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REVIEW

Nanosuspension-Based Drug Delivery Systems for Topical Applications

Mohamed Mahmud E Aldeeb[1,2,*], Gofarana Wilar[3,*], Cecep Suhandi[1,*], Khaled M Elamin[4,*], Nasrul Wathoni[1,*]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, 45363, Indonesia; [2]Department of Pharmaceutics, Faculty of Pharmacy, Elmergib University, Alkhoms, 40414, Libya; [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, 45363, Indonesia; [4]Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, 862-0973, Japan

*These authors contributed equally to this work

Correspondence: Nasrul Wathoni, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, 45363, Indonesia, Tel +62-22-842-888-888, Email nasrul@unpad.ac.id

Abstract: Nanosuspensions have garnered recent attention as a promising strategy for mitigating the bioavailability challenges of hydrophobic drugs, particularly those characterized by poor solubility in both aqueous and organic environments. Addressing solubility issues associated with poorly water-soluble drugs has largely resolved the need to enhance drug absorption and bioavailability. As mucosal formulations and topical administration progress in the future, nanosuspension drug delivery, straightforward formulation techniques, and versatile applications will continue to be subjects of interest. Nanosuspensions have undergone extensive scrutiny in preparation for topical applications, encompassing ocular, pulmonary, and dermal usage. Among the numerous methods aimed at improving cutaneous application, nanocrystals represent a relatively recent yet profoundly intriguing approach. Despite the increasing availability of various nanosuspension products, primarily designed for oral administration, only a limited number of studies have explored skin permeability and drug accumulation in the context of nanosuspensions. Nevertheless, the scant published research unequivocally underscores the potential of this approach for enhancing cutaneous bioavailability, particularly for active ingredients with low to medium solubility. Nanocrystals exhibit increased skin adhesiveness in addition to heightened saturation solubility and dissolution rate, thereby augmenting cutaneous distribution. The article provides a comprehensive overview of nanosuspensions for topical application. The methodology employed is robust, with a well-defined experimental design; however, the limited sample size raises concerns about the generalizability of the findings. While the results demonstrate promising outcomes in terms of enhanced drug delivery, the discussion falls short of addressing certain limitations. Additionally, the references largely focus on recent studies, but a more diverse inclusion of historical perspectives could offer a more holistic view of the subject.

Keywords: nanosuspension, nanotechnology, topical, dermal

Introduction

Over the past two decades, there has been a notable emergence of cutting-edge advancements in the realm of pharmaceutical research and development. The automation of the drug discovery process, facilitated by technologies such as high-throughput screening, combinatorial chemistry, and computer-aided drug design, has resulted in the generation of a substantial array of highly effective drug candidates.[1] Regrettably, a considerable proportion of these promising medications grapple with the challenge of low water solubility, with approximately 40% of drugs in the developmental pipeline encountering solubility issues.[2,3] High-throughput screening techniques have contributed to the identification of an increasing number of drugs exhibiting limited water solubility.

The constrained solubility of these compounds poses a significant impediment during the initial phases of screening for pharmacological activity, as well as throughout the formulation development and clinical testing processes.[4] Preparing one of these substances for preclinical investigations and pharmacological activity assessments is

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a prerequisite well in advance of their commercialization.[5] Consequently, it becomes evident that the adoption of innovative technical strategies is imperative to augment the bioavailability of drugs characterized by low solubility. The predominant challenge faced by the pharmaceutical industry resides in the development of novel formulation procedures and drug delivery technologies tailored to effectively address the solubility limitations associated with therapeutic candidates. These concerns frequently intersect with issues pertaining to low oral bioavailability.[6–8] To attain optimal bioavailability for these medications, it is imperative to ensure their rapid absorption post-oral administration. The intravenous route represents an additional viable means of administration.[9]

Furthermore, various formulation strategies have been devised to address the challenges posed by poorly soluble medications, often referred to as "particular strategies".[10] The effectiveness of these approaches hinges on the specific chemical characteristics exhibited by molecules, encompassing factors such as their solubility in different organic solvents and distinctive attributes related to molecular size or configuration, such as molecules designed for incorporation into cyclodextrin ring structures.[2,11] Undoubtedly, a more rational approach would involve the implementation of a "universal formulation approach" that can be applied to a wide range of molecules.

Micronization, the process of reducing the size of drug powders within the range of 1 to 10 μm, is a widely employed technique in pharmaceutical formulations aimed at augmenting the oral bioavailability of drugs. The formulation process discussed here is commonly employed with the objective of enhancing the oral bioavailability of pharmaceutical compounds.[12,13] The insufficient solubility of frequently used medicines often restricts the effectiveness of micronization. Addressing the unresolved issues related to the bioavailability of drugs categorized under the biopharmaceutical specification class II, which are characterized by limited solubility, necessitates more than solely increasing the surface area to enhance dissolution rates.[14]

Building upon the concept of micronization, nanonization has emerged as a subsequent development. Since the 1990s, the field of nanosystems has advocated for the utilization of nanocrystals, rather than microcrystals, to improve the oral bioavailability of pharmaceuticals. Additionally, nanocrystals that can be dispersed in water, known as nanosuspensions, have found application in intravenous administration and pulmonary delivery of medications.[15]

Over the past two decades, drug nanocrystal technology has emerged as a prominent advancement in the pharmaceutical industry. One of the key advantages of developing drugs with low solubility lies in the technique it employs, which results in the creation of "nanosuspensions". These nanosuspensions are essentially the dispersion of drug nanocrystals within a liquid medium, typically water.[16–21] Nanosuspensions primarily comprise drug nanoparticles, typically ranging in size from 100 to 1000 nm. To maintain stability, these nanoparticles are supported by a small quantity of surface-active compounds.[3,22]

The skin serves as a crucial site for the painless and non-invasive administration of therapeutic substances, allowing for the control of their release and circumventing first-pass metabolism. Upon dermal absorption, medications can exert their effects locally, regionally, or systemically at various target sites.[23,24] These considerations have led to the perception that drug delivery through the skin is a compelling yet challenging research area. The principal obstacle is overcoming the skin's remarkable impermeability. The skin is a complex tissue that shields the body from invading pathogens, withstands chemical and physical assaults, and regulates essential functions such as temperature regulation.[25] Its various anatomically distinct layers consist of the stratum corneum (SC), the accessible epidermis, and the dermis. The primary physical barrier is situated in the SC, composed of protein-rich dead cells (corneocytes with cornified cytoskeletal components and corneodesmosomes) and lipid-rich structures (lamellar sheets comprising roughly equimolar amounts of free fatty acids, cholesterol, and long-chain ceramides).[5,26] The nucleated epidermis also contributes to the barrier through desmosomes, cytoskeletal components, tight junctions, and adherens junctions. Hence, the overall design of the skin, rather than a specific element, determines its efficacy as a protective barrier. The skin's appendages, including sweat glands, pilosebaceous units, and hair follicles, emanate from the dermis or subcutaneous fat tissue, introducing significant discontinuities within this tight framework.[27,28]

Drugs administered to the skin's surface access the skin through two pathways: the trans-appendageal and transepidermal routes, facilitated by passive diffusion. The skin's appendages provide an alternative route for delivering medications into the skin, potentially making drug delivery through the stratum corneum (SC) less challenging.[29] While this route was previously considered of minimal significance due to its relatively small area, current research

on follicular penetration has shed light on the potential importance of hair follicle pathways in the skin penetration process and the reservoir for topically administered chemicals. Nanosuspensions are colloidal dispersions of nanosized drug particles in an aqueous vehicle. When applied to dermal surfaces, nanosuspensions offer several potential mechanisms for improved drug delivery:

1-Increased Surface Area: The reduced particle size in nanosuspensions provides a larger surface area for drug contact with the skin, facilitating enhanced absorption.

2-Improved Penetration: Nanoparticles can penetrate the stratum corneum (the outermost layer of the skin) more effectively than larger particles. This improved penetration may lead to increased bioavailability of the drug.

3-Targeting Specific Skin Layers: Nanosuspensions can be designed to target specific layers of the skin or even specific cells, allowing for more controlled and targeted drug delivery.

4-Facilitated Drug Solubility: Nanosuspensions can enhance the solubility of poorly water-soluble drugs, potentially leading to better absorption through the skin.

5-Interaction with Skin Appendages: Nanoparticles may interact with hair follicles, sweat glands, or other skin appendages, facilitating localized drug delivery.[30,31] As illustrated in Figure 1. Nanosuspensions have found diverse applications in topical formulations, offering advantages such as improved bioavailability and enhanced drug penetration. While limitations exist, ongoing research and technological advancements continue to address and overcome these challenges, making nanosuspensions increasingly promising for topical drug delivery. Nanosuspensions in topical drug delivery aim to address challenges associated with drug solubility, penetration, stability, and controlled release. Various methods are employed to achieve nanosized particles, each offering specific advantages depending on the characteristics of the drug and the desired application.

Furthermore, several dermatological conditions, such as acne, alopecia, and various skin tumors, are closely associated with the sebaceous glands and hair follicles. A molecule can traverse the epidermis through one of two pathways: transcellularly, by passing through the corneocytes, or intercellularly, by moving through the lipid domains that separate the corneocytes.[4,32] Although it is generally accepted that the intercellular route serves as the primary

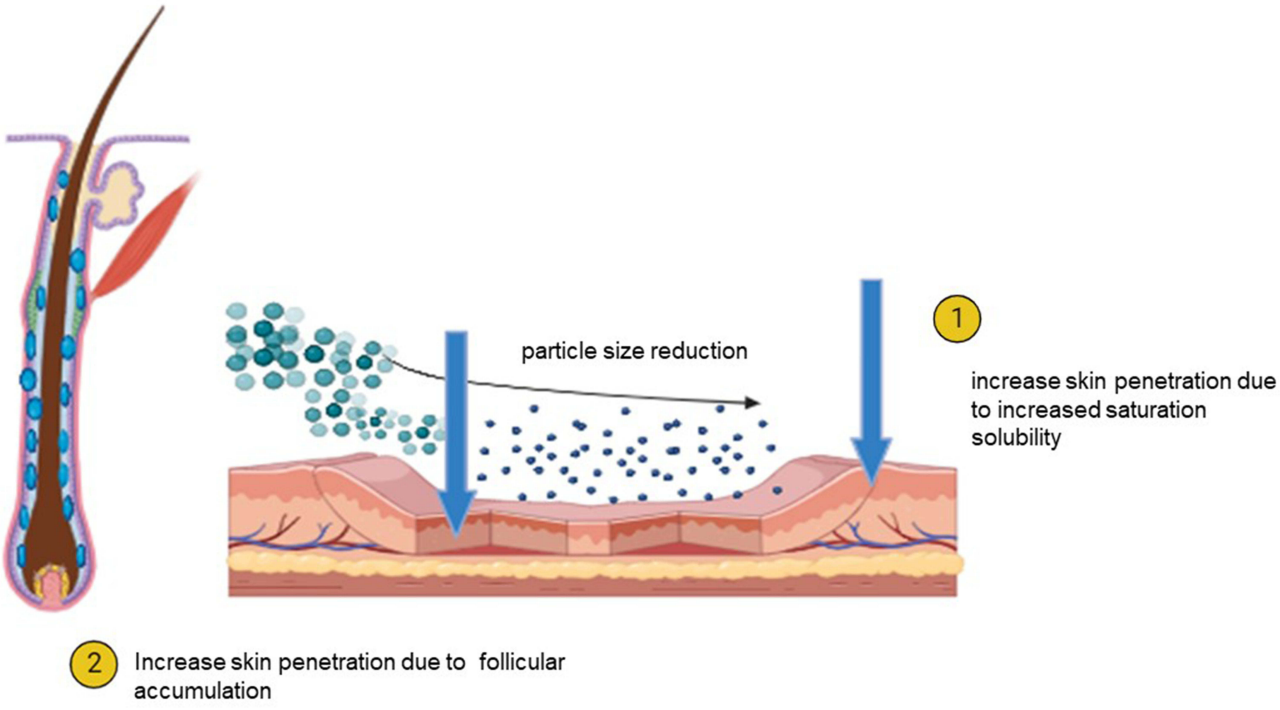

Figure 1 Schematic representation of proposed mechanism of increased dermal penetration of drug nanosuspension. Created with BioRender.com.

pathway for drug penetration, this route is significantly restricted by the formidable barrier created by the outermost SC layer.[33,34.]

The current investigation involved a literature review where articles were gathered through searches on Google Scholar, PubMed, and Scopus, utilizing keywords such as nanosuspension, topical application, dermal, and drug delivery system. The selection of libraries was based on specific inclusion and exclusion criteria. Inclusion criteria encompassed literature providing insights into the use of nanosuspension-based drug delivery systems for topical applications, published between the years 2000 and 2023. Exclusion criteria involved review papers, and publications without full-text availability.

Preparation Methods for Nanosuspension

In the pharmaceutical field, researchers have devised a variety of techniques for the formulation of nanosuspensions. These methods can be broadly categorized into three main classifications: bottom-up technology, top-down technology, and a hybrid approach that integrates elements from both.[35] Furthermore, advanced research efforts have yielded successful development of various preparation strategies, including supercritical fluid technology, an emulsification-solvent evaporation approach, and a melt emulsification approach.[19,31] As illustrated in Figure 2, specific methods for preparing topical nanosuspensions for topical application can involve individual approaches or a combination thereof, such as the "smart crystal" technique, which combines high-pressure homogenization (HPH) and wet milling. Additionally, nanosuspensions can be prepared using a combination of two methods: anti-solvent preparation and HPH.

High-Pressure Homogenization Method

The cavitation process is executed through the high-pressure homogenization (HPH) method, where a solution is expelled from cavities or fissures and then applied to disperse drug crystals, relying on the application of substantial shear forces.[36] Microfluidization and piston-gap homogenization represent two homogenization concepts frequently employed in conjunction with specific homogenizers that adhere to these principles.[5,19]

According to the jet-stream theory of microfluidization, the coarse suspension accelerates within the homogenizing chamber due to high-speed forces like collision, shear, and cavitation, resulting in a reduction in particle size. This process involves two types of chambers: "Z" and "Y". In "Z"-type chambers, the suspension repeatedly changes

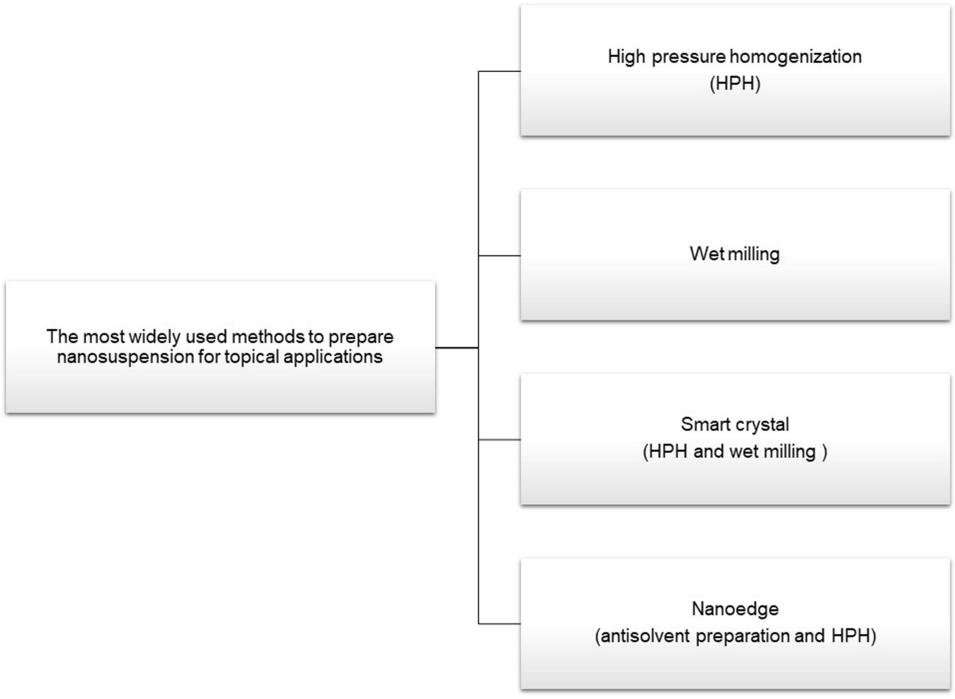

Figure 2 Main methods of nanosuspension preparation for topical applications.

direction, leading to particle collisions and shear forces. In "Y"-type chambers, the suspension flow is divided into two streams, colliding head-on.[13,22,37]

In the second type of homogenizer, referred to as piston gap homogenization, the coarse suspension rapidly passes through a very narrow gap. All these procedures employ pressures ranging from 350 MPa to 500 bar. Smaller particle nanosuspensions (NSs) can usually be produced by increasing the pressure and the number of cycles.[26] It involves initially agitating a freshly prepared coarse suspension containing the active pharmaceutical ingredient (API) and a stabilizer for a specific duration. Subsequently, this mixture is passed through a narrow gap under high pressure, with various processing parameters influencing the particle size alteration, ultimately resulting in the formation of a nanosuspension.

Media Milling Nanocrystals

Pearl ball mills or high-shear media mills are employed for the production of nanosuspensions. The mill consists of a recirculation chamber, a milling chamber, and a milling shaft. The drug is then directed through the mill, which contains small grinding balls or pearls, within an aqueous solution.[38,39] These balls move through the interior of the grinding jar, striking the sample on the opposite grinding jar wall as they rotate at a very high shear rate, while the temperature is carefully controlled. A substantial reduction in particle size is achieved through the combined effects of friction and impact.[12,20,40] The milling medium or balls are crafted from materials such as strongly cross-linked polystyrene resin with excellent abrasion resistance, zirconium oxide, or aluminum oxide sintered in ceramic. A piece of equipment suitable for achieving a particle size below 0.1 μm is a planetary ball mill (PM100 and PM200; Retsch GmbH and Co., KG, Haan, Germany). Wet milling was utilized to produce a nanosuspension of Zn-Insulin with an average particle size of 150 nm.[10,41] The initially prepared coarse suspension of API and stabilizer is recirculated through the grinding chamber until the desired particle size range is attained.

Smart Crystal Method

The smart crystal method focuses on a specific nanosuspension formulation designed for dermal applications. The description highlights findings from this formulation as an example, emphasizing that this approach is unique and not representative of other methods used in the study or field. In essence, the summary underscores the specificity of the smart crystal method in the context of nanosuspension formulations for dermal use. This method combines low-energy pearl milling and high-pressure homogenization, as exemplified in the creation of rutin nanocrystals. When compared to a 5% solution of a water-soluble rutin-glycoside derivative, a nanosuspension containing 5% rutin in the form of non-dissolved nanocrystals exhibited 500-fold greater antioxidant activity when applied to the skin of human volunteers. This increased activity can be attributed to the higher solubility of active rutin as a nanocrystal, leading to a greater concentration gradient between the dermal formulation and the skin.[1,29,42] Consequently, this phenomenon indirectly enhances the drug's absorption rate.

Rutin that has already permeated the skin is swiftly replaced by molecules from the rapidly dissolving nanocrystals. Furthermore, the original lipophilic rutin molecule is believed to penetrate the skin more effectively than the hydrophilic rutin-glycoside derivative due to its higher affinity for the target sites.[43–46]

Nanoedge Method

Nanoedge technology operates on the principle of combining precipitation and homogenization. In this method, the drug is initially dissolved in an organic solvent. Subsequently, this solution is blended with a miscible anti-solvent for precipitation. The drug undergoes precipitation due to its limited solubility in the water-solvent mixture. This precipitation process is accompanied by high-shear processing, achieved through a combination of rapid precipitation and high-pressure homogenization.

Factors Affecting the Stability and Efficacy of Nanosuspension

Stabilizers

The primary role of a stabilizer is to ensure the thorough wetting of drug particles in order to prevent Ostwald's ripening and the agglomeration of nanosuspensions, thereby promoting the physical stability of the formulation. This is achieved by serving as a steric or ionic hindrance.[7,13,22] The type and quantity of stabilizer employed have a substantial impact on both the physical stability and in vivo behavior of the nanosuspension. Various stabilizers, including lecithins, povidones, cellulosics, polysorbates, and poloxamers, have been used in nanosuspension development thus far. In the quest for creating a nanosuspension suitable for parenteral administration and capable of withstanding autoclaving, lecithin has emerged as the preferred stabilizer.[16,47]

Organic Solvents

When emulsions or microemulsions are utilized as templates, organic solvents become necessary in the nanosuspension formulation. In such cases, it is advisable to opt for pharmaceutically acceptable, less hazardous, water-miscible solvents like methanol, ethanol, chloroform, and isopropanol. Partially water-miscible solvents such as ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate, and benzyl alcohol are also preferred over conventional hazardous solvents like dichloromethane in the formulation process.[31,48]

Co-Surfactants

When developing nanosuspensions with the aid of microemulsions, the selection of a suitable co-surfactant becomes a critical consideration. This is because the choice of co-surfactants can significantly affect the internal phase uptake and drug loading within a specific microemulsion composition, ultimately impacting the phase behavior. Although literature references often mention bile salts and dipotassium glycyrrhizinate as potential co-surfactants, other solubilizers, such as transcutol, glycofurol, ethanol, and isopropanol, can be employed in the formulation of microemulsions without introducing any undue risks.[19,28]

Other Additives

Nanosuspensions can incorporate various additives, which encompass buffers, salts, polyols, osmotic agents, and cryoprotectants. These aforementioned additives fulfill multiple roles aimed at enhancing the stability and efficacy of the nanosuspension.

Buffers assume a crucial role in the maintenance of precise pH levels, while salts contribute to system stability by providing ionic strength. Polyols, on the other hand, act as stabilizers, preventing particle aggregation. Osmotic agents are tasked with regulating the solution's osmolarity to ensure compatibility with cellular structures. Finally, cryoprotectants are employed to safeguard the nanosuspension during freezing and thawing procedures.[48,49]

Post-Production Processing

Post-production processing becomes essential in nanosuspensions when the drug candidate is highly susceptible to hydrolytic cleavage or chemical degradation. Additionally, processing may be warranted if even the most effective stabilizer fails to maintain the nanosuspension's stability over an extended period, or if certain routes of administration pose limitations.[6,17,40]

Given these considerations, techniques such as lyophilization or spray drying can be employed to generate a dry powder comprising nanoscale drug particles. When opting for either of these unit operations, a judicious decision must be made, taking into account the drug's characteristics and cost-effectiveness. In general, spray drying proves to be a more cost-effective and practical choice compared to lyophilization.[24,30]

Parameters Evaluated Reflecting Characteristics of Nanosuspension

Color, Odor, and Taste

Prior to commencing the formulation process of oral dosage forms, it is imperative to account for particular considerations. Particle size, crystal structure, and subsequent alterations in particle dissolution can all potentially lead to flavor

discrepancies, particularly in the case of active ingredients. Changes in taste, odor, and color may also serve as indicators of chemical instability.[7,22]

Particle Size and Its Distribution

The particle size distribution plays a significant role in determining the physiochemical characteristics of the formulation, encompassing factors such as saturation solubility, dissolution rate, and physical stability. To assess the particle size distribution, several methods are available, including photon correlation spectroscopy (PCS), laser diffraction (LD), and the Coulter Counter Multisizer.[40,48,49]

PCS boasts a measurement range spanning from 3 nm to 3 µm, while the LD method covers a range of 0.05 to 80 µm. It's worth noting that the LD approach yields a relative size distribution, whereas the Coulter Counter Multisizer provides an accurate count of individual particles. Given that the smallest capillaries have diameters of 5–6 µm, it's essential that particles intended for intravenous (IV) therapy are smaller than 5 µm, as larger particles could potentially cause capillary blockages and embolisms.[17,50]

Zeta Potential

The zeta potential serves as an indicator of suspension stability. In cases where stability relies solely on electrostatic repulsion, a zeta potential of ±30 mV is requisite for maintaining stability. However, when a combination of electrostatic and steric stabilization is employed, a zeta potential of ±20 mV would suffice.[4,16,18,32]

Crystal Morphology

Methods such as X-ray diffraction analysis in conjunction with either differential scanning calorimetry or differential thermal analysis can be employed to elucidate the polymorphic transformations induced by high-pressure homogenization in the crystal structure of the drug.[7,33] As a result of the high-pressure homogenization process, nanosuspensions have the potential to undergo modifications in their crystalline structure, potentially transitioning into an amorphous state or adopting alternative polymorphic patterns.[8,20,21]

Solubility and Dissolution Rate

Nanosuspensions offer a significant advantage over other methods by simultaneously enhancing both dissolution rate and saturation solubility. Distinct physiological solutions must be employed to determine these two crucial parameters.[30,35] Saturation solubility and dissolution rate assessments are valuable for predicting the in vitro behavior of the composition. The reduction in particle size leads to an increase in dissolution rate, resulting in elevated dissolution pressure.[37,51]

Density

A critical parameter to consider is the specific gravity or density of the formulation. A decrease in density often indicates the presence of entrapped air within the formulation's structure, and its over time. To assess density accurately at a specific temperature, it is advisable to employ a well-mixed and uniform formulation and utilize precision hydrometers for these measurements.[2,17,52]

pH Value

To mitigate "pH drift" and the coating of electrode surfaces with suspended particles according to (Shrestha et al, 2014), it is essential to measure the pH of an aqueous formulation at a specific temperature, and this measurement should only be conducted once equilibrium has been achieved following settling. It is crucial not to introduce electrolytes into the external phase of the formulation to maintain pH stability.[15,22]

Droplet Size

The droplet size distribution of microemulsion vesicles and nanosuspension are both colloidal systems used in pharmaceutical and other industries for drug delivery and formulation, can be assessed using either the light scattering method or

electron microscopy. For dynamic light scattering, a spectrophotometer is employed, which utilizes a neon laser with a wavelength of 632 nm.[39,52]

Viscosity

The viscosity of lipid-based formulations with different compositions can be assessed at various shear rates and temperatures using a Brookfield-type rotating viscometer. However, not all nanosuspensions are lipid-based. Nanosuspensions can be formulated using various types of stabilizers and carriers, including polymers, surfactants, and other non-lipid-based materials. Lipid-based nanosuspensions are just one type of formulation used to create stable and effective nanosized drug particles. For this measurement, it is necessary to immerse the samples in a thermal bath that maintains the sample chamber of the instrument at 37°C.[16,23]

Physical Stability

The primary role of the stabilizer is to ensure comprehensive coverage of the drug particles, thereby preventing Ostwald ripening and agglomeration in the nanosuspension and ultimately offering a physically stable formulation by serving as either a steric or ionic barrier[1,51] Stabilizers such as cellulosics, poloxamers, polysorbates, lecithin, polyoleates, and povidones are frequently utilized in nanosuspensions. When developing parenteral nanosuspensions, lecithin may be a suitable choice.[6,29]

Biological Performance

Irrespective of the chosen route and method of administration, establishing an in vitro/in vivo correlation and closely monitoring the drug's in vivo performance are fundamental components of certain drug delivery systems. However, they may not be as applicable or straightforward for intravenous formulations due to the rapid and direct entry of the drug into the systemic circulation, bypassing many of the factors that make IVIVC relevant for oral formulations. This holds especially true for intravenously administered drugs, given the significant implications for their in vivo behavior. The drug's performance in vivo is heavily reliant on organ distribution, which, in turn, is contingent upon its surface characteristics, including surface hydrophobicity and interactions with plasma proteins. To address these considerations, nanosuspensions are utilized.[10,18] It is well-established that the quality and quantity of protein absorbed following intravenous injection of nanoparticles is an interaction of nanoparticles with proteins in the bloodstream, play a pivotal role in determining their ultimate destination within the body. Consