals for improvement dissolution and oral absorption of poorly soluble drugs

Gautam Kumar Ray[1], Amiya Kumar Prusty[2*], Sanjeev Kumar[3], Chinmaya Sahoo[4], Rajat Kumar Prusty[5], Sangita Ghosh[6], Sourav Kumar Sahoo[7]

[2*]Faculty of Pharmacy, C.V. Raman Global University, Bhubaneswar. -752054

[1,3,4,5,6,7]Ph.D. Research Scholar, Faculty of Pharmacy, C.V. Raman Global University, Bhubaneswar, Odisha, India-752054

Corresponding author email : amiyaprusty@gmail.com

Received: 26th May, 2024; Revised and Accepted: 17th Jun, 2025; Available Online: 25th July, 2025

Abstract

Nanocrystal formulation key elements of consideration; stabilizers; the importance of key processing parameters will be outlined. The physicochemical characteristics of nanoparticles (e.g., size and polydispersity index (PDI), Zeta potential (ZP), morphology, crystalline structure, drug release profiles, and stability) and their absorption and lymph uptake mechanism in the gastrointestinal (GI) tract are tabulated in Table1. About 40-70% of new drug candidates exhibit poor solubility in aqueous media, resulting in slow dissolution and absorption from the GI tract and lower bioavailability; thus, nanocrystal technology provides a solution to this problem. Thus, it can be concluded that pharmaceutical nanocrystals are an interesting technology that has many applications and has shown success in the clinic to achieve successful delivery of water-insoluble drug substances. The review elaborates scientific principles behind nanocrystal technology, including size reduction, surface energy, dissolution kinetics, and formation of nanocrystals by top-down approach (milling and grinding), bottom-up approach (precipitation, anti-solvent crystallization, and emulsion-based methods), and hybrid or combination approach (SmartCrystal). Nanocrystal formulation considerations, importance of stabilizers, and critical processing parameters are discussed in brief, and the applicability of Quality by Design (QbD) to develop robust and reproducible nanocrystal formulation. Physicochemical characterization of nanocrystals, like Particle size (PS) and polydispersity index, ZP, morphology, crystalline state, dissolution performance, and stability, as well as mechanisms of absorption and lymphatic uptake from the GI tract, are summarized. Commercial examples, recent advances in oral nanocrystal dosage forms, scale-up and manufacturing challenges, regulatory issues, and new approaches in nanocrystal drug delivery systems are reviewed. In conclusion, pharmaceutical nanocrystals are an attractive drug delivery technology with broad applicability and clinical success in achieving successful delivery of water-insoluble drug substances.

Keywords: Nanocrystals, Poorly water soluble drugs, dissolution enhancement, oral bioavailability, Quality by Design

How to cite this article: Ray GK, Prusty AK, Kumar S, Sahoo C, Prusty RK, Ghosh S, Sahoo SK. Formulation and Evaluation of Nanocrystals for Improvement Dissolution and Oral Absorption of Poorly Soluble Drugs. Int J Drug Deliv Technol. 2026;16(72s): 1314-1334. DOI: 10.25258/ijddt.16.72s.143

1. Introduction

Poor aqueous solubility remains one of the most prominent issues facing today's drug development, accounting for as much as 40% of all marketed drugs and around 70-90% of newly discovered drugs being poorly water soluble. The Oral absorption of most drugs is dependent upon their solubility and dissolution rate in GI fluids. Thus, poor solubility can often lead to slow dissolution, incomplete absorption, significant patient-to-patient variability, and diminished efficacy[1]. The dimensions of such particles are on the scale of 100-1000 nm, and these are made solely from the active pharmaceutical ingredients (APIs) in a crystalline, free-flowing form, kept from agglomerating by the addition of other agents. Various techniques for enhancing the solubility and dissolution rate of poorly soluble drugs have been reported, e.g., size reduction, solid dispersion, formation of salts, cyclodextrin inclusion complexes, co-crystals, SEDDS, micellar solutions, co-solvents and surfactants, etc. Most of these methods suffer from various disadvantages like very poor encapsulation efficiency, poor physicochemical properties, and limitations of scale-up and suitable drug loading range.

Poor aqueous solubility reduces the quantity of dissolved drug present in gastrointestinal contents, consequently impairing absorption of drugs; other causes for this problem are possible: the wettability of the drug, the dissolution rate; poor stability of the drug; effect of food; metabolism during first-pass hepatic; transporters mediating efflux of the drugs from cells[2].

High dosages therefore become necessary to attain a therapeutic level of a drug, thus increasing potential for toxicity and patient compliance problems. Several methods for improving the solubility and dissolution rate of poorly soluble drugs have been reported, for example, particle size (PS) reduction, solid dispersion, formation of salts, cyclodextrin inclusion complexes, co-crystals, self-emulsifying drug delivery systems (SEDDS), micellar solutions,

and co-solvents and surfactants, etc. Many such methods pose challenges like very poor drug loading capacity, instability of physicochemical nature, and difficulty during scale-up, and a restricted range of suitable drugs. It has inspired an evolution towards better, faster methods.

Pharmaceutical nanocrystals have arisen as an encouraging means for tackling difficulties faced in the dissolution of these poorly soluble drugs, along with increasing gastrointestinal absorption and oral availability. The size of such particles ranges from 100 nm to 1000 nm, and they are entirely comprised of the APIs in a crystalline state, but are prevented from clumping through the use of stabilisers. Because the surface area is much greater and wettability and solubility are improved, they disperse and dissolve faster according to the Noyes-Whitney equation. In this review, this review discusses pharmaceutical nanocrystal technology as a modern approach to overcome these shortcomings and achieve enhanced dissolution and absorption of poor water-soluble drugs[3]. The review covers the fundamental aspects of nanocrystal technology, choice of excipients for their formulation, preparation methods, Quality by Design (QbD) based process optimization, and characterization. This study also discusses the mechanism of increased drug dissolution and gastrointestinal absorption, their therapeutic applications, current marketed products, and considerations for manufacturing and regulatory requirements, along with prospects. Our purpose is to summarize the state-of-the-art and provide a valuable reference to researchers and formulation scientists for the successful design and commercialization of nanocrystal-based oral drug delivery.

2. Poorly Soluble Drugs: Challenges in Oral Drug Delivery

Poor aqueous solubility is considered to be the main problem for successful oral delivery of a lot of drug products. Oral drug delivery success depends on the rate at which a drug dissolves in GI fluid, followed by permeability through the intestinal barrier. Consequently, the poor solubility of the drugs leads to inadequate or inconsistent absorption in the GI tract.

For drugs in BCS class II and class IV, poor aqueous solubility often leads to slow dissolution as the rate-limiting process of bioavailability. Other than solubility-related parameters, other formulation and physiological variables also hinder effective oral drug delivery, including slow dissolution, extensive first-pass metabolism, food-dependent absorption, and variability among individuals, etc. For many orally administered drugs, improvement of their dissolution rate remains the main goal in pharmaceutical formulation development[4].

2.1 Solubility-Limited Absorption

After oral administration, poorly soluble drugs will first dissolve in GI fluids before getting into the bloodstream. The slower dissolution rates of poorly soluble drugs mean a smaller amount of dissolved drug concentration for absorption through the intestine. The drug with higher permeability might still suffer poor absorption due to poor dissolution. Due to this, poor bioavailability of the drug, slow onset of action, increased interindividual variability, and poor therapeutic effects might occur with the oral administration of poorly soluble drugs[5].

2.2 Dissolution Rate Limitations

Dissolution rate limits overall drug absorption via the oral route. As depicted by the Noyes-Whitney equation, the dissolution rate depends on the surface area of particles and the concentration gradient between the surface and solution[6].

2.3 First-Pass Metabolism

Most orally absorbed drugs pass first the intestinal wall and the liver, where they may be metabolized into inert products or active metabolites before they are introduced into the systemic circulation. This so-called “first pass metabolism” might drastically decrease the total amount of drug reaching the systemic circulation, particularly in the case of those compounds with a high extraction ratio from the liver. The enhanced dissolution rate itself is unlikely to reduce first-pass metabolism, but, in the case of rapid and complete dissolution and subsequent quick absorption of the drug from the GI tract, a greater fraction of the dose will reach the systemic circulation before undergoing extensive metabolism. Additionally, the formulated nanocrystal can also facilitate some alternate absorptive pathways such as absorption via the lymphatic system, thus decreasing the overall first-pass effect to a certain extent[7].

2.4 Food Effects

Gastrointestinal contents in the form of food can influence the oral absorption and hence bioavailability of poorly soluble drugs significantly. Administration of food affects the time taken to empty gastric contents, motility of intestinal fluids, secretion of bile, and pH of GI fluid, and composition of the luminal contents, which affect solubility and absorption of the drug. A fat-rich diet tends to improve absorption of lipophilic poorly soluble drugs by stimulation of bile salt secretion, whereas drugs given under fasted state are much less absorbed[8]. Food interaction leading to poor bioavailability also dictates poor therapy for these drugs, and complicates their dosing schedule, thus necessitating the design of food-neutral formulations.

2.5 Variability in Oral Bioavailability

The amount of an orally administered drug that gains access to the systemic circulation without metabolic alteration is referred to as oral bioavailability. A higher intra-individual variability in poorly soluble drugs is found because of differences in gastro- intestinalphysiological conditions, stomach pH, transit time in the gastrointestinal tract, release of bile secretion, activity of enzymes and transporters, age of individuals, disease conditions, food pattern, etc. Apart from the formulation-related factors such as PS, shape of crystals, presence of multiple polymorphic forms, and excipients used for formulation, these factors significantly influence the process of oral absorption of the drug, which may ultimately lead to a greater variation in oral bioavailability[9].

3. Pharmaceutical Nanocrystals: Fundamentals and Classification

Pharmaceutical nanocrystals consist of 100-1000 nm crystalline particles of APIs without any significant formulation components or carriers to prevent aggregation by means of a minimal amount of stabilizers, such as polymers or surfactants, at the particle surface. They represent the drug carrier-free system where no carriers have been introduced, and the crystalline nature of the drug molecule has been maintained at the same time, enhancing its dissolution by means of a dramatically enhanced surface area of the drug. Consequently, reduction in PS not only improves saturation solubility, dissolution velocity, and interaction with biological fluid, which in turn increases the oral absorption and oral bioavailability of poor water-soluble drugs. Due to these benefits, the use of nanocrystal technology as a formulation strategy for BCS class II and class IV drugs has gained widespread popularity[10].

3.1 Characteristics

Pharmaceutical nanocrystals are particles within a range of about 100-1000 nm having various desirable physicochemical characteristics that contribute to enhanced performance of drugs in oral delivery. They have the advantage of sub-micron dimensions, which lead to a substantially high specific surface area, rapid dissolution rate, improved dissolution, and thus significantly enhance the solubility. Because they consist of a crystalline form of the drug, they provide high loading capacities while having a limited need for stabilizers.

They provide wetting and improve saturation solubility and further help the attachment with the gastrointestinal tract, hence resulting in greater dissolution. Depending on the dosage form requirements, nanocrystals are usually developed as suspensions, tablets, capsules, pellets, or orally disintegrating tablets. Due to versatility, ease of manufacturing scale-up, scalability of production process, compatibility with most excipients, and their applicability to both new drug molecules and reformulation of existing molecules, these have gained significant attention[11].

3.2 Nanoparticles vs. Nanocrystals

Though these nanoparticles and nanocrystals have been used as one. But there are many differences both structurally as well as functionality aspects. The term nanoparticles usually refers to systems based on a carrier wherein the drug gets loaded, encapsulated, embedded, dispersed, or encapsulated within the matrix of the carrier, which is usually a polymer, lipid, or organic material. On the other hand, nanocrystals are formed of a solid drug that has a fine layer of stabilizers such as surfactants or polymer adsorbed on the surface, and no carrier matrix has been introduced in such cases. Therefore, the formulation of drug nanoparticles possesses good drug loading, limited chemical components, and minimal risk of toxicity of carriers and, most importantly, increased bioavailability in comparison to other known nanocarrier formulations. Nanoparticles are used for targeted drug delivery, controlled drug delivery systems, and to prevent drug degradation[12].

3.3 Types of Nanocrystals

Pharmaceutical nanocrystals can be classified broadly depending on its content, size range, manufacturing process, and application into pure drug nanocrystals and surface modified nanocrystals depending upon their content whereas classified into different based on the methods of preparation such as down-top (media milling), up-down(precipitation) and combination (SmartCrystal technology); Also depending on the dose form, nanocrystals are formulated as capsules, tablets, suspension, pellets etc[13].

3.4 Advantages and Disadvantages of Nanocrystals

Nanocrystals provide numerous advantages over the existing conventional approaches to increase solubility. Nanocrystals, having a high specific surface area and low PS, exhibit drastically increased solubility and faster dissolution rate, leading to improved absorption and oral bioavailability. Also, they ensure high drug loading capacity because of free from carriers; the formulations contain simplified composition and can be produced with ease, with scale-up of production using common and scalable equipment, has been approved clinically for several drugs. However, because of the high surface energy of these nanomaterials, they tend to aggregate and crystallize, particularly during processing and storage. Therefore, the selection of a proper stabilization system is a must. Other issues like Ostwald ripening, polymorphism, and growth also

need to be considered in the long-term stability of nanocrystals[14]. Scale-up, drying process, regulatory considerations, and QC are other challenges for nanocrystal formulation development[15].

4. Scientific Basis of Nanocrystal Technology

4.1 Noyes–Whitney Equation

It can be largely attributed to the increased dissolution performance for drug nanocrystals from the Noyes-Whitney equation that correlates the dissolution rate of a drug to drug properties. This relationship has demonstrated the dependence of dissolution rate on the surface area and the concentration difference. DC/dt is the dissolution rate, D is the diffusion coefficient, A is the surface area of the drug particle, Cs is the saturation solubility, C is the drug concentration in the solution, h is the thickness of the diffusion layer, and V is the volume in which dissolution occurs (Eq. 1)[16].

$$\frac{dC}{dt} - \frac{DA\,(Cs-C)}{hV} \text{--- (1)}$$

In particular, PS reduction to the nanoscale greatly increases the available surface area of the particles, and thus greatly improves the rate of drug dissolution as well as the absorption after oral administration. As a result, nanocrystal technology could efficiently address dissolution-limited absorption associated with poorly soluble drugs.

4.2 Ostwald–Freundlich Equation

The Ostwald-Freundlich equation was used to describe the increase in saturation solubility with the decrease in PS. When the PS falls in the nanoscale range, with a decrease in size, an increase in surface curvature leads to increased surface free energy, thus leading to increased molecular motion on particle surfaces. Due to increased surface energy, the saturation solubility value is higher in the case of larger crystals; for instance, where S is saturation solubility of nanoparticles, S is the saturation solubility of bulk, is surface tension, M is molecular weight, V is molar volume, r is particle radius, R is the gas constant, and T is absolute temperature[17]. Decreasing the radius causes an increase in saturation solubility, which is reflected as a stronger concentration gradient, resulting in a faster dissolution rate and an increase in absorption (Eq.2).

$$In\frac{S}{S\alpha} - \frac{2\gamma MV}{rRT} \text{--- (2)}$$

4.3 Kelvin Equation

Particle curvature effects on surface energy and vapor pressure were explained in more detail by the Kelvin equation. Because nanocrystals are characterized by surfaces of very high curvature, the particles will have a higher thermodynamic energy as compared to larger particles[18]. This high surface energy increases the apparent solubility of drugs and allows quick dissolution in the gastrointestinal environment (Eq.3).

$$In\frac{P}{Po} - \frac{2\gamma V}{rRT} \text{--- (3)}$$

Where P is the vapor pressure of the curved particle, Po is the vapor pressure of a flat surface, γ is the surface tension, Vm is the molar volume, r is the radius of the particle, R is the universal gas constant, and T is the absolute temperature. Originally, it was used to explain vapor pressure, but it can be used theoretically to explain increases in thermodynamic activity and improved dissolution of nanodrug crystals.

4.4 High Saturation Solubility

A primary advantage of nanocrystal technology is to enhance the apparent saturation solubility of poor water-soluble drugs. Nanocrystal particles, on a nanometer-scale, present a larger surface curvature and higher surface free energy, thereby increasing interaction with the dissolution medium. This elevated thermodynamic activity causes an increase in the local dissolved drug concentration at the particle surface, creating a larger concentration gradient that drives dissolution diffusion across the GI membrane. Increased saturation solubility is particularly significant for the absorption of poorly water-soluble drugs of BCS Class II and Class IV, where dissolution is a rate-limiting factor[19].

4.5 Increased Dissolution Rate

Compared to conventional microcrystals, nanocrystals exhibit rapid dissolution of the drug because of their high surface area and improved wettability. According to the Noyes-Whitney equation, decreasing PS results in increased available surface area for contact with the solvent, leading to an accelerated rate of dissolution. Furthermore, increased saturation solubility generated due to nanosized particles can also be regarded as a contribution to increasing the driving force for absorption. A combination of large surface area and improved saturation solubility can produce very rapid drug dissolution[20].

4.6 Enhanced Adhesiveness

The extremely small PS of nanocrystals with high surface energy causes their enhanced adhesion on gastrointestinal mucosa. This prolonged interaction with the gastrointestinal epithelium surface provides sufficient residence time for the nanoparticles on the mucosal lining and enhances drug dissolution and absorption. Stabilizers used to prepare nanocrystals, like hydrophilic polymers and surfactants, enhance the wettability of the particle and help closer contact

with the mucus layer, thus increasing surface-to-mucus interaction, leading to an improvement in drug permeability, and thereby preventing loss of undissolved drug during passage in the GI tract[21].

4.7 Improved Gastrointestinal Residence

Pharmaceutical nanocrystals also possess the characteristic property of enhanced gastrointestinal residence due to their nanosized dimensions and good mucoadhesion characteristics, thus increasing time and opportunity for drug dissolution and absorption in the GI tract. Interaction of nanoparticles with the intestinal mucus prolongs the clearance of nanoparticles from the intestinal surface and ensures prolonged local drug concentration on absorptive epithelium. Rapid dissolution of drug from nanocrystals creates a supersaturated microenvironment that promotes prolonged absorption of dissolved drug before precipitation in the GI tract[22].

5. Materials Used in Nanocrystal Formulation

To optimize nanocrystal formulation, several factors need to be considered. These include selection of the correct formulation materials for PS reduction, physical stability, and dissolution rate. Despite most being formed primarily with the APIs, they require other components called excipients (stabilisers, cryoprotectants, surface modifiers) which perform critical roles: preventing particles from clumping together to remain nano-sized, enabling redispersibility, aiding with passage through the stomach and into the intestine. The drug's own properties such as solubility, crystallinity, melting point, hydrophobic or hydrophilic properties and structure all play a role in whether the formulation strategy is viable, so the proper choice of formulation materials can ultimately decide the success of an oral, injected or even topically administered formulation.

5.1 Drug Candidates

Designing the best nanocrystal requires selecting the best possible drug. Generally, the best drugs are very poorly water-soluble (BCS class II), yet they still manage to pass through the membranes of the intestines (high membrane permeability) and/or they are BCS Class IV drugs. Only small amounts of the drug are required for activity; High oral bioavailability (dose dependent and absorption process dependent; dissolution dependent oral absorption – systemic absorption is determined by the rate of dissolution of the drug); Drugs which are high lipophilicity, intrinsically low aqueous solubility and reasonable chemical stability can benefit greatly from nanonisation since size reduction greatly increases surface area and consequently, surface phenomena like dissolution rate and saturation solubility increase without modification of the molecule structure which contributes to oral bioavailable drug. There have been many successful nanocrystal drugs already, e.g., cancer, antifungal, inflammation, anti-diabetes, anti-viral, cardiovascular[23].

5.2 Stabilizers

Since particles of nano-size have a very high surface area to volume ratio and are inherently unstable, several materials are added to prevent them from clumping or 'growing' while they are stored. This process, referred to as stabilisation, depends on a variety of factors such as the drug being used, how it's processed and what form it will take after manufacturing. Ideally, a stabilizer should be biocompatible, form a tight bond with the drug's surface, not be toxic and be compatible with standard pharmaceutical production methods[24].

5.2.1 Poloxamers

Poloxamers (Pluronic F68 and F127) are non-ionic polymers widely used as stabilisers in both oral, injected and topical nanocrystal applications. This type of polymer, which has both polyethylene oxide (PEO) and polypropylene oxide (PPO) components, can form an outer layer of steric protection around particles. Poloxamers also help reduce interfacial tension, the 'energy' at the surface of two different materials (e.g., the drug and the liquid medium), assist with PS reduction, improve solubility and help to maintain a stable physical dispersion over time[25].

5.2.2 Polyvinylpyrrolidone (PVP)

This polymer readily attaches to the crystal surface, often via hydrogen bonds. PVP can prevent Ostwald ripening – the growth of large crystals at the expense of small ones – and can improve the ability of the nanoparticles to wet easily in water and disperse rapidly. There are different grades of PVP, such as PVP K30 and K90, available to tailor formulation to a patient's needs[26].

5.2.3 Hydroxypropyl Methylcellulose (HPMC)

As a cellulosic derivative, HPMC creates a barrier of hydration or hydrophilicity around the nanoparticle to stop them from aggregating. It is also useful for suspending particles and reducing settling. Because of its capacity to form gels or films, and for being readily 'sticky' to mucous membranes, it's useful in a wide range of formulation types, including oral solids and films. Different forms and weights of HPMC exist for different applications.

5.2.4 Soluplus

The nature of soluplus being an amphiphilic copolymer; it has two different parts: hydrophobic and hydrophilic ones. This double action enables it not only to deliver solid stabilization for poorly soluble drugs, but also to facilitate solubility. Soluplus can also help to inhibit Ostwald ripening,

improve drug wetting, promote release after dissolution, and increase oral uptake by maintaining supersaturation in the intestinal environment. It has become increasingly popular in recent years as a combined stabilising and solubilising system.

5.2.5 Polyethylene Glycol (PEG)

The PEG molecules adsorb onto the particle surface, creating steric hindrance to aggregation, and improving wettability and dispersibility. Being a polymer, the length of its chains (molecular weight) can be varied to adjust the properties of the suspension or formulation. It also shows very good tolerability for use in humans and has been used in many applications, including drug delivery.

5.2.6 Tween (Polysorbates)

Tween compounds, particularly Tween 20 and 80 (which are non-ionic surfactants), are common stabilisers in many different types of formulations. For nanocrystals, their primary role is to reduce the surface tension between the drug particles and the dispersion liquid, thus aiding their breakdown into nanoparticles and helping to prevent re-aggregation. They can also improve the solubility, wettability and overall physical stability of nanosuspensions, and are commonly employed in orally administered and injectable drug products.

5.2.7 Lecithin

Lecithin is a natural compound (a phospholipid) and an increasingly popular choice for oral, injectable and even nutraceutical nanocrystal formulations. Its strong binding to hydrophobic drug surfaces, combined with its amphiphilic nature, leads to both physical steric stabilisation and improved wetting. Lecithin also aids in interaction with biological interfaces and can potentially influence the passage of nanocrystals across membranes to increase absorption.

5.3 Cryoprotectants

Cryoprotectants are important in the process of lyophilization of nanocrystal suspensions, during which they avoid size changes and permanent aggregations of particles. When frozen, ice crystal formation exerts high pressure on the particles that causes particle swelling and melting with the consequent change in size and growth. Mannitol, trehalose, sucrose, glucose, lactose, and sorbitol can form a solid and non-crystalline, amorphous barrier around nanoparticles when frozen, so that they are protected against mechanical forces exerted by ice crystals, thus preserving original physical and chemical properties. Besides this improved re-dispersibility of nanoparticles following the re-hydration and stabilization during storage, the formulation with these cryoprotectants also helps in the formation of the lyophilized powder form, which may be directly formulated in tablets, capsules, and sachets[27].

5.4 Surface Modifiers

These compounds not only stabilize the nanoparticles, but also alter physicochemical and biological characteristics, i.e., improve their wettability, mucoadhesion, permeation through the mucus layer and/or cellular uptake and gastrointestinal residency, and can even provide the basis of targeted drug delivery systems. PEG works to prolong circulation time in the bloodstream through both steric stabilization and engagement of phagocytes. Chitosan will increase the mucoadhesion and the permeability through intestinal epithelia. The addition of HA facilitated cellular uptake through receptor-mediated targeting (RHAMM receptor on HA); albumin helped in biocompatibility and in further targeting towards specific cell types such as cancer cells, and various targeting ligands (e.g., peptides, polysaccharides, and antibodies) are also used to increase the targeting specificity[28].

6. Preparation Methods of Pharmaceutical Nanocrystals

The primary processes of pharmaceutical nanocrystallization are a top-down approach, a bottom-up approach, and a combinatorial (SmartCrystal) approach that may be used individually or in combination depending upon the physicochemical properties of the drug and desired product characteristics. In top-down technology, the overall concept is the breakdown of micrometer-sized drug crystals to the nanometer range with any method like media milling, high-pressure homogenization, wet bead milling, microfluidization, etc without loss of crystallinity. However, in the bottom-up method, the crystallisation process is modified by molecules or solutions such as anti-solvent crystallisation, solvent evaporation, controlled crystallisation, and supercritical fluids. It gives more control over size parameters. The advantage of combining the two techniques via Combination (SmartCrystal) Technology results in an increase in process efficiency, improvement of PSD, saving of time and energy, as well as enhanced drug loading and excellent stability (Figure 1). For this reason, it is the most commonly used method in large-scale production of nanocrystalline drugs[29].

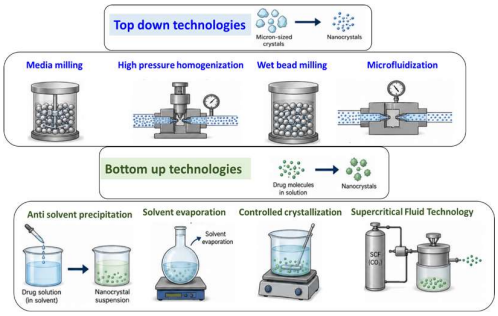

Fig.1. various techniques in the preparation of pharmaceutical nanocrystals

7. Quality by Design (QbD) Approach for Nanocrystal Development

Processes shall continue to be monitored and improved during the product lifecycle. The knowledge obtained from such studies is applied to find optimum formulations and process parameters to determine the design space wherein the nanocrystals of consistent quality can be manufactured. All have been investigated with respect to their potential effects on the CQA. The QTPP of the nanocrystal formulation is defined at first stage. It determines the final quality and functionality of the nanocrystal based on different characteristics (PS, solubility, bioavailibility, stability, therapeutic response etc.). From the QTPP, the Critical CQAs, or the measurable parameters of product quality, are identified.

Next, potential Critical Material Attributes (CMAs) (i.e., attributes of drug substance and excipients like polymorphism, excipient grade, surfactant used) and Critical Process Parameters (CPPs) (i.e., processing variables that can influence the CQAs like milling time, homogenization pressure, processing speed, temperature and concentration of stabilizing agents) are identified and risk-assessed for their potential impact on CQAs. A comprehensive risk assessment is performed to determine factors that present the most significant risks to product quality and process consistency. Finally, relationships between CMAs, CPPs and CQAs are explored through statistically based experiments, such as design of experiments (DoE) – including fractional factorials, BBD and CCD. The knowledge generated through these studies is used to optimize formulation and process parameters to identify a design space where consistent production of nanocrystals with desired quality attributes is achievable (Figure 2). Results in a well-defined nanocrystal product demonstrating high, consistent quality with enhanced solubility, improved bioavailability, and reliable performance and manufacturability. Continued process monitoring and continuous improvement will be maintained throughout the product lifecycle[30].

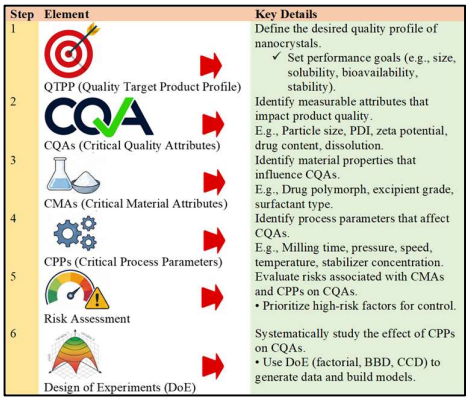

Fig.2. Quality by Design for Robust Pharmaceutical Nanocrystal Products

8. Formulation Optimization Strategies

A rational design of the formulation and the process parameters can be utilized to prepare stable, well-crystallized nanoparticles with tailor-made size, solubility, and oral absorption. Many parameters have a great impact on the quality characteristics of nanocrystal formulations; these are PS, type and concentration of stabilizers; drug: stabilizer ratio, Milling parameters, Number of cycles, homogenization, scale-up parameters, etc. The actual elaboration of drug formulations nowadays utilizes the QbD approach, where DoE (Design of Experiments) can be a tool to identify key variables (formulation), interactions, and define conditions providing the right control, scalability, and readiness for the regulatory file[31].

8.1 PS Optimization

Among various physical properties of pharmaceutical nanocrystals, PS is the most important factor as it determines the oral absorption, saturation solubility, dissolution rate, and physical stability of drugs. The smaller the PS in the range of 100-500 nm, the larger will be the surface area, and as per the Noyes-Whitney equation, dissolution rate will be enhanced due to increased specific surface area of the drug. Conversely, particles of extremely small size, due to higher surface free energy, exhibit a greater tendency to aggregation and Ostwald ripening. Thus, an ideal PS range needs to be found that has a beneficial effect on dissolution and ensures physical stability over the long term. Factors like milling time, homogenization pressure, processing temperature, and concentration of stabilizer need to be optimized for the desirable PS[32].

8.2 Stabilizer Selection

The stabilizer plays an important role in ensuring the physical stability of the nanoparticles during processing, storage, and administration. A good stabilizer should be able to effectively adsorb onto the particle surface, provide electrostatic and/or

steric repulsions to inhibit aggregation, and maintain adequate Zeta potential (ZP) to achieve good colloidal stability. Stabilizer choice is governed by the physicochemical properties of the drug, method of preparation, surface characteristics of the particles, and the intended formulation. Common stabilizers used include poloxamers, PVP, HPMC, PEG, Soluplus, Tween 80, SDS, and lecithin. In most cases, combinations of polymers and surfactants demonstrate enhanced performance over individual stabilizers, improving PS distribution, dissolution properties, and storage stability[33].

8.3 Drug-to-Stabilizer Ratio

It is a significant formulation parameter that affects PS reduction, dispersion stability, and dissolution performance. Inadequate amounts of stabilizer may not provide adequate coverage on the drug surface, resulting in aggregation and crystal growth. Excessive use of stabilizer may increase viscosity, hinder the process of milling, cause issues in further downstream processes, and add unnecessary cost. Therefore, optimization of the drug-to-stabilizer ratio is crucial for proper coverage on the drug particle surface and for optimal size reduction and dissolution. Statistical experimental designs like factorial designs, BBD, or CCD are used for this purpose.

8.4 Milling Parameters

In the case of top-down methods, parameters during milling such as time, speed of milling, size of bead, material of bead, load of bead, drug concentration, and process temperature are crucial for reducing PS. Increased milling intensity can promote PS reduction. On the other hand, longer milling time may cause heat generation, crystal defects, polymorphism transition, and contamination from milling materials. By optimization, size reduction can be obtained effectively with the original crystalline nature and physical character of drugs preserved[34].

8.5 Homogenization Cycles

In high-pressure homogenization, the best-known and often the most widely applied technique of obtaining nanodrugs or nanoparticles that have a high dissolution rate and low solubility is used. The drug suspension is fed into the gap under enormous pressure at high speed and is sheared, cavitated, and impacted to reduce the PS. The pressure of homogenization, number of cycles, temperature, and the initial PS affect the characteristics of nanocrystals. Although more homogenization cycles result in further PS reduction and improved uniformity, excess cycles can increase the energy expenditure, time of processing, and possibly affect the stability of the drug. Hence, the objective of process optimization is to achieve the desired PS within the minimum number of cycles.

8.6 Scale-Up Considerations

Successful industrialization and commercialization of pharmaceutical nanocrystals depend on developing scalable and reproducible processes that produce products of consistent quality. Scale-up is critical and requires maintenance of similar equipment characteristics, milling or homogenization intensity, batch size, energy input, process temperature, and time in larger batches to achieve comparable PS distribution and drug performance across different scales. It is essential to establish an understanding of the process parameters through process modeling and PAT tools to implement QbD principles and ensure robustness and compliance. The challenges during scale-up include proper equipment selection, wear and tear of the equipment, process contamination, aggregation during drying, and batch-to-batch consistency[35].

9. Characterization of Pharmaceutical Nanocrystals

Ensuring Quality, Stability and Performance of Pharmaceutical Nanocrystals. Since the technology for drug delivery using nanocrystals involves reducing the PS of the API while retaining its crystalline form, a variety of analytical techniques are applied to evaluate PS, surface charge, morphology, crystallinity, dissolution characteristics, and storage stability of the formulation. This technique provides an invaluable source of information regarding formulation quality, consistency of manufacturing, and drug product performance, and its one essential condition for translation and approval for commercialisation of a product[36].

9.1 PS and Polydispersity Index (PDI)

For pharmaceutical nanocrystals, PS is the key factor influencing solubility, oral absorption rate, and physicochemical stability. The size of oral formulations is typically below 500nm (100-1000nm). In addition, PS can be easily assessed using dynamic light scattering (DLS) or Photon Correlation Spectroscopy (PCS). Apart from PS, PDI assesses the uniformity of PS distribution and is defined as the ratio of the width of the size distribution to the mean size. Typically, PDI below 0.30 reflects a narrow and homogeneous distribution, whereas higher PDI values can be indicative of potential stability issues[37].

9.2 ZP

ZP can be utilized as an indicator of the surface charge of nanocrystal suspensions and hence the electrostatic stabilization mechanism. Electrostatic stabilization in formulations of nanocrystals, as explained previously, can provide effective stability only if particles possess a high magnitude of ZP. ZP can be evaluated via electrophoretic light scattering

(ELS). In general, formulations solely dependent on electrostatic stabilization possess greater stability if ZP > 30 mV, whereas sterically stabilized formulations can be stable at lower values of ZP. The high magnitude of ZP leads to maximum repulsive forces between dispersed particles, thereby preventing them from aggregating, sedimenting, or ripening[38].

9.3 Morphology (SEM, TEM and AFM)

The morphology, general shape, and size of the nanoparticles can be examined by TEM or SEM, which gives qualitative validation for their production. Surface topographic information of individual nanocrystals can be found using Atomic Force Microscopy (AFM). The combination of the above mentioned methods allowed confirmation of the presence of nanocrystals in the sample, examination of their shape, the quality of their surface, and estimation of the degree of agglomeration[39].

9.4 Crystal Structure

This spectrum is so sensitive to chemical structure that from an analysis of the FTIR spectrum of the final formulation and also their raw materials, it may allow a possible confirmation of the chemical interaction or no chemical modification to the active ingredient. Because this method responds to vibrations, requires very little sample preparation, and has very high detection sensitivity, this technique offers a powerful new route to exploring the solid-state properties of nanocrystals. It can also be utilized to evaluate the stability of the polymorphs, whether there is an alteration of the melting point for the original drug, vanishing of the melting point, or the depression of the melting point suggestive of a mixture of polymorphs, and the absence of a melting point showing amorphization. XRD data is useful for information on the crystal lattice, symmetry, and atom arrangement. It has been employed for the investigation of the preservation of the original crystalline form of nanocrystals or amorphization during production[40].

DSC can be used for evaluation of the melting point, glass transition temperature, and other thermal events and thus can provide a distinction between amorphous and crystalline forms of formulation. DSC is a measure of total heat of melting, thus enabling calculation of crystallinity. It may also be used to assess the stability of polymorphs by evaluating if there is any change in the melting point of the original substance, disappearance of the melting point, or melting point depression, indicating the presence of a mixture of polymorphs or amorphization. The FTIR spectroscopic technique can provide insight into intermolecular interactions between drug and excipients, particularly hydrogen bonding. Since it is very sensitive to chemical structure, analysis of the FTIR spectrum of the final formulation in conjunction with its constituents can provide confirmation of the presence of chemical interactions or absence of chemical modifications of the APIs.

Raman spectroscopy technique can also be used for identifying the polymorphism, various crystalline forms, and amorphous contents. High sensitivity to vibrational modes and low requirement for sample preparation make this technique a promising technique to study the solid-state properties of nanocrystals.

9.5 Saturation Solubility

It is expected that reduction of the drug PS down to the nano-meter range would enhance solubility. This phenomenon has been described by the Ostwald-Freundlich equation, where increased surface area due to nano-sizing results in higher surface free energy, which increases the apparent saturation solubility. The equilibrium solubility is measured by incubating an excess amount of nanocrystals in a given medium and then quantifying the dissolved drug concentration using HPLC or UV spectroscopy[41].

9.6 Dissolution Studies

It is often believed that improving drug solubility and increasing surface area by manufacturing nanocrystals leads to a significant increase in dissolution rate. Dissolution behavior is typically evaluated using an *in vitro* release test using one of the pharmacopeial dissolution apparatuses under specified conditions. The drug dissolution profiles are frequently described using various kinetic models.

9.7 Wettability

Wettability of poorly soluble drugs usually affects their dissolution rate in the gastrointestinal environment as the hydrophobic surface of such particles resists wetting by water-based GI fluids. Improving the wettability of drug particles can enhance their dissolution and improve absorption by increasing the rate of wetting. The wettability of nanocrystals could be enhanced by their nanoscale size and their coating with hydrophilic polymers. Contact angle measurement is one of the ways to determine the wettability of particles[42].

9.8 Stability Studies

It's another parameter required for a safe and repeatable pharmacological profile of the drug. Chemical & mechanical & physical stability tests of nanocrystal formulation under storage conditions. It involves measuring PS, PDI, ZP, crystal form of the formulation, drug concentration, and rate of drug release.

9.9 Drug Content

Accurate measurement of drug content is important to ensure the consistency of dosage units.

It is usually achieved by using UV spectroscopy or High-Performance Liquid Chromatography (HPLC) following dissolution of a weighed amount of nanocrystal product[43].

9.10 Redispersibility

For dried formulation (e.g., spray-dried, freeze-dried), rapid redispersibility of nanocrystals without irreversible aggregation and recovering the original PS distribution is vital for subsequent formulation and administration.

10. Mechanisms of Improved Dissolution and Oral Absorption

These DOE methods, such as factorial, Box-Behnken Design (BBD), and Central Composite Design (CCD), may be applied to examine consistent factors and their relationship with responses, develop a predictive model, and find an appropriate design space. Hence, optimization techniques are being used to achieve desired quality attributes in the given design space, thereby enabling efficient nanoformulations of enhanced solubility, improved oral bioavailability, and repeatable manufacturing processes. In essence, QbD results in continued process improvements, reduction of development time and process and manufacturing variability, and facilitates efficient scaling and regulatory approval of nano-drug products. A comprehensive risk assessment is performed by identifying the critical influence of CMAs and CPPs on CQAs, whereby high-risk parameters are designated for careful control. DoE approaches such as factorial, BBD, and CCD are used to evaluate systematic relationships between factors and responses, develop predictive models, and determine a suitable design space. Based on this, optimization methods are applied to realize the specified quality characteristics within the determined design space, thus leading to robust nanocrystal formulations with higher dissolution rates, better oral bioavailability, and a reproducible manufacturing process. QbD leads to ongoing process improvement, decreased development time and manufacturing variability, and supports the successful scale-up and approval of nanocrystal-based pharmaceutical products[44].

11. Oral Bioavailability of Nanocrystals

Hence, the success of drug nanocrystal-based formulation hinges on tailoring and optimizing these parameters that correlate to both the formulation and Physiology of the gastrointestinal tract so as to enhance drug dissolution and absorption of the drug from the GI tract, which finally results in increased oral bioavailability and homogeneous action *in vivo*. PS plays the main and most important part; making size of particle of nano-scale range improves the rate of dissolution, saturates solubility, and very significantly increases the surface area of drug particles. Certain innate attributes of the medicine, like its inherent water solubility, lipophilicity, absorption, passage from one site to another (permeability), and the total dosage, are going to cause impacts on absorption of medicine in the alimentary system. ZP (surface charge) has a significant impact on sustaining the colloidal dispersion and also in preventing aggregation, which aids in the interaction with intestinal mucosal cells and the mucus layer, thereby affecting absorption. Stabilizers of both type and concentration are critical for protecting the stability of the nanocrystals, avoiding Ostwald ripening and aggregation, thereby increasing wetting efficiency. Crystalline morphology of the drug, or crystal habit, or crystal form, is important to affect surface energy, solubility, dissolution rate, and also stability; appropriate crystal form selection is therefore a requirement for desirable performance. Some intrinsic properties of the drug, such as its intrinsic aqueous solubility, lipophilicity, permeability, and the dose of the drug, will affect absorption from the gastrointestinal tract. Finally, physiological conditions such as varying pH, the pattern of gastric emptying, intestinal propulsion, and presence of intestinal fluid, the amount of mucus, enzymes, bile salts, and the presence of food will certainly affect the behavior of the drug in the stomach and intestines. Thus, the success of formulation based on drug nanocrystals involves optimizing these parameters related to the formulation and Physiology of the gastrointestinal tract to achieve enhanced dissolution and improved absorption from the gastrointestinal tract, thereby enhancing the overall oral bioavailability and uniform therapeutic action[45].

12. *In vitro*, *Ex vivo* and *In vivo* Evaluation

In vitro studies form the initial stage for evaluation of pharmaceutical nanocrystals. The major focus in this section includes determination of dissolution rates, membrane permeability, and physicochemical stability under simulated physiological conditions. These tests offer a fast, relatively inexpensive, and highly reproducible method for obtaining initial information about the performance of formulations before proceeding to biological studies.

12.1 Dissolution Studies

The most significant performance characteristic for *in vitro* evaluation of nanocrystals is their dissolution rate since the absorption rate for poorly water-soluble drug products is typically rate-limited by the dissolution process. The test typically uses United States Pharmacopeia (USP) Dissolution Apparatus I (Basket) or Apparatus II (Paddle) with set parameters, i.e., at 37±0.5ºC, and aqueous media resembling gastric (simulated gastric fluid, SGF) or

intestinal fluids (simulated intestinal fluid, SIF). Because nanocrystals are much more energetic due to high surface area and have increased surface energy to overcome repulsive forces and the interface energy of interaction with biological fluids, these formulations exhibit faster and more extensive dissolution rates compared with conventionally micronized drug particles. To study the release kinetics of drugs from nanocrystals, data from dissolution profiles are fitted to various kinetic equations, such as zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas equations[45].

12.2 Permeation Studies

Nanocrystals' primary effect is to enhance drug dissolution; however, they could also enhance drug permeation due to maintaining a high concentration gradient between the luminal compartment and the intestinal tissue. *In vitro* permeability studies [either cell cultures like Caco-2 cell monolayers or MDCK cell monolayers or PAMPA model, etc.] will predict the permeability of the drug, the rate of absorption, and the amount of drug absorption. Thus, they may indirectly hint at which underlying permeation mechanism (simple passive diffusion, carrier-mediated transport, or intracellular uptake) may be responsible for nanoparticle uptake. Enhanced permeability usually relies on improved dissolution and prolonged supersaturation[46].

12.3 Stability Studies

In vitro stability studies address the capacity of a nanocrystal formulation to remain physicochemically stable over time during storage and under simulated physiological conditions, such as in simulated gastric or intestinal fluids. Characterization of parameters like PS, PDI, ZP, drug loading, crystallinity, and dissolution rate over a time period can be carried out to assess the stability of the formulations. Studies on the aggregation or crystal growth properties in simulated physiological fluids predict how the formulations would perform after oral administration in a biological system. Stability evaluation is also performed at accelerated conditions to establish the shelf life of the formulations.

12.4 *Ex vivo* Evaluation

Ex vivo models employ freshly excised animal tissues, bridging the gap between simplified *in vitro* systems and the complexity of *in vivo* environments. These systems provide valuable information about drug permeability and intestinal absorption without the inconvenience of *in vivo* administration in animals or humans.

In vitro permeability studies across isolated animal intestinal segments (from rats, rabbits, pigs, etc.) represent classic *ex vivo* models for assessing intestinal absorption of various drug formulations, including nanocrystals. Intestinal segments are mounted on Ussing chambers filled with a physiological buffer. After incubation with the drug solution, samples from the basolateral side of the epithelium are periodically collected to determine the quantity of the drug permeated. This approach can effectively reveal the ability of nanocrystals to enhance permeability through factors such as increased surface area available for dissolution, improved wettability, and formulation of a sustained supersaturated drug solution close to the absorptive surfaces of the intestine[46].

12.4.1 Everted Sac Studies

One of the most extensively used *ex vivo* models for the study of gastrointestinal drug absorption is the everted intestinal sac technique. A segment of small intestine is inverted and filled with a physiological buffer, and then it is incubated in an aqueous solution of the nanocrystal formulation under controlled conditions (37 C, 30 minutes to several hours). Drug transport into the serosal fluid is quantitatively determined over time. This *ex vivo* model has been useful for investigating intestinal drug absorption, permeability, and regional variation of absorption, carrier-mediated transport, and influence of formulation components on drug uptake and, thus, is valuable for preliminary evaluation of nanocrystal-based orally administrable formulations[46].

12.4.2 Franz Diffusion Studies

The Franz diffusion cell was developed as a model for studying skin permeation but has also been widely applied for evaluating drug permeation across excised intestinal segments. A biological membrane separates two compartments: the donor compartment is filled with a nanocrystal formulation, whereas the receptor compartment contains a physiological buffer solution. Periodically, samples from the receptor compartment are removed and analyzed for the concentration of permeated drug to calculate permeation rates and diffusion coefficients. The method allows for rapid evaluation of permeation and estimation of a drug's permeability through an intestinal wall[47].

12.5 *In vivo* Evaluation

These three methodologies (*In vitro*, *ex vivo*, and *in vivo* assessment) were complementary to characterize the behaviour of nanocrystal drug delivery systems and are therefore crucial for an appropriate and predictable therapeutic result of drug product development on the basis of nanocrystal technology. Commonly, one would compare the AUC from a nanocrystal drug product versus the AUC from a non-nanocrystal form of that drug. These measures give an idea of the rate of distribution of the drug into the body, its delivery to

target tissues, and its distribution and accumulation at the target tissues. This is really significant for developing oral bioavailability nanocrystal dosage forms. This data is then used to derive several pharmacokinetic parameters to define the pharmacokinetic profile. C_{max} is the maximum plasma concentration, T_{max} is the time to peak plasma concentration, AUC is the area under the plasma concentration-time curve, t½ is the elimination half-life, and MRT is the mean residence time. These are all utilised in the description of the pharmacokinetic profile. Rodents, rabbits, dogs, or non-human primates are usually used for conducting pharmacokinetic studies using oral administration of nanocrystal formulations. Plasma levels are measured after the orally dose administrations at various time intervals and using appropriate analytical methods (e.g., HPLC or LC-MS/MS) that measure the drug concentration in the plasma. Calculation of several pharmacokinetic parameters, such as the peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}), the area under the plasma concentration-time curve (AUC), the elimination half-life (t½), and the mean residence time (MRT), is made from this data and used to characterize the pharmacokinetic profile. In this comparison of pharmacokinetic profiles, the enhanced parameters should result in higher AUC, C_{max}, and faster T_{max} compared to the respective conventional dosage form of the same drug. Oral bioavailability is usually demonstrated by comparing the AUC of a drug administered in nanocrystal formulation with the AUC of the same drug administered from conventional formulations. Absolute bioavailability of the drug is obtained by comparing the AUC of the orally administered nanocrystal formulation with the AUC of the intravenously administered dose of the same drug. Normally, higher AUC, C_{max}, and relatively higher absolute bioavailability values of the drug from nanocrystal dosage forms are obtained compared to comparable formulations, which may be explained by fast dissolution, increased saturation solubility, enhanced residence time in the gastrointestinal tract, and greater absorption through the intestinal wall. This is very important to establish the clinical significance of nanocrystal dosage forms in orally available forms. Tissue distribution of the orally administered nanocrystal formulation can provide evidence about drug fate in different tissues after oral administration in an animal model at specified time points after sacrifice of animals. Drug levels are determined in important organs, such as liver, spleen, lungs, kidney, heart, and contents of the gastrointestinal tract, as well as blood plasma and tissue. These findings provide a measure of the extent and rate at which drugs are distributed into the body, reach target tissues, and accumulate at those tissues. Tissue distribution at the site of action is especially important for drugs that target specific tissues (e.g., for cancer therapies) and can explain the improvement in efficacy without increased dose and may explain the reduced risk of systemic exposure of the organs. These three approaches, namely *in vitro*, *ex vivo,* and *in vivo* evaluations, are complementary in the characterization of the behaviour of nanocrystal drug delivery systems and are essential to ensure adequate and predictable therapeutic outcomes with respect to the development of drug products based on the nanocrystal technology[48].

13. Dosage Forms Based on Nanocrystals

Nanoparticle technology allows nanocrystals to be formulated into various oral dosage forms. These include oral suspensions, tablets, capsules, films, pellets, granules, and sachets.

14. Therapeutic Applications

Use of pharmaceutical nanocrystals for formulation into nutraceuticals has also been widely studied, as the nanocrystal approach provides a viable solution to circumvent poor water solubility and bio-limitation (natural) of absorption of many popular nutraceuticals, namely curcumin, resveratrol, quercetin, Coenzyme Q10 and a host of other bioactive phytochemicals, thus enabling potent oral absorption and ensuing biological effects. Hence, as a result of the above-mentioned advantages of the formulation of pharmaceutical nanocrystals, one of the most important and worthwhile formulation techniques for developing oral delivery systems for a variety of diseases and enhancing patient compliance was found. In the preparation of antiviral agents, nanocrystals increased the solubility and dissolution rate of poorly water-soluble drugs, resulting in increased antiviral efficacy at low doses; they improved the bioavailability and antiviral action of the drugs. Application in antiviral drug formulation of drugs In designing antiviral drugs nanocrystals not only improved solubility and dissolution of insoluble drugs, and thus potentially increased the therapeutic effects at reduced doses, but could increase the bioavailability of antivirals as well.

Nanocrystals improve the poor water solubility of azoles like fluconazole and itraconazole to enhance drug dissolution of the drugs; increase the oral absorption of the drugs; and remarkably increase the therapeutic activity against deep systemic fungal infection and skin fungal infection. Antidiabetic drugs formulated as nanocrystals improve intestinal absorption and minimal interpatient variability and exhibit better glucose-lowering effects. NSAID formulation nanocrystals enhanced the drug dissolution and increased the rate of pain relief. Systemic and gastrointestinal toxicity might also be enhanced. Poorly water-soluble antihypertensive drugs available in the market are formulated as nanocrystals to improve their oral bioavailability,

constancy of plasma drug concentration, and their therapeutic activity. Poorly water-soluble CNS drugs are formulated into nanocrystal formulations to enhance their oral absorption. In some cases, it can be even better penetrating blood-brain barrier by enhanced solubility and bioavailability, leading to enhanced therapeutic activity. In antiviral drug formulation, nanocrystals enhance solubility and dissolution of poorly soluble drugs, which may increase the therapeutic activity with reduced dose; for example, nanocrystals can improve the bioavailability and antiviral activity of drugs.

The application of pharmaceutical nanocrystals in nutraceuticals has been explored widely, as they can overcome poor water solubility and the inherent bio-limitation of absorption of nutraceuticals such as curcumin, resveratrol, quercetin, Co-enzyme Q10, and many other bioactive phytochemicals to enhance their oral bioavailability and the following pharmacological response. Due to these above-mentioned benefits of the pharmaceutical nanocrystals technology, it is one of the important formulation strategies to design oral formulations for the treatment of a variety of diseases, and patient compliance was also increased[49].

15. Commercially Available Nanocrystal Products

Clinical approval and market acceptance of the pharmaceutical nanocrystal technology have already demonstrated that the problem of the low solubility and low oral bioavailability of the poorly water-soluble drugs could be effectively solved by using the technology. There are also a few nanocrystal formulations already available that can be taken orally or injected, or put into the eyes. The approval and commercial success of other drugs such as Rapamune (sirolimus), Emend (aprepitant), Tricor (fenofibrate), Megace ES (megestrol acetate), and Invega Sustenna (paliperidone palmitate), and many others based on nanocrystal-based technologies serve as a validation and the embodiment of clinical success for this technology[50]. These formulations are aimed at achieving increased rate of dissolution, better absorption, and enhanced therapeutic efficacy along with reduced patient variation. These formulations are produced primarily using top-down approaches, specifically wet media milling and high-pressure homogenization. These processes are well established for producing stable nanocrystalline suspensions at a manufacturing scale. Through these efforts, drug development has moved forward significantly; drug performance enhancement and oral delivery through nanocrystals have proved to be successful for BCS Class II and IV drugs. Further innovation in the manufacturing processes, stabilization of drug nanosuspensions, and regulatory guidelines for their development will continue to expand the global market for the development and marketing of nanocrystal-based products.

16. Scale-Up and Manufacturing Challenges

Consistency in product attributes, including PS, PS distribution, crystal habit, and physicochemical stability, is critically important when the production scale is increased from bench to pilot and commercial. This requires strict process reproducibility where variations in processing parameters such as milling/homogenization times and pressure, temperatures, the presence of stabilizing agents, and processing conditions (solvents, temperature) have the potential to adversely affect nanocrystal quality. The equipment used to manufacture the nanocrystal needs to be appropriately selected with appropriate process control and scaled appropriately with process design, but not at the cost of quality and consistency. It also needs to be robust and energy efficient to consistently produce the required characteristics, minimizing contamination, heating, and wear. The formulation type will determine if sterility is a necessary part of the manufacturing process[51]. If used for injection (parenteral, intravenous), ophthalmic, or other sterile applications, nanocrystals should be produced via aseptic process technology. Alternatively, sterile filters can be used for sterilisation, where the nanocrystal formulation can be sterilised after processing using steam or gamma radiation, while trying to prevent aggregation or growth of the crystal structure. After producing the nanosuspension, the particles will need to be dried if they are to be presented as a solid product, a form that is easier to handle and package for distribution and long-term storage, using methods such as spray drying, freeze drying, fluidized-bed drying or vacuum drying with the incorporation of the appropriate cryo/drying agent. However, these processes can induce aggregation and require careful selection and optimisation to prevent irreversible loss of redispersibility. Process scale-up with Process Analytical Technology (PAT) and QbD for continuous manufacturing of pharmaceutical nanoparticles. This has attracted considerable interest due to improved process control, real-time monitoring, efficiency gains (reduced time and costs) and better predictability in scale-up than conventional batch processes. Managing and controlling critical parameters (CPPs) and attributes (CQAs) are improved with such integrated approaches.

17. Stability Considerations

17.1 Pharmaceutical Nanocrystals – Stability Considerations

Long-term stability is arguably the single most important aspect determining successful development, commercialization, and clinical use of pharmaceutical nanocrystals. Due to their very small

size and extremely high surface area, nanocrystals are characterized by high surface free energy and, consequently, are more unstable in a thermodynamic sense as compared to the macro-crystals. Various physicochemical stability challenges, such as aggregation, Ostwald ripening, crystal growth, and polymorphic transformations, may occur in nanocrystals during production, handling, packaging, and storage of the nanocrystal product, potentially altering the dissolution performance, oral absorption, and quality of the formulation. Therefore, suitable formulation strategies (e.g., choice of stabilizer, drying method, storage condition, etc.) should be adopted to address all these physical instabilities of drug nanocrystals[52].

17.2 Aggregation

Aggregation is one of the most common issues faced while formulating pharmaceutical nanocrystal formulations. As they have a high surface energy and a tendency to associate with each other, it is understandable why aggregations are formed by van der Waals forces. This, in effect, increases the size of the nanocrystals. An increase in the PS (which occurs as a consequence of the agglomeration of fine drug particles) brought about a reduction in the effective surface area of drug particles, which, in turn, led to a reduction in dissolution rate, solubility in saturated media, and consequently also led to a reduction in oral bioavailability. It also causes sedimentation of nanosuspensions and difficulty redispersion of dried nanocrystals after drying. Stabilizers such as poloxamers, PVP, HPMC, PEG, Tween surfactants, SDS, and lecithin are commonly added to provide steric or electrostatic (or electrosteric) stabilization against aggregation. The concentration of stabiliser and processing parameters need to be optimized to provide long-term colloidal stability to the suspension[53].

17.3 Ostwald ripening

Ostwald ripening is a thermodynamically driven phenomenon of increase in the PS as the smaller nanocrystals dissolve and redeposit on larger ones due to their different surface free energies. Small particles are thermodynamically less stable, and their saturation solubility is higher compared to large particles. Consequently, molecules diffuse from small crystals to larger crystals, thereby reducing their size while increasing the size of larger crystals. In due course of time, the average PS increases, thus leading to an increase in PS distribution, a decrease in dissolution rate, and reduced bioavailability. Ostwald ripening is best retarded by selection of suitable stabilisers, use of drugs with lower solubility, optimal PS distribution of the initially formulated nanoparticles, and by proper storage conditions (temperature). A combination of polymer and surfactants has also been used to prevent Ostwald ripening effectively.

17.4 Crystal growth

Crystal growth (also known as Ostwald ripening; sometimes, crystal growth is used interchangeably for any process which leads to the growth of nanocrystals) refers to the increase in size of nanocrystals by deposition of molecules on the crystal surface during storage or processing. While aggregation results in adherence of particles without altering their structure, crystal growth leads to the growth of an individual crystal. Crystal growth can occur if there is a slight residual supersaturation, temperature fluctuations, or prolonged storage, even with adequate stabilisers. Increased PS of nanocrystals can also reduce their specific surface area available for dissolution, thereby minimizing the enhanced dissolution property achieved with nanocrystal technology[54].

17.5 Storage conditions

Appropriate storage conditions play a significant role in maintaining physical and chemical stability of pharmaceutical nanocrystal formulations throughout their intended shelf-life. Temperature, humidity, light, oxygen, and physical stress can impact physical and chemical characteristics of drug nanocrystals. For instance, higher temperatures can enhance the rate of Ostwald ripening and crystal growth, whereas high humidity can lead to particle aggregation. Drugs susceptible to light or oxidation should be protected accordingly[55]. Pharmaceutical nanocrystal formulations are generally stored in tight, light-resistant containers under conditions of controlled temperature and humidity in accordance with regulatory guidelines, such as International Council for Harmonisation (ICH) stability guidelines. For solid formulations, protective agents such as cryo- or lyoprotectants can be added to maintain redispersibility. Various quality tests such as PS and distribution, PDI, ZP, drug content, crystallinity, dissolution profile, and redispersibility can be performed to confirm the long-term stability and quality of nanocrystal products[56].

18. Regulatory Aspects

The regulatory approval process for pharmaceutical nanocrystal formulations is extremely strict. To guarantee reliable quality, safety, and performance for drug nanocrystal formulations, the formulation process needs to conform to the strict requirements of regulatory agencies around the world. For example, the US FDA considers that nanotechnology offers opportunities to develop superior formulations for drugs of low water solubility, but demands extensive particle characterisation for size, size distribution, crystal morphology, polymorphic form, surface properties, dissolution behaviour, stability and batch-to-batch reproducibility.

Similarly, the European Medicines Agency requires the thorough assessment of medicinal products based on nanomaterials, considering physicochemical properties, the manufacturing process, storage and stability conditions, pharmacokinetics, safety aspects and clinical efficacy to provide a guarantee of quality and therapeutic equivalence. Additionally, globally harmonised International Council for Harmonisation (ICH) guidelines provide the regulatory backbone for product development and approval; particularly ICH Q8, Q9, Q10, Q11, Q12, Q1, and Q2 offer clear regulatory guidance regarding product development and manufacture, quality risk management, pharmaceutical quality systems, and stability testing. Nanocrystal-based product development adheres to these general guidelines, although not yet exclusively addressed in a specific document. Key product attributes required for routine control of nanocrystal-based drug product development include regular characterisation of PS and size distribution, polydispersity index, ZP, crystalline status, crystal form, morphology, drug content, assay, dissolution profile, saturation solubility, residual solvents, microbiological properties,redispersibility, stability(physical and chemical), and batch-to-batch variability. Generic products necessitate demonstrating therapeutic equivalence and thus require comprehensive bioequivalence data that can either consist of *in vitro* dissolution profiles compared with those of the reference product, or a pharmacokinetics study, or, when needed, *in-vivo* clinical bioequivalence studies[57]. The utilisation of QbD, PAT and the latest regulatory risk-based approval principles ensures the robustness and the reproducibility of nanocrystal-based drug product manufacturing processes and facilitates smoother regulatory approval[58].

19. Recent Advances and Emerging Trends

Over the last decade, pharmaceutical nanocrystal technology has witnessed immense development. It has evolved from being just PS reduction to the development of highly performant multifunctional drug delivery platforms. A synergistic advancement in material science, nanotechnology, computational modelling, and pharmaceutical engineering has helped design nanocrystals with higher stability, target-site delivery, sustained release profile, and better patient compliance[59]. Modern strategies, including surface functionalization, mucoadhesive systems, targeted nanocrystals, hybrid nanocarriers, Artificial Intelligence (AI)-driven formulation optimization, 3D printing, continuous manufacturing, and personalised medicine, are shaping the future of nanocrystals into versatile drug delivery systems for next-generation oral delivery[60].

Table 1: Representative patents related to pharmaceutical nanocrystal technology

Patent No. and year	Assignee	Novelty	Importance
US5846550; 1998	Elan Corporation	Wet milling	Enhancing dissolution and bioavailability
US6015543; 2000		Surface-modified nanocrystals	more bioavailability
US6187326; 2001		Nanocrystals	Enhancing dissolution and bioavailability
US6410019; 2002		Nanoparticles	For poor water-soluble drugs
US6582716; 2003		Nanoparticles	Stability with less particle aggregation
US7083795; 2006		Nanocrystals	Enhancing dissolution and bioavailability
WO2015070214A1; 2015	AbbVie Inc.	Nanocrystals	Enhancing dissolution and bioavailability
WO2021176427A1; 2021	Various innovators	Nanocrystals	Enhancing dissolution and stability

Table 2: Commercially marketed nanocrystal-based pharmaceutical products

Brand	Main component	Company	Therapeutic Indication	Benefit of Nanocrystal Formulation
Emend®	Aprepitant	Merck	Nausea and vomiting	Good dissolution

			during cancer therapy	and absorption
Focalin XR	Dexmethylphenidate	Novartis	hyperactivity disorder	Improved dissolution with sustained therapeutic performance.
Megace ES	Megestrol	Par Pharmaceutical	Anorexia/cachexia in cancer patients	More bioavailability
Rapamune	Sirolimus	Pfizer	Prevents body immune response when an organ transplant is done	More bioavailability
Tricor	Fenofibrate	AbbVie	Hypertriglyceridemia	Uninterrupted bioavailability even in the presence of food
Zanaflex Capsules	Tizanidine HCl	Acorda Pharmaceuticals	Muscle spasticity	More dissolution
Invega Sustenna	Paliperidone palmitate	Janssen	Schizophrenia	Controlled release
TriCor	Fenofibrate	AbbVie	Dyslipidemia	Good bioavailability

19.1 Surface Functionalized Nanocrystals

The technique of surface functionalization of pharmaceutical nanocrystals has become a major tool in augmenting biological performance. The nanocrystal surface is chemically modified using polymers, surfactants, polysaccharides, peptides, antibodies, or other ligand moieties. Such modification increases stability, provides site-specific targeting, improves wettability of particles, enhances mucus penetration, promotes greater uptake into cells, and sustains residence of the drug at the desired location to increase overall therapeutic effect. This approach enhances the efficacy of nanocrystal drugs by targeting the site-specific cells or receptors and thereby reducing the off-site toxicity[61]. Commonly used coating materials for nanocrystals include polyethylene glycol (PEG), chitosan, hyaluronic acid, albumin, folic acid, etc.

19.2 Mucoadhesive Nanocrystals

Mucoadhesive nanocrystals are designed to retain drug particles within the gastrointestinal system for prolonged periods. To enhance mucin affinity, the nanocrystals are coated or incorporated with mucoadhesive polymers like chitosan, carbopol, sodium alginate, HPMC, or polycarbophil. The increased contact of nanocrystals with the mucosal surface causes slower transit time in the GI tract and thereby maintains higher local drug concentration. Such prolonged residence in the stomach can promote enhanced drug dissolution, improved absorption, and consequently increased bioavailability, especially for those drugs having a narrow absorption window and poor solubility. These formulations find applications in cancer, antiviral, and peptide-based drug delivery[62].

19.3 Targeted Nanocrystals

A drug that delivers to the organ, tissue, or cell of the body specifically. The strategy of targeting can be divided into[63]:

Passive targeting

Passive accumulation of nanodrug at specific sites by the enhanced permeability and retention (EPR effect) phenomenon, especially at tumors.

Active targeting

Nanocrystals are conjugated to the targeting ligand (antibody, peptide, folic acid, transferrin, aptamer, carbohydrates, etc.) which have affinity towards the receptors of specific cells and lead to the enhanced uptake of nanocrystals into cells by endocytosis; resulting in increased local concentration of the drug at target site with decreased side effects. Many targeting nanocarriers have undergone clinical studies in different diseases like cancer, infectious & inflammatory diseases.

19.4 Hybrid Nanocrystals

Having two or more nanocarriers associated in tandem results in hybrid nanocrystals that harness the benefits of both systems together and synergize the merits thereof. Such nanocrystals can be linked with liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, hydrogels, and self-emulsifying systems to stabilize the drug and modify the release rate of the drug. This

has increased the total therapy efficacy due to improved drug solubility from the nanoparticle nature of nanocarriers and sustained drug release from the shell coating of other nanocarriers[64]. This approach finds promising application for poorly soluble anti-cancer agents, anti-inflammatory drugs, and delivery of central nervous system agents.

19.5 AI-Assisted Formulation Design

AI and ML have been adopted in several research areas, with pharmaceutical formulation development being one of them. By harnessing huge amounts of formulation data, AI and ML can predict how the various parameters in formulation affect multiple nanocrystal parameters, including PS, size distribution, ZP, and drug release[65].

19.6 3D Printing

3D printing, or to give it its correct term, additive manufacturing, is a rapidly developing novel technology and can serve as a viable means of developing future patient-tailored, drug-loaded, orally active nanocrystal medicines. Such nanocrystal drug ink or filament can be manufactured by a 3D printing method with specified geometric and size dimensions, with specific drug concentrations capable of yielding a correct dosage, a distinct dissolution profile, and a satisfactory mechanical property for the drug in the combinational product or other desired dosage forms[66].

19.7 Personalized Medicine

A long-awaited future in pharmaceutical drug delivery has long been described in the realm of “personalized medicine.” Personalized medicine would be a kind of new wave of medicine based on individual patient differences, their genetic profile, stage of illness, and expected drug response. And perhaps most importantly for a new wave of delivery, 3D printable nanocrystal technologies could, with assistance of someAI driven approaches to formulate nanocrystals with specific drug(s) and to fabricate tailored 3D printed oral dosage forms, allow one to customize the doses of an orally administered therapy for an individual, and even for specific patient populations that have been profiled, thereby opening new avenues for better and safer individual drug therapies via the oral route[67-74].

20. Conclusion

Pharmaceutical Nanocrystal technology is one of the established successful techniques over the past 2 decades to improve oral administration of poorly water-soluble drugs. Formation of drug nanoparticles in the range of nano size (10-1000 nm) in their crystalline state (nanocrystals), in which there would be increased surface area, increased saturation solubility, increased dissolution velocity, increased wetting property, and improved oral bioavailability of the drug without affecting the drug's identity. Nanocrystals can achieve high drug loading, have less need for carriers, offer design flexibility, and can be applied to various dose forms; hence, they represent an alternative to many established methods of solubility enhancement. Improvements in the design of manufacturing processes like wet media milling, high-pressure homogenization, antisolvent precipitation, and hybrid technologies associated with the QbD approach have led to a significant advance in their process robustness, scale-up capabilities, and reproducibility of quality. Physicochemical characterization and stability of nanocrystals remain vital for consistent quality and therapeutic performance. Challenges, namely particle aggregation, Ostwald ripening, polymorphism, and issues of drying-induced instability, scale-up, and the regulatory landscape, are some of the issues that can affect their commercial success. Novel approaches such as surface functionalization, AI-guided formulation design, continuous manufacturing processes, and personalized medicine offer new opportunities. In the future, nanocrystals in conjunction with pharmaceutical science, materials engineering, and regulation are believed to further pave the way for a promising route to safely and effectively treat with the help of less soluble drug molecules.

References

[1] Pardhi VP, Verma T, Flora S, Chandasana H, Shukla R. Nanocrystals: an overview of fabrication, characterization and therapeutic applications in drug delivery. Current pharmaceutical design 2018;24:5129-46.

[2] Shegokar R, Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. International journal of pharmaceutics 2010;399:129-39.

[3] Chogale MM, Ghodake VN, Patravale VB. Performance parameters and characterizations of nanocrystals: a brief review. Pharmaceutics 2016;8:26.

[4] Purohit D, Sharma S, Lamba AK, Saini S, Minocha N, Vashist N, et al. Nanocrystals: A deep insight into formulation aspects, stabilization strategies, and biomedical applications. Recent Patents on Nanotechnology 2023;17:307-26.

[5] Shah S, Parmar B, Soniwala M, Chavda J. Design, optimization, and evaluation of lurasidone hydrochloride nanocrystals. AAPS PharmSciTech 2016;17:1150-8.

[6] Al Hazzaa SA, Rajab NA. Cilnidipine nanocrystals, formulation and evaluation

for optimization of solubility and dissolution rate. Iraqi Journal of Pharmaceutical Sciences 2023;32:127-35.

[7] Kumar A, Valamla B, Thakor P, Chary PS, Rajana N, Mehra NK. Development and evaluation of nanocrystals loaded hydrogel for topical application. Journal of Drug Delivery Science and Technology 2022;74:103503.

[8] Li J, Wang Z, Zhang H, Gao J, Zheng A. Progress in the development of stabilization strategies for nanocrystal preparations. Drug delivery 2021;28:19-36.

[9] Peltonen L, Strachan C. Understanding critical quality attributes for nanocrystals from preparation to delivery. Molecules 2015;20:22286-300.

[10] Witika BA, Aucamp M, Mweetwa LL, Makoni PA. Application of fundamental techniques for physicochemical characterizations to understand post-formulation performance of pharmaceutical nanocrystalline materials. Crystals 2021;11:310.

[11] Jain S, Patel K, Arora S, Reddy VA, Dora CP. Formulation, optimization, and in vitro–in vivo evaluation of olmesartan medoxomil nanocrystals. Drug delivery and translational research 2017;7:292-303.

[12] Patel V, Mehta TA. Betamethasone dipropionate nanocrystals: investigation, feasibility and in vitro evaluation. AAPS PharmSciTech 2022;23:197.

[13] Sun Y, Chen D, Zhao Y, Zhou K, Zhang B, Wang H, et al. Exploitation of nanocrystal suspension as an effective oral formulation for oxfendazole. Drug Delivery and Translational Research 2022;12:1219-29.

[14] Seo S, Kim G-Y, Kim M-H, Lee KW, Kim M-J, Chaudhary M, et al. Nanocrystal formulation to enhance oral absorption of silybin: preparation, in vitro evaluations, and pharmacokinetic evaluations in rats and healthy human subjects. Pharmaceutics 2024;16:1033.

[15] Lu Y, Wang Z-h, Li T, McNally H, Park K, Sturek M. Development and evaluation of transferrin-stabilized paclitaxel nanocrystal formulation. Journal of controlled release 2014;176:76-85.

[16] Rizwan M, Damodharan N. Mathematical modelling of dissolution kinetics in dosage forms. Research Journal of Pharmacy and Technology 2020;13:1339-45.

[17] Eslami F, Elliott JA. Role of precipitating solute curvature on microdrops and nanodrops during concentrating processes: the nonideal Ostwald–Freundlich equation. The Journal of Physical Chemistry B 2014;118:14675-86.

[18] Galvin K. A conceptually simple derivation of the Kelvin equation. Chemical engineering science 2005;60:4659-60.

[19] Sood R, Tomar D, Kaushik P, Sharma P, Rani N, Guarve K, et al. Enhanced solubility and increased bioavailability with engineered nanocrystals. Current Drug Therapy 2024;19:638-47.

[20] Yu Q, Wu X, Zhu Q, Wu W, Chen Z, Li Y, et al. Enhanced transdermal delivery of meloxicam by nanocrystals: Preparation, in vitro and in vivo evaluation. Asian journal of pharmaceutical sciences 2018;13:518-26.

[21] Zhou Y, Du J, Wang L, Wang Y. Nanocrystals technology for improving bioavailability of poorly soluble drugs: a mini-review. Journal of nanoscience and nanotechnology 2017;17:18-28.

[22] Sathali AHA, Prakash JCS. Formulation and Evaluation of Amisulpride Nanocrystal Tablets. Research Journal of Pharmacy and Technology 2015;8:1294.

[23] Khan BA, Rashid F, Khan MK, Alqahtani SS, Sultan MH, Almoshari Y. Fabrication of capsaicin loaded nanocrystals: Physical characterizations and in vivo evaluation. Pharmaceutics 2021;13:841.

[24] Garhy DM, Ismail S, Ibrahim HK. Preparation and Evaluation of Curcumin Nanocrystals for Topical Administration: Cell Line Analysis and Histopathological Examination. Journal of Pharmaceutical Innovation 2026;21:388.

[25] Tuomela A, Hirvonen J, Peltonen L. Stabilizing agents for drug nanocrystals: effect on bioavailability. Pharmaceutics 2016;8:16.

[26] Koczkur KM, Mourdikoudis S, Polavarapu L, Skrabalak SE. Polyvinylpyrrolidone (PVP) in nanoparticle synthesis. Dalton transactions 2015;44:17883-905.

[27] Hassan AS, Soliman GM. Rutin nanocrystals with enhanced anti-inflammatory activity: Preparation and ex vivo/in vivo evaluation in an inflammatory rat model. Pharmaceutics 2022;14:2727.

[28] Ahmed IS, Elnahas OS, Assar NH, Gad AM, El Hosary R. Nanocrystals of fusidic acid for dual enhancement of dermal delivery and antibacterial activity: in vitro, ex vivo and in vivo evaluation. Pharmaceutics 2020;12:199.

[29] Liu Y, Li Y, Xu P, Shen Y, Tang B, Wang Q. Development of abiraterone acetate nanocrystal tablets to enhance oral bioavailability: formulation optimization, characterization, in vitro dissolution and pharmacokinetic evaluation. Pharmaceutics 2022;14:1134.

[30] Babu GN, Muthukaruppan M, Ahad HA. Azadirachta indica Fruit Mucilage Aided Mucoadhesive Microspheres of Acyclovir for Drug Entrapment and Mucoadhesive Time Assets with Design-Expert Software. Indian Journal of Pharmaceutical Education & Research 2025;59.

[31] Chinthaginjala H, Ahad HA, Bhargav E, Pradeepkumar B. Central composite design aided formulation development and optimization of clarythromycin extended-release tablets. Indian J Pharm Educ Res 2021;55:395-406.

[32] Babu GN, Menaka M, Ahad HA. Neem fruit mucilage-aided mucoadhesive microspheres of acyclovir using 32 factorial design with design-expert software. Appl Biol Res 2022;24:17.

[33] Panchal S, Ahad HA, Srinivas H, Ramachandra GB, Gangadharaiah M, Srinivas S. Breaking barriers in ocular drug delivery: Unveiling the role of ocular inserts as controlled release systems. Research Journal of Pharmaceutical Dosage Forms and Technology 2024;16:245-50.

[34] Mundarinti SHB, Ahad HA. Impact of Pistacia lentiscus Plant Gum on Particle Size and Swelling Index in Central Composite Designed Amoxycillin Trihydrate Mucoadhesive Microspheres. Indian Journal of Pharmaceutical Education & Research 2023;57.

[35] Yadiki MN, Suggala VS, Reddy Puchalapalli DS, Ahad HA. Temperature and Exposure Time Impact on the Extraction of Opuntia ficus-indica and Opuntia dillenii Cladodes on% Yield as a Response: Screening using Design Expert Software. Global Journal of Medical Pharmaceutical & Biomedical Update 2022;17.

[36] Babu GN, Muthukaruppan M, Ahad HA. Impact of Azadirachta indica Fruit Mucilage on particle size and swelling index in Central Composite Designed Acyclovir mucoadhesive microspheres. Baghdad Science Journal 2023;20:0425-.

[37] Chinthaginjala H, Ahad HA, Samhitharao B, Yasaswini VS, Madana G, Varam NJ, et al. Formulation and In Vitro Evaluation of Fast Disintegrating Tablets of an Anti-Inflammatory Drug using Natural Super Disintegrating Agents.(2). Int J Life Sci Pharma Res 2021;2:9-18.

[38] Nikam N, Kolekar Y. Formulation and Evaluation of Herbal Shampoo: A Comparative Study By DoE/QbD Approach. Journal of University of Shanghai for Science and Technology 2022;24:273-87.

[39] Mohanty SS, Selvammathy R, Ahad HA, Kumar ES. Combined use of glucagon-like peptide-1 receptor agonists and sodium-glucose cotransporter 2 inhibitors for cardiovascular protection. Indian Journal of Pharmacology 2026;58:293-306.

[40] Ryntathiang M, Ingtih M, Sankar SS, Ahad HA, Kumar ES. Endovascular Thrombus Management in Acute Deep Vein Thrombosis: The Expanding Role of Catheter-Directed Thrombolysis. Annals of phlebology 2026;24:1-10.

[41] Jyotheesh G, Mahalingan K, Gandhi S, Mannamanavar MR, Ahad HA. Review on polymer selection and optimization of matrix tablets using systematic formulation strategies. Indian Journal of Biochemistry and Biophysics (IJBB) 2026;63:770-81.

[42] Veed HKK, Kumar ES, Valappil NV, Kotian AC, Prasad P, Ahad HA. A Central Composite Design Optimized Diclofenac Potassium Loaded Niosomal Gel: Fabrication and Evaluation. INDIAN JOURNAL OF PHARMACEUTICAL EDUCATION AND RESEARCH 2026;60:S584-S96.

[43] Nikhil Kumar G, Satheesh Kumar E, Banoth TNS, Sugunarajan Latha A, Melehalli Shivaraju B, Abdul Ahad H. Harnessing liposome technology for precision medicine: design, delivery, and clinical impact. Nanomedicine Journal 2025.

[44] Abdul Ahad H, SP R, Sugali Banoth T, Ganugapenta NK. Development and In Vitro Characterization of a HPMC K4M and Guar Gum-Based Triple-Layer Matrix Tablet System for Chondroitin Sulphate and Diclofenac Sodium. Bulletin of Pharmaceutical Sciences Assiut University 2025.

[45] Venkatesh R, Ahad HA, Sreedhara A, Gowda SR, Krishnappa C, Nagesh PH. Innovative Cloud Solutions: Reshaping the Future of Healthcare and Pharma Industries with Enhanced Efficiency, Collaboration and Patient-Centric Paradigms. Asian Journal of Pharmaceutical Research 2025;15:163-70.

[46] Satheesh Kumar E, Ahad HA. In Vivo Pharmacokinetic Evaluation of a Gastro-retentive Triple Drug Delivery System Utilizing Natural Polymers for Enhanced Helicobacter pylori Eradication. Bulletin of Faculty of Pharmacy Cairo University 2025;63:6.

[47] Singh R, Ahad HA, Rakesh S, Kumar GN, Mohanraja P, Jyotheesh G. Enhancing environmental health: The role of chemical flocculation in wastewater remediation. Research Journal of Pharmaceutical Dosage Forms and Technology 2025;17:41-52.

[48] Gowda SR, Ahad HA, Kumar ES, Sreedhara A, Venkatesh R. A Deep Dive into Microspheres-A Comprehensive Review for Researchers Uncovering Triumphs, Innovations and Future Directions. Asian Journal of Research in Pharmaceutical Science 2024;14.

[49] Ndongwe T, Makoni PA, Matlou GG, Nhlapo TA, Corvis Y, Siwe-Noundou X, et al. Predicting and validating surfactant performance in nanocrystal stabilization using multi-criteria decision analysis and solubility parameter modeling. European Journal of Pharmaceutical Sciences 2026:107479.

[50] Yanamadala Y, Muthumula CMR, Khare S, Gokulan K. Strategies to enhance nanocrystal formulations for overcoming physiological barriers across diverse routes of administration. International Journal of Nanomedicine 2025:367-402.

[51] Jain A. Formulation and In-Vitro evaluation of Nanocrystal formulation of poorly soluble drugs. Journal of Drug Delivery and Therapeutics 2020.

[52] Romero GB, Keck CM, Müller RH, Bou-Chacra NA. Development of cationic nanocrystals for ocular delivery. European Journal of Pharmaceutics and Biopharmaceutics 2016;107:215-22.

[53] Abduljabbar A. Nanocrystal-Loaded Orally Disintegrating Films for Pediatric Oncology: Formulation, Stability, and Therapeutic Outcomes. Journal of Nanostructures 2026;16:794-807.

[54] Ullah N, Khan S, Ahmed S, Govender T, Faidah HS, de Matas M, et al. Dexibuprofen nanocrystals with improved therapeutic performance: fabrication, characterization, in silico modeling, and in vivo evaluation. International journal of nanomedicine 2018:1677-92.

[55] Fouziya B, Hindustan AA, Dontha SC, Jagarlamudi SV, Reddy UC, Reddy PN. Fabrication and evaluation of cefpodoxime proxetil niosomes. Asian Journal of Pharmacy and Technology 2022;12:109-12.

[56] Kumar P, Singh AK, Almalki WH, Alruwaili NK, Alzahrani A, Alhamyani A, et al. Development, Characterization, In Vitro, Ex Vivo, and Stability Evaluation of a Miconazole Nitrate Nanocrystal-loaded Hydrogel for Topical Application. Current Medicinal Chemistry 2026;33:835-59.

[57] Chinthaginjala H, Bogavalli V, Hindustan AA, Pathakamuri J, Pullaganti SS, Gowni A, et al. Nanostructured lipid carriers: a potential era of drug delivery systems. Ind J Pharm Edu Res 2024;58:21-33.

[58] Pernochi Scerbo JM, Ribotta J, Palma SD, Formica ML. Management of Ocular Diseases Using Nanocrystal-based Formulations. Nanocrystals: A Universal Tool for the Formulation of Poorly Soluble Drugs: Springer; 2026. p. 213-46.

[59] Eltahir AKAE, Ahad HA, Haranath C, Meharajunnisa B, Dheeraj S, Sai BN. Novel phytosomes as drug delivery systems and its past decade trials. Research Journal of Pharmaceutical Dosage Forms and Technology 2023;15:51-4.

[60] Patil R, Pardhi E, Mehra NK. Recent Advancements in Nanocrystals: Emerging Trends in Drug Delivery Systems. Crystal Growth & Design 2025;25:8795-824.

[61] Abdul AH, Bala AG, Chintaginjala H, Manchikanti SP, Kamsali AK, Dasari RRD. Equator assessment of nanoparticles using the design-expert software. International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN) 2020;13:4766-72.

[62] Haranath C, Vamsi KS, Qarmout YA, Ahad HA, Chandana S. Impact of Vroman's Effect on Pharmacodynamics and Pharmacokinetics on Nanoparticulate Drug Delivery Systems. Journal of Young Pharmacists 2022;14.

[63] Haranath C, Poojitha N, Ahad HA, Yarra S, Eranti B. Recent advances in lipid based nanovesicles for transdermal drug delivery. Journal of medical pharmaceutical and allied sciences 2022;11:5217-20.

[64] Ahad HA, Chintaginjala H, Rahamathulla S, Rupasree A, Kumar AS, Pallavi BP. Pathfinder Nanosponges for Drug Targeting by Factorial Design: A Glance Review. Research Journal of

Pharmaceutical Dosage Forms and Technology 2021;13:341-4.
[65] Kumar LS, Ahad HA. Quality by design based quercetin hydrate nanoemulsions for enhanced solubility by reducing particle size. Indian Journal of Pharmaceutical Education and Research 2023;57:965-70.
[66] Teja AST, Ahad HA, Chinthaginjala H, Baba P, Fakruddin GAK, Tarun K. A Primer for Pharma Professionals on 3D Printing: Fundamentals and Applications. Journal of Young Pharmacists 2023;15:7-12.
[67] Chen L, Wang Y, Zhang J, Hao L, Guo H, Lou H, et al. Bexarotene nanocrystal—Oral and parenteral formulation development, characterization and pharmacokinetic evaluation. European Journal of Pharmaceutics and Biopharmaceutics 2014;87:160-9.
[68] A. K. Prusty*and S. K. Sahu, Biodegradable Nanoparticles - A Novel Approach for Oral Administration of Biological Products, International Journal of Pharmaceutical Sciences and Nanotechnology, 2009, 2(2),503-508. doi.org/10.37285/ijpsn.2009.2.2.2
[69] Prasanna Parida, Amiya Kumar Prusty, Bikash Ranjan Jena, Diptimayee Jena, Chinmaya Sahoo, Biodegradable Polymer-based Nanoparticles: A Breakthrough in Oral Protein Drug Delivery, Nanoscience & Nanotechnology-Asia; 2025,15(4), e22106812388555. DOI:10.2174/0122106812388555250724094310.
[70] Prasanna Parida, Amiya Kumar Prusty, Chinmaya Sahoo, Sujit Dash, Bikash Ranjan Jena, Kirtimaya Mishra, 2025. Ionic gelation technique using biodegradable polymer for stabilized and sustained oral delivery of nano protein, Advancement in Animal Handling and Generative AI for Pre-clinical Studies Pages:22-27Publisher-CRC Press.ISBN-9781041140818, DOI:10.1201/9781003672869-3
[71] Prasanna Parida, Amiya Kumar Prusty, Rajat Kumar Prusty, Bikash Ranjan Jena, Debabrata Panda, Sanjoy Das, 2025. Overcoming hurdles in oral protein and peptide drug delivery: Strategies for future prospective, advancement in Animal Handling and Generative AI for Pre-clinical Studies. Pages 28-41, Publisher CRC Press. ISBN-9781041140818, DOI:10.1201/9781003672869-4
[72] S. K. Sahu and A. K. Prusty*, Design and evaluation of a nanoparticulate system prepared by biodegradable polymers for oral administration of protein drugs, Die Pharmazie 2010; 65, 824-829.
[73] A. K. Prusty* and S. K. Sahu, Development and evaluation of insulin incorporated nanoparticles for oral administration, ISRN Nanotechnology, 2013, 1-6. doi.10.1691/ph.2010.0188.
[74] Parida, P., Prusty, A.K. Optimization of Streptokinase-Loaded Chitosan-Alginate Nanoparticles Using Box Behnken Design for Oral Delivery. BioNanoSci. 16, 126 (2026). https://doi.org/10.1007/s12668-025-02350-2