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*Review*

# A Review of Pharmaceutical Nano-Cocrystals: A Novel Strategy to Improve the Chemical and Physical Properties for Poorly Soluble Drugs

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**Abstract:** Nowadays, many commercial drugs have poor solubility and bioavailability. Cocrystals are formulated to modulate active pharmaceutical ingredients' properties with improved solubility, dissolution, and bioavailability compared to their pristine individual components in the pharmaceutical industry. Nano-cocrystals, crystals in the nano range, can further enhance these properties because of not only the cocrystal structure, but also the large surface to volume ratio of nanocrystals. Even though there are many studies on cocrystals, the research of pharmaceutical nano-cocrystals is still in the initial stage. Thus, it is necessary to conduct a systematic study on pharmaceutical nano-cocrystals. In this review, the possible preparation approaches of nano-cocrystals have been reported. To have a comprehensive understanding of nano-cocrystals, some analytical techniques and characterizations will be discussed in detail. In addition, the feasible therapeutic application of nano-cocrystals will be presented. This work is expected to provide guidance to develop new nano-cocrystals with commercial value in the pharmaceutical industry.

**Keywords:** active pharmaceutical ingredients; pharmaceutical nano-cocrystals; preparation approaches; characterization; therapeutic application



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## 1. Introduction

In the pharmaceutical industry, active pharmaceutical ingredients (APIs) in the solid state are most commonly administered to patients. Many pharmaceutical companies and scientists are endeavoring to develop novel drugs with good solubility, dissolution rate, mechanical properties, hygroscopicity, optimal physical stability and chemical stability. At present, it is a fact that approximately 60% of commercial drugs have poor aqueous solubility issues [1]. Such difficulty is a major driving force for scientists to pursue approaches for improving the pharmaceutical and biological properties of drug products. Cocrystals have great potential applications in solubility enhancement. Research has shown that drug molecules of cocrystals display 4–20 times improvement in solubility [2]. Furthermore, the cocrystal formation can improve the physical properties without changing the original pharmacological characteristics of pure drug molecules [3,4]. In recent years, cocrystals have attracted much attention from researchers and pharmaceutical companies because of these desired properties [5–7]. Kouya prepared TAK-020/gentisic acid cocrystals with enhanced dissolution rate by adopting the solid grinding method and the slurry method [8]. Shaikh formulated a cocrystal of theophylline and 4-aminobenzoic acid using the melt granulation method through adding hydrophilic binder, which can be stored for 14 days under the condition of 50°C, 75% RH (Relative Humidity) [9]. David's team thought that highly soluble cocrystals can transform to the most stable drug forms in solution [10].

Many efforts have been devoted to study the improvement of the mechanical and pharmaceutical properties of poorly soluble drugs in past decades. Cocrystals emerged over

time and became a popular method for drug modifications [11]. However, there is still a big challenge to develop appropriate cocrystals for therapeutic products in the pharmaceutical industry because of the very poor oral absorption. Nano-cocrystals means crystals in the nanometer range, which can further improve the solubility of drugs compared to cocrystals. In addition, some authors also reported that nano-drugs are in fact typically dispersed to improve their stability. Not only the cocrystals' structure, but also the nano-scale particles with increased surface area can exert their effects in improving the dissolution rate and bioavailability of poorly soluble natural products [12]. Nano-cocrystals can be prepared by several techniques, many of which come from techniques applied in nanocrystal manufacture. Usually, there are two methods to manufacture nanocrystals, including a top-down technique, i.e., ball milling by using shear forces to reduce the particle size to nanometers, and a bottom-up technique, i.e., precipitation involving nucleation and crystal growth [13,14]. However, due to the lack of systematic studies on the preparation of nano-cocrystals and criteria for parameters' optimization, the application of nano-cocrystals in the pharmaceutical industry is facing enormous challenges. Thus, systematic works should be performed to expand the application of pharmaceutical nano-cocrystals in the medical field.

In this review, we studied the application of nano-cocrystals in the pharmaceutical area. The formulation preparation of nano-cocrystals will also be discussed in this work. In addition, the characterization techniques of pharmaceutical nano-cocrystals will be investigated in detail. Additionally, prospects will be proposed to provide guidance for the development of nano-cocrystals with specific and desired pharmaceutical properties in the near future.

