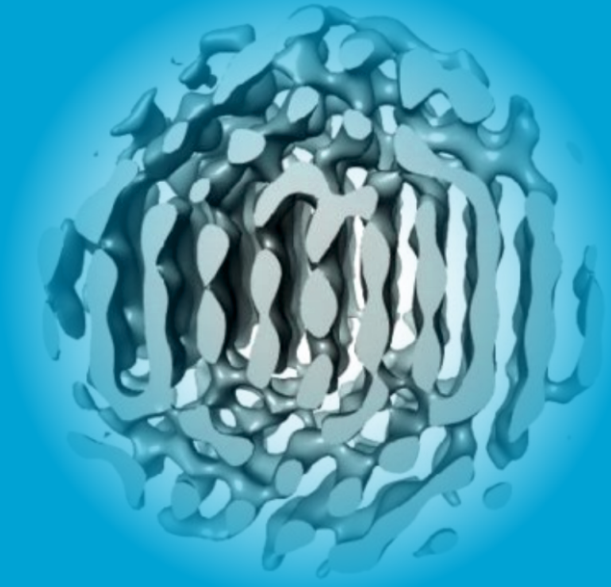


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Prof. Dr. Kun HARISMAH
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**DESIGN AND DEVELOPMENT OF NANOCRYSTALLINE
DRUG DELIVERY SYSTEMS FOR IMPROVED
SOLUBILITY AND BIOAVAILABILITY
OF POORLY WATER-SOLUBLE DRUGS:
A PHARMACEUTICAL PERSPECTIVE**

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PREFACE

Poor aqueous solubility of many therapeutic compounds is a significant challenge in contemporary drug development, especially for oral drug delivery systems in which fast and reproducible dissolution of the drug is required to achieve maximum drug absorption and hence optimal effect. With almost 40% of drugs currently on the market and up to 90% of drug candidates being discovered as Biopharmaceutical Classification System (BCS) Class II and IV—defined as low solubility and/or low permeability—there has never been a greater requirement for efficient formulation approaches. Of the numerous strategies for enhancing solubility created over the years, nanocrystalline drug delivery systems have proved to be a potent and effective one. They are composed of pure drug particles minimized to nanometer sizes, commonly ranging from 100–1000 nm, and are stabilized with surfactants or polymers to avoid aggregation and achieve a colloidal dispersion stability. The nanometer size of the drug particles provides a very much enhanced surface area, resulting in an enormous enhancement of dissolution rate and saturation solubility, as dictated by the Noyes–Whitney and Ostwald–Freundlich equations. Consequently, nanocrystalline drugs significantly improve drug absorption rate and extent in the gastrointestinal tract, ultimately enhancing bioavailability and therapeutic responses. The development and designing of nanocrystalline drug delivery systems involve a multidisciplinary approach drawing on pharmaceutical, chemical, and engineering sciences. There are two main methodologies used in the production of

nanocrystals: top-down and bottom-up approaches. Top-down methods like wet media milling and high-pressure homogenization break down large drug crystals into the nanoscale with the aid of mechanical forces. Top-down methods are common in industry because they are scalable and reproducible. Bottom-up methods like antisolvent precipitation and controlled crystallization entail the creation of nanocrystals from molecularly dissolved drug under controlled levels of supersaturation. Hybrid techniques integrating the two approaches are also under investigation to maximize particle size and stability with minimal energy input and processing time. The selection of the production method is a function of the physicochemical characteristics of the drug, the desired product attributes, and manufacturing viability. Stabilization of nanocrystals is an important process in their development. Nanocrystals will aggregate from high surface energy without stabilization, leading to loss of nanoscale benefits. The use of proper stabilizers like nonionic surfactants (e.g., poloxamers, Tweens), polymers (e.g., PVP, HPMC), and cryoprotectants is critical to maintain particle stability during processing, storage, and administration. These stabilizers not only avoid agglomeration but also control redispersibility, mucoadhesion, and drug release kinetics. In addition, drying processes like freeze-drying or spray drying can be utilized to transform liquid nanosuspensions into solid forms without losing particle size and functionality. Comprehensive characterization is indispensable for ensuring the quality, safety, and efficacy of nanocrystalline formulations. Techniques such as dynamic light scattering (DLS), scanning and transmission electron microscopy

(SEM/TEM), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and zeta potential analysis are employed to assess critical attributes such as particle size, morphology, crystallinity, thermal behavior, and stability. These parameters directly influence dissolution performance, bioavailability, and product shelf life. Nanocrystalline drug delivery systems enhance solubility, dissolution, and bioavailability, making them crucial in pharmaceutical development for improved drug performance, patient compliance, and therapeutic efficacy.

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TABLE OF CONTENTS

PREFACE	iv
INTRODUCTION	1
1. Nanocrystalline Technology: Tackling Solubility Challenges	4
2. Challenges in Delivering Poorly Water-Soluble Drugs	7
3. Design and Development of Nanocrystalline Drug Delivery Systems	11
4. Materials Employed in Nanocrystalline Drug Delivery Systems.....	15
4.1. Active Pharmaceutical Ingredients (APIs).....	16
4.2. Stabilizers and Excipients.....	17
4.3. Polymers and Polymeric Materials.....	19
5. Methods for Preparing Nanocrystalline Drug Delivery Systems..	20
5.1. Top -Down Approaches.....	21
5.1.1. High-pressure homogenization (HPH).....	21
5.1.2. .Bead milling.....	25
5.1.3.Jet milling.....	29
5.2. Bottom -Up Methods.....	33
5.2.1. Solvent evaporation.....	34
5.2.2. precipitation.....	38
6.Characterization of Nanocrystalline Drug Delivery Systems.....	45

7. In Vitro and In Vivo Assessment of Nanocrystalline Drug Delivery Systems.....	52
8. Nanocrystalline Drug Delivery Systems: Toxicity and Biocompatibility.....	57
9. Regulatory Considerations for Nanocrystalline Drug Delivery Systems.....	61
10. Clinical Applications of Nanocrystalline Drug Delivery Systems.....	66
11. Case Studies: Nanocrystalline Drug Delivery Systems in Practice.....	70
12. Future Directions for Nanocrystalline Drug Delivery Systems.....	75
13. Limitations and Challenges of Nanocrystalline Drug Delivery Systems.....	79
14. Patent and Intellectual Property Implications for Nanocrystalline Drug Delivery Systems.....	84
15. Comparison with Other Drug Delivery Systems.....	88
16. Conclusion.....	93
REFERENCES.....	94

INTRODUCTION

Nanocrystalline drug delivery systems have proven to be revolutionary in the field of pharmaceutical sciences, providing a hopeful solution for improved solubility, bioavailability, and therapeutic activity of drugs with poor water solubility. Drug development has encountered serious challenges during the last several decades in delivering drugs through effective administration because of the poor dissolution properties in an aqueous medium. With almost 40% of newly discovered chemical compounds having low water solubility, the urgency for novel drug delivery methods has increased^[1]. Nanocrystalline formulations, through the decrease in particle size to nanometer dimensions, have transformed how drugs interact with biological systems and resulted in enhanced therapeutic benefit. Nanocrystals are pristine drug particles downsized to nanometric dimensions, and their usual range is from 10 to 1000 nanometers, stabilized by surfactants or polymers to avoid agglomeration. They are unlike other nanoscale drug delivery systems like polymeric nanoparticles or liposomes in that they consist completely of drug substance with no other carrier materials. Their minute size causes an enhanced surface area, which enormously augments drug dissolution, hence the bioavailability, particularly of Biopharmaceutics Classification System (BCS) class II and IV drugs that are poor solutes. One of the main benefits of nanocrystalline drug delivery is the capability to overcome solubility issues without changing the molecular architecture of the drug^[2,3]. Conventional techniques like salt formation, co-solvency, and complexation are usually limited by drug instability or formulation issues. Yet, it is simple to formulate nanocrystals in different dosage forms, such as tablets, capsules, injectables, and ophthalmic solutions, based on the desired application of pharmaceuticals. Nanocrystal formation is brought about through a range of top-down and bottom-up methods. The top-down methods are media milling and high-pressure homogenization, which consist of deaggregating larger drug particles into nanoscale structures. Media milling applies high-energy impact forces produced by grinding beads to decrease particle size, while high-pressure homogenization

makes the drug suspensions pass through small channels at elevated pressures in order to reduce size. Conversely, bottom-up strategies like precipitation and supercritical fluid technology entail the controlled coming together of molecules to create nanocrystals in a manner that is uniform in size distribution and stable. The physicochemical characteristics of nanocrystals also contribute greatly to their enhanced pharmacokinetic and pharmacodynamics. The higher surface area of nanocrystals improves the dissolution rate, thus facilitating quicker absorption of high drug levels and rapid onset of action. This is especially helpful for poorly water-soluble drugs, where the traditional formulations cannot produce a desirable therapeutic plasma level. Nanocrystals also have increased adhesion to biomembranes, which enhances permeation and distribution in tissues. Nanocrystalline drug delivery systems have proven to be of vast potential to enhance oral bioavailability. Most of the poorly soluble drugs, when given in their traditional form, have poor absorption and low systemic availability. Nanocrystalline drug formulations overcome this limitation by increasing drug solubility in the gastrointestinal tract, leading to consistent and effective absorption. In addition, stabilization of nanocrystals with surfactants avoids aggregation and confers long-term stability, essential for sustaining activity over a period of time. Besides oral delivery, nanocrystals have shown impressive benefits in parenteral drug delivery. Injectable nanocrystal formulations have provided the opportunity to administer drugs with poor solubility without using cytotoxic organic solvents or high amounts of surfactants. Not only does this enhance patient safety but also the bioavailability of the drug by targeting active drug particles directly into systemic circulation. Injectable formulations based on nanocrystals have been formulated for anticancer drugs, antibiotics, and anti-inflammatory drugs, where there is an urgent need for instant and effective drug activity^[4,5,6,7]. Ophthalmic drug delivery is also an area where nanocrystalline systems have come to the fore. Most ophthalmic drugs are plagued by low corneal penetration and elimination, resulting in inefficient therapeutic effects. Nanocrystals increase drug retention at the ocular surface, increasing drug residence time and enhancing absorption into ocular tissues. This becomes especially useful in the treatment of eye diseases

like glaucoma, conjunctivitis, and dry eye syndrome, where a sustained release of the drug is necessary for therapeutic effect. Transdermal drug delivery has also seen advances with the introduction of nanocrystalline formulations. Traditional topical products tend to fail due to poor skin penetration owing to the lipophilic stratum corneum. The reduced size and increased solubility of nanocrystals allow for easier skin penetration, resulting in improved drug deposition in the dermis. This is beneficial in the treatment of dermatological diseases like psoriasis, eczema, and local pain, where successful drug penetration is important. The use of nanocrystals is also present in pulmonary drug delivery. Inhalable nanocrystals are designed to treat respiratory disorders like asthma, chronic obstructive pulmonary disease (COPD), and tuberculosis. The submicron size of the particles promotes deeper penetration into the lungs and direct delivery of the drug into the affected alveolar areas. This targeted therapy reduces systemic side effects and increases the concentration of drug locally, making inhalable nanocrystals a novel option for respiratory treatment. Safety and stability of nanocrystalline drug products are critical factors in their formulation. Stability problems like aggregation, Ostwald ripening, and recrystallization may influence the effectiveness of nanocrystals in the long term. Thus, the selection of stabilizers, process conditions, and storage conditions is essential in preserving the physicochemical stability of nanocrystals. Sophisticated characterization tools like dynamic light scattering, X-ray diffraction, and differential scanning calorimetry are used to evaluate particle size distribution, crystallinity, and thermal stability to ensure sound formulation development. Although nanocrystalline drug delivery systems have many benefits, they also have difficulties in large-scale production, regulatory acceptance, and cost-effectiveness. Manufacturing processes such as high-pressure homogenization and media milling require specialized equipment and stringent quality control measures, increasing production costs^[8,9,10,11]. Additionally, regulatory agencies demand comprehensive safety and efficacy data before approving nanocrystalline formulations for commercial use. Overcoming these challenges will demand ongoing advances in formulation technology, increased manufacturing scalability, and less complex regulatory

avenues. In summary, nanocrystalline drug delivery systems are a major innovation in pharmaceutical technology, overcoming the shortcomings of drugs that are poorly soluble by improving dissolution, bioavailability, and therapeutic efficacy. Their cross-application across routes of administration, such as oral, parenteral, ophthalmic, transdermal, and pulmonary, speaks to their promise in contemporary drug development. With continuous research and development, nanocrystalline formulations are poised to revolutionize the practice of drug delivery with novel solutions for a variety of therapeutic opportunities^[12,13,14].

1. Nanocrystalline Technology: Tackling Solubility Challenges

Nanocrystalline technology has become a groundbreaking approach to tackle several hurdles faced in a variety of scientific and industrial sectors, especially in pharmaceuticals, material sciences, and chemical engineering. One of the main challenges in these fields is solubility, which plays a crucial role in determining the bioavailability, effectiveness, and stability of compounds, particularly when it comes to drug formulation. With its unique capability to work with materials at the nanoscale, nanocrystalline technology offers an effective solution to the long-standing solubility issues that have complicated the creation of effective treatments and products. To get a clearer picture of how nanocrystalline technology addresses solubility concerns, it's essential to first grasp the core issue. Solubility refers to how well a substance—like a drug or chemical compound—dissolves in a solvent, usually water, to create a consistent solution^[15,16,17]. How quickly and extensively a compound dissolves in a solvent can greatly influence its bioavailability, which is particularly important for oral medications. Many compounds, especially those that are hydrophobic or not easily soluble in water, have a tough time dissolving in the gastrointestinal system, leading to limited absorption into the bloodstream. This can lower the effectiveness of oral drug formulations and often necessitates higher doses, increasing the risk of side effects and affecting patient compliance. Nanocrystalline technology offers a way to resolve these solubility issues by breaking down a compound into nanoparticles, which typically range from 1 to 100 nanometers in diameter. At this

size, the physical and chemical properties can change significantly compared to larger particles, often exhibiting better solubility, bioavailability, and stability. The smaller size leads to a higher surface area-to-volume ratio, allowing better interaction between the drug and the solvent, which results in quicker and more complete dissolution, ultimately enhancing bioavailability^[18,19,20,21]. One major strength of nanocrystalline technology is its ability to modify material properties at both the atomic and molecular levels. Reducing a substance to the nanoscale alters its thermodynamic properties, including its surface energy, solubility, and the rate at which it dissolves. Nanocrystals have more atoms or molecules exposed on their surfaces, enabling more effective interactions with solvents. Plus, nanoparticles usually demonstrate greater stability in solutions, helping to prevent problems like aggregation or recrystallization that can occur with bigger particles. These improved dissolution characteristics make nanocrystals especially beneficial for drugs that face low solubility and poor bioavailability, such as certain active pharmaceutical ingredients (APIs). Creating nanocrystalline formulations involves transforming bulk materials into nanoparticles^[22,23,24,25]. Several methods can be employed to produce nanocrystals, such as milling, high-pressure homogenization, and solvent evaporation. Milling uses mechanical grinding to reduce particle size, whereas high-pressure homogenization forces the material through a narrow space under pressure to break it down into finer particles. Solvent evaporation typically involves dissolving the material in a solvent and then carefully evaporating that solvent to leave behind the nanocrystals. Each of these methods comes with its own set of advantages and challenges, but they all leverage the concept of reducing particle size to enhance material solubility. Nanocrystalline technology has made a significant impact in the pharmaceutical arena, transforming how oral drug formulations are developed^[26,27,28,29]. Many drugs, particularly those that are poorly soluble, face notable bioavailability challenges. For example, compounds like curcumin, paclitaxel, and diazepam have very limited solubility in water, leading to unpredictable absorption and diminished therapeutic benefits when taken orally. By creating nanocrystals from these substances, their solubility can be greatly improved, leading to

better absorption rates in the gastrointestinal tract. This can result in more reliable and effective therapeutic outcomes and might even reduce the necessary dosage. Beyond pharmaceuticals, nanocrystalline technology is making notable advances in other industries, including food science, cosmetics, and materials engineering. In food science, enhancing the solubility of vitamins and antioxidants using nanocrystals can improve their bioavailability and stability in food products. In the cosmetics realm, nanocrystals enhance the delivery and absorption of active ingredients in skincare items, making them more effective in treating skin issues. In material science, the unique qualities of nanocrystalline materials are leveraged for their mechanical, optical, and electronic properties, creating new opportunities for the development of high-performing materials. One of the standout benefits of nanocrystalline technology is its capacity to improve the solubility of hydrophobic compounds, which are among the toughest to formulate^[30,31,32,33]. These compounds often aggregate or crystallize when exposed to water, leading to poor dissolution and bioavailability. By reducing their size to the nanoscale, the surface area increases, allowing the compounds to disperse more effectively in aqueous environments. This improved solubility not only boosts drug absorption but also enables the creation of more efficient formulations that can be given in lower doses, decreasing the risk of side effects. Moreover, the use of nanocrystals can help tackle the challenges posed by polymorphism, where a compound can take on multiple crystal forms, each exhibiting different solubility traits. Some forms may not dissolve well, while others do. Nanocrystal technology allows for control over which crystal form is utilized, ensuring that the most soluble variant is selected for drug formulations. This can lead to more consistent and reliable performance, ultimately enhancing the therapeutic effectiveness of a drug. Despite the many advantages, there are still challenges to consider with nanocrystalline technology. One key issue is the stability of nanocrystals in suspension. Because of their small size and high surface energy, they tend to aggregate over time, which can diminish their effectiveness by reducing surface area and affecting dissolution rates. To address this, various stabilizing agents like surfactants, polymers, or other excipients are often employed to prevent

aggregation and improve the long-term stability of nanocrystal suspensions. Another factor to keep in mind is the potential toxicity of nanocrystalline materials. Generally viewed as safe, their tiny size and large surface area can sometimes lead to unexpected interactions with biological systems. So, it's crucial to thoroughly evaluate the safety of nanocrystalline formulations through preclinical and clinical studies to ensure they don't have harmful effects. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA), are keeping a close eye on how nanomaterials are used in drug formulations to make sure they meet safety standards before they get approved for use. In summary, nanocrystalline technology holds great promise for addressing the age-old challenge of solubility in a range of scientific and industrial fields. By shrinking particle sizes down to the nanometer scale, nanocrystals show improved solubility, bioavailability, and stability, making them invaluable in developing new drugs and other products. The ability to manipulate materials on a nanoscale opens up new possibilities for enhancing the performance of various compounds, especially those struggling with solubility. As the technology advances, it's likely that nanocrystalline formulations will play a vital role in modern pharmaceutical development and beyond, pushing the boundaries of nanotechnology further^[34,35,36].

2. Challenges in Delivering Poorly Water-Soluble Drugs

Delivery of poorly water-soluble drugs remains one of the most significant issues in drug development. About 40% of commercially available drugs and almost 90% of drug candidate drugs in development are plagued by poor solubility, resulting in poor bioavailability, variable therapeutic response, and formulation issues. Resolution of these issues relies on the understanding of physicochemical properties of drugs, formulation techniques, and innovations in drug delivery technology. One of the main issues in poorly water-soluble drug delivery is their slow dissolution rate. Solubility is a direct predictor of the absorption of a drug in the GI tract, with aqueous solubility being one of the most significant factors in ensuring adequate availability of the drug in systemic circulation. Low-solubility drugs are more prone to exhibit incomplete or variable absorption due to the decreased availability of

the drug in the GI tract, resulting in less than optimal therapeutic response. The Biopharmaceutics Classification System (BCS) classifies drugs into four categories according to solubility and permeability, with Class II and Class IV drugs plagued by serious issues of solubility. BCS Class II drugs are poorly soluble but highly permeable, and Class IV drugs are poorly soluble and poorly permeable, making their formulation extremely challenging. Pharmacokinetic variability is another serious issue. Poorly water-soluble drugs are more likely to exhibit erratic absorption profiles, which are affected by factors such as pH-dependent solubility, food effect, and gastric emptying rate^[37,38,39,40]. This variability causes fluctuating drug plasma concentrations, and drug failure or toxicity is more probable. For example, pH-dependent solubility drugs can be poorly soluble in the stomach but more soluble in the intestine, with unpredictable absorption. Additionally, food availability can increase or decrease drug solubility, and hence formulation development and dosing regimen becomes more challenging. Particle size reduction is a general approach to enhance the dissolution rate and solubility of poorly water-soluble drugs. Techniques such as micronization and nanonization are aimed at enhancing surface area for dissolution, and hence the bioavailability. However, limitations such as stability concerns, nanoparticle agglomeration, and potential changes in drug crystallinity are present, which affect its dissolution behavior. Additionally, the high-energy process of nanoparticle formation may lead to drug degradation or alteration of drug properties, which need careful optimization. Lipid-based formulations have also been found to be an effective solution for the problem of solubility. Formulations such as self-emulsifying drug delivery systems (SEDDS) and lipid nanoparticles enhance drug solubility and permeability by enabling dissolution in the lipid phase prior to introduction into the aqueous environment of the GI tract. Lipid-based systems also enable lymphatic transit, avoiding first-pass metabolism and increased bioavailability. However, limitations such as stability, scalability, and regulatory challenges limit their wider applications. Additionally, lipid formulations must be specially designed so that phase separation, precipitation, or drug degradation does not take place over time. Amorphous solid dispersions (ASDs) are

another sophisticated formulation method for enhancing the solubility of poorly water-soluble drugs. By converting the crystalline drug to the amorphous form, ASDs enhance dissolution and bioavailability rates. Polymers such as hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) are generally used to stabilize the amorphous form and prevent recrystallization. Although they are beneficial, ASDs have limitations such as physical and chemical instability, potential hygroscopicity, and lack of scalability for large-scale production. Maintenance of the amorphous structure for the whole product shelf life is required for uniform drug performance. Cyclodextrins were widely researched as solubilizers of weakly water-soluble drugs^[41,42,43]. These cyclic oligosaccharides include hydrophobic drugs in the form of inclusion complexes, resulting in increased apparent solubility and dissolution rate. Cyclodextrins are a versatile means of increasing drug solubility without disturbing the chemical architecture of the drug. Their utility, however, is limited by restrictions on their potential toxicity, proficiency of complexation, and need for high concentration in certain cases. Approval of cyclodextrin-based drugs also requires extensive safety evaluations, which are part of the complexity of their construction. Nanotechnology-based drug delivery systems, including polymeric nanoparticles, liposomes, and dendrimers, have been studied for their ability to improve the solubility and bioavailability of weakly water-soluble drugs. These systems enable targeted drug delivery, controlled release, and improved stability. However, their development from the lab to the clinic is restricted by numerous challenges, including scalability, reproducibility, and regulatory challenges. The high cost of particle preparation and toxicity and long-term safety issues also restrict their commercialization^[44,45,46,47]. Moreover, the interaction of nanoparticles with biological membranes and immune response must be extensively explored to ensure therapeutic efficacy and safety. Co-crystals have been identified as a new approach to modulating the physicochemical properties of weakly water-soluble drugs. By including co-formers in crystalline forms, co-crystals improve solubility and dissolution rates without compromising drug stability. Pharmaceutical co-crystals can be engineered to maximize solubility without disturbing the molecular

architecture of the drug, making it an interesting approach for formulation development. However, their stability, regulatory status, and manufacturability are active research and optimization topics. The role of surfactants in increasing drug solubility and dissolution is established. Surfactants reduce surface tension, improve wetting, and cause micelle formation, and thus improve drug solubilization. Polysorbates, sodium lauryl sulphate, and bile salts are the most widely used surfactants. While surfactants offer a convenient means of enhancing solubility, the risk of irritancy, toxicity, and incompatibility with other formulation components necessitates proper choice and optimization. Current developments in solid-state chemistry and computational modeling have also assisted in the resolution of solubility issues. Predictive modeling allows researchers to identify potential solubility-improving strategies early on in drug development, reducing the number of experimental tests. Additionally, solid-state transformations such as salt formation and polymorph screening provide further means of enhancing drug solubility^[48,49,50,51]. Salt formation, indeed, has been applied extensively to enhance the solubility of weakly acidic and basic drugs. Stability, hygroscopicity, and the choice of suitable counterions, however, are challenges that must be overcome to make this strategy a success. Although breakthroughs in avoiding the pitfalls of poorly water-soluble drugs have been made, regulatory hurdles remain a major obstacle. New drug products must be subjected to rigorous testing of safety, efficacy, and stability for approval. Regulatory bodies such as the FDA and EMA have stringent guidelines for bioavailability studies, choice of excipients, and manufacturing processes. The complexity of new drug delivery systems may necessitate additional tests to demonstrate their superiority over standard products, increasing development time and expense. In summary, the delivery of poorly water-soluble drugs is faced with multiple challenges such as limited dissolution rates, variable pharmacokinetics, stability concerns, and regulatory concerns. While a number of formulation approaches such as particle size reduction, lipid-based systems, amorphous solid dispersions, nanotechnology, and co-crystals have been promising, each approach has its limitations. Increased research and technological development

will be required to design effective drug delivery platforms that optimize bioavailability and therapeutic response. The intersection of computational modeling, new excipients, and new manufacturing processes will be necessary to solve the problem of solubility, ultimately leading to better patient outcomes and increased treatment possibilities^[51,52].

3. Design and Development of Nanocrystalline Drug Delivery Systems

Nanocrystalline drug delivery systems are a cutting-edge solution in medicinal chemistry, putting into use the potential of nanotechnology to overcome most of the challenges long prevailing in the way of effective drug formulation. Nanocrystalline drug delivery systems are specifically engineered to overcome significant challenges like low solubility, poor bioavailability, and fast elimination of drugs from the body. Nanocrystals with particle size between 1 and 100 nanometers have enormous potential to enhance the solubility and bioavailability of low water-soluble drugs. Design and development of nanocrystalline drug delivery systems involve complex considerations ranging from the choice of appropriate materials to the adjustment of particle size, surface properties, and the entire formulation to deliver maximum therapeutic effect and safety. Poor solubility of most active pharmaceutical ingredients (APIs) is one of the significant challenges to the pharmaceutical industry today. According to the Biopharmaceutics Classification System (BCS), most of the drugs belong to Class II and Class IV, which are marked by poor solubility and, in Class IV, poor permeability as well. For the drugs belonging to Class II and Class IV, their dissolution rate is the rate-determining step in the absorption in the gastro-intestinal tract, which may result in poor bioavailability. To improve the therapeutic efficacy of such drugs, appropriate improvement in their solubility and dissolution rate is necessary. Nanocrystals, with their very small size and high surface area, have been found to be an acceptable solution to this problem, offering an enormous improvement in the dissolution and absorption of such poorly soluble drugs. Designing a nanocrystalline drug delivery system begins with the choice of the active pharmaceutical ingredient

(API) to be formulated as nanocrystals. The choice of API is extremely critical, since the growth of nanocrystals is best for poorly water-soluble drugs. These drugs have low dissolution rates in the gastrointestinal tract, leading to erratic pharmacokinetics and non-optimised therapeutic response. By reducing the size of the drug particles to the nanoscale, the surface area of the drug increases exponentially, thereby leading to fast dissolution in aqueous media. This large surface area also increases the rate of absorption of the drug through the intestinal membrane, increasing the bioavailability of the drug^[53,54]. Once the API is chosen, the next step is to choose the best method of preparing the nanocrystals. Several methods have been developed for preparing nanocrystals, each having its own advantages and limitations. High-pressure homogenization, milling, solvent evaporation, and precipitation techniques are the most popular methods. High-pressure homogenization is one of the most popular methods, where the drug is suspended in a liquid medium and then forced under high pressure through a narrow orifice. This causes the fragmentation of the particles into nanometer-sized crystals. Milling, however, is done by grinding the drug in wet or dry form, breaking the particles into smaller particles. Both the processes yield formation of nanosized crystals, which are further processed into final drug delivery system. Other very crucial consideration in the development of nanocrystalline drug delivery systems is the addition of stabilizers or surfactants to avoid agglomeration of the nanocrystals. Due to their small size and high surface energy, nanocrystals tend to agglomerate or cluster over a period of time, which decreases their efficacy. To solve this issue, stabilizers such as polymers, surfactants, and excipients are added in the formulation to provide stability to the nanocrystals. These stabilizers are adsorbed onto the surface of the nanocrystals, preventing aggregation and enhancing the long-term stability of the drug delivery system. Choice of the stabilizer is crucial, as it must be biocompatible, non-toxic, and able to provide stability to the nanocrystals in the formulation and in vivo conditions. The surface properties of the nanocrystals are crucial to the function of the drug delivery system^[55,56]. The charge and hydrophilicity of the nanocrystals can have an important role in their interaction with biological systems. For example,

positively charged nanocrystals can interact with the negatively charged cell membranes, which could enhance the uptake of the cells. Similarly, the surface modifications can be used to enhance the solubilization and stability of the nanocrystals in physiological conditions. These modifications are performed by coating the nanocrystals with agents such as polyethylene glycol (PEG), lipids, or polysaccharides, which can enhance the biocompatibility and half-life of circulation of the nanocrystals in blood. Nanocrystalline drug delivery systems can be formulated to achieve specific release profiles, and thus controlled or sustained release of the drug can be attained. This is particularly useful for drugs requiring long duration of therapeutic action or for drugs having a short half-life in the body. By controlling the release of the drug from the nanocrystals, it is possible to achieve a more stable and sustained drug concentration in the blood, which can improve patient compliance and reduce side effects. Release rate can be controlled by factors such as the size of the nanocrystals, the nature of the stabilizer used, and the composition of the general drug delivery system. In certain cases, the nanocrystals are suspended in a polymer matrix or lipid system, which also controls the release of the drug^[57,58]. The bioavailability and pharmacokinetics of nanocrystalline drug delivery systems are key factors that must be optimally developed. Nanocrystals have the potential to enhance bioavailability by increasing solubility and dissolution rate of drugs and enhancing absorption of drugs in the gastrointestinal tract. However, high bioavailability needs good nanocrystal formulation to impart stability and functionality in vivo. Nanocrystals can also be used in drug delivery systems to improve drug targeting to organs or tissues. This may be achieved through surface modification to enable receptor-mediated targeting or use of nanoparticles that possess the ability to cross biological barriers, such as the blood-brain barrier, to deliver the drug to the target site. Once the nanocrystals are formulated and integrated into a drug delivery system, rigorous in vitro and in vivo testing must be performed to assess their functionality. In vitro assessment typically involves the determination of the dissolution profile, particle size distribution, stability, and drug release kinetics of the nanocrystalline drug formulation. This is achieved through techniques such as dynamic light scattering (DLS),

scanning electron microscopy (SEM), and application of high-performance liquid chromatography (HPLC). In vivo assessment, however, quantifies the pharmacokinetics, bioavailability, and therapeutic activity of the nanocrystal-based drug delivery system. Animal testing is also widely employed to determine the ADME of the drug, as well as screen for side effects or toxicity. Nanocrystalline drug delivery systems have several benefits over traditional drug preparations^[59,60]. The most significant benefit is the improvement of the solubility and bioavailability of weakly water-soluble drugs. By decreasing the particle size to the nanoscale range, the surface area of the drug is exponentially increased, which improves dissolution and absorption. This can lead to increased therapeutic efficacy and more consistent pharmacokinetics, which is particularly crucial for drugs that have variable absorption in the gastrointestinal tract. Additionally, use of nanocrystals can reduce the need for high doses of the drug, which can reduce the risk of side effects and toxicity. Nanocrystals also possess the property of targeted delivery of the drug, allowing selective delivery of the drug to the target organ or tissue. This is particularly significant for drugs administered in cancer treatment, where the ability to target the site of the tumor directly can increase therapeutic effects while reducing damage to healthy tissue. Additionally, nanocrystalline drug delivery systems can be engineered to deliver sustained or controlled release profiles, reducing the need for frequent dosing and improving patient compliance^[61,62]. By designing the size, surface properties, and release profile of the nanocrystals cleverly, an enormously broad range of drug release profiles can be achieved that can be designed to suit the individual needs of the patient. While there are many advantages of nanocrystalline drug delivery systems, there are a few challenges that need to be overcome in the process. One of the most significant issues is the long-term stability of the nanocrystals and their potential toxicity in vivo. Though nanocrystals are comparatively safe, their high surface area and nanoparticle size sometimes lead to unforeseen interactions with biological systems. One needs to analyze the biocompatibility of the nanocrystals so that they do not create harmful effects on the body. Moreover, storage-induced aggregation of nanocrystals can lead to reduced efficacy, and steps need to be

formulated to prevent this from occurring^[63,64]. Another problem that has to be tackled is the regulatory approval of nanocrystalline drug delivery systems. Due to the innovative nature of nanotechnology, regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require an enormity of safety and efficacy data before they approve nanocrystal-based products for clinical use. This can put a long lag on the development and commercialization of nanocrystalline drug delivery systems. However, as nanotechnology science advances further, regulatory procedures are being formulated to address the inherent issues of nanomaterials, and it is expected that the approval process in the future will be less burdensome. In total, the design and synthesis of nanocrystalline drug delivery systems are a revolutionary idea in pharmaceutical science, with the potential to address genuine issues such as poor solubility, low bioavailability for orally administered drugs, and uncontrolled release of the drug. By exploiting the unique features of nanocrystals, such as the ability to flow freely in the body, these systems have the potential to enhance drug efficacy, reduce side effects, and offer more predictable pharmacokinetic profiles. However, the development of nanocrystalline drug delivery systems must tackle earnestly parameters such as particle size, surface features, stability, and release rates. With ongoing research in this area, it is possible that nanocrystalline drug delivery systems will emerge as an increasingly significant component of the therapy of numerous diseases, especially those characterized by poorly soluble drugs or those necessitating targeted therapy^[65,66].

4. Materials Employed in Nanocrystalline Drug Delivery Systems

The development of nanocrystalline drug delivery systems (NDDS) has revolutionized the pharmaceutical industry considerably, particularly in the formulation of drugs with low solubility. Nanotechnology is exploited in such systems to enhance the solubility, bioavailability, and therapeutic activity of drugs, enabling the administration of highly active drugs that would otherwise lack a significant clinical function. The principal idea behind drug delivery by nanocrystals is the reduction of the size of drugs to the nanoscale, which makes the surface area large

and allows for the faster dissolution, absorption, and bioavailability. Nevertheless, the successful implementation and use of nanocrystalline drug delivery systems are highly reliant upon the choice of appropriate materials for the preparation and stabilization of the nanocrystals. The constituents in NDDS are required to be selected with extreme care depending upon a number of factors such as biocompatibility, stability, function, and capacity to augment the desired therapeutic outcome. These constituents are categorized into a number of different types such as the active pharmaceutical ingredients (APIs), stabilizers or excipients, and the drug delivery vehicles or matrices^[67,68].

4.1 Active Pharmaceutical Ingredients (APIs)

The starting point for any nanocrystalline drug delivery system is the active pharmaceutical ingredient, which is the drug meant to cure the illness for which the system is intended. The major attribute that will assess the appropriateness of an API for employment in nanocrystalline systems is its solubility. Drugs characterized by low water solubility, or limited bioavailability, are the best candidates for nanocrystalline formulation. These drugs tend to be members of Biopharmaceutics Classification System (BCS) Classes II (low solubility, high permeability) and IV (low solubility, low permeability). Most such drugs are not well absorbed in the gastrointestinal tract, and their clinical efficacy is compromised due to their inability to dissolve quickly enough to be absorbed optimally. Finer particle size reduction to the nanometer scale increases the surface area and, with it, the rate of dissolution, which in turn affects their bioavailability. Nanocrystals of APIs enhance the extent and rate of dissolution and promote better absorption during systemic circulation^[69,70]. Drugs that have been successfully formulated as nanocrystals include anticancer drugs (e.g., paclitaxel), diabetes (e.g., glibenclamide), and infectious disease (e.g., antifungals) drugs. Moreover, highly lipophilic or poorly soluble in water drugs, such as curcumin or cyclosporine A, also greatly benefit from nanocrystal formulation since their dissolution is significantly enhanced when formulated in this form. By minimizing particle size of the API, nanocrystals overcome the problem of poor solubility, leading to improved therapeutic effects^[71,72].

4.2. Stabilizers and Excipients:

Although the nanocrystals played a crucial role in improving dissolution and bioavailability, the intrinsic instability of the nanoparticles because of their reduced particle size and enormous surface area complicates drug delivery system formulation as a stable entity. The nanocrystals tend to flocculate or aggregate with time, and this can result in the loss of their characteristic properties like high surface area and improved solubility. Hence, stabilizers and excipients form crucial parts of nanocrystalline drug delivery systems since they avoid particle agglomeration, regulate the size of the particles, and provide stability to the nanoparticles throughout formulation and storage^[73,74]. The most frequently used stabilizers in nanocrystalline formulations are surfactants, polymers, and cryoprotectants. Surfactants are amphiphilic molecules that get adsorbed on the surface of nanoparticles, forming a steric or electrostatic barrier that avoids particle aggregation. Surfactant selection is of extreme importance to the physical and chemical stability of the nanocrystals and must be biocompatible and non-toxic. Surfactants such as polyvinyl alcohol (PVA), polysorbates (Polysorbate 80), and cetyltrimethylammonium bromide (CTAB) are some of the surfactants commonly applied in formulations of nanocrystals. These surfactants are selected on the grounds that they adsorb on the surface of the nanoparticles and impart steric hindrance to keep particles apart from each other. Polymers are another type of stabilizer that are commonly applied in nanocrystal formulation^[75,76]. They further stabilize against aggregation by creating a physical layer on the nanocrystals and enhancing the stability of dispersion of the particles in the solution. These polymers are usually polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and hydroxypropyl methylcellulose (HPMC). PEG, in specific, is also widely utilized as it can inhibit protein adsorption and suppress immune system recognition, thereby prolonged circulation times of nanoparticles in the bloodstream. In addition, PEGylation (PEGylation of nanocrystals) has also been found to enhance the stability and solubility of hydrophobic drugs. Cryoprotectants are excipients applied in nanocrystal products to stabilize the particles for freeze-drying or

lyophilization, usually applied to store and prepare nanocrystal suspensions. Cryoprotectants like trehalose, mannitol, and sucrose also prevent ice crystal formation when the nanocrystals are frozen, which would otherwise destroy the nanocrystal structure. The excipients also maintain the stability and integrity of the nanocrystals upon long-term storage so that the solubility-enhancing effect is retained. Drug Delivery Vehicles and Matrices: Besides the stabilizers, the selection of proper drug delivery vehicles or matrices is another important feature of the design of nanocrystalline drug delivery systems. These systems offer a platform for loading the nanocrystals and establishing how the drug will be delivered to the target location. The selection of drug delivery vehicle is based on several factors, such as the route of administration intended, the drug nature, and the release profile desired. For oral delivery of drugs, which is the most popular administration route, the nanocrystals are usually formulated into solid dosage forms like tablets or capsules, or liquid formulations like suspensions. The most important problem of oral drug delivery is the guarantee of the stability of the drug in the gastrointestinal tract together with the required release and absorption^[77,78]. Here, the use of nanocrystals in solid formulations like solid dispersions or inclusion complexes has been successful in promoting drug solubility. Solid dispersions are usually developed by dispersing the nanocrystals in a carrier material like polymers or surfactants that helps in increasing the drug's dissolution rate. Likewise, inclusion complexes of nanocrystals and cyclodextrins may enhance lipophilic drug solubility. Besides oral drug delivery, nanocrystalline drug delivery systems are also being explored for parenteral (injection) and transdermal (skin) delivery. For parenteral formulations, the nanocrystals may be introduced into lipid-based carriers like liposomes or solid lipid nanoparticles (SLNs) or polymeric nanoparticles. Liposomes are bilayer lipid vesicles in the shape of spheres and are utilized as drug carriers for both hydrophilic and lipophilic drugs. If nanocrystals are encapsulated within liposomes, the stability and solubility of nanocrystals increase, and the drug may be delivered in a controlled manner. Liposomal preparations have been reported to enhance the pharmacokinetics of a number of poorly soluble drugs through controlled release and minimizing systemic toxicity.

Solid lipid nanoparticles (SLNs) are another potential vehicle for nanocrystalline drug delivery. SLNs are made up of solid lipid cores that can be used to entrap drugs, and they possess features like biocompatibility, controlled release, and capability to deliver lipophilic drugs^[79,80]. SLNs may be formulated to deliver the drug slowly over a long time, especially when the drug needs prolonged action or for those possessing a short half-life. In transdermal drug delivery, nanocrystalline systems are frequently placed in lipid-based systems or hydrogels. The aim of transdermal delivery is to deliver the drug sustainably across the skin barrier, eluding the first-pass metabolism associated with oral delivery. Nanocrystalline formulations are advantageous in this regard because they can improve the permeability of the skin and facilitate the controlled release of the drug. Lipid-based carriers, such as nanoemulsions or microemulsions, are commonly used to encapsulate nanocrystals for transdermal delivery, enhancing the penetration of the drug through the skin.

4.3. Polymers and Polymeric Materials:

Polymers are widely utilized for the development of nanocrystalline drug delivery systems because of their properties like biodegradability, biocompatibility, and control over drug release. Polymers may be utilized to encapsulate the nanocrystals, stabilize them, and regulate their release pattern. The most widely used biodegradable polymers are poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and poly(lactic acid) (PLA), which find wide applications in the development of sustained-release drug delivery systems. These polymers break down over time, delivering the drug in a controlled fashion. The addition of nanocrystals to these polymer matrices facilitates improved dissolution and bioavailability, especially for drugs with poor water solubility. Aside from biodegradable polymers, mucoadhesive polymers are also commonly used in nanocrystalline drug delivery systems targeted for mucosal delivery, like the nasal or oral cavity. Mucoadhesive polymers like chitosan or carbopol can engage with the mucosal membranes, extending the in vivo time of residence of the drug and facilitating its absorption. This is particularly advantageous for drugs that must be absorbed via the mucosal

membranes, providing a compromise to systemic delivery. Nanocrystalline drug delivery systems are an emerging area of pharmaceutical research that holds the potential to solve existing problems such as poor solubility, low bioavailability, and uncontrolled pharmacokinetics. Materials employed in these systems are crucial to their effectiveness, ranging from the active pharmaceutical ingredients (APIs) that constitute the nucleus of the formulation to the stabilizers, excipients, and drug delivery vehicles used to provide the system with stability, bioavailability, and controlled release. All of these materials need to be judiciously chosen and perfected to provide the desired therapeutic response while guaranteeing biocompatibility, stability, and safety. With developing nanotechnology, the opportunities for the creation of increasingly advanced and effective drug delivery systems will continue to grow, presenting new avenues for the treatment of many diseases and conditions. The materials described herein will be at the heart of the developing evolution of nanocrystalline drug delivery systems and their translation to clinical use^[81,82].

5. Methods for Preparing Nanocrystalline Drug Delivery Systems

Nanocrystalline drug delivery systems (NDDS) represent a cutting-edge strategy in pharmaceutical technology, designed to boost the solubility and bioavailability of drugs that don't dissolve well. By reducing drug particles to the nanoscale (ranging from 1 to 100 nanometers), these systems significantly increase their surface area, which enhances how quickly they dissolve and are absorbed. This approach is particularly useful for drugs that have low water solubility—known as Class II and Class IV drugs, according to the Biopharmaceutics Classification System—since these often have poor bioavailability when taken orally. Creating nanocrystalline drug delivery systems involves several preparation methods, each tailored to achieve specific features for a particular drug, such as better solubility, stability, and controlled release. Generally, these methods fall into two categories: top-down and bottom-up techniques. Each of these comes with its own set of advantages and challenges, depending on the type of drug, the desired formulation, and the therapeutic goals^[83,84].

5.1. Top-Down Approaches

Top-down techniques for creating nanocrystals focus on mechanically breaking down larger drug particles into tiny nanometer-scale pieces. These methods generally use high-energy mechanical forces to reduce particle size, making them efficient for generating nanocrystals from bulk drug materials. One of the key benefits of top-down methods is their versatility; they can be applied to a broad range of drugs, particularly those with low solubility. The most common top-down techniques include:

- high-pressure homogenization
- bead milling
- jet milling

5.1.1. High-pressure homogenization (HPH): It is a common technique in the pharmaceutical field, particularly for creating nanocrystalline drug delivery systems. This method is great for decreasing particle size down to the nanoscale, which is especially helpful for drugs that don't dissolve well in water and have low bioavailability. When the particle sizes of these drugs are reduced, their surface area increases, ultimately improving how fast they dissolve and get absorbed by the body. By shrinking drug particles down to the nanometer range, HPH significantly boosts their solubility and bioavailability, making it an effective approach for enhancing the therapeutic effects of drugs that struggle with water solubility. The HPH process involves pushing a drug suspension or dispersion through a narrow opening at high pressure, which generates strong mechanical forces that help break down the particles. The combination of these forces with cavitation, shear, and turbulence leads to a smaller, more uniform nanocrystal dispersion that's suitable for both oral and parenteral administration. Essentially, the technique relies on applying very high pressure to a liquid formulation containing the drug particles. Usually, the suspension is pressurized to between 500 and 2000 bars. Once pressurized, it's forced through a tiny nozzle, creating a high-speed jet that accelerates the liquid and generates shear forces on the particles. This results in the drug particles breaking into smaller sizes,

bringing them down to the nanometer scale. The suspension often goes through the homogenizer multiple times to attain the desired particle size distribution^[85,86]. One of the standout benefits of high-pressure homogenization is its ability to create nanocrystals with consistent size distributions, which is key for ensuring reliable therapeutic performance. Having uniform particle sizes is crucial for achieving a predictable dissolution rate, ultimately leading to improved bioavailability and better regulation of drug release profiles. The process is versatile, allowing for the creation of both hydrophilic and hydrophobic drug nanocrystals, making it applicable to a wide range of pharmaceutical products. With the increased surface area from smaller particle sizes, poorly soluble drugs can dissolve more quickly, improving absorption in the gastrointestinal tract, which is crucial for oral delivery. Plus, HPH is scalable, meaning it can be used in large-scale industrial production while ensuring quality control and consistency in the final products. However, HPH does come with challenges. A significant drawback is the energy consumption required to generate the high pressures needed for reducing particle size. This energy input can produce heat, which might destabilize sensitive drugs, risking degradation or loss of effectiveness. To handle this, the process is typically carried out under controlled temperatures, often utilizing cooling systems to prevent overheating. Additionally, high pressure might occasionally lead to the formation of microparticles instead of true nanocrystals, particularly if the process isn't optimized or if the suspension lacks sufficient stabilizers^[86,87]. Therefore, properly formulating the drug suspension is vital for achieving the desired nanosize and preventing particle clumping post-process. Stabilizers or surfactants are essential for maintaining nanocrystal stability after homogenization. Nanocrystals can be quite unstable due to their high surface energy, meaning they tend to aggregate or reform into larger particles without adequate stabilization. To avoid this, surfactants or polymers are often incorporated during homogenization to help keep the nanocrystals stable and prevent aggregation. Choosing the right stabilizing agents is crucial; they must be biocompatible, effectively stabilize the particles, and not interfere with the drug's pharmacological properties. In some cases, a combination of stabilizers might be

necessary to achieve both optimal particle size reduction and stability. The number of homogenization cycles also plays a crucial role in shaping the final characteristics of the nanocrystals. Typically, the suspension goes through the homogenizer several times, with each cycle breaking down the particle sizes even further. The required number of cycles will vary based on the target final particle size, the type of drug, and the conditions during formulation. Generally, more cycles result in smaller particles and better uniformity, though this increases processing time and energy use. Finding the right balance between the number of cycles, energy efficiency, and product quality is key for optimizing the homogenization process in large-scale manufacturing. It's also worth noting that high-pressure homogenization can be slower compared to other particle reduction methods like jet milling or bead milling, especially when dealing with super fine nanocrystals. As the particle sizes shrink, the suspension becomes thicker, making it tougher to push through the homogenizer. This can extend processing times and limit throughput, which could be an issue for industrial-scale production. Moreover, the equipment for HPH tends to be pricey and requires regular upkeep to ensure consistent performance. The high energy needs and costs can make this technique less appealing in certain scenarios, particularly when compared to other more energy-efficient methods^[89,90]. Still, high-pressure homogenization is a valuable method for creating nanocrystalline drug delivery systems, especially when used alongside other formulation strategies. For example, employing suitable surfactants, solvents, or stabilizers in the pre-formulation stage can enhance process efficiency and product quality. Research continues to refine the homogenization process to increase particle size reduction efficiency, cut down on energy consumption, and tackle challenges related to heat-sensitive drugs and particle aggregation. Innovations in equipment, like microfluidic devices for producing nanoemulsions and nanocrystals, may also help alleviate some issues tied to high-pressure homogenization. In summary, high-pressure homogenization is a crucial technique for preparing nanocrystalline drug delivery systems, boasting advantages like scalability, consistent particle size distribution, and improved drug solubility and bioavailability^[92]. While

it does face challenges, such as high energy use, potential heat issues, and the need for stabilizing agents, it remains highly effective for enhancing the properties of poorly soluble drugs. By fine-tuning the process and addressing its limitations, high-pressure homogenization can keep playing a vital role in advancing drug delivery systems, offering significant benefits in treating diseases involving hard-to-soluble drugs.

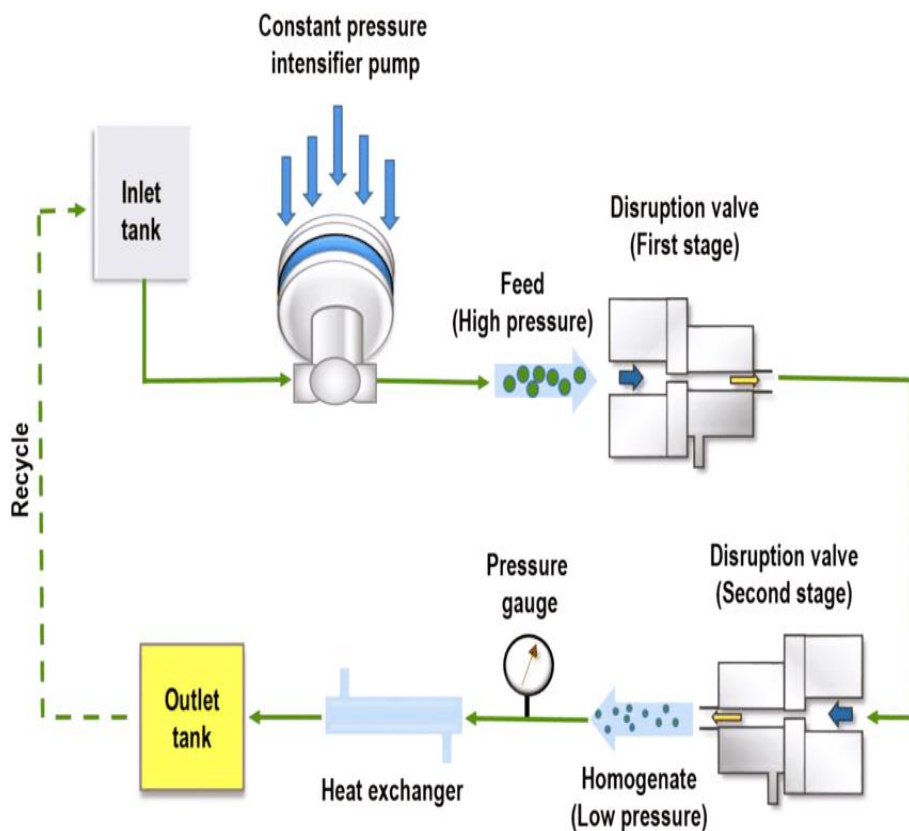


Figure 1: The high-pressure homogenization method, which breaks down cells and recovers intracellular contents, is depicted in this diagram. The material is first fed into the system from an inlet tank that is under pressure from an intensifier pump. After that, the substance passes successively through two disruption valves, which cause the cells to burst under intense pressure. Before the homogenate is cooled in a heat exchanger and gathered in an outlet tank, a pressure gauge

keeps an eye on the system. To ensure effective operation, there is also a recycle loop for reprocessing some material. For efficient cell disruption and product recovery, this technique is essential in the biotechnology and pharmaceutical sectors.

5.1.2. Bead milling: Bead milling, or pearl milling, or wet milling is a widely used technology for making nanocrystalline drug delivery systems, especially for minimizing the size of drug particles to the nanometer range. This process has assumed a very prominent role in the pharmaceutical sector as it can generate extremely stable nanosuspensions of poorly water-soluble drugs, thus enhancing their solubility and bioavailability. Bead milling is based on the concept of mechanical size reduction, wherein the drug particles undergo tremendous shear forces due to the motion of the grinding beads or pearls through a suspension. The enormous energy developed during this process fragments the drug particles into reduced sizes and brings them down to the nanoscale. This reduction in size results in an increase in the surface area of the particles, which enhances better dissolution rates and thus increases the absorption and bioavailability of the drug in the body. Bead milling has been found to be effective for the synthesis of a wide range of nanocrystals of hydrophobic and hydrophilic drugs, and it has found extensive use in preparing oral, parenteral, and topical drug delivery systems. In bead milling, a drug suspension is generally prepared by suspending the drug in a liquid vehicle that can be either aqueous or organic based on the drug's solubility. The grinding beads or pearls, usually of materials such as zirconium, glass, or ceramic, are incorporated into the suspension. The mill works through agitating the suspension, causing the beads to travel back and forth inside a close chamber. The grinding beads strike against the drug particles, producing shear forces and mechanical energy that shatter the drug particles into smaller pieces. The strength of the shear force is also a function of the agitator speed, bead size and material, and suspension viscosity. With the beads constantly impacting the drug particles, they break down the particle size to the nanometer range after several hours of milling. Particle size and suspension uniformity are important parameters during the preparation of nanocrystalline drug

delivery systems because they determine the dissolution rate of the drug, stability, and bioavailability. One of the most important advantages of bead milling is that the process can be applied to a broad spectrum of drugs, such as hydrophilic and hydrophobic drugs. In the case of hydrophobic drugs, which are notoriously challenging to dissolve in water, bead milling offers a pragmatic solution by enhancing the surface area of the drug particles. It is especially useful for drugs with poor aqueous solubility since increasing their surface area will speed up their rate of dissolution, resulting in greater absorption and bioavailability. In addition, bead milling can be used to generate nanocrystals with a limited size distribution, which is critical to maintaining a consistent drug release profile. Particle size uniformity guarantees that the drug will act in a predictable way in the body, improving the reproducibility of the therapeutic response. Bead milling also has the benefit of being a relatively gentle process relative to other size-reduction methods like high-pressure homogenization or jet milling. Since the drug particles are not exposed to severe conditions such as heat or high pressure, bead milling can be a better choice for thermolabile drugs. It is, therefore, a perfect choice for drugs that can be degraded by heat or high shear, since the process is under control and the temperature and mechanical forces applied are comparatively mild. In addition to this, bead milling is also capable of producing a very stable nanosuspension with low aggregation of particles, which is a major issue in the production of nanocrystals. This stability is usually attained by incorporating stabilizers or surfactants in the suspension during the process of milling in order to maintain the drug particles from aggregating or flocculating. Despite all these benefits, bead milling does have some challenges. One of the greatest problems is the risk of contamination. Through the passage of the milling beads, they can become worn down and break apart, discharging little pieces of the bead material into the formula. Contamination of this type can have an impact on the quality and safety of the final product, especially for parenteral preparations where foreign particles may induce severe adverse effects. To overcome this problem, it is necessary to choose beads that are non-reactive and biocompatible, and that are carefully inspected for wear and tear during the milling process^[95,94]. The second

challenge is the necessity of controlling the milling parameters, like bead size, milling time, and suspension viscosity, in a very precise manner. These parameters can have a strong impact on the size of the final particles and on the distribution of the nanocrystals. For instance, beads that are larger will have higher efficiency in breaking up larger particles but can produce more heat, which can result in instability within heat-sensitive drugs. In the same way, the milling duration should be optimized to take care of grinding the drug particles to the desired particle size without over-milling to create amorphous particles instead of crystalline particles. A further disadvantage of bead milling is the possibility of the creation of high-viscosity suspensions as the particle size decreases. When the size of the drug particles diminishes and the surface area gets larger, the suspension can increase its viscosity, causing it to be harder to process. Increased viscosity can cause longer milling times, less throughput, and greater energy consumption, which might render the process less efficient and economically friendly, especially in mass production. To counteract this, the drug concentration within the suspension is generally maintained at optimal levels, and formulation may be balanced by addition of solvents or other excipients to keep the viscosity in working limits. Bead milling also involves careful planning of the selection of grinding media. The bead size and material must be critically selected to achieve maximum efficiency in the milling process. Smaller beads (with diameters between 0.1 and 1.0 mm) are usually employed for generating smaller particles, but larger beads can be used to reduce the size of larger drug particles more efficiently. Beads made of different materials can also influence the efficiency of the size reduction, the degree of contamination, and the rate of size reduction. Zirconium oxide and stainless steel are widely used milling bead materials because they are wear-resistant and long-lasting, but other materials such as glass and ceramic beads can also be used, depending on the nature of the formulation requirements. The performance of the bead milling process is highly dependent upon the conditions under which milling is carried out and also upon formulation conditions. For instance, the speed of the agitator, the type of bead, and the duration of the milling process all contribute to the final particle size and distribution. The

formulation of the drug suspension also plays a critical role in determining the success of the milling process. The selection of solvent, stabilizers, and surfactants may affect the solubility of the drug, the suspension's viscosity, and product stability. Besides, the temperature and pH of the suspension may also affect the behavior of the drug particles during milling and need to be strictly controlled to produce the desired outcome. In summary, bead milling is a very efficient and common method for preparing nanocrystalline drug delivery systems. It provides several benefits, such as flexibility, gentle processing conditions, and the capacity to generate stable nanosuspensions of hydrophilic and hydrophobic drugs. Nonetheless, the method also contains some drawbacks, such as the risk of contamination, requiring detailed control of milling parameters, and difficulty in handling suspension viscosity^[96,97]. By maximizing the milling conditions and choosing the correct formulation, bead milling can be a critical tool in the creation of nanocrystals that increase the solubility and bioavailability of poorly soluble drugs and improve their therapeutic effect and offer new drug delivery opportunities.

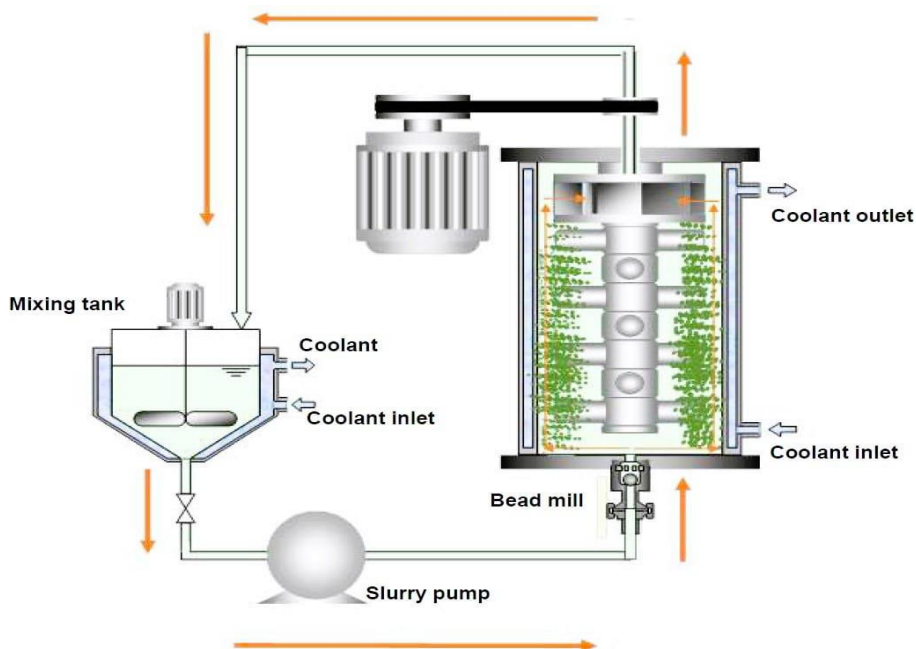


Figure 2: This system is an example of a bead mill configuration used for material dispersion and fine grinding. It begins with a mixing tank that prepares the initial material blend and uses a coolant inlet to maintain the ideal temperature. With the help of coolant inlets and outlets to regulate the temperature, the mixture is subsequently transferred by a slurry pump to the bead mill, where it is ground and dispersed through beads. The procedure guarantees accuracy in reaching the required material consistency, which makes it crucial for sectors like the manufacturing of paint, ink, and pharmaceuticals.

5.1.3. Jet milling: It is a popular technique for creating nanoparticles, especially when developing nanocrystalline drug delivery systems. It's essentially a size reduction process that employs high-velocity air or gas streams to break down substances into smaller particles, thus enhancing the solubility and bioavailability of drugs that don't dissolve well in water. The core idea behind jet milling is to propel particles using high-pressure jets of air or gas, causing them to collide and shrink in size. Unlike other milling methods that utilize grinding media like

beads or pearls, jet milling minimizes contamination risks by relying solely on particles colliding with one another at high speeds, which results in fragmentation. The strong impact and shear forces from these collisions reduce particle sizes down to the nanometer range, which is vital for improving how quickly drugs dissolve and are absorbed. The process starts with a dry powder that gets fed into a jet mill where it encounters high-pressure air or gas. This material then experiences powerful air jets that push the powder particles through nozzles or a specially designed chamber. The pressure can be as high as several bars, directing the particles into a confined space. During this process, they collide with each other, breaking apart into smaller sizes. Besides collision forces, the jet milling method also generates shear forces and turbulent airflow, which further aids in breaking down agglomerates and reducing the size of the primary particles to nanoscales. The final distribution of particle sizes can vary based on several factors, including how long milling occurs, the air pressure used, and the flow rate—all of which need to be precisely controlled for a consistent nanoparticle distribution. One big advantage of jet milling is that it operates as a dry process. Unlike bead milling or high-pressure homogenization, which might need solvents or dispersing agents, jet milling works without any liquid medium. This aspect notably reduces the risk of incorporating harmful additives or contaminants, a crucial consideration in pharmaceutical applications. Furthermore, this method can handle a wide range of materials, whether hydrophilic or hydrophobic, making it a flexible choice for creating nanocrystals. Particularly, for poorly water-soluble drugs that typically have low bioavailability, jet milling effectively increases the particle surface area, which boosts the drug's dissolution rate and absorption. Because of this, it's often used in formulating oral drug delivery systems, especially for drugs that struggle to dissolve. Another significant benefit of jet milling is that it doesn't involve any grinding media. Traditional milling techniques, like bead milling, can lead to wear and contamination over time. Jet milling eliminates this concern by relying on the collisions of particles for size reduction, leading to a cleaner operation. Additionally, since it's a dry process, there's no need for solvents, simplifying the formulation steps and avoiding the complications of removing any residual solvents post-

milling. That said, jet milling has its downsides. One of the main challenges is the high energy requirement to generate the necessary air pressure for effective size reduction. This means that substantial energy is needed to speed up the air or gas, which can make the process costly to operate. There's also potential for heat generation during jet milling, which might not be suitable for heat-sensitive drugs, as the temperature increase could degrade active pharmaceutical ingredients (APIs). To address this, cooling systems or temperature controls are often integrated to maintain the stability of these sensitive drugs. Another limitation of jet milling is its relatively low throughput when compared to other size-reduction methods. It works well for small batches of drug material, but can become inefficient when scaling up for mass production. This limited capacity means it might not always be the most economical choice for producing large quantities of nanoparticles. Additionally, the process depends heavily on precise factors like gas pressure, flow rate, and nozzle design, all of which must be finely tuned to achieve the desired particle size and distribution. Fluctuations in these aspects can result in inconsistencies in the final product, potentially impacting the reproducibility and quality of the drug delivery system. Despite the hurdles, jet milling remains an essential technique for crafting nanocrystals, especially in situations where contamination must be kept to a minimum and a solvent-free approach is essential. Creating drug nanoparticles without grinding media or solvents makes jet milling particularly appealing for the pharmaceutical field, especially in developing oral drug delivery systems. The dry nature of the process also allows for easy inclusion of nanoparticles into tablets, capsules, or powders for inhalation, offering versatility in the final dosage form. In terms of size distribution, jet milling consistently produces nanoparticles with a relatively tight size range, which is key to ensuring the reliability and performance of the drug. The ability to adjust milling conditions—like air pressure, nozzle setup, and feed rate—grants control over the eventual particle size, ensuring it falls within the desired limits for optimal therapeutic effects. Plus, this approach can be adapted to various drug types, from simple organic compounds to complex biologics, making it versatile for a wide range of pharmaceutical applications. An important factor in jet milling is also

the design of the milling equipment^[99,100]. Different jet mill types, such as fluidized-bed jet mills, spiral jet mills, and opposed-jet mills, offer unique benefits based on specific applications. For instance, fluidized-bed jet mills can utilize a fluidized state to keep particles suspended for more effective size reduction, while spiral jet mills utilize a spiral chamber to yield high-velocity collisions. Each jet mill type has its own pros and cons, and selecting the right equipment depends on various factors like the drug's characteristics, desired particle size, and how scalable the process needs to be. In summary, jet milling is a highly effective approach for generating nanocrystalline drug delivery systems, especially for drugs with poor water solubility. Its capability to create dry, solvent-free nanoparticles without risking contamination from grinding media makes it a compelling option for pharmaceutical uses. However, it does have limitations—such as high energy consumption, potential heat issues, and comparatively low throughput—that can affect its cost-effectiveness in large-scale operations. Nevertheless, jet milling continues to be a vital method for enhancing drug solubility and bioavailability, playing a crucial role in the advancement of innovative drug delivery systems.

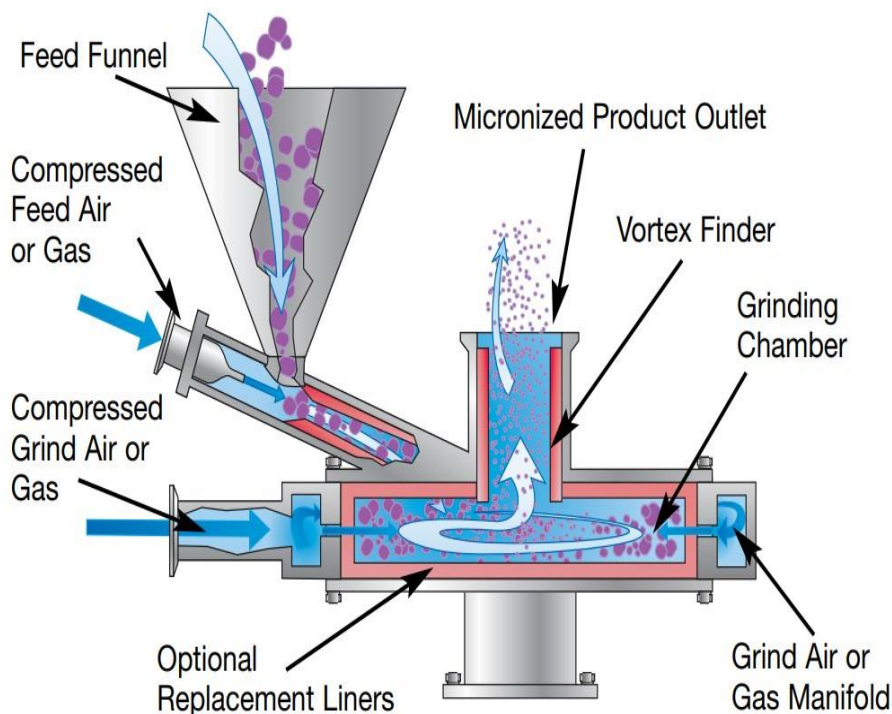


Figure 3: A jet mill is a machine used to reduce material particle sizes and grind materials extremely finely. It works by putting the material through a feed funnel and mixing it with gas or compressed air. When this mixture enters the grinding chamber, the material is broken up into finer particles by a vortex created by high-velocity air streams. Optional liners can preserve the grinding chamber's effectiveness, and the micronized product is collected at the outlet. This technology is widely used in industries like chemicals and pharmaceuticals that need precise particle sizes.

5.2. Bottom-Up Methods: Bottom-up method, on the other hand, includes the creation of nanocrystals from lower units such as molecules, ions, or nanoparticles through processes including crystallization or precipitation. As opposed to top-down methods that disassemble bulk material, bottom-up approaches construct the nanostructures from a molecular or atomic scale, offering an alternative

method of nanocrystal creation. The method most commonly used for the synthesis of bottom-up nanocrystals is

- solvent evaporation

- precipitation

5.2.1. Solvent evaporation: Solvent evaporation is a common method used in the production of nanocrystalline drug delivery systems, especially for the formulation of nanoparticles, nanocrystals, and other colloidal drug carriers. The method is of particular use for poorly soluble drugs that need increased dissolution and bioavailability. The basic principle involved in solvent evaporation is dissolving the drug in an appropriate organic solvent and then evaporating the solvent under controlled conditions to result in precipitation of the drug as nanocrystals or nanoparticles. This method enables the production of nanoscale drug particles with high surface area, hence increasing the dissolution rate and, as a result, drug absorption in the body. Solvent evaporation method starts by the formulation of the drug in a solvent that can dissolve the active pharmaceutical ingredient (API). The selection of a solvent is important and is based on the drug's physicochemical characteristics, including its solubility, polarity, and molecular makeup. Ethanol, acetone, chloroform, and dichloromethane are examples of typical solvents that can be used for the process. After dissolving the drug in the solvent, the second step is the controlled evaporation of the solvent, usually done under reduced pressure or gentle heat. When the solvent is evaporated, the drug precipitates out as nanoparticles, which are then separated, washed, and dried to produce the end product^[101,102]. The process may be performed with different techniques, such as rotary evaporation, spray drying, or vacuum solvent evaporation. One of the most important benefits of solvent evaporation is that it is capable of generating nanoparticles with a high surface area and a narrow particle size distribution, which are essential in order to enhance the solubility and bioavailability of weakly soluble drugs. By decreasing the particle size to the nanorange, the surface area of the drug is drastically enhanced, resulting in an increased rate of dissolution and improved absorption in the gastro-intestinal tract. This is especially

useful for drugs with poor solubility since increasing their surface area increases their ability to dissolve in biological fluids, thus contributing to their increased therapeutic efficacy. Moreover, the solvent evaporation method can also be utilized to deliver a vast number of drug delivery systems such as polymeric nanoparticles, lipid nanoparticles, and drug-loaded microspheres, making it flexible in drug formulation. A benefit of solvent evaporation is that it can also be employed to prepare stable nanoparticles, which are less likely to agglomerate in comparison to drug particles of larger size. The tiny dimensions of the nanoparticles prohibit them from settling or aggregating, a typical problem in larger drug particles or emulsions. Furthermore, the process may be tailored to adjust the size and surface characteristics of the nanoparticles by manipulating different parameters, e.g., the composition of the solvent, evaporation rate, and temperature. This permits the particle properties to be refined to the point that they are within the specifications required for drug release, stability, and bioavailability. Nevertheless, the solvent evaporation technique has certain drawbacks which must be borne in mind when preparing nanocrystalline drug delivery systems. One of the main challenges is that the solvents employed in the process may be toxic. Organic solvents, though efficient at dissolving a broad spectrum of drugs, are toxic or irritating to human tissues when residues are left behind in the end formulation. It is, hence, paramount that all solvent residues are eliminated in the process of evaporation to guarantee the safety of the end product. This is particularly important for drugs that are intended for parenteral or oral administration, where the presence of residual solvents could lead to adverse effects or compromise the safety of the drug delivery system. The efficiency of the solvent evaporation process can also be affected by the volatility of the solvent used^[103,104]. Some solvents evaporate more readily than others, which can lead to inconsistencies in the evaporation process and the final particle size. For example, solvents with high volatility may cause the drug to precipitate too quickly, leading to the formation of larger particles or aggregates instead of well-dispersed nanoparticles. On the contrary, low-volatility solvents can take longer evaporation times, and this can lead to poor control of the final particle size as well as longer processing

times. Hence, proper choice of the solvent and optimization of evaporation conditions are required in order to obtain the required drug characteristics. The solvent evaporation can also be fairly slow, particularly when working with high volumes of solvent or high amounts of drug material. The requirement for controlled pressure and temperature conditions, together with the slow evaporation of the solvent, may make the process slow, which may be undesirable for large-scale production. In addition to that, the removal of the residual solvents is an important step since the residual solvent can have an impact on the stability and safety of the end product. In other cases, further purification or washing procedures could be needed to confirm that all traces of solvent are removed, and this would increase the complexity of the process. Furthermore, the solvent evaporation process might not apply to all drugs. For example, unstable drugs in organic solvents or drugs sensitive to heat might degrade while undergoing solvent evaporation. In such instances, the drug can become inactive pharmacologically, and the process will not be viable for such molecules. To counter this drawback, the process might need to be altered in some form, like using low-boiling-point solvents or gentle conditions of evaporation. Nonetheless, even with these adjustments, solvent evaporation might not be the most suitable approach for extremely heat- and organic solvent-sensitive drugs. The process of nanoparticle fabrication via solvent evaporation can also be affected by the drug's nature. High molecular weight and complicated molecular structures of some drugs do not readily dissolve in typical organic solvents, allowing it to be challenging to obtain the required particle size of the drug. Under these circumstances, co-solvents or surfactants could be incorporated to enhance the process of dissolution and ensure that stable nanoparticles are formed. The use of excipients, however, might bring in some added complexity to the formulation and would impact the release profile, bioavailability, and safety of the drug delivery system. Thus, proper choice of excipients is essential to avoid any interference with the therapeutic attribute of the drug. Even with these difficulties, solvent evaporation continues to be a popular and successful process for the production of nanocrystalline drug delivery systems, especially with the drugs that are poorly soluble^[104,105]. By

lowering the size of the drug particles to the nanometer scale, solvent evaporation increases the solubility and bioavailability of drugs, rendering them more efficient in the treatment of a range of medical conditions. The technique is versatile and makes it possible to prepare various forms of drug delivery systems, including polymeric nanoparticles, lipid nanoparticles, and drug-loaded microspheres. While there are drawbacks to solvent evaporation, including the possible toxicity of solvents, the requirement for precise optimization of evaporation conditions, and the slow process rate, these drawbacks can usually be overcome by thoughtful formulation development and process optimization. In summary, solvent evaporation is a convenient and efficient method for the production of nanocrystalline drug delivery systems that increase the solubility and bioavailability of poorly soluble drugs. Through the proper choice of solvent, the adjustment of evaporation conditions, and the elimination of traces of solvent, this method can be employed to obtain stable, homogeneous nanoparticles with enhanced therapeutic activity. In spite of its drawbacks, solvent evaporation is still a crucial technique in pharmaceutical formulation, especially for drugs that need increased solubility for better absorption and therapeutic effects.

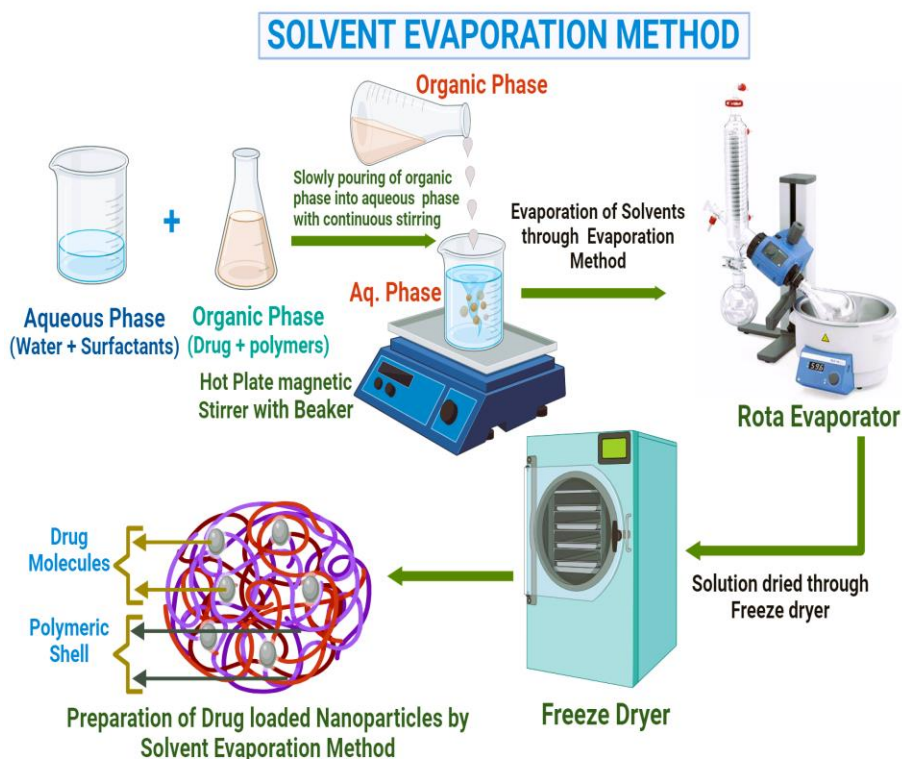


Figure 4: A key method in targeted drug delivery, the solvent evaporation method is shown in this image for creating drug-loaded nanoparticles. With the help of a magnetic stirrer on a hot plate, it begins by mixing an aqueous phase that contains surfactants with an organic phase that contains medications and polymers. Using a rota evaporator, the mixture is subjected to solvent evaporation and then freeze-drying to produce nanoparticles with drugs encapsulated in a polymeric shell for stability and controlled release for therapeutic uses.

5.2.2. Precipitation: Precipitation is a commonly employed method in pharmaceutical formulation, especially in the production of nanocrystalline drug delivery systems. Precipitation is largely utilized to enhance the solubility and bioavailability of weakly water-soluble drugs by converting them to nanoparticles or nanosuspensions. The

precipitation process entails the transformation of a solubilized drug to its solid state in a process that is controlled and where the solubility of the drug in a solvent is surpassed, resulting in the formation of crystals or precipitation of the drug. By regulating the conditions under which the precipitation takes place, it is easy to manufacture nanoscale particles with improved properties, including greater surface area, better dissolution rate, and improved bioavailability. The fundamental basis of precipitation is to change the conditions of solubility of a drug, resulting in its separation from a solvent. This can be done by modifying parameters such as temperature, pH, solvent ratio, or ionic concentration, which affect the solubility of the drug. If the solubility limit is exceeded, the drug begins to precipitate in the form of solid particles, which later combine to form larger structures or precipitate as individual particles, depending upon the procedure and conditions applied. In the case of nanocrystals, the aim is to manage the size, morphology, and stability of the formed precipitates so that they fall in the desired nanometer range. Such particles possess a considerably greater surface area than larger particles, thus facilitating rapid dissolution as well as improved absorption within the body. Precipitation can be performed based on numerous techniques, including solvent evaporation, anti-solvent precipitation, and co-precipitation. Of these, precipitation by anti-solvent is the most widely employed process in the fabrication of nanocrystalline drug delivery systems. In this process, a drug solution is formulated in an appropriate solvent, and an anti-solvent is added, which is one in which the drug is extremely poorly soluble^[106,109]. The introduction of the anti-solvent lowers the solubility of the drug in the solution so that it precipitates out as nanoparticles. Through the adjustment of the rate of addition of the anti-solvent, temperature, and mixing regimen, the size and properties of the resulting particles of the drug can be accurately fine-tuned. The main advantage of this method is that it allows for the rapid formation of nanoparticles, and it can be used for a wide variety of drugs and solvents. Another important factor in the precipitation process is the use of stabilizers or surfactants. These materials are incorporated into the formulation to stop the freshly formed nanoparticles from aggregating or developing into large particles, which would be counterproductive to

forming a nanocrystalline system. Stabilizers, including polymers, surfactants, or ligands, adsorb onto the particle surface and form a steric or electrostatic barrier that stops the particles from coalescing. This is important as particle aggregation may result in loss of benefits related to nanosizing, for example, higher surface area and better rate of dissolution. In certain instances, a blend of stabilizers and surfactants is employed to create more effective stabilization and control over particle size and distribution. The process of precipitation can be improved or optimized by varying different parameters, including the concentration of the drug, the solvent to anti-solvent ratio, and the temperature at which the process is performed. For instance, raising the concentration of the drug in the solvent may result in increased precipitation rate since more drug molecules are available to aggregate into particles. This, however, may also enhance chances of particle agglomeration, which is not desired. Thus, optimization of such parameters has to be done with care to attain the preferred particle size and size distribution. As with temperature, the drug's solubility in the solvent and anti-solvent can also be affected by the rate and extent of precipitation. Lower temperatures tend to bring about slower precipitation, which will give greater control over the particle size, while increasing the temperature may produce faster nucleation and smaller particle production. One of the significant benefits of precipitation is how simple and inexpensive it is. In contrast to more sophisticated methods, such as bead milling or high-pressure homogenization, precipitation does not involve costly equipment or multi-step processing. It can be easily carried out in the standard laboratory equipment, which makes it a convenient option for small-scale research work as well as large-scale commercial manufacturing. The fact that particle size and morphology can be controlled by simple variations in solvent composition and other conditions makes precipitation an attractive method for the production of nanocrystals of different drugs. Precipitation does have some limitations, though. One of the most important limitations is the generation of nanoparticles with a narrow size distribution. It is not always easy to create uniform particle sizes in the precipitation process, particularly with anti-solvent precipitation techniques. Without adequate control of the precipitation conditions, particles can coagulate,

or a broad distribution of particle size can occur, resulting in an erratic drug release profile. This is especially undesirable for drugs that need to be accurately dosed and release controlled. In order to solve this problem, further steps, like filtration or centrifugation, can be employed to eliminate big particles or aggregates, but this might make the process more complicated. Another drawback is that the use of solvents and anti-solvents is possible, which need to be expelled from the final product. Remaining solvents can be harmful or irritative, particularly in drug products being used for parenteral administration. Hence, it is important to ensure the removal of any residual solvents totally, and that may involve further operations such as washing, filtration, or vacuum drying. The selection of solvents and anti-solvents also has to be carefully made, as they can impact the drug's solubility and the stability of the end product. Unless selected properly, solvents may weaken the drug or disrupt the intended particle properties of the nanoparticle. The precipitation method is also restricted by drug solubility characteristics. Although precipitation is a great option for drugs of poor solubility, it might not work for very soluble drugs in the selected solvent. In such a situation, other methods, like solid dispersion or co-precipitation using a carrier, would be required to obtain the desired particle size reduction. In addition, pH- or temperature-sensitive drugs can also experience stability problems during the precipitation process since these conditions may change the properties of the drug or cause drug degradation^[107,108]. Although these limitations exist, precipitation is still an invaluable and highly utilized technique for nanocrystalline drug delivery systems development. The capability of influencing the particle size and surface characteristics of nanoparticles makes it a good method to enhance the solubility, dissolution rate, and bioavailability of drugs with poor solubility. Moreover, its ease of use and low cost make it a popular method for both small-scale lab experiments and bulk industrial production. With optimized processing parameters and the inclusion of stabilizers to avoid particle agglomeration, precipitation can be a successful approach to produce high-quality nanosuspensions and nanocrystals that improve the therapeutic activity of poorly soluble drugs. Hybrid methods in the context of nanocrystalline drug delivery systems signify the union of two or more distinct methods to overcome

the drawbacks of single methods and obtain better outcomes in terms of particle size decrease, stability, and bioavailability improvement of poorly soluble drugs. These methods are becoming more crucial in drug development, as they allow for more accurate control of the physicochemical properties of the drug, which is an important factor in enhancing its therapeutic efficacy. Hybrid methods usually involve the combination of physical processes like precipitation or milling with chemical processes like the use of surfactants or polymers to form nanocrystals or nanoparticles with optimum size, stability, and release properties. Through combining multiple techniques, hybrid methods hope to solve issues like the production of uniform particle size, avoiding agglomeration, and being able to increase production to make it useful on an industrial scale. One of the most popular hybrid methods is the union of precipitation and milling^[110]. Precipitation, frequently employed for the synthesis of nanocrystals, involves dissolving the drug in a solvent, then adding an anti-solvent to cause the drug to precipitate out in the form of nanosized particles. But precipitation itself may produce particles that are not of equal size or may degrade with respect to aggregation over time. In order to counteract this, milling processes, be it bead milling or jet milling, can be utilized following precipitation. Here, mechanical energy is used to further decrease particle size and disintegrate any aggregates, leading to a more equal dispersion of nanoparticles. When precipitation is followed by milling, size and shape of the nanoparticles are able to be better controlled, as well as the aggregation risk minimized. The hybrid process not only enhances the size distribution but also the stability and solubility of the drug, so that it is more bioavailable when taken. Approximately a second example of a hybrid process is solvent evaporation with high-pressure homogenization. Solvent evaporation is a widely accepted method in nanocrystal synthesis, where a drug is dispersed in a solvent and the solvent is evaporated in controlled conditions such that the drug precipitates as nanoparticles. Although solvent evaporation is an effective method for the production of nanoparticles, it at times produces particles with non-uniform shapes and a large size distribution. High-pressure homogenization is a mechanical technique in which the drug suspension is subjected to high pressures to be passed

through a small gap, which generates intense shear forces that reduce the particle size. When solvent evaporation is coupled with high-pressure homogenization, the drug particles can be more efficiently reduced to the nanoscale with improved control of size and distribution. This hybrid process not only improves the quality of the nanocrystals but also enhances formulation stability and scalability. Another hybrid technique involves the integration of supercritical fluid technology with other size reduction technologies. Supercritical fluid technology, such as the supercritical antisolvent (SAS) process, employs a supercritical fluid (a fluid above its critical temperature and pressure) as an antisolvent to precipitate the drug in nanoparticulate form^[50,46]. The employment of supercritical fluids enables nanoparticles to be formed without the use of organic solvents, which may be toxic or lead to contamination. Nevertheless, supercritical fluid-based techniques in some instances are complicated and challenging to upscale for commercial production. To overcome this, hybrid techniques integrating supercritical fluid technology with other processes like precipitation or milling may be utilized. For instance, supercritical fluids in combination with bead milling provides controlled particle size reduction in a scalable fashion as well as removal of solvents, rendering the process eco-friendly and efficient. Besides these mechanical hybrid methods, there are chemical hybrid methods as well that merge precipitation or nanoparticle synthesis with the use of stabilizers, surfactants, or polymer carriers. Stabilizers are also usually needed in nanoparticle formulations to avoid aggregation and ensure long-term stability. Surfactants, being surface-active molecules, can lower the surface tension between drug particles and the medium in which they are dispersed, enabling better dispersion and stabilization of the nanoparticles. Such stabilizers and surfactants are added either during the precipitation step or following particle formation, depending on the final formulation desired. Hybrid methods that combine precipitation with stabilizer or surfactant addition provide greater control over the size, surface charge, and overall stability of the nanoparticles. This not only enhances the drug's solubility and bioavailability but also its capacity to be stable in suspension, excluding the formation of aggregates that would affect the drug's efficacy. Hybrid

methods are also applied in conjunction with lipid-based formulations. Lipid nanoparticles, such as liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), have found broad application in drug delivery because they can encapsulate hydrophilic or lipophilic drugs. Production of lipid nanoparticles, however, at times leads to a variable size distribution or unsatisfactory drug release profiles. By integrating lipid-based formulations with other methods, like solvent evaporation or precipitation, hybrid approaches can be utilized to enhance the encapsulation efficiency, particle size control, as well as the drug release rates. For example, solvent evaporation methods may be utilized to formulate drug-loaded lipid nanoparticles, which may in turn be processed using high-pressure homogenization or other size-reduction processes to further narrow the particle size and make it more evenly distributed. This combination method extends the functionality of lipid nanoparticles with a very efficient solution to both hydrophilic and hydrophobic drugs. Hybrid methods are among the key advantages due to their versatility. It is possible, through mixed techniques, to design the drug delivery system according to specific therapeutic requirements. For instance, a hybrid approach can be employed to design nanoparticles for the targeted delivery of drugs wherein modifications on the surface or the addition of targeting ligands may be incorporated during the preparation step. Such modifications enable the nanoparticles to bind to specific receptors or tissues, thus enhancing the therapeutic action of the drug while reducing off-target effects. In addition, the pharmacokinetics of the drug can be enhanced with the use of hybrid methods, including prolonging the release time or regulating the rate at which the drug is released. This is especially relevant to drugs that need extended or controlled release over time, since hybrid approaches can be used to optimize the release profile. Although hybrid methods have many benefits when it comes to enhancing the performance of nanocrystalline drug delivery systems, there are also challenges involved in using them^[85,89]. One of the key issues is that the process is complex because it tends to involve the mixture of several different techniques, each with different parameters which must be accurately controlled. Integration of precipitation with milling or homogenization, for example, demands very controlled

conditions such as temperature, pressure, and composition of the solvent so that the required particle size and properties are obtained. Also, hybrid methods can be time- and resource-intensive to use compared to employing a single method, and this could raise the cost of production. Another limitation is the possibility of interaction among the various ingredients employed in the hybrid approach, including the drug, stabilizers, and excipients, which can impact the stability and bioavailability of the resultant formulation. Notwithstanding these limitations, hybrid strategies are still a promising technique for designing sophisticated nanocrystalline drug delivery systems. They provide the scope for combining the advantages of various techniques, which results in better control over particle size, stability, and drug release characteristics. These methods also give the room for designing drug delivery systems that are optimized to meet special therapeutic requirements, be it for solubilizing, improving bioavailability, or sustaining delivery. With the ongoing advancement in pharmaceutical technology, hybrid methods will increasingly become pivotal in the manufacture of efficient and effective drug delivery systems for various drugs. The process of preparing nanocrystalline drug delivery systems is a very dynamic and fast-growing field of pharmaceutical technology. Through both top-down and bottom-up approaches, pharmaceutical researchers are able to make nanocrystals that greatly increase the solubility and bioavailability of poorly soluble drugs, thereby improving their therapeutic efficiencies. Techniques such as high-pressure homogenization, bead milling, and jet milling provide potent means of reducing bulk drugs to nanometer-sized particles, whereas solvent evaporation and precipitation permit controlled crystal growth of nanocrystals from molecular precursors. In spite of the attendant difficulties of their production, they continue to be developed, offering improved solutions to drug delivery issues that have long constrained the efficacy of some therapies^[36,59].

6. Characterization of Nanocrystalline Drug Delivery Systems

Characterization of nanocrystalline drug delivery systems is an important process to ensure that these systems are desirable in terms of the conditions for drug delivery, stability, and therapeutic action.

Nanocrystals, which are literally drug particles whose diameters are on the order of nanometers, have several benefits in drug formulations in terms of increased solubility, improved bioavailability, and better control over drug release. In order to maximize these advantages, one needs to clearly define the properties of nanocrystalline drug delivery systems in a thorough and complete context. It includes a range of methods to analyze the physical, chemical, and biological properties of the nanocrystals, all of which have an impact on the function of the drug delivery system. The particle size and size distribution is the first part that needs to be taken into account when characterizing nanocrystalline drug delivery systems. The nanocrystal size determines the drug's solubility and bioavailability. Nanosizing a drug generally increases its surface area, thus its dissolution rate and hence its absorption in the body. The particle size is generally measured by methods like dynamic light scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). DLS is also useful in establishing the hydrodynamic size of nanoparticles suspended, giving information on particle distribution, while SEM and TEM provide high resolution images which may give precise particle morphology and structure. Another significant parameter is particle size distribution because a tight size distribution guarantees uniformity of the formulation that is important to ensure consistent therapeutic performance. Besides particle size, surface charge or zeta potential is yet another significant property to define. Zeta potential gives information regarding the stability of nanoparticle dispersions by projecting the extent of electrostatic repulsion among particles. A positive or negative high zeta potential would generally indicate well-dispersed and stable particles because the repulsive forces avoid particle aggregation^[56,25]. Electrophoretic light scattering is employed to measure the zeta potential, which is a critical measure of the colloidal stability of the nanocrystalline product. A too-low zeta potential will cause the particles to aggregate over time and will compromise the drug delivery system's performance. On the other hand, a high enough zeta potential is important to keep the nanoparticles dispersed and not aggregated, thereby preserving the bioavailability of the desired drug. The surface features and morphology of nanocrystals are also

crucial in controlling their performance in drug delivery vehicles. The surface features regulate the interaction of the nanoparticles with biological membranes, cell uptake, and the stability in blood circulation. Morphological examination by methods like SEM and TEM enables the visualization of particle surface texture, shape, and size. The nanocrystal shape—whether spherical, needle-like, or irregular—can dictate the way they interact with the biological environment. For example, rod-shaped or elongated particles could have varying uptake and release profiles compared to spherical nanoparticles. Surface functionalization is also significant, as surface modifications like polymer coating, surfactant coating, or coating with targeting ligands may affect the solubility, stability, and biological interactions of the nanocrystals. Crystallinity and phase behavior of nanocrystals is another significant aspect of characterization since these influence the solubility and dissolution rate of the drug directly. Nanocrystalline drug products are generally manufactured to increase the solubility of sparingly soluble drugs by transforming them into their nanocrystalline state with an enormously increased surface area compared to bulk drug particles. The drug crystallinity can be quantified through X-ray powder diffraction (XRD), which gives insight into the crystalline structure of the drug at the molecular level^[46,100]. XRD analysis allows for the identification of the drug's crystalline phases and the degree of crystallinity. In many cases, drugs in nanocrystalline form may exhibit a different crystallographic form compared to the bulk form, which can enhance their solubility. The amorphous composition of the formulation can also be quantitated, as amorphous drugs are more soluble than crystalline drugs. Differential scanning calorimetry (DSC) is another method to evaluate thermal behavior of the nanocrystals. DSC analyzes heat flow involved in phase transitions, e.g., melting, crystallization, and glass transitions that are useful in understanding stability and crystallinity of the drug. A sharp melting point is characteristic of high crystallinity, while broad or non-existent peaks could be indicative of amorphous or semi-crystalline nature. This information is important for knowing the stability of the nanocrystals when in storage and how they function in the body. Amorphous or partially amorphous nanocrystals tend to have higher

dissolution rates, which can be beneficial to improve drug bioavailability. The dissolution rate and solubility of the nanocrystalline formulation are likely the most straightforward assessments of its performance as a drug delivery system. Nanocrystals are normally made to enhance the dissolution rate of poorly soluble drugs by enhancing their surface area. The nanocrystalline drug's dissolution profile is quantifiable using a dissolution test system, in which the drug is immersed in a solution, and the drug release rate is observed over a period of time. The dissolution rate of nanocrystals is higher than that of bulk drug particles because of the smaller size and larger surface area of nanocrystals. Finally, the formulation can be subjected to conditions mimicking the gastrointestinal tract to determine the performance of nanocrystals in vivo. Faster dissolution of nanocrystals enhances their absorption in the gastrointestinal tract, resulting in higher bioavailability, particularly for water-soluble drugs with low solubility. Stability is another parameter worth considering in characterizing nanocrystalline drug delivery systems. Nanocrystals, as with all colloidal systems, are susceptible to physical instability, which may lead to aggregation of particles, size variation, or loss of drug content. Stability testing of nanocrystalline products includes both short-term and long-term stability tests. These tests generally measure changes in particle size, surface charge, and drug content under conditions of storage such as temperature, humidity, and light exposure. Stability is usually assessed with accelerated stability test methods, which expose the formulation to severe conditions to forecast how it will perform with the passage of time. For instance, the nanocrystals can be exposed to high temperatures or freeze thaw cycles to see whether they are stable over time^[60,58]. Also, drug stability in the formulation can be tested to see whether the active pharmaceutical ingredient degrades over time, which is vital for maintaining long-term drug efficacy. In vitro release testing is important for ascertaining the performance of nanocrystalline drug delivery systems in controlled or sustained drug release. These experiments mimic the environment that the drug will experience when it is administered, usually with a release medium that mimics the physiological environment of the gastrointestinal tract. The rate of release can be examined through a number of different methods,

including the diffusion cell method, in which the nanocrystals are positioned inside a membrane that divides two compartments, one being the release medium and the other being a receptor solution. The release of the drug over a period is then quantified. Through such studies, researchers are able to assess if the drug release is occurring appropriately, which is important for maintaining therapeutic efficacy and for preventing toxicities due to bursts in release. In vivo studies are also integral in characterization of nanocrystalline drug delivery systems, especially in learning about their pharmacokinetic profile. Animal models or clinical trials are employed to evaluate the absorption, distribution, metabolism, and excretion (ADME) of the drug. Direct proofs of the bioavailability and therapeutic effectiveness of the nanocrystals are obtained through in vivo studies, providing information on how the formulation enhances drug absorption and targeting at the site of action. The plasma drug concentration is usually measured over time and the pharmacodynamic effects of the drug are evaluated. Imaging methods, including fluorescence microscopy or magnetic resonance imaging (MRI), can also be employed to monitor the distribution of nanocrystals throughout the body^[21,12]. This allows researchers to learn how the nanocrystals behave in tissues, whether they have the ability to pass through biological barriers, and if they can target particular locations, like tumors or inflamed tissues. Toxicological testing is also a critical component in the characterization process since the safety of the nanocrystalline preparation needs to be extensively examined before being applied in clinical practice. Toxicity of nanocrystals varies based on their size, surface characteristics, and formulation ingredients. The toxicological analysis of nanocrystals usually examines the biocompatibility of the nanocrystals, such as their cytotoxicity, hemotoxicity, and ability to cause inflammatory or allergic reactions. These evaluations can be performed in vitro with cell cultures or in vivo with animal models to determine the possible risks to human health. Conclusion, characterization of nanocrystalline drug delivery systems is a comprehensive and multifaceted process that encompasses a range of techniques to examine particle size, surface charge, shape, crystallinity, solubility, stability, release profiles, and safety. All these factors are critical in ensuring the efficacy and safety of the drug

delivery system. Using a combination of physical, chemical, and biological characterization techniques, researchers will be in a prime position to guarantee that nanocrystalline drug formulations are up to standard for increased bioavailability, controlled release, and enhanced therapeutic efficacy. Accurate characterization is necessary to transfer nanocrystalline formulations from the laboratory to the clinic, where they may provide great advantages for patients, especially in the treatment of disease by poorly soluble or hydrophobic drugs.

Physicochemical Characterization

└─► Particle Size & Distribution

- Method: Dynamic Light Scattering (DLS), Laser Diffraction
- Purpose: Measures average size and polydispersity index (PDI)
- Importance: Affects dissolution rate and bioavailability

|

└─► Zeta Potential

- Technique: Electrophoretic Light Scattering
- Function: Determines surface charge of particles
- Significance: Forecasts physical stability (repulsion against aggregation)

|

└─► Morphology & Surface Characteristics

- Technique: Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM)
- Function: Reveals shape, size, surface roughness
- Significance: Impacts dissolution and interaction with biological membranes

|

—► Crystallinity and Polymorphism

| • Technique: Powder X-ray Diffraction (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR)

| • Relevance: Determines crystalline/amorphous form and polymorphic change

| • Significance: Solubility and stability rely on crystalline structure

—► Thermal Behavior

| • Method: DSC, Thermogravimetric Analysis (TGA)

| • Purpose: Measures melting point, degradation temperature

| • Importance: Formulation stability and processing conditions are guided by it

---► Saturation Solubility

| • Method: Shake Flask Method

| • Purpose: Determines maximum dissolution rate in chosen media

| • Importance: Verifies dissolution rate increase relative to raw drug

|► In Vitro Dissolution Rate

| • Method: USP Dissolution Apparatus (Type I or II)

| • Purpose: Ascertains drug release as a function of time

| • Importance: Forecasts in vivo drug absorption and bioavailability

—► Redispersibility (in case of dried nanocrystals)

- | • Method: Rehydration and DLS or SEM
- | • Purpose: Assesses return to original particle size ability
- | • Importance: Important in lyophilized or spray-dried formulations
- |
- Stability Testing
- | • Method: Storage at ICH conditions, regular testing
- | • Purpose: Monitors physical and chemical stability
- | • Importance: Ensures long-term usability and quality of formulation

Flowchart 1: This flow describes a methodical way to assess nanocrystals for drug delivery, with an emphasis on their stability, solubility, size, surface charge, morphology, and crystallinity. Integrating this data to maximize formulation quality and therapeutic efficacy is the ultimate objective.

7. In Vitro and In Vivo Assessment of Nanocrystalline Drug Delivery Systems

Assessment of nanocrystalline drug delivery systems (NDDS) by in vitro and in vivo methods is an important step towards the development and optimization of pharmaceutical formulations for enhancing the bioavailability, solubility, and therapeutic effectiveness of poorly soluble drugs. Nanocrystalline preparations, including the downsizing of drug particles to nanometer dimensions, have received intense interest for their promise of circumventing the solubility constraints inherent in most drugs and enhancing their pharmacokinetics and pharmacodynamics. In vitro and in vivo testing of these systems enables scientists to evaluate a number of performance parameters, such as drug release, stability, bioavailability, and safety, and this gives vital

information in bringing these formulations to clinical uses. In vitro assessment of nanocrystalline drug delivery systems is mainly concerned with the investigation of the physicochemical properties of the drug formulation under laboratory-controlled conditions. These assessments enable a comprehensive knowledge of the release profile, solubility, stability, and other desirable properties of the nanocrystals in conditions simulating the physiological environment^[58,25]. The initial objectives of in vitro testing are to confirm that the nanocrystalline formulation has maximum drug release and stability properties and to forecast how it will behave inside the body prior to conducting expensive and time-consuming in vivo studies. The dissolution rate and release pattern of the nanocrystalline drug product is the initial and most basic component of in vitro analysis. One of the major benefits of nanocrystals is that they can improve the solubility of drugs with low water solubility by providing more surface area for dissolution. The dissolution profile of the drug would generally be monitored using a dissolution test apparatus, in which the drug is subjected to a dissolution medium mimicking physiological conditions, e.g., simulated gastric fluid (SGF) or simulated intestinal fluid (SIF). This technique assists in analyzing the rate at which the drug gets released from the nanocrystals and the quantity released over time. The release profile can be determined by collecting samples after a set interval of time and quantifying the drug's concentration in solution. The rate of dissolution is typically compared to that of the bulk drug to find out the enhancement in solubility offered by the nanocrystals. Besides dissolution testing, the release mechanism of the drug is another area of major interest in in vitro assessment. Nanocrystals are typically formulated to deliver the drug in a controlled or sustained manner. In order to realize the release mechanism, researchers might use mathematical modeling of release data. Models such as zero-order, first-order, and Higuchi's model are commonly used to describe the release kinetics of drugs from nanocrystalline formulations. The selection of an appropriate release model is essential for understanding the rate at which the drug is delivered and determining whether the formulation will provide an immediate or sustained therapeutic effect. Peppas-Sahlin's model, for instance, is commonly used to investigate

the release mechanism in systems where drug release is controlled by diffusion and erosion processes. This is especially pertinent for nanocrystalline systems that could be subject to physical transformations like swelling or degradation of the matrix with time. Another important in vitro testing is the stability studies of nanocrystalline drug delivery systems^[78,53]. Because of their nanoscale size, nanocrystals are more prone to physical instability in the form of aggregation, precipitation, or crystallization upon aging. These instabilities would result in particle size changes, zeta potential changes, and drug content changes, which can have adverse effects on the performance of the nanocrystalline product. Stability studies are normally conducted under accelerated conditions, for example, high temperature, humidity, and light exposure, to mimic long-term storage conditions. Throughout these studies, the nanocrystals are periodically monitored for variations in size distribution, shape, surface charge (zeta potential), and drug loading. The physical form of the drug (e.g., crystalline vs. amorphous) can also be tracked by methods like X-ray diffraction (XRD) or differential scanning calorimetry (DSC) to verify that the nanocrystals have the desired physicochemical characteristics. Particle size and surface charge (zeta potential) are also characterized routinely in in vitro studies since they influence the stability and solubility of the nanocrystals directly. Dynamic light scattering (DLS) is typically employed to measure the hydrodynamic size of the particles suspended in solution, which indicates the stability and dispersion characteristics of the nanocrystals. A limited particle size is necessary to optimize surface area and enhance solubility, with high zeta potential being necessary to keep the nanocrystals in solution and from aggregating. These features are also crucial to maintaining the functionality of nanocrystalline formulations in vivo. After the in vitro analysis has established the desired drug release, stability, and particle characteristics, in vivo testing is needed to establish the drug's pharmacokinetics, pharmacodynamics, and safety of the nanocrystalline formulation. In vivo testing gives a broader view regarding how the drug is absorbed, distributed, metabolized, and excreted (ADME) in a living organism and its overall therapeutic efficacy. In vivo testing is usually conducted using animal models, but

clinical trials can be required for human use^[22,69]. The main aim of in vivo testing is to compare the nanocrystalline formulation with traditional drug formulations in terms of bioavailability, targeting of the drug, and therapeutic activity. Bioavailability of a drug is defined as the proportion of the dose administered, which enters the systemic circulation as active drug. Nanocrystalline formulations are especially useful for poorly soluble drugs, since particle size reduction enhances the surface area and dissolution rate, and hence the absorption of the drug in the gastrointestinal tract. In vivo bioavailability experiments are usually conducted by pharmacokinetic evaluation, where the plasma or blood concentration of the drug is determined at specific time points following administration. The area under the curve (AUC) is computed to find out the overall drug exposure, and other parameters like C_{max} (the maximum concentration) and T_{max} (the time to achieve maximum concentration) are also examined. By comparing the pharmacokinetic profiles of conventional formulations with nanocrystalline formulations, researchers are able to assess the enhancement in bioavailability. Drug distribution and targeting are also assessed in vivo to find out how effectively the nanocrystals are delivered to their site of action. This is especially crucial with respect to targeted drug delivery, where nanocrystals are engineered to target certain tissues or organs, like tumors or inflammation sites. Real-time tracking of nanocrystal distribution can be done using in vivo imaging methods like fluorescence imaging, magnetic resonance imaging (MRI), or positron emission tomography (PET). These methods enable researchers to view the drug uptake and distribution across various tissues, giving useful information regarding the performance of the formulation in a living system. By altering the surface of nanocrystals with targeting ligands or antibodies, the formulation can be engineered to selectively target certain cells or tissues, enhancing the therapeutic index and reducing off-target effects. Pharmacodynamic action of nanocrystalline drug delivery systems is assessed using in vivo efficacy studies, which measure the time-dependent biological effect of the drug. In these studies, animal models of the disease condition to be targeted are usually employed, and the therapeutic effect of the nanocrystals is compared with that of standard forms or placebo therapy^[25,68].

Treatment response in most instances is quantified in terms of alterations in disease markers, tumor volume, or symptoms, based on the targeted disease. The potential of nanocrystals to improve the efficacy of drugs is especially relevant for conditions that need a high drug concentration at the site of action, such as cancer, inflammation, or infection. Safety and toxicity are paramount factors during *in vivo* assessment. Although nanocrystals possess numerous benefits, their dimensions and physicochemical nature may, on occasion, create some unforeseen toxicological effects. Toxicological investigations are therefore paramount to guarantee that the formulation is safe for use in humans. Such investigations are normally aimed at evaluating the acute toxicity, chronic toxicity, immunotoxicity, hemotoxicity, and genotoxicity of the nanocrystals. Histopathological investigations of organs and tissues may be performed to identify any indicators of organ damage or inflammation. Finally, blood examinations and clinical chemistry tests are employed to monitor the influence of the nanocrystals on several physiological parameters, including liver and kidney function. The nanocrystalline formulation is also tested for its biocompatibility to guarantee that the formulation does not trigger adverse immune reactions or other long-term health problems. Apart from the general safety investigations, degradation and mechanisms of clearance are major determinants for the safety and long-term effectiveness of nanocrystalline formulations. Nanocrystals, based on their composition, can be broken down by the biological system and eliminated from the body. Metabolic pathways and elimination rates of the nanocrystals are tested to ascertain that they are not sequestered in organs or tissues, which would lead to potential toxicity or side effects. Half-life studies and elimination pathways of the nanocrystals assist in establishing whether the drug will be in circulation long enough to achieve its therapeutic benefits or if it will be cleared from the body quickly^[78,53]. In summary, *in vitro* and *in vivo* analysis of nanocrystalline drug delivery systems delivers essential information on the release profile of the drug, stability, bioavailability, distribution, targeting, pharmacodynamics, safety, and efficacy of the product. With the help of a mix of lab-based assays and animal experiments, scientists can get a clear idea of the behavior of nanocrystals in both the controlled

lab environment and inside a living system. This dual assessment of in vitro and in vivo then confirms that the nanocrystalline compositions are safe, potent, and can deliver therapeutic effects to patients, especially when dealing with poorly soluble or hydrophobic drugs. With progressive development of nanocrystal formulations, these assessments will continue to be central to the translation of new drug delivery technology from the lab to the clinic.

8.Nanocrystalline Drug Delivery Systems: Toxicity and Biocompatibility

Toxicity and biocompatibility of nanocrystalline drug delivery systems (NDDS) are perhaps the most important among these factors that need to be thoroughly analyzed prior to the use of these systems in a clinical setting. While nanocrystals bring significant benefits for enhancing the bioavailability and solubility of sparingly soluble drugs, their nanosize and resultant enormous surface area endow them with special physicochemical characteristics that can pose undesirable risks. Since nanocrystalline systems aim to increase the efficiency of drug delivery by improving solubility, increasing surface area, and providing controlled or sustained release, it is necessary to determine their safety as regards possible toxicity, immunogenicity, and biocompatibility. Knowledge of the safety profiles of such preparations is crucial not only to ensure therapeutic effectiveness but also to avoid any unwanted adverse effects when given to patients. Toxicity is used to indicate the capacity of a substance to induce damage to biological systems. For nanocrystalline drug delivery systems, in addition to the drug itself, toxicity can result from a range of reasons such as excipients present in the formulation, the physical characteristics of the nanocrystals like particle size, surface charge, and surface modification, and administration. The tiny nature of nanocrystals enables them to pass through biological membranes and tissues with greater ease compared to large particles, which exposes them to increased risks of undesired interactions with proteins, cells, and organs. While the larger surface area of nanocrystals can improve drug dissolution and bioavailability, it can also result in increased exposure to toxicological effects. The cytotoxicity of nanocrystalline drug delivery systems has been one of

the most extensively researched topics in their toxicity. It is the property that allows the nanocrystals to harm or kill cells, particularly those which constitute healthy tissue. There are a number of reasons for cytotoxicity, such as the nanocrystal concentration, particle size, shape, surface charge, and the chemical structure of the drug delivered. Small particle-sized nanocrystals (commonly between 10 to 500 nanometers) are able to more easily interact with cell membranes and therefore damage or even modulate cell function. For example, smaller nanocrystals possess a greater surface area that could enhance their reactivity with cellular materials, maybe triggering oxidative stress, inflammation, or even cell death. This cytotoxicity may be evaluated by in vitro cell culture experiments, where the nanocrystals are treated with various cell types (e.g., epithelial cells, fibroblasts, endothelial cells) and the corresponding effects on cell viability, proliferation, and morphology are determined. The surface properties and size of nanocrystals have a great impact on their cytotoxic activity. Particles that are too small can be endocytosed by cells with ease, leading to accumulation within the cell and cytotoxicity. Particles that are too large, however, can be less readily internalized by cells, lowering their toxicity but perhaps diminishing their therapeutic potential. Surface charge or zeta potential also plays an important role in predicting the interactions of nanocrystals with biomembranes. Nanocrystals with strong positive or negative surface charge are more stable in suspension and possess improved dispersion quality^[36,98]. Yet charged particles can also interact with cell membranes in a way that might cause cellular stress, endocytosis, or membrane damage, depending on charge and cell type involved. Aside from cytotoxicity, nanocrystals can also induce genotoxicity, which involves damaging the genetic material of a cell that can give rise to mutation or cancer. The capacity of nanocrystals to penetrate the nucleus of cells and engage with DNA raises a concern, particularly for drugs with known genotoxicity. Genotoxicity tests generally include assessing the capacity of nanocrystalline formulations to induce chromosomal damage or mutations. Typical in vitro tests used to measure genotoxicity are the Ames test, the micronucleus test, and the Comet assay that quantify DNA damage, chromosomal aberrations, or gene mutations caused by the nanocrystals. These experiments are

important to ensure that the nanocrystals do not cause undesirable genetic changes that would result in cancer or other genetic diseases. The immunotoxicity of nanocrystalline drug delivery systems is equally pertinent. The response of the immune system to nanoparticles is unpredictable, as nanocrystals may interact with several immune cells, such as macrophages, dendritic cells, and lymphocytes. These interactions may result in immune activation, allergies, and chronic inflammation. When nanocrystals are given to the body, they tend to be treated as foreign bodies, eliciting an immune response. This immune response may result in hypersensitivity or inflammation and may cause adverse effects. In a few instances, nanoparticles may become covered by proteins in the blood, a phenomenon referred to as the protein corona, which may change their biological identity and their interactions with immune cells. In addition, the surface functionalization of nanocrystals (e.g., the attachment of targeting ligands, surfactants, or polymers) can also affect their immunotoxicity. In vitro and in vivo tests are normally carried out to determine the activation of immune responses, e.g., release of pro-inflammatory cytokines and induction of immune cell proliferation^[85,82]. These tests ensure that the nanocrystals do not cause unwanted immune reactions upon administration. A second area of toxicity related to nanocrystalline drug delivery systems involves hemotoxicity, defined as toxic effects on blood cells and the circulatory system. Hemotoxicity may be caused by the interaction between nanocrystals and blood constituents, including red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. When nanoparticles are introduced into the circulatory system, they may either interact with blood cells directly, resulting in hemolysis (red blood cell rupture), or trigger an immune response that influences the activity of blood cells. Hemolysis tests are performed to evaluate if nanocrystals induce red blood cell destruction that would lead to anemia and other conditions. Nanoparticles also impact blood clotting processes, which could lead to excessive bleeding or clot formation. The possibility of such side effects is determined by studying the coagulation factors and the counts of blood cells in animal models. The findings from these tests assist in establishing whether the nanocrystals can be safely distributed in the blood without affecting the

system to any harm. Biocompatibility is another essential aspect in determining the safety of nanocrystalline drug delivery systems. Biocompatibility is the capacity of a material to function with a proper host response when used in a biological system. For nanocrystals, biocompatibility encompasses their capacity to interact with tissues within the body without inducing toxic responses or immune rejection. Biodegradable nanocrystals, derived from biodegradable materials like biodegradable polymers or lipids, may enhance the formulation's biocompatibility. These nanocrystals would degrade into non-toxic breakdown products over time, minimizing the risk of tissue or organ accumulation, which may result in delayed toxicity. Biodegradable nanocrystals can be cleared safely from the body, usually via renal or hepatic pathways, without any adverse effects. The biocompatibility assessment also includes knowledge of the interaction of nanocrystals with different organs and tissues following administration^[36,94]. Animal tissue distribution studies assist in the evaluation of whether the nanocrystals deposit in organs like the liver, spleen, or kidneys, which might signify possible toxicity or accumulation. These studies also assess whether nanocrystals are able to pass through biological barriers, like the blood-brain barrier, which may be favorable for certain applications (e.g., for targeted drug delivery to the brain) but could also be harmful depending on the nature of the nanocrystals. Long-term investigations must establish whether repeated exposure to the nanocrystals results in chronic toxicity, organ impairment, or immune responses. The clearance of nanocrystalline formulations from the body is also a key factor for the assurance of biocompatibility. Nanocrystals with poor clearance profiles may become tissue-bound and result in long-term toxicities. The mechanisms of clearance by nanocrystals are greatly determined by their shape, size, surface characteristics, and the composition of materials they are formulated from. The main pathway of renal clearance is for small particles of nanoparticles, whereas larger ones are generally cleared via the reticuloendothelial system (RES), mostly by the liver and spleen. The half-life of blood-borne nanocrystals is a crucial parameter, which decides their persistence in circulation before they are cleared from the system by the body. In vivo toxicology tests are essential for testing the safety of nanocrystalline

drug delivery systems. In vivo toxicology testing involves administering the nanocrystals to animal models and observing different endpoints, like body weight, organ weight, histopathological alterations, blood parameters, and behavioral changes. They are usually conducted at a range of dose levels in order to ascertain the acute and chronic toxicity of the formulation. The chronic toxicity studies, in specific, are vital for determining the long-term safety of nanocrystals since they identify the possible adverse effects arising from the prolonged exposure^[94,2]. Animal models are also employed to determine the immunotoxicity, genotoxicity, and hemotoxicity of the product, giving an overall safety profile. In summary, although nanocrystalline drug delivery systems present high potential for enhancing the therapeutic activity of poorly soluble drugs, their biocompatibility and toxicity need to be carefully examined to certify their safety for humans. The nanocrystals' small size, big surface area, and surface characteristics have the potential to cause toxicity, such as cytotoxicity, genotoxicity, immunotoxicity, and hemotoxicity. This risk can, however, be minimized by proper design and rigorous testing with a range of in vitro and in vivo tests. Biocompatible and biodegradable formulations provide a further safety aspect to ensure that the nanocrystals will be safe in clinical use. Good toxicological analysis and biocompatibility testing will lead nanocrystalline drug delivery systems to the forefront of next-generation drug delivery technology, providing more effective and safer treatments to patients.

9. Regulatory Considerations for Nanocrystalline Drug Delivery Systems

The creation and marketing of nanocrystalline drug delivery systems (NDDS) have gained a lot of interest in the pharmaceutical sector due to their capability to tackle issues related to poorly soluble drugs. By shrinking drug particles down to the nanometer range, these systems significantly boost drug solubility, bioavailability, and overall therapeutic effectiveness. But even with these exciting benefits, navigating the regulatory environment for nanocrystalline formulations can be tricky. There are several regulatory factors to consider, including safety, efficacy, quality assurance, manufacturing processes, and

clinical trials, all of which must adhere to the strict guidelines laid out by regulatory bodies like the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and various global agencies. Understanding these regulations is vital for the successful development, approval, and market presence of nanocrystalline drug products. A key regulatory consideration for nanocrystalline drug delivery systems is the safety evaluation of the nanocrystals. Because of their small size and expansive surface area, nanocrystals possess unique physicochemical traits that can impact their behavior in biological systems in ways that we still don't fully grasp. This includes how they interact with cells, tissues, proteins, and other biological molecules, which can lead to concerns about toxicity, immune reactions, and overall biocompatibility. Regulatory authorities require thorough preclinical and clinical data to assess the potential risks of these nanocrystals. Gathering safety information typically requires both in vitro and in vivo studies aimed at determining possible cytotoxicity, genotoxicity, immunotoxicity, and other negative effects. Safety assessments for nanocrystalline systems usually involve toxicological studies aimed at understanding the risks of adverse reactions in human subjects. These studies often focus on how nanocrystals are absorbed, distributed, metabolized, and eliminated by the body (a process known as ADME). It's crucial to find out if nanocrystals build up in organs, if they cause tissue damage, or if they provoke an immune response. Regulatory agencies need this information to weigh the benefits of nanocrystalline delivery systems against any potential hazards. Long-term toxicity assessments are especially critical, as they can highlight chronic effects that might occur from prolonged exposure. In addition, biocompatibility testing is necessary to confirm that the formulation does not yield negative effects within biological systems. Essentially, nanocrystals should be biocompatible, meaning they shouldn't provoke toxicity, inflammation, or unwanted immune reactions when introduced into the body. Along with safety, efficacy is another major consideration that regulatory authorities emphasize when evaluating nanocrystalline drug delivery systems^[6,9]. Since nanocrystals are meant to enhance the solubility and bioavailability of poorly soluble drugs, regulatory bodies require solid proof that these improvements lead to better therapeutic

outcomes. To demonstrate the efficacy of nanocrystalline formulations, sponsors must carry out clinical trials to see if the nanocrystals lead to improved drug absorption, higher plasma concentrations, and better therapeutic effects compared to traditional drug formulations. Efficacy trials typically involve randomized, controlled clinical studies in which the nanocrystalline drug is compared to a placebo or a standard drug version. Primary endpoints for these studies often include improvements in clinical symptoms, biomarkers, or disease progression, depending on what condition is being treated. The design of clinical trials must consider the distinct pharmacokinetic and pharmacodynamic characteristics of nanocrystalline formulations to accurately assess their therapeutic potential. Quality control for nanocrystalline drug delivery systems is another area of significant concern for regulatory agencies. Creating nanocrystals involves complex formulation processes that can lead to variations in the size, shape, and surface characteristics of the particles. Regulatory authorities like the FDA and EMA insist that nanocrystalline formulations adhere to rigorous quality control standards to ensure they are consistent, reproducible, and safe. This involves stringent testing of critical physicochemical properties such as particle size, size distribution, surface charge (zeta potential), morphology, and the drug's physical state (whether it is crystalline or amorphous). Additionally, assessing the stability of the nanocrystalline formulation is crucial to ensure the product maintains its efficacy and safety throughout its shelf life. Stability studies usually evaluate factors like drug degradation, changes in particle size, and the risk of aggregation or precipitation. Moreover, the manufacturing processes for nanocrystalline drug delivery systems need to be robust and scalable to meet the demands of mass production. Nanocrystals are generally produced using methods such as high-pressure homogenization, bead milling, or solvent evaporation, which require careful monitoring of parameters like temperature, pressure, and the types of excipients used. Regulatory agencies need these manufacturing processes to be clearly defined and validated to ensure they consistently produce the desired product. Good Manufacturing Practice (GMP) guidelines must be followed to ensure that nanocrystals are produced in a controlled, sterile environment,

reducing the likelihood of contamination. Manufacturers must also show they have quality control measures in place to oversee the production process and ensure the nanocrystals meet the necessary specifications at all stages of manufacturing. The regulatory classification of nanocrystalline drug delivery systems is another important factor. In many countries, nanocrystals are categorized as nanomedicines, which face specific regulations regarding their development, approval, and marketing. However, the classification can differ based on the intended use, composition, and formulation. In some situations, nanocrystals might be recognized as new chemical entities (NCEs), necessitating extensive preclinical and clinical data to prove safety and efficacy. In other scenarios, if the nanocrystal formulation includes a drug that's already been approved, it might be treated as a new dosage form of an existing drug, potentially requiring fewer clinical studies for regulatory approval. How a regulatory agency classifies a product has implications for the development process, including the extent of preclinical and clinical testing needed. In the U.S., the FDA has established guidelines for developing and approving nanomedicines, including nanocrystalline drug delivery systems. The FDA's Center for Drug Evaluation and Research (CDER) handles new drug applications (NDAs) for nanocrystal formulations and requires a comprehensive data set that covers pharmacology, toxicology, clinical efficacy, and manufacturing quality of the nanocrystal-based drug. The FDA also stresses the importance of nonclinical studies, such as pharmacokinetic and pharmacodynamic assessments, to understand how the nanocrystals perform in vivo and their potential benefits in treatment. Additionally, the FDA Nanotechnology Task Force has set out specific considerations for nanotechnology-based drug products, including guidelines for physicochemical characterization, toxicity evaluations, and suitable clinical trial designs. In the European Union, the EMA has similar guidelines for approving nanocrystalline drug delivery systems. The EMA's Committee for Medicinal Products for Human Use (CHMP) oversees the evaluation of nanomedicines, requiring sponsors to submit quality, safety, and efficacy data for nanocrystal formulations to ensure they meet the agency's stringent standards. The EMA also offers detailed guidance on the clinical

development of nanomedicines, highlighting the necessity for appropriate toxicological testing and clinical trial designs that are tailored to the specific characteristics of nanocrystalline formulations. The international regulatory environment for nanocrystals continues to evolve, given that nanotechnology is a relatively new area, and regulatory bodies are still fine-tuning their guidelines to address the challenges posed by nanomaterials. As more nanocrystal-based drug delivery systems proceed through clinical trials and gain approval for commercial use, it's likely that regulatory agencies will keep updating their policies to stay aligned with the advancements in nanomedicine. One major challenge in regulation is the inconsistency in nanotechnology regulations across different regions. While the FDA and EMA have set specific guidelines for nanomedicines, other areas might have different requirements or may not have fully developed regulatory frameworks for nanotechnology-based drugs. This patchwork of regulations can pose problems for manufacturers looking to market nanocrystal formulations in various regions. Efforts for regulatory harmony, such as those led by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), aim to tackle these challenges by promoting consistency in the evaluation and approval of nanocrystal-based drug products across multiple markets. To sum up, in order to guarantee that these novel formulations satisfy the safety, effectiveness, and quality requirements for clinical use, the regulatory considerations for nanocrystalline drug delivery systems are complex and demand close attention. To address the particular difficulties presented by nanocrystals, including their physicochemical characterization, toxicological testing, and clinical evaluation, regulatory bodies like the FDA and EMA have created particular guidelines. Manufacturers are required to follow strict quality control procedures and provide thorough preclinical and clinical research to prove the safety and effectiveness of their nanocrystalline formulations. To ensure that drug delivery systems based on nanocrystals are safe and effective for patients, regulatory bodies will probably update and improve their guidelines as the field of nanomedicine develops further^[58,69,25].

10. Clinical Applications of Nanocrystalline Drug Delivery Systems

Nanocrystalline drug delivery systems (NDDS) are a forceful utility in the field of pharmaceutical science as they provide ample advantages in the delivery of poorly soluble and bioavailable drugs. NDDS systems make use of nanocrystals, small crystalline particles of drugs with diameters usually between 10 to 500 nanometers, to increase the dissolution, solubility, and bioavailability of drugs. The distinct physicochemical attributes of nanocrystals, including their large surface area, small size, and the flexibility to tailor their surface properties, render them extremely beneficial for enhancing drug delivery efficacy. The therapeutic areas for clinical use of nanocrystalline drug delivery systems are vast, ranging from cancer therapy to neurological diseases, cardiovascular ailments, and infectious diseases. The potential of these systems to overcome the shortcomings of traditional drug delivery systems has created new opportunities for the treatment of diseases. This thorough investigation is dedicated to the clinical applications of NDDS and discusses their possible advantages, shortcomings, and continued improvements that may influence their future application in clinics^[26,49]. The greatest benefit of nanocrystalline formulations is the enhancement of poorly soluble drugs' solubility and bioavailability. A number of drugs, especially those which are lipophilic in nature, have poor solubility in water, thereby greatly reducing their absorption from the gastrointestinal tract, and consequently their therapeutic activity. Traditional approaches to improving drug solubility, including the incorporation of solubilizers or complexing agents, are often restricted in terms of stability, efficiency, and safety. Nanocrystalline drug delivery systems provide a promising solution to these issues by decreasing the particle size of the drug to the nanometer range, which enlarges the surface area accessible for dissolution. The increased surface area contributes to increased rates of dissolution, hence enhancing the oral bioavailability of the drug. This is especially significant in the case of oral delivery, the most frequently used method of drug administration. One of the most prominent clinical uses of nanocrystalline drug delivery systems is cancer treatment. The limited

solubility of many anticancer drugs has the potential to greatly reduce their efficacy in treating tumors. Nanocrystals have widely been explored due to their potential in enhancing the solubility and bioavailability of different chemotherapy drugs, thus their therapeutic action. For instance, chemotherapeutic agents like paclitaxel and docetaxel, which are commonly utilized for cancer treatment, can be designed in the form of nanocrystals to enhance their solubility and minimize the requirement for toxic excipients like Cremophor EL in conventional products. Nanocrystalline preparations of such drugs can improve their tumor targeting, which results in more efficient treatment and decreased systemic toxicity. The enhanced solubility and bioavailability also enable the lower dosing, minimizing the side effects of chemotherapy, including nausea, fatigue, and organ toxicity. Additionally, nanocrystals can be formulated to encapsulate hydrophobic anticancer drugs so that the drug is released under controlled conditions at the tumor site and efficacy is increased while side effects are reduced. Nanocrystalline drug delivery systems are also being researched for treating neurological disorders^[98,64]. The blood-brain barrier (BBB), a selective permeability barrier that defends the brain against harmful substances, is a major obstacle in the delivery of drugs to the central nervous system (CNS). Most therapeutic compounds for neurological disorders, such as Alzheimer's disease, Parkinson's disease, and brain tumors, are poorly soluble and encounter difficulty in crossing the BBB. Nanocrystals present the solution by increasing the solubility of CNS drugs and their ability to cross the BBB. Due to their small size, nanocrystals are able to invade the endothelial cells of the BBB via passive diffusion or active transport. Nanocrystals can also be surface-functionalized with targeting ligands that are bounded to particular receptors present on the BBB, thus increasing their potency to deliver drugs directly to the brain. For example, nanocrystalline drug formulations of compounds like dopamine for Parkinson's disease or donepezil for Alzheimer's disease are in the process of being formulated to enhance their solubility and brain-targeted delivery. These drug formulations not only enhance drug bioavailability in the brain but also reduce peripheral side effects, enhancing the overall therapeutic responses of patients with

neurological disorders. Also, the clinical utility of nanocrystalline drug delivery systems encompasses the treatment of infectious diseases. Antimicrobial resistance (AMR) is a rising worldwide health issue, and access to effective antimicrobial therapies is more urgent than ever before. Nanocrystalline formulations have been found to have potential in enhancing the solubility and efficacy of antibiotics, antifungals, and antiviral agents. For instance, pharmaceuticals such as ciprofloxacin, amphotericin B, and azole antifungals can be formulated as nanocrystals in order to increase their solubility, stability, and therapeutic effects. Moreover, nanocrystals can also enhance the local concentration of the drug at the infection site and provide improved treatment. Nanocrystalline systems also have the ability to facilitate the controlled delivery of antimicrobial agents, which maintains the therapeutic level of the drug for a prolonged duration and minimizes the dosing frequency. This method not only enhances the effectiveness of antibiotics but also minimizes the chance of drug resistance by providing a sustained drug concentration at the infection site. Cardiovascular diseases (CVD) are one of the other disease conditions where there are possible clinical uses of nanocrystalline drug delivery systems. Most drugs employed in CVD treatment, including statins, antiplatelet drugs, and angiotensin-converting enzyme (ACE) inhibitors, are plagued with poor solubility, which restricts their bioavailability and effectiveness^[1,2]. By designing these drugs as nanocrystals, their solubility can be increased, and hence therapeutic efficacy. As such, nanocrystalline formulations of atorvastatin or simvastatin have been used to enhance the dissolution rate of these drugs, thus enhancing their absorption and decreasing the time required for achieving therapeutic levels in the blood. In addition, nanocrystalline products can be designed to provide sustained or controlled release, especially for those drugs utilized in the management of chronic diseases such as hypertension, heart failure, and hyperlipidemia. The method lessens the frequency of dosing, improves patient compliance, and sustains the therapeutic effect throughout the duration. The clinical use of nanocrystalline drug delivery systems is not limited to the treatment of ocular disorders. Ocular drugs used for diseases like glaucoma, cataracts, and age-related macular degeneration

(AMD) frequently encounter issues regarding poor ocular bioavailability. Traditional ocular drug delivery routes, e.g., eye drops or injectables, tend to experience poor drug retention coupled with rapid clearance from the eye, leading to suboptimal therapeutic effects. Nanocrystals present a number of benefits in this regard, such as enhanced ocular penetration and the capacity to deliver higher drug concentrations at the target site for more extended periods^[85,28]. Nanocrystalline drug formulations of ocular therapeutic agents like timolol for glaucoma have been reported to increase drug absorption across the corneal membrane, enhancing intraocular bioavailability as well as therapeutic efficacy. Also, the controlled release of medicines from nanocrystalline preparations can lower the frequency of treatment, especially for patients with chronic eye diseases. Long-term administration of medicines is necessary in the case of chronic diseases, and because of this, nanocrystalline drug delivery systems are extremely useful as they have sustained and controlled release characteristics. These systems enable gradual release of the drug over a long period of time, which can maintain the drug levels within the body at therapeutic levels, enhance compliance of the patient, and decrease side effects caused by high peak drug levels. Controlled nanocrystal release may be accomplished by multiple mechanisms such as the application of polymers, lipid-based carriers, or hydrogels to entrap the drug or by adjusting the particle size and surface chemistry of the nanocrystals themselves. For instance, the application of nanocrystals for the controlled delivery of antidiabetic, anti-inflammatory, and antihypertensive drugs has demonstrated potential in optimizing disease management through sustained drug levels and less frequent dosing despite the promising future of nanocrystalline drug delivery systems, there are a number of challenges that require solution before wide applicability in clinical practice. One of the biggest challenges is scalability in nanocrystal manufacturing. Lab-scale manufacturing of nanocrystals is quite easy, but scaling up to commercial manufacturing and ensuring consistency in particle size, distribution, and stability is challenging. The toxicity of some nanocrystalline formulations is also a cause of concern. Although nanocrystals in general provide enhanced drug solubility and bioavailability, due to their small size and high

surface area they are potentially toxic, with possible cytotoxicity, genotoxicity, and immunotoxicity, particularly if they become deposited in tissues or organs^[34,39]. Thus, comprehensive toxicological studies and biocompatibility testing have to be conducted to prove the safety of nanocrystalline drug delivery systems. In summary, nanocrystalline drug delivery systems can be used across a variety of clinical conditions in different therapeutic fields, which include oncology, neurology, infectious diseases, cardiovascular diseases, ocular diseases, and chronic diseases. The potential of nanocrystals to enhance the solubility, bioavailability, and controlled release of drugs renders them an exciting drug delivery platform for the improvement of drugs with poor aqueous solubility. As manufacturing scalability, toxicity, and regulatory issues continue to pose challenges, advancing nanocrystalline drug delivery systems presents great potential to further enhance the treatment of many diseases and enhance patient outcomes.

11. Case Studies: Nanocrystalline Drug Delivery Systems in Practice

The advent and utilization of nanocrystalline drug delivery systems (NDDS) are a breakthrough in the field of pharmaceutical science, providing a new method of addressing the issues of poorly soluble drugs. With ongoing prominence in the field of nanomedicine, an increasing number of case studies provide compelling evidence on how these systems are used in real life. These case studies do not just highlight the scientific and technical innovations of nanocrystal formulations but also offer useful insight into their actual effectiveness in real-world applications towards enhanced drug delivery and therapeutic benefits. A number of priority examples illustrate the effective use of nanocrystalline drug delivery systems for the treatment of a wide range of diseases, ranging from cancer and cardiovascular ailments to neurological conditions and infectious diseases. Through these case studies, we can know the potential, challenges, and future prospects for the extensive applications of nanocrystals in clinical settings^[37,31]. The most notable case study among the applications of nanocrystalline drug delivery systems is the formulation of paclitaxel nanocrystals for cancer therapy. Paclitaxel, an anticancer chemotherapy

drug, is poorly soluble in water, making drug formulation difficult and restricting its bioavailability. Formulations of paclitaxel traditionally utilized solubilizing agents like Cremophor EL, which, although efficient at increasing solubility, had the potential to induce acute hypersensitivity reactions and other side effects in patients. In response to this, nanocrystalline paclitaxel formulations were created that greatly enhanced its solubility and bioavailability while avoiding the use of toxic excipients. The Nanotax® formulation, which is a paclitaxel nanocrystal product, was marketed as a nanocrystalline-drug delivery system that allows for the administration of higher doses without the Cremophor EL side effects. Experiments proved that this preparation not only increased the solubility and bioavailability of paclitaxel but also supported its therapeutic activity and enabled more efficient treatment of solid tumors. In clinical trials, paclitaxel nanocrystals have been found to possess higher tumor response rates and less systemic toxicity compared to conventional paclitaxel formulations and thus offer a promising alternative for cancer treatment. Another interest case study is the formulation of griseofulvin nanocrystals as an antifungal drug for the treatment of fungal infections. Griseofulvin, an antifungal drug mainly employed to treat dermatophyte infection, is less water-soluble, resulting in less absorption in the gastro-intestinal tract. This poor bioavailability usually requires the administration of high doses, thus enhancing the risk of side effects. In an attempt to maximize the bioavailability of griseofulvin, scientists came up with nanocrystalline formulations of the drug. These formulations lower the particle size of griseofulvin into the nanometer range, enhance the dissolution rate of the drug, and enable quicker absorption. In clinical trials, griseofulvin nanocrystals resulted in substantially better therapeutic responses in patients with fungal infections since the nanocrystals are quickly absorbed and yield increased plasma levels of the drug at decreased doses. This better drug delivery not only increases the efficacy of griseofulvin but also minimizes the occurrence of side effects of high-dose therapy^[48,42]. This case report indicates the potential of the nanocrystalline formulations to enhance the delivery of antifungal drugs, eventually leading to more effective treatment for patients with problematic infections. The application of nanocrystalline drug delivery

systems is also picking up pace in treating neurological disorders, wherein the delivery of drugs to the brain is a major challenge. The blood-brain barrier (BBB), which will only allow the passage of specific substances into the brain, hinders many drugs from reaching therapeutic concentrations in the central nervous system (CNS). One of the more notable case studies here is that of donepezil nanocrystals for the management of Alzheimer's disease. Donepezil is an acetylcholinesterase inhibitor that is employed in the treatment of the cognitive symptoms of Alzheimer's disease. Nonetheless, the drug's low bioavailability and poor solubility hinder its clinical efficacy. In an effort to overcome this, scientists created nanocrystalline formulations of donepezil that not only improved its solubility but also helped in crossing the BBB. Experiments proved that nanocrystalline donepezil has improved absorption, leading to increased drug levels in the brain as well as better cognitive performance among patients suffering from Alzheimer's disease. This case study emphasizes the potential of nanocrystalline drug delivery systems to improve drug delivery to the brain, which has the promise of improved control of neurological diseases. In the cardiovascular disease (CVD) arena, atorvastatin nanocrystals provide another example of the success of nanocrystalline drug delivery. Atorvastatin, one of the most widely used statins for controlling cholesterol and preventing cardiovascular events, is also poorly soluble, and this impacts its absorption and bioavailability. Researchers have come up with nanocrystalline formulations for atorvastatin that enhance its solubility and allow faster absorption in the bloodstream. Clinical trials have indicated that atorvastatin nanocrystals offer increased plasma levels and reach therapeutic levels sooner compared to standard formulations. This rapid absorption leads to better therapeutic efficacy, helping patients achieve optimal cholesterol control. Moreover, the nanocrystalline formulation enables the use of lower doses, minimizing the risk of side effects such as muscle pain and liver enzyme elevation^[51,59]. The example of atorvastatin nanocrystals illustrates how nanotechnology can be used to optimize drug delivery for the management of chronic diseases such as hyperlipidemia in terms of both therapeutic response and patient compliance. Another prime case study of nanocrystalline drug delivery

is the formulation of doxorubicin nanocrystals for cancer treatment. Doxorubicin, an anthracycline anticancer agent, is a very potent anticancer compound, effective against several cancers such as breast cancer, leukemia, and lymphoma. It is, however, restricted in its clinical application due to its low solubility and dose-dependent cardiotoxicity. The nanocrystalline drug delivery of doxorubicin corrects these drawbacks by enhancing its solubilization, bioavailability, and controlled release, decreasing the frequency of side effects and improving the therapeutic activity of the drug. Doxorubicin nanocrystals also enhance the drug's pharmacokinetics, allowing it to remain in the blood for extended durations and concentrate at the tumor site via enhanced permeability and retention (EPR) effects. Clinical trials have demonstrated that doxorubicin nanocrystals exhibit greater antitumor efficacy and reduced side effects when compared with traditional formulations, indicating that they have potential as a cancer treatment. Ketoconazole, an antifungal agent, is one of the other instances where nanocrystalline formulations have been effectively utilized. Ketoconazole is employed for the treatment of a wide range of fungal infections but is plagued by low solubility and poor bioavailability, making it less therapeutic if administered orally. Nanocrystalline ketoconazole formulations dramatically increase its solubility, enabling increased plasma levels and more efficacious therapy. These preparations also minimize the side effects of high doses, as they enable the drug to be absorbed better and in minimal doses. This case study illustrates the usefulness of nanocrystalline drug delivery systems in the therapy of infectious diseases, where enhancing the solubility and bioavailability of antifungals can result in more efficacious treatments with fewer side effects. Nanocrystalline formulations are also proving to be useful in ocular disease treatment, notably with drugs like timolol employed in the treatment of glaucoma. Timolol, a non-selective beta-blocker, is routinely employed to reduce intraocular pressure in glaucoma patients. Because it has poor solubility in aqueous environments and is cleared quickly from the eye, its effectiveness is compromised when given in conventional formulations^[65,66]. By decreasing the particle size of timolol to the nanometer range, scientists have produced nanocrystalline formulations

that improve the solubility and retention time of the drug in ocular tissues. Clinical trials have shown that nanocrystals of timolol enhance intraocular bioavailability, increase the duration for which therapeutic levels of drug are maintained, and decrease dosing frequency in comparison to standard timolol eye drops. This technology has the ability to greatly enhance the treatment of glaucoma and other eye diseases, providing more efficient and convenient treatment to patients. Although these case studies show the huge possibilities of nanocrystalline drug delivery systems in improving the efficacy of poorly soluble drugs, there are challenges that must be overcome. One of the most significant barriers to the clinical translation of nanocrystal formulations is the process of manufacturing. Manufacturing nanocrystalline drug products on a commercial scale with uniform particle size and quality can be expensive and time-consuming. It is essential to have scalable, affordable manufacturing methods to facilitate the widespread use of nanocrystals in the clinical setting. Furthermore, although nanocrystals provide impressive solubility and bioavailability improvements, their toxicity and long-term safety must be explored in depth. Although promising, the special properties of nanocrystals, including their high surface area, could evoke concerns regarding cellular toxicity, immune responses, and organ accumulation. Stringent preclinical and clinical evaluation is required to confirm that nanocrystalline drug delivery systems are safe for human application. In summary, drug delivery systems based on nanocrystals have been an excellent solution for countering the difficulties in the delivery of insoluble drugs. From cancer, cardiovascular conditions, neurological conditions, eye diseases, and infections, case studies in the field show that nanocrystals provide substantial gains in solubility, bioavailability, and drug efficacy. Such advances translate to better treatments, fewer side effects, and improved compliance. Nonetheless, to maximize the potential of nanocrystalline products, more research and development must be pursued to streamline processes, ensure safety, and resolve regulatory hurdles. With continued advancements in the field, nanocrystalline drug delivery systems can potentially change how we treat numerous diseases, leading to greater success for patients all over the world^[72,71].

12.Future Directions for Nanocrystalline Drug Delivery Systems

Nanocrystalline drug delivery systems (NDDS) are a new and dynamic field in pharmaceutical sciences, and their prospects are very promising in terms of revolutionizing the delivery of drugs to patients. The principle behind nanocrystals is to shrink the size of particles of drugs to nanometer levels, thus making them more soluble, bioavailable, and therapeutically efficient. This technology has already proven effective in enhancing the pharmacokinetics of highly insoluble drugs and allowing the creation of new therapies in a broad range of medical conditions. Yet, the full potential of nanocrystalline drug delivery systems remains to be unleashed, and future development will probably base itself on achievements made so far while addressing a number of challenges. The future of NDDS is likely to be influenced by the evolution in nanotechnology, personalized medicine, and regulatory approaches that will unlock new horizons in drug delivery. One of the foremost directions for nanocrystalline drug delivery systems in the future is the improvement in nanocrystal formulation methods. Existing technologies, including high-pressure homogenization, bead milling, and jet milling, have succeeded in miniaturizing drug particles to the nanometer range. These processes are, however, expensive, time-consuming, and challenging to scale for large-scale industrial manufacturing^[82,88]. As demand for nanocrystalline products grows, the need for more efficient, scalable, and affordable manufacturing processes will become increasingly important. Future breakthroughs in continuous processing technologies, including microfluidic-based manufacturing or solvent-free processing technologies, may have significant effects on the scalability of nanocrystals. Furthermore, green chemistry methods that reduce the employment of toxic chemicals and solvents in the formulation process will most likely have a significant part in future NDDS development. The continuous search for newer milling methods and the refinement of existing production techniques will also help enhance the reproducibility, consistency, and quality of nanocrystalline drug products. Another area of crucial importance where future advances will be sought is the increased drug targeting.

Although nanocrystals have shown enhanced solubility and bioavailability, one of the issues that still remains is the delivery of these drugs to the targeted site of action, diminishing side effects and enhancing therapeutic potency. Active targeting and passive targeting are methodologies that can be utilized for targeting nanocrystals towards specific tissues or cells. In active targeting, the surface of nanocrystals is modified in order to bind targeting ligands, e.g., antibodies, peptides, or small molecules, that have affinity with receptors present on target cells. Targeting ligands may, for instance, be designed to selectively bind with cancer cells so that drug delivery to tumor tissue may be targeted and minimal exposure to normal tissues achieved. Passive targeting, however, takes advantage of the enhanced permeability and retention (EPR) phenomenon in tumors and inflamed tissues, through which the malfunctioning blood vessels trap the nanocrystals in the region. As nanotechnology evolves, we can anticipate that increasingly complex targeting systems will become available that increase the specificity of drug delivery to targeted organs or cellular locations. This will lead to more effective treatments for complicated diseases, like cancer, neurological disorders, and autoimmune disorders. Aside from targeted drug delivery, another future direction that seems very promising for nanocrystalline drug delivery systems is the controlled and sustained delivery of drugs^[99,96]. One of the most potential opportunities in this field is that one can engineer nanocrystals to release their drug payload in a controlled fashion over an extended time. This controlled release strategy has the potential to decrease the frequency of dosing, enhance patient compliance, and sustain the therapeutic drug levels within the system for extended periods. Various methods may be utilized for the purpose, such as the employment of biodegradable polymers, lipid carriers, or hydrogels capable of encapsulating nanocrystals and controlling the delivery of the drug. Responsive drug delivery systems, which deliver drugs upon exposure to external stimuli like pH, temperature, or light change, are also being investigated as a method for enhanced release profiles of nanocrystals. By combining nanocrystals with advanced drug release technologies, future NDDS could provide a new paradigm for the treatment of chronic diseases, such as diabetes, cardiovascular

diseases, and cancer, where long-term drug delivery is crucial. The integration of personalized medicine with nanocrystalline drug delivery systems will be another significant development in the future. Personalized medicine is intended to tailor treatment according to patient-specific characteristics, including genetic profiles, disease, and drug response. Nanocrystals, with their potential to enhance drug solubility, bioavailability, and targeted delivery, can be a central component in the creation of personalized treatments. For instance, the application of nanocrystals in conjunction with biomarkers may be used to provide more targeted drug delivery so that the patient gets the most effective treatment required for their individual needs. In cancer, for example, customized formulations using nanocrystals might be made to home in on tumor-specific antigens, thus maximizing drug delivery at the tumor site and minimizing side effects in the general circulation. In addition, nanocrystals can be designed to bypass clinical heterogeneity commonly found in patient groups, e.g., differing drug absorption rates or drug resistance. Targeted nanocrystal-based therapies may transform the treatment of many diseases by providing individualized treatments that maximize therapeutic effects in conjunction with reducing undesirable side effects. With advancing nanocrystalline formulations, one potential of extraordinary interest is to develop combination therapies. For a majority of diseases, especially cancer and chronic diseases such as cardiovascular disease, combination therapy is employed to address more than one pathway and enhance the efficacy of treatment. Nanocrystals provide a singular opportunity to combine combination therapies in one delivery system^[100,101]. By encapsulating multiple drugs in nanocrystals or mixing them with other therapies like gene therapies or immunomodulators, it might be possible to combine therapeutic benefits with less complex treatment regimens. For instance, drug-eluting nanocrystals would be able to release a chemotherapeutic agent and a targeted biological therapy simultaneously, increasing synergistic effects and minimizing the possibility of drug resistance. The advancement of combination nanotherapy can not only enhance the efficacy of current medications but also provide new opportunities in the treatment of diseases that are challenging to treat today. In addition, multifunctional nanocrystals is

another developing field, where nanocrystals are engineered to carry out more than one function at the same time. These multitasking nanocrystals may be designed to merge drug delivery with additional therapeutic approaches, including photothermal therapy, photodynamic therapy, or magnetic resonance imaging (MRI). For instance, nanocrystals may be coated with magnetically responsive nanoparticles to facilitate magnetic targeting of drug delivery to a particular target, or they may be tagged with fluorescent dyes to facilitate real-time observation of drug distribution within the body. This multifunctionality has the potential to greatly improve the accuracy and effectiveness of treatments, particularly for complicated diseases such as cancer, where localized drug delivery and monitoring are paramount to successful treatment. Along these lines, nanocrystals may be engineered not only to deliver medicine but also to offer diagnostic and therapeutic capabilities in a single platform, opening the doors to theranostics, an area that combines therapy and diagnostics on one platform. The biocompatibility and safety of nanocrystalline drug delivery systems will remain a predominant concern in their future development. As nanotechnology approaches clinical use, the long-term implications of nanocrystals for human health and the environment should be carefully assessed. A key research area will be determining how nanocrystals interact with the immune system and whether they present any risk of toxicity, inflammation, or immune stimulation. While the high surface area and small size of nanocrystals present great therapeutic benefits, the reverse may occasionally be true in the sense of causing cytotoxicity or genotoxicity unless controlled with care. Research in the future will have to address the issues of nanocrystal biocompatibility, reducing any side effect, and the development of a strategy for safe clearance from the body. The employment of biodegradable materials in nanocrystals is one of the encouraging methods of addressing issues of accumulation and long-term toxicity. Regulatory aspects for nanocrystalline drug delivery systems is another key area that will define the future of this technology. With nanocrystals shifting from the lab to the clinic, there will be a requirement of proper and consistent regulatory guidelines to guarantee their safety, efficacy, and quality. Regulatory bodies like the FDA and

EMA are already in the process of framing guidelines for assessing nanomedicines, but these guidelines will have to grow as nanocrystals gain more acceptance^[102,110]. This also involves framing standardized test procedures for assessing the physicochemical characteristics, stability, and biological interactions of nanocrystals. The future of NDDS will also be driven by harmonization of global regulatory requirements, with the nanocrystals being able to be developed and commercialized globally with minimal restrictions. Finally, the future of nanocrystalline drug delivery systems is enormously promising, with the capability to transform the management of many diseases. Progress in nanotechnology, such as better formulation methods, targeting mechanisms, drug release, and customized approaches to medicine, will further propel the development of NDDS. Combination therapies and multi-functional nanocrystals provide new avenues for enhancing cure and quality of life in patients. Simultaneously, sustained emphasis on biocompatibility, safety, and regulatory clearance will be crucial for the widespread use of nanocrystals in the clinic. As these hurdles are overcome, nanocrystalline drug delivery systems will be a mainstay of future medicine, enabling patients to receive more effective, targeted, and personalized treatments.

13.Limitations and Challenges of Nanocrystalline Drug Delivery Systems

Nanocrystalline drug delivery systems typically refer to the systems that offer enhanced solubility, bioavailability, and targeted drug delivery through the process of reducing the size of drugs to the nanoscale. Over the last few decades, NDDS have attracted high interest based on their ability to provide solutions for many issues related to drug delivery systems. However, despite their numerous advantages, nanocrystalline drug delivery systems face several challenges and limitations that must be addressed to fully realize their potential in clinical applications. These challenges stem from the complexity of nanocrystal formulations, issues related to the manufacturing and scaling-up processes, concerns about safety and toxicity, and regulatory hurdles that hinder their widespread use. One of the fundamental challenges in the development of nanocrystalline drug

delivery systems is the instability of nanocrystals. Nanocrystals are highly sensitive to changes in environmental conditions such as temperature, pH, and ionic strength. This susceptibility stems from the large surface area to volume ratio of nanocrystals, which causes them to be susceptible to physical transformations such as aggregation or recrystallization upon aging. In water solution, for instance, nanocrystals are readily aggregating into bulk particles, and this results in their loss of beneficial characteristics including increased dissolution rates and bioavailability. This clustering tends to be caused by the higher surface energy of the particles, leading to instability in the system. Aside from aggregation, nanocrystals can recrystallize, with the smaller particles growing into bigger crystals, rendering their nanoscale advantage useless. Preventing aggregation and recrystallization is therefore a big issue in the preparation of NDDS. Several stabilizers, including surfactants, polymers, and lipid layers, are commonly employed to counteract such problems, but long-term stability of the formulations is still a problem. Another significant drawback of nanocrystalline drug delivery systems is concerned with their scalability and production. Industrial production of nanocrystals involves very specialized equipment and processes that not only cost a lot but also take a long time. Standard methods of preparing nanocrystals, like high-pressure homogenization, bead milling, and jet milling, are sometimes hard to scale up without affecting the quality and homogeneity of the end product. High-pressure homogenization, for instance, necessitates accurate regulation of pressure and flow rate to attain equal-sized particles, which may be hard to accomplish when handling bulk material. In addition, the requirement of high volumes of solvents in certain manufacturing processes is a concern of cost, environmental effects, and safety of the manufacturing process. It will be essential to create more efficient, scalable, and sustainable manufacturing methods for the broad implementation of nanocrystalline drug delivery systems into clinical practice. Also, the process of designing nanocrystals to satisfy the particular demands of every drug adds to the complexity, as various drugs might need different stabilizers or preparation conditions, further disturbing the manufacturing process^[2,8]. Toxicity is yet another major challenge that

should be addressed with utmost care while creating nanocrystalline drug delivery systems. Although nanocrystals provide several advantages related to solubility and bioavailability, the small particle size and high surface area of such systems could also produce negative interactions with biological systems. The potential for nanocrystals to be toxic lies in their capacity to penetrate biologic barriers, such as cellular membranes, which can lead to cell uptake and organ accumulation in organs like the liver, spleen, or kidneys. These interactions have the capacity to induce inflammatory reactions, immune responses, or oxidative stress, which may lead to cytotoxicity. Surface chemistry of nanocrystals, such as the nature and extent of stabilizing agents or surfactants employed, also determines the safety and biocompatibility of such systems. In case the stabilizing agents leach or are themselves toxic, these pose extra threats. Therefore, the long-term safety and biocompatibility of nanocrystalline drug delivery systems need to be comprehensively investigated in preclinical and clinical studies. Animal studies and human clinical studies are imperative to determine the possible toxicity of these systems, such as any negative effects that can occur as a result of accumulation of nanocrystals within the body or interaction with certain cell types. The biological fate of nanocrystals in vivo is a further significant challenge to understand. After injection, nanocrystals can be identified and removed by the body's immune system, more specifically the reticuloendothelial system (RES), which includes cells in the liver, spleen, and bone marrow that assist in eliminating foreign substances. The rapid removal of nanocrystals from the blood can restrict their therapeutic effectiveness, since they might not circulate long enough to target the affected tissue or organ. In addition, the body residence time of nanocrystals can be affected by various parameters, including particle size, shape, charge, and coating. Nanocrystals with a very small size may be cleared rapidly, whereas particles with a larger size may not permeate biological barriers or may get accumulated in organs, and therefore can have toxicity. To overcome these challenges, attempts have been made to alter the surface characteristics of nanocrystals by encapsulating them with polymers, lipids, or other biocompatible substances for enhancing their circulation time and minimizing their

immunogenicity. Still, there is a difficulty in obtaining an optimal compromise between stability, circulation time, and target-specific delivery^[99,100]. Another drawback of nanocrystalline drug delivery systems is the difficulty in targeting a specific tissue or cell. Whereas nanocrystals have the possibility of providing benefit in solubility and bioavailability, getting them to the appropriate location of action is still a major challenge. The ability to target specifically diseased tissue, including cancerous cells or inflamed tissue, while avoiding normal tissue requires advanced targeting approaches. Active targeting, in which nanocrystals are ligand-functionalized to bind to particular receptors on target cell surfaces, is another method that has been successful. The method can be difficult to execute, though, because receptor-ligand interactions are complex and the cells present in tumors or other ailing tissues may be heterogeneous. Passive targeting based on the EPR effect is also effective in certain conditions but not under all situations, especially in tissues where there is not a significant presence of leaky vasculature. Moreover, despite advanced targeting approaches, making sure the nanocrystals arrive at the target location in appropriate concentrations to deliver therapeutic effects is an intricate challenge. Regulatory issues also present immense challenges to the development and approval of nanocrystalline drug delivery systems. Regulatory bodies like the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have strict regulation requirements for the approval of novel drug delivery systems, including nanotechnology-based ones. Yet there is still no clear and coherent set of regulations specifically aimed at nanocrystalline drug delivery systems. The physicochemical characteristics of nanocrystals that are distinct from other particles, including size, large surface area, and charge, may necessitate novel regulation schemes to evaluate their safety and efficacy^[33,66]. Specifically, nanocrystal toxicity testing and long-term safety should be addressed in depth. Existing regulations frequently do not include thorough guidelines about how the biological effects of nanocrystals should be assessed, and thus clinical development and approval are delayed. Furthermore, the processes through which nanocrystals are manufactured need to be of high quality so that the reproducibility and consistency of the final product is

guaranteed. This again is a challenge for pharmaceutical manufacturers because they have to invest in process validation, which is within regulatory compliance. Until more precise nanocrystal regulations are in place, NDDS development and commercialization are likely to encounter major obstacles. Lastly, patient acceptability and cost considerations are further hindrances to the universal adoption of nanocrystalline drug delivery systems. The technical complexity of nanocrystal formulations and their unique manufacturing processes tend to make them more expensive to produce than conventional drug formulations. These additional expenses could be transferred to the patient, which could make nanocrystalline treatments less available, especially in low-resource environments. In addition, even though nanocrystalline preparations could have desirable characteristics, for example, enhanced bioavailability and targeted delivery, patients and clinicians might be slow to embrace these treatments because of reluctance based on safety, effectiveness, and delayed side effects. Making nanocrystal-based therapies cost-effective and proving them to be better in terms of clinical benefit than current treatments will be important to obtaining patient acceptance and achieving widespread application. In summary, although nanocrystalline drug delivery systems possess a multitude of benefits over traditional drug delivery systems, they are not without enormous challenges and limitations. Problems associated with the stability of nanocrystals, scalability of production, possible toxicity, biological disposition, target delivery, approval from regulatory agencies, and expense need to be resolved to achieve the full potential of this technology. These hurdles will be overcome through ongoing research and development and also through interactions among academia, industry, and regulatory bodies to develop good guidelines for safe and effective use of nanocrystals in clinical practice. As these problems are addressed, nanocrystalline drug delivery systems are going to play an increasingly significant role in current medicine, providing new solutions for the treatment of many diseases.

14. Patent and Intellectual Property Implications for Nanocrystalline Drug Delivery Systems

Nanocrystalline drug delivery systems (NDDS) are an ever-emerging field with immense potential, with remarkable advancements being made in the formulation of new drugs that improve solubility, bioavailability, and therapeutic effect. As the technologies continue to evolve, strong intellectual property (IP) protection is also becoming increasingly necessary to protect investments made by pharmaceutical companies, researchers, and innovators. Patents are also important in safeguarding the property rights of developers and allowing the commercialization of such systems, thereby supporting additional innovation in the area^[55]. Intellectual property concerns are nevertheless sophisticated and multidimensional, for they cover not just patent law but also issues involving regulatory approval, international patent planning, and possible patent infringement or conflict of patent rights. The dynamic nature of the nanotechnology sector and the intricate nature of nanocrystalline systems provide both challenges and opportunities with respect to IP protection. This discussion explores the different patent and intellectual property issues concerned with nanocrystalline drug delivery systems, the significance of filing and owning patents in this new technology. The initial significant concerns with IP protection for NDDS are the nature of nanocrystalline drug delivery systems from a patenting point of view. Nanocrystals are merely drug particles brought down to the nanoscale level, and their physicochemical properties such as increased solubility, bioavailability, and rate of dissolution make them extremely desirable in pharmaceutical uses. From an IP aspect, all of these following aspects of nanocrystalline formulations can be patented: the composition of the drug itself, the process of formulation, and even the final product itself. The formulation is the particular drug compound and any excipients incorporated into the formulation, including stabilizers, surfactants, or polymers that can be added to improve the stability and performance of the nanocrystals. The process of formulating, which involves the techniques applied to miniaturize the drug to the nanoscale (including high-pressure homogenization, bead milling, or solvent evaporation), is

another area of NDDS potentially patentable. Lastly, the final product itself, such as the particular drug in its nanocrystalline state, can also be patented if it is novel, inventive, and useful. A major problem in patenting NDDS is the assessment of novelty^[22,55]. Since nanotechnology is an evolving and newly emerging technology, most patents for NDDS tend to be developed from previous researches and advances, so it would be difficult to determine what exactly constitutes an original and new innovation. Especially, a prior art search is needed to ascertain if a specific nanocrystal composition, process, or formulation has been previously disclosed in the public sphere or patented by others. Novelty in nanocrystalline systems may rely on the fact that one can prove that the formulation or preparation method is considerably different from the preceding technologies. For example, if a novel preparation method for nanocrystals notably enhances the bioavailability of the drug or solves particular stability issues not addressed before in previous formulations, then the approach can be deemed novel. In the case of the composition of nanocrystals, factors such as the use of novel stabilizers or specific types of excipients could be patentable, especially if they are shown to provide a significant improvement in the performance of the drug. Patentability also hinges on the demonstration of inventive step or non-obviousness. This is particularly relevant in the context of NDDS, where incremental improvements in formulation or manufacturing processes are common. For example, researchers might find a new stabilizer or an improved technique for preparing nanocrystals that enhances solubility, but such innovation needs to be demonstrated to be non-obvious to a person skilled in the art. Determination of obviousness then can become a point of conflict, particularly in very competitive areas such as nanotechnology, where several research groups might be working on similar ideas and developing overlapping technologies^[77,11]. Under these circumstances, proving that the invention represents a sufficient inventive advance over the state of knowledge is essential for obtaining patent protection. Courts tend to look into whether the remedy for a problem was obvious to persons in the field or if it involves a new and surprising insight, which is needed for a patent grant. In nanocrystalline drug delivery systems, utility is also a significant requirement for

patentability. The usefulness of a patentable invention has to be visibly proved, i.e., the nanocrystalline process or product has to display functional application in the desired use—in this case, enhanced delivery and effectiveness of pharmaceuticals. This is especially true in the case of filing patents for novel drug forms or new delivery systems, where it is required to demonstrate that the system delivers concrete therapeutic advantages like enhanced solubility, bioavailability, or targeting efficacy in order to gain patent protection. Moreover, the clinical or preclinical information demonstrating the utility of the invention is also crucial for determining the functionality of the nanocrystalline delivery system. Applicants for patents need to demonstrate, through experimental evidence, that the nanocrystalline drug delivery system offers a recognisable benefit over conventional drug delivery systems, which can involve improved therapeutic effects, decreased dosing frequency, or an improved safety profile. Apart from the difficulty in proving novelty, inventive step, and utility, patentability of nanocrystalline drug delivery systems may be made difficult by conflicts between formulation patents and method patents. As noted above, patents may be applied for the drug formulation itself, formulation preparation procedure, or both. But pharmaceutical patents for formulations or compositions are more challenging to defend due to the presence of broad patent claims that might protect a broad range of formulations, including those formulated by other scientists. To overcome the problem, method patents are often sought, which define a particular process of making the nanocrystals. Method patents usually have a narrower range of protection but are essential in protecting the proprietary nature of a pioneering approach or manufacturing method. Firms and innovators in NDDS commonly apply formulation patents as well as method patents to obtain broad protection^[66,44]. Once a patent for a nanocrystalline drug delivery system has been issued, it offers an exclusivity time frame in which the patent owner can inhibit others from making, using, or selling the invention covered by the patent without authorization. But getting a patent does not preclude commercial success or the launch of a product. One of the biggest hurdles for companies interested in developing and commercializing nanocrystalline drug delivery systems is the regulatory path. Regulatory

approval of nanocrystalline drug products is difficult and region by region. Regulatory authorities, including the U.S. Food and Drug Administration (FDA) or European Medicines Agency (EMA), have set guidelines for the approval of new drug products, but nanocrystals tend to fall into a gray area since they deviate dramatically from conventional drug formulations. The approval of nanocrystalline systems is thus not only patent-protected but also subject to the agency's assessment of the product's stability, efficacy, and safety. On the global scene, patent strategy is a crucial factor. Owing to the competitiveness of the pharmaceutical sector and growing interest in drug delivery systems based on nanocrystals, it is critical to obtain international patent protection in order to avoid entry by competitors with similar products. This usually entails the filing of patent applications in various jurisdictions, including large pharmaceutical markets like the United States, Europe, Japan, and growing markets such as China and India. A PCT (Patent Cooperation Treaty) application is usually the initial step towards obtaining international protection, enabling inventors to pursue patent rights across multiple countries by submitting a single application. Still, filing for patents in many nations is expensive and takes time, as the applicant has to meet every nation's patenting demands and legal procedures. The capacity to acquire and uphold patents across jurisdictions is fundamental to attaining a competitive edge, and corporations have to consider very carefully in which jurisdictions they should submit their patent applications depending on the commercial appeal of their product. Another issue that concerns patenting nanocrystalline drug delivery systems is the possibility of patent infringement. Patent infringement is when a competing entity makes, uses, or sells a patented invention without the approval of the patent owner^[13,16]. Patent infringement in the case of NDDS may be complicated, especially when it comes to method patents and formulations that have similarities with preceding technologies. When a company or researcher infringes on a current patent, they may face legal issues, such as lawsuits, injunctions, and financial compensation. Since nanocrystals are a fast-moving area, there are common overlapping claims in patents and conflicting issues regarding the scope of protection. To prevent potential infringements, businesses must

perform stringent freedom-to-operate searches to find existing patents and ascertain if their product or technology violates any of these patents. In other instances, patent cross-licensing or licensing agreements might be required to settle patent conflicts and enable firms to utilize existing patented technologies. Lastly, the financial cost of patent litigation and the prospect of legal conflict pose extraordinary financial risks for firms that are developing nanocrystalline drug delivery systems. The expense of defending a patent or bringing a patent infringement suit can be too expensive, and it can take away productive resources from research and development. This is especially a concern for small businesses or start-ups that may not have much in the way of resources. As a consequence, pharmaceutical companies frequently try to hedge financial risk from patent conflicts by merging with larger companies, entering into licensing arrangements, or through patent pools that share intellectual property rights. Proper patent strategy and management are necessary to reducing these risks and ensuring intellectual property developed by nanocrystalline drug delivery systems is properly protected. In summary, nanocrystalline drug delivery system development and commercialization are heavily reliant on intellectual property issues, and patents are an important instrument for safeguarding innovation and allowing firms to achieve a strategic edge. The intricacies of patenting NDDS, such as novelty, inventive step, and patentable subject matter issues, need special thinking and strategic planning. Patent protection brings exclusivity, but it needs to be supplemented by effective regulatory measures, international patent filings, and risk management strategies to prevent patent infringement and ensure that innovative technologies reach the market. With the changing nature of nanotechnology and the increasing significance of nanocrystalline formulations in drug delivery, intellectual property will remain crucial to determining the future of this technology^[110,102].

15.Comparison with Other Drug Delivery Systems

Nanocrystalline drug delivery systems (NDDS) are among the leading advances in pharmaceutical technology, with clear benefits in enhancing the solubility, bioavailability, and therapeutic efficiency of sparingly soluble drugs. The systems are a quantum leap ahead of

conventional drug delivery technologies since they leverage the special physicochemical characteristics of materials at the nanoscale. At the wider level of drug delivery, there is a need to see how nanocrystalline drug delivery systems relate to other traditional and new drug delivery systems. Some of the existing systems include solutions, tablets, liposomes, micelles, solid lipid nanoparticles (SLNs), and polymeric nanoparticles, among others. Every delivery system has certain advantages and disadvantages, and it is important to know these variations to establish the optimal way of delivering a particular drug in the most effective and efficient fashion. Nanocrystalline drug delivery systems are particularly aimed at meeting the challenge of poor water solubility, which is one of the principal drawbacks in the development of many therapeutic agents. Poor solubility is a serious problem since drugs that are poorly soluble in water cannot be effectively absorbed by the gastrointestinal tract due to poor bioavailability. Conventional formulations such as solid dosage forms like tablets and capsules tend to be plagued by these solubility problems, rendering them ineffective for poorly soluble drugs in aqueous media. By compacting the size of the drug particles to the nanometer range, NDDS is able to greatly increase the surface area of the drug, making it dissolve faster and, hence, become more bioavailable. The small size of nanocrystals means that they dissolve faster in the body, making them absorbed faster and leading to better therapeutic efficacy. This is one of the main benefits of NDDS over conventional dosage forms, which do not generally get to the same extents of solubility improvement. Conversely, the most common way of delivering drugs is still with tablets and capsules, which are the most common method because they are simple, inexpensive, and easy to administer. These formulations generally depend on the drug's dissolution in the gastrointestinal tract, which is slow and wasteful for poorly soluble drugs. In most instances, for the drugs to defeat solubility, high doses of the drug must be given, resulting in dosing difficulties and side effects. In addition, the bioavailability of drugs delivered through traditional delivery systems is regularly hindered by first-pass metabolism, gastric pH, and enzymatic breakdown. By contrast, nanocrystalline drug delivery systems have the capability to reduce most of these problems by

increasing solubility while reducing the necessity for high doses. Nonetheless, although they are easy to formulate and produce, conventional drug delivery systems tend not to achieve the same level of solubility enhancement as nanocrystalline formulations. Besides improving bioavailability, NDDS are also especially effective in targeted drug delivery, a principle that seeks to deliver the therapeutic agent to the desired location of action, thereby reducing systemic exposure and side effects. Conventional drug delivery systems are incapable of selectively targeting specific cells or tissues. Targeted delivery has been achieved with the help of newer drug delivery technologies like liposomes and polymeric nanoparticles. Liposomes, for example, are lipid bilayer vesicles that are spherical in shape and are capable of encapsulating both hydrophilic and hydrophobic drugs. These systems are engineered for enhanced drug solubility, drug stabilization against degradation, and targeting cells or tissues through surface modifications, including PEGylation or the attachment of ligands. While liposomes have been found useful in some drug delivery contexts, they also have challenges surrounding stability, manufacturing complexity, and expense. In addition, liposomes tend to be big in size, and this can restrict their function to deliver drugs effectively to specific tissues or traverse cellular barriers. In comparison, nanocrystals, due to their smaller size, can more easily permeate biological barriers, such as the blood-brain barrier, and provide more efficient drug delivery to specific tissues, especially when combined with targeted surface modifications. Polymeric nanoparticles represent another modern drug delivery system that is gaining significant attention. These nanoparticles are composed of biocompatible and biodegradable polymers that can encapsulate a wide variety of drugs. Polymeric nanoparticles provide some benefits, for example, controlled drug release, enhanced stability, and surface modification for targeting specific cells or tissues. Unfortunately, there are some challenges that polymeric nanoparticles pose, like drug loading capacity, batch-to-batch reproducibility, and toxicity by the degradation products of the polymers. Whereas polymeric nanoparticles are suitable for most drugs, care must be exercised in controlling their size and composition to achieve the best drug release

profiles and avoid side effects. In contrast, nanocrystals do not need encapsulation, as the drug itself is miniaturized down to the nanoscale to provide a more straightforward formulation process. Moreover, the physical characteristics of nanocrystals, including size and surface area, can be more precisely controlled and tuned for a particular application, giving an upper hand over polymeric nanoparticles in some situations. Another interesting drug delivery system is solid lipid nanoparticles (SLNs), which consist of solid lipids and are capable of carrying lipophilic as well as hydrophilic drugs. SLNs provide sustained release of the drug, stability against degradation, and enhanced stability over conventional formulations. They are especially effective for the delivery of lipophilic drugs, which might not be efficiently delivered via regular means. SLNs do, however, have some limitations in that they have limited drug loading capacity, lack stability when stored, and risk having drug leakage during circulation^[1,9,12]. Nanocrystals, on the other hand, do not have the same limitations concerning drug leakage since the drug exists in the crystalline state, which is more stable by nature. Moreover, the manufacturing process of nanocrystals can be easier and more scalable than SLNs, thus an even more viable choice for high-volume production. Micelles, a self-assembled delivery system produced by surfactants in water, are yet another vital drug delivery system that has come into the limelight over the last few years. Micelles are usually employed to deliver hydrophobic drugs through encapsulation in the hydrophobic interior of the micelle. They have a number of benefits, including improved hydrophobic drug solubility and selective tissue targeting by surface functionalization. Micelles are, however, subject to limitations like instability, low capacity for drug loading, and the possibility of premature release of the drug trapped within them. Nanocrystals, on the other hand, provide improved stability, improved drug loading, and more uniform drug release patterns, especially for drugs with poor aqueous solubility. The ease of nanocrystal formulation and control over their physical characteristics also render them more suitable for some uses than micelles. Compared to traditional systems like solutions and solid dosage forms, nanocrystalline drug delivery systems have several advantages in terms of bioavailability, drug solubility, and pharmacokinetic profiles.

Nanocrystals can be engineered to increase the drug dissolution rate, thus promoting faster absorption and increased bioavailability. Conventional solutions, while they might be more highly dissolvable than solid dosage forms, tend to lack the stability and extended release characteristics of nanocrystalline systems. With drugs that are poorly soluble, nanocrystalline systems can circumvent much of the problem associated with conventional solutions, including requiring huge amounts of solvent or running the risk of being readily cleared from the system^[34,59]. Furthermore, the controlled release capabilities of nanocrystalline systems allow for sustained therapeutic effects without the need for frequent dosing, making them more advantageous than both solutions and traditional oral dosage forms for certain drugs. While nanocrystalline drug delivery systems present several advantages, they are not without their challenges. Manufacturing complexity, stability concerns, and high production costs are all limitations that must be addressed when developing nanocrystalline formulations. The preparative processes employed in making nanocrystals, i.e., bead milling and high-pressure homogenization, are often costly and challenging to integrate with commercial processes. The stability of nanocrystalline products may also depend on the process of formulation, type of stabilizers added, as well as environmental factors like temperature and humidity. For certain drugs, the high surface area of nanocrystals can cause aggregation or recrystallization, diminishing the advantages of the nanocrystalline form. Notwithstanding these challenges, improvements in nanocrystal manufacturing technologies and the synthesis of new stabilizers are enhancing the feasibility and efficacy of NDDS. In summary, nanocrystalline drug delivery systems hold great promise as a better alternative to conventional drug delivery systems as they counteract issues like inadequate solubility of drugs, low bioavailability, and the requirement for sustained drug release. When viewed in relation to other contemporary drug delivery vehicles such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, and micelles, nanocrystals offer singular benefits with regard to simplicity, stability, and drug loading potential. Yet every drug delivery vehicle presents special benefits as well as limitations, and the best delivery vehicle will ultimately depend on the drug in question as well

as the desired therapeutic outcome. As nanotechnology continues to develop, nanocrystalline drug delivery systems will increasingly play a critical role in the development of innovative, successful treatments for a broad array of illnesses. The future challenge will be to overcome the remaining obstacles to their general clinical adoption, especially in the areas of manufacturing, stability, and scalability, while at the same time addressing concerns over regulation and safety^[88,99].

16. Conclusion

In wrapping things up, nanocrystalline drug delivery systems are a groundbreaking leap in pharmaceutical technology. They tackle major issues like poor solubility and bioavailability that can limit how well many drugs work. These systems bring several advantages over traditional methods, such as quicker dissolution rates, better bioavailability, and the capability for controlled, long-lasting drug release. When you compare them to other advanced delivery systems like liposomes, polymeric nanoparticles, and solid lipid nanoparticles, nanocrystals really shine due to their simplicity, stability, and impressive drug loading capacity. They're particularly effective for poorly soluble drugs, which can be tricky to deliver with conventional systems. That said, there are still hurdles to overcome, including the complexities of manufacturing, challenges with stability, and scalability issues. Processes like high-pressure homogenization or bead milling can be expensive and need some tweaking for large-scale production. Plus, keeping nanocrystalline formulations stable over time is essential to ensure they keep working effectively. Fortunately, ongoing advancements in stabilizers and production methods are making it easier to create nanocrystal formulations. Overall, nanocrystalline drug delivery systems hold great promise for enhancing the treatment of various diseases by improving drug solubility and bioavailability. As research moves forward, these systems are set to become vital in developing next-generation therapies, aiming to boost efficacy and reduce side effects, which will ultimately benefit both patients and healthcare outcomes.

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DESIGN AND DEVELOPMENT OF NANOCRYSTALLINE DRUG DELIVERY SYSTEMS FOR IMPROVED SOLUBILITY AND
BIOAVAILABILITY OF POORLY WATER-SOLUBLE DRUGS: A PHARMACEUTICAL PERSPECTIVE