## 2. Pharmaceutical Cocrystal

### *2.1. Definition of Cocrystal and Nanocrystal*

The definition of cocrystal has always been the subject of extensive debate at a chemical and legal level [15]. In general, the accepted definition is that cocrystals are solids that are crystalline single phase materials composed of two or more molecules in a stoichiometric ratio [16], which are neither solvates nor simple salts. The so-called pharmaceutical cocrystal refers to the combination compounds of the API and cocrystal former (CCF) assembled via hydrogen bonds or other non-covalent interactions, wherein the pure states of the API and CCF are mainly reported in the literature as solid reagents, with the only exception of very few papers in which liquid components are involved at room temperature, according to the works reported by Mazzeo, Bacchi and Capucci et al. [17–19]. The CCF, one of the components in the formation of a pharmaceutical cocrystal, contains physiologically acid-base salts and non-ionized molecules, such as food additives, preservatives, pharmaceutical excipients, vitamins, minerals, amino acids and other active molecules, and even other APIs, etc. These components can combine with APIs by hydrogen bonding, π-π stacking, van der Waals interactions and other non-covalent bonds in the same crystal lattice [20]. From a chemical point of view, the API molecule itself does not decrease their therapeutic effects and it still maintains the original pharmacological effects, while the solubility, bioavailability, and stability of cocrystals have been greatly improved. Especially, for some oral pharmaceutical cocrystals, pharmaceutical and chemical properties showed significant improvement [21,22].

Pharmaceutical nanocrystals represent solid drug nanoparticles which are stable when dispersed in surfactants or polymer materials. The drug-loading of pharmaceutical nanocrystals is close to 100%. Pharmaceutical nanocrystals with special functionality can be obtained by adding functional groups in stabilizers composed of surfactants and polymers [23]. The pharmaceutical nanocrystal is a novel dosage form which can solve the solubility and dissolution issues of insoluble drugs, improving their oral bioavailability and distribution in vivo. Currently, marketed drug nanocrystals include sirolimus tablets, paclitaxel albumin nano-suspension, etc. [24,25].

### *2.2. Application of Pharmaceutical Nano-Cocrystal*

Pharmaceutical nano-cocrystals can greatly improve the delivery properties of poorly soluble drugs, which was a big challenge faced by the pharmaceutical industry for a long time. Study on the formulation of pharmaceutical nano-cocrystals has a good market value. Large-scale production of nanocrystals can be achieved through techniques such as precipitation, media milling, and high-pressure homogenization [26–28]. At present, many market products of nano-cocrystals have been prepared by adopting these techniques. Some reported nano-cocrystals have been listed in Table 1.

**Table 1.** Some reported pharmaceutical nano-cocrystals and preparations.

| Nano CoCrystals | Preparation | Nano Size | Advantages | Ref. |
|---|---|---|---|---|
| 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX) and 2,4,6-trinitrotoluene (TNT) | Spray drying method | 50 nm to 200 nm | High energy and low sensitivity | [29]. |
| Baicalein-nicotinamide (BE-NCT) | High pressure homogenization | 251.53 nm | Improving dissolution rate (2.54-fold) and bioavailability(6.02-fold) of BE | [12]. |
| 4-aminosalysilic acid and sulfamethazine | High-pressure homogenization (HPH) and high-power ultrasound | - | Enhanced the dissolution of sulfamethazine; stable for 6 months when stored at room temperature | [30]. |
| Ezetimibe | Solvent evaporation method and anti-solvent method | 226.4 ± 53 nm | Showed 18.8-fold increase in the dissolution efficiency | [31]. |
| Lamivudine (3TC) and zidovudine (AZT) | Wet media milling top-down approach | 271.0 ± 92.0 nm | Nanosuspensions exhibit a reduced side effect; increased adherence to therapy and reduced resistance to therapy | [32]. |
| Caffeine and 2,4-dihydroxybenzoic acid | Using "top-down" media milling | <100 nm | Enhanced dissolution rates; improved bioavailability and efficacy of medication | [33]. |
| Caffeine/Oxalic acid 2:1 and Caffeine/Glutaric acid 1:1 cocrystals | Spray flash evaporation process | About 60 nm | Enhanced stability, high solubility, high bioavailability, optimized drug up-take | [34]. |
| Hydroxypropyl methylcellulose and sodium dodecyl sulfate | Wet milling | <300 nm | Improve the absorption of poorly soluble drugs | [22]. |

The application of appropriate stabilizers and proper co-formers can endow nanocrystals with long-term stability and commercial feasibility. Nanocrystals showed good therapeutic applicability when administered by oral, intravenous, pulmonary, ocular, and dermal routes. In addition, the drug molecules with nanostructure can be targeted to specific regions.

#### 2.2.1. Drug Delivery

Oral administration is considered to be the safest and most comfortable route of drug delivery [35]. The nanocrystals with large surface area can significantly increase the saturation solubility of drugs, thereby increasing the dissolution rate. It is reported that the increase rate in the oral bioavailability of the nanocrystal tablets can reach 21% [36]. Studies have shown that itraconazole-adipic acid nano-cocrystal has higher oral bioavailability than amorphous formulations [21]. The oral bioavailability improvement of phenazopyridine-phthalimide nano-crystals is 2.44 times higher than that of coarse suspension [37]. In Muller's team's work, the nanocrystals were fixed in a solid PEG (polyethylene glycol) matrix, and the nanocrystals were ground into fine powder, then directly compressed into tablets or filled in capsule shells [38]. The improved oral bioavailability of poorly soluble drugs can be directly incorporated into tablets, capsules or hot-melt solid matrices by adopting this novel drug delivery system.

Intravenous delivery is considered to be the most appropriate administration route with low dosage and excellent bioavailability. However, during formulation development, the usage of toxic solvents and excipients limits the widespread application of the intra-

venous delivery route [39,40]. When nanocrystals are used for intravenous administration, it is not necessary to add any harmful excipients. Thus, nanocrystals are considered to be an ideal candidate for intravenous administration. In the current market, nanocrystals have been successfully prepared for intravenous administration such as asulacrine [41], melasrol [42], oridonin [43], itraconazole [39], diazepam [44], etc.

Ophthalmic drug delivery is a challenging task [45], especially for some insoluble drugs such as hydrocortisone prednisolone [46]. and flumerolone [47]. However, this problem can be solved by using nanocrystals for ophthalmic drug delivery. Ali et al. used microfluidic nanoprecipitation and wet milling to manufacture hydrocortisone nanocrystals. The prepared nanosuspensions can keep the sustained drug action for 9 h compared to 5 h for the drug solution. This work showed that drug nanosuspensions have potential for ophthalmic delivery [26]. By incorporating nanocrystals into an in situ gelling system composed of poloxamer and polycarbophil, an advanced ophthalmic drug delivery system for forskolin (an intraocular antihypertensive agent) has been developed. The prepared nanosuspension/hydrogel systems have better efficacy in reducing intraocular pressure (31%, 12 h) than the effect of traditional eye suspension (18%, 4–6 h) [48]. Hence, this study proved that the nanosuspension is able to control drug release and shows great potential for glaucoma therapy.

#### 2.2.2. Targeted Nanocrystals

Nowadays, many patients are suffering from cancer. It is estimated that the number of patients with cancer will reach 13.2 million in 2030 [49]. Chemotherapy is an effective way to treat cancer. However, this therapy has the deficiency of drug resistance, which limits its wide application. In order to overcome this issue, several specific cytotoxic drugs for cancer cells are constantly being developed. However, many of them have poor solubility and poor bioavailability in vivo. Nano-crystals with good solubility and bioavailability have been used as chemotherapy drugs for targeted cancer treatment. By using pressure homogenization techniques, Meghna prepared nanosuspension PIK75 using the high pressure homogenization technique. The results showed that nanosuspension PIK75 has a saturated solubility of 11-fold improvement and enhanced stability in plasma [50]. Zheng synthesized silybin nanosuspension by using the high pressure homogenization (HPH) method to treat prostate cancer [51]. Nevirapine suspensions modified by nanocrystals exhibited improved targeting ability, enhanced bioavailability and prolonged residence time of therapeutic drugs at the target site [52]. Thus, targeted nanocrystals exert good functions for the treatment of cancer, as shown in Figure 1.

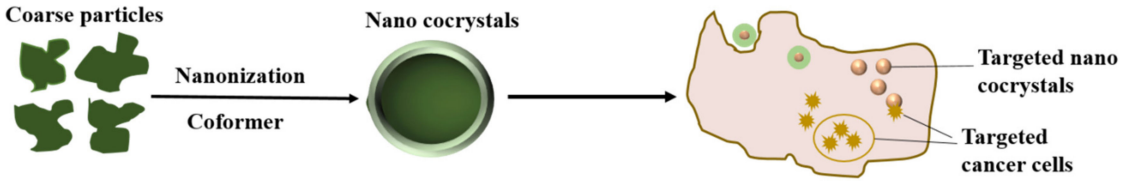


**Figure 1.** Nano cocrystals exert functions on targeting cancer cells.

## 3. Preparation Method of Pharmaceutical Nano-Cocrystals

Typically, preparation methods of pharmaceutical nano-cocrystals include top-down synthesis and bottom-up techniques. The common methods of the top-down technique are the ball milling method using shear forces to manufacture the particles with nano size, and high pressure homogenization. Precipitation is one of the bottom-up techniques involving nucleation and crystal growth processes. The synthesized approaches have been sketched in Figure 2.

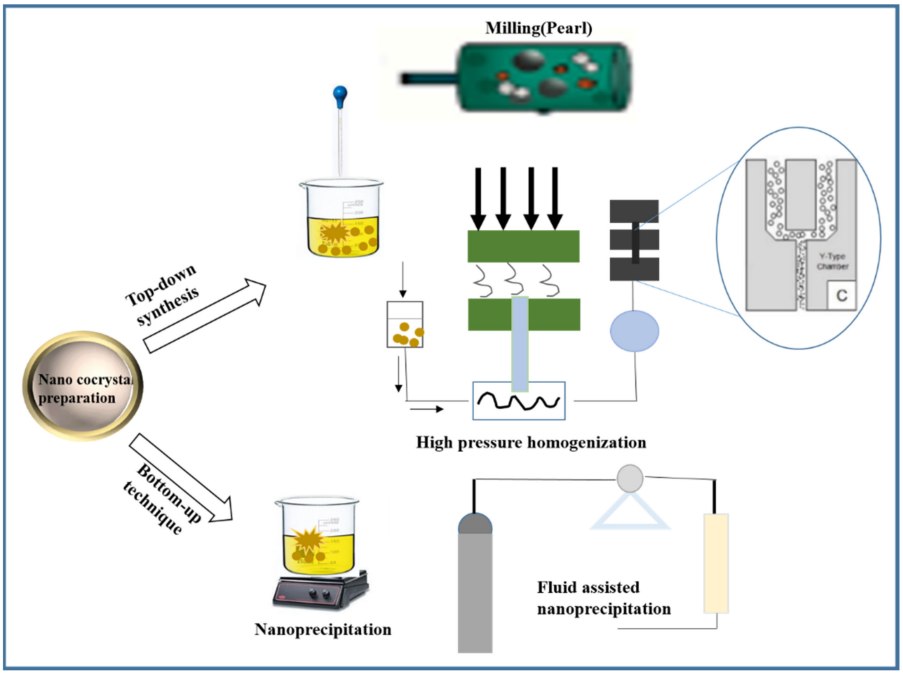


**Figure 2.** Schematic of pharmaceutical nano-cocrystals formation.

### *3.1. Top-Down Synthesis*

#### 3.1.1. High Pressure Homogenization

High pressure homogenization (HPH) is widely applied to obtain nanosized crystals, and it has potential in the preparation of pharmaceutical nanosized crystals to increase the dissolution rate and improve drug bioavailability. By using poloxamer 188 as a stabilizer, nano-cocrystals consisting of BE(Baicalein) nanocrystals and BE-NCT(Baicalein-nicotinamide) have been produced using the HPH method [12]. Using different stabilizers, praziquantel has been developed by adopting the HPH technique. After screening, two promising formulations were obtained by combining poloxamer 188 with polyvinylpyrrolidone or maltodextrin [53]. Even though the HPH method can successfully prepare nanosized crystals, this technique is still immature for wide application. High energy requirements, being time-consuming, as well as uniformed nano-particle size are the main defects of HPH.

#### 3.1.2. Milling

Solid-state milling: Solid-state grinding refers to the cocrystal solid materials mixed in an appropriate stoichiometric ratio, then crushed with a mortar, pestle, a ball mill or a vibration mill. During the preparation process, there is no need to use any solvent. Typically, the grinding time is about 30–60 min. Many cocrystals can be produced by using the solid-state milling method. Solid-state milling can reduce the particle size easily, but increase the specific surface area. Compared with the method of preparing cocrystals by dissolution, it exhibits excellent performance in increasing selectivity. In addition, it is easy to operate and results in the rapid preparation of cocrystal products. In Andrew's work, he introduced small quantities of solvent to increase solid-state cocrystallization kinetics or to provide polymorph control, which is of particular significance in the pharmaceutical field, and successfully obtained organic cocrystals by adopting the solid-state grinding approach [54]. Braga reported that the prepared cocrystals can potentially improve the solubility and thermal stability in "old" solid-state chemistry fields [55]. Jug found that solvent-free grinding is an effective method to prepare a solvent-free cyclodextrin inclusion complex [56]. Solid-state grinding is an alternative method which can successfully achieve the preparation of nano-cocrystals, but there are still some disadvantages such as formation and aggregation for particles in the micrometer range [57].

Liquid-assisted milling: This is a method to enhance the polymorphism of the crystal system by adding a small amount of solvent during the grinding process [58]. The role of the solvent is to accelerate the catalysis actions. Its advantage lies in improving the performance of the product and controlling the production of polymorphs while increasing the crystallinity of the product. This method is particularly suitable for the formation and preparation of cocrystals. For time consideration, this method can enhance the crystallization rate, and is especially appropriate for materials with poor crystallization performance in pure grinding [59]. This method is a good choice for the preparation of cocrystals with high purity. It has selectivity in the formation of polymorphic cocrystals. By adding solvents with different polarity, the crystalline polymorph form can be converted to another organic component. However, liquid-assisted grinding also has limitations, including small-scale production, high energy consumption, and poor performance in terms of product purity. For the purpose of liquid-assisted grinding, ethanol was added into a grinding container for the construction of terahertz containing a two-component cocrystal by grinding phenazine and mesaconic acid together in Nguyen's study [60]. Zhou conducted and synthesized new 1:1 cocrystals of resveratrol with 4-aminobenzamide and isoniazid by liquid-assisted grinding [61].

### *3.2. Bottom-Up Technique*

Anti-solvent precipitation: The precipitation approach is commonly used to prepare nano-cocrystals, especially under the diverse combination system of soluble and insoluble components during cocrystal preparation. Due to the different solubilities of cocrystal components in aqueous medium, the successful preparation of stable nano-cocrystals is a big challenge [62]. Thereby, the anti-solvent system combining stabilizer is selected and added to obtain stable nano-cocrystals with desired particle size. Due to the different solubilities of cocrystals in solvent and anti-solvent, the high probability of cocrystal precipitation will be seen when an anti-solvent is added to the system [63]. A lack of systematic study in the selection of anti-solvents has limited the application of this method in the medical field. Thus, the selection of anti-solvents is the key step in the preparation of nano-cocrystals. Generally speaking, to assure sufficient interaction during crystallization, the selected solvent should follow this rule: it should have solubility of both drugs and co-formers. Studies have shown that during the preparation of carbamazepine-nicotinamide nano cocrystals, when water was used as an anti-solvent, carbamazepine precipitated alone and there was no cocrystal formation because of the high solubility of nicotinamide in water. In contrast, the system containing n-hexane used as an anti-solvent can successfully prepare carbamazepine-nicotinamide nano cocrystals [64]. John demonstrated using a combination of multiple solvent selections (acetone, hexanes, chloroform) and Span-85 surfactants to prepare pharmaceutical nano cocrystals with enhanced dissolution rates and bioavailability [33].

### *3.3. Electrohydrodynamic Atomization (EHDA) and Spray Drying Method (SD)*

Due to the simplicity and potential scalability, electrohydrodynamic atomization, which is also called electrospraying, and spray drying have been widely used to manufacture nano cocrystals for medical usage. Meanwhile, it is not necessary to add any suspension stabilizers and dry products to ensure the physicochemical stability of nanoparticles for a long time [65].

EHDA is a versatile technique based on the application of electrically charged fluid, which comes from the electrospinning technology for the production of microfibers or nanofibers [66,67]. By controlling the process parameters, this method has good reproducibility and can easily operate in a continuous way. Compared to other conventional methods such as nano-precipitation technology, EHDA is a one-step method to produce nanoparticles with narrow size distribution, and it is not necessary to use any surfactants and stabilizers [68–70]. In addition, the product is collected in the form of dried powder without any residual solvent. Generally speaking, EHDA facilitates the synthesis of pure

drug particles, especially in the formation of nanocrystals, microcrystals and cocrystals [71]. Wang et al. used the electrospray method followed by annealing at high temperature to prepare nanocrystals of carbamazepine, which is a poorly soluble drug for continuous drug manufacture [72]. Electrospray is adopted to treat the solutions of various concentrations in methanol to obtain carbamazepine particles with a diameter in the range of 320 nm, and the water solubility increased by 26.4%. The average particle size increases with the flow rate, which is in good agreement with the theoretical model [73]. During the electrospray process, the rapid evaporation of solvent is beneficial for the formation of cocrystals owing to the increased rate of nucleation and growth [71]. Although EHDA can be operated at high temperature and low pressure, the process parameters can be adjusted to achieve the balance of solvent evaporation and the formation of crystalline particles. This technology is still not mature. Whether it can completely replace the high pressure homogenization technology is still a question.

Spray drying is a continuous, economical, and scalable process to produce dry powder formed by the atomization of liquid feed under an atomizer [74,75]. SD has been widely used in the food, cosmetics, and pharmaceutical fields, and it has been used to prepare pharmaceutical cocrystals as well [75–77]. Conventional spray-dryers are unable to produce drug particles smaller than 2 μm [78,79], while the Nano Spray Dryer B-90 successfully produces drug nanoparticles. The SD technique is an attractive approach to fabricate pharmaceutical cocrystals. Li's team mixed 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX, 2.4 g) and 2,4,6-trinitrotoluene (TNT, 0.6 g) and dissolved the mixture in acetone (100 mL) to form a co-solution. Then, the B-290 Mini Spray Dryer was used to successfully obtain cocrystals of HMX and TNT with high energy and low sensitivity by the spray drying method [29]. In Pawar's work, the cocrystal product of sfavirenz-glutaric acid showed improved solubility and dissolution rate, leading to faster absorption and better bioavailability of the active drug by the spray drying technique [80]. Nano-cocrystals of caffeine/oxalic acid (2:1), caffeine/glutaric acid (1:1), TNT/CL-20 (1:1), and HMX/Cl-20 (1:2) were prepared by adopting the spray flash evaporation process [34]. Compared with liquid formulations, the solid products obtained by spray drying technology are more physically and chemically stable because they have little agglomeration, degradation and other phenomena related to the existence of solvents [75]. However, systematic research about controlling the crystalline/amorphous structure of desired products, as well as their size, size distribution, and morphology, is a big challenge when using the spray drying method to prepare nano-cocrystals.

## 4. Advanced Characterization Techniques

### *4.1. X-ray Diffraction (PXRD, SCXRD)*

PXRD, SCXRD are very important means to identify the crystal structure. X-ray analysis technology has been widely used in the analysis of the crystal structure of APIs [81,82]. Nugrahani et al. prepared the ionic cocrystal of monohydrate and tetrahydrate of diclofenac sodium-L-proline [83]. The hydrate form was characterized by single crystal X-ray diffraction, and the results showed that the solubility of the hydrate was higher than that of the cocrystal consisting of sodium salt of diclofenac and the anhydrous diclofenac-L-proline. During the drying process, the release of water led to the dissociation of cocrystals into a physical mixture of diclofenac and L-proline. Buol et al. described the cocrystallization of the nootropic drug and nefiracetam for the first time. The large cocrystal screening of 133 cocrystals prepared by liquid-assisted grinding was operated and 13 new cocrystals were identified though single crystal X-ray diffraction characterization [84]. Wroblewska et al. introduced a new co-former of 1-hydroxy-4,5-dimethylimidazole 3 oxide. They used high-resolution solid-state NMR to study the formation of cocrystals of barbituric acid and thiobarbituric acid during ball milling. These technologies of 13C CP/MAS, 15N CP/MAS and 1H Very Fast MAS NMR combined with single crystal X-ray diffraction to study the structure of new co-formers and cocrystals. The effects of the polymorphic and tautomeric forms of barbituric acid/thiobarbituric acid on cocrystallization were evaluated.

At a concentration of up to 100 μM, the cocrystal is not cytotoxic in HeLa and 293T cells, indicating that 1-hydroxy-4,5-dimethyl-imidazole 3 oxide has good biocompatibility [85].

### *4.2. Thermal Analysis*

The main applications of DSC (Differential scanning calorimetry) include phase transition definitions, melting point, glass transition, Curie point, crystallinity determination, kinetic studies, drug transformation, physical stability test, and purity control [86]. DSC is the most common method of thermal analysis, mainly because it is fast, simple, and easy to operate [87]. The DSC test is a powerful tool for screening polymorphisms and studying the stability of relative polymorphisms. Thermogravimetric analysis (TGA) can measure the quality of the sample during heating and detect the structure of a solvent or hydrate. Rohani studied the DSC of cocrystals of theophylline and nicotinamide prepared at different grinding times because theophylline (271.4 °C) and nicotinamide (128.2 °C) have different melting points. Nicotinamide melted first, then new crystals nucleated and epitaxially grew at higher temperature. When the temperature is further increased, TP-NCT (theophylline−nicotinamide) crystals completely melted [88].

### *4.3. Molecular Vibration Spectroscopy (IR, Raman)*

Molecular vibration spectroscopy is a branch of molecular spectroscopy, which can be divided into infrared (IR) and Raman spectroscopy. For different crystals, the bond length and bond angle are different, and the vibration and rotation energy are also different. Thus, vibration spectroscopy can be used to distinguish different crystals. For different crystals, the IR spectrum exhibits differences in the frequency of band adsorption, peak shape, peak position and peak intensity [89]. Common sample preparation methods for IR include KBr pellets, the shake-flask method, the film method, the liquid membrane method, etc. At present, the KBr pellets method is the most commonly used method for analyzing drug/food crystals. IR spectroscopy is a simple and rapid way to distinguish crystal forms. In Guo's study, rod shaped nanocrystals and spherical-like nanocrystals were prepared by sonoprecipitation and bead milling. Then, they used IR combined with other characterizations to study the chemical structure of lovastatin, and they found there is no structure change during the preparation process [90]. Wijayasinghe et al. [91]. used DSC and FT-IR spectroscopy to determine the crystalline properties of freeze-dried lactose with or without lactic acid. The FT-IR spectrum showed that a hydration layer composed of lactic acid and $H_3O^+$ ions was distributed into the surface of the lactose molecules through strong H bonds, resulting in a change in the structure of lactose, creating unfavorable conditions for lactose crystallization. This work suggested that the partial or complete removal of lactic acid from acid whey may improve the crystallization ability of lactose and promote the development of acid whey.

Raman spectroscopy is an analytical method in low frequency mode based on Raman scattering to observe the vibration, rotation and other vibrations of materials [92]. For some non-polar groups, there is no obvious absorption peak in IR, while the absorption peak can be observed in Raman spectroscopy. The measured sample does not require special treatment, so the crystal structure of the sample will not change during the sample preparation process, which is conducive to crystal structure analysis. Chen introduced a new method for continuous polymer coating of drug crystals based on solid hollow fiber cooling and crystallization. Through the analysis of Raman, DSC and XRD characterizations, the results showed that uniform coating and free-flowing drug/product can be obtained without the loss of pharmacological properties and controlled release characteristics [93].

### *4.4. Microscopy Techniques (TEM, AFM)*

The direct observation of crystals through a microscope is a key analytical method for studying crystals in drugs and food. Thus far, five microscope techniques have been applied in this field: polarizing microscope (PLM), hot stage microscope (HSM), atomic force microscope (AFM), transmission electron microscope (TEM), and scanning tunneling

microscope (STM). Compared to PXRD characterization, AFM and TEM are more sensitive techniques which can be successfully used to test materials with nanometer scale. In the pharmaceutical and food fields, AFM and TEM have been widely applied for the analysis of crystals owing to their high sensitivity. Ricarte et al. [94] used TEM to study the crystalline of the solid dispersion of griseofulvin/hydroxypropyl methyl cellulose acetate succinate. TEM results showed real space images and electron diffraction patterns, and identified the griseofulvin crystals in the spray-dried solid dispersion. This work proved that TEM is a promising technique for characterizing crystalline in solid dispersions—even a small degree of crystallization. Hübner et al. [95] used atomic force microscopy (AFM) to study the size and structure of n-CL-20/HMX nano cocrystals. This work demonstrated that AFM is an effective method for analyzing crystal morphology and studying the interaction mechanism between nanocrystals and other substances.

### *4.5. Solid-State NMR Spectroscopy*

Solid-state NMR spectroscopy can be used to analyze the dynamics behavior and chemical environment of atoms in crystals. Therefore, solid-state NMR spectroscopy is an important tool to analyze and determine the crystal forms. In Pinon's work, [96]. solid-state NMR spectroscopy enhanced by dynamic nuclear polarization was used to study the polymorphs and solvates of organic solids. The NMR technique was applied to analyze the effects of three polymorphs and one hydrated form on samples of theophylline, a drug for curing asthma. Solid-state NMR spectroscopy can provide useful information in the study of drug/food crystal forms. Common characterizations and analytical techniques used to test pharmaceutical nano-cocrystals will be listed in Table 2 along with their advantages.

**Table 2.** Commonly used characterizations and analytical techniques to test the chemical and physical properties of pharmaceutical nano-cocrystals.

| Characterization Techniques | Analysis Object | Advantages |
| --- | --- | --- |
| SCXRD | Analysis of internal structure of crystals, such as unit cell dimensions, bond lengths, angles, and details of crystal packing); analysis of the charge density; chirality test of molecules; real-time monitoring | Maintain the pristine structure of samples; easy sample preparation; rapid test technique. |
| PXRD | Analysis of phase purity and crystallinity | High sensitivity; easy sample preparation; rapid test technique; maintain the pristine structure of samples. |
| DSC,TGA | Test solid state properties | Simple and fast; has excellent accuracy; easy to operate; wide range of temperatures; real-time process monitoring. |
| IR, Raman | Test the properties of solid state | Easy to operate; provide information on chemical structure and interactions. |
| SEM, TEM,AFM | Characterization of particle shape, morphology, as well as the particle size | Easy to operate; provide available information on shape and morphology of samples, even in a nanometer scale. |
| NMR | Solid state properties | Important method for analysis and determination of crystal forms in multiply crystals system; analysis of the dynamics behavior and chemical environment of atom. |

Note: SCXRD—Single Crystal X-ray Diffraction; PXRD—Powder X-ray Diffraction; DSC—Differential Scanning Calorimetry; TGA—Thermal Gravimetric Analysis; SEM—Scanning Electron Microscope; TEM—Transmission Electron Microscope; AFM—Atomic Force Microscopy.

## 5. Conclusions and Prospect

Pharmaceutical nano-cocrystals have been used as a novel approach to improve the solubility, stability and bioavailability of drugs. Pharmaceutical nano-cocrystals will be the focus of drug discovery research in the future. Currently, few studies have been re-

ported on pharmaceutical nano-cocrystals, especially a lack of systematic studies on the preparation and characterization of pharmaceutical nano-cocrystals. This review comprehensively introduced common preparation methods of nano-cocrystals, and discussed the characterization technologies used to analyze the nano-cocrystals in detail. Although the conventional technology for preparing nano-cocrystals by high pressure homogenization is mature, it needs to be operated under high pressure conditions, which results in a significant increase in energy consumption. As it is not necessary to add any stabilizers and desiccants, electrohydrodynamic atomization and the spray drying method have great potential for preparing nano-cocrystals. Due to the restriction of the crystal size imposed in pharmaceutical nano-cocrystals, the crystal structure can only be investigated via PXRD or ED since the typical dimension of single crystal for SCRD is rather large. However, direct structure determination could remain a difficult task; in this case, spectroscopic and thermal analyses may diagnose the formation of intermolecular interaction. For pharmaceutical nanocrystals, the solid-state characterizations (spectroscopy and thermal analysis) were not possible to directly determine the crystal structure of these organic nanocrystals because of their small size. More analytical works should be studied for nano-cocrystals, since we need to confirm the formation and interaction of nano-cocrystals' structure.

At present, several research directions in the formulation design of pharmaceutical nano-cocrystals have been reported here. We are confident in predicting that pharmaceutical nano-cocrystals will become more popular for a broader commercial market in the pharmaceutical industry in the future.

1. Structural analysis. Study of the molecular conformation and functional groups of drug molecules can provide assistance in the design and preparation of specific cocrystals, which has always been a huge challenge due to the complexity of the chemical structure of APIs.
2. Selection of co-formers. When preparing the cocrystals, the selection of co-formers is an important step. Usually, medicinal excipients, common salt-forming ligands, food additives, and other active pharmaceutical ingredients are the preferred co-formers.
3. Development of advanced analytical techniques. Analytical technique is an effective means to detect the internal structure of drug molecules. Appropriate characterization may provide practical guidelines for the preparation of new nano-cocrystals, as well as aiding in studying the interaction mechanism of nano-cocrystals.

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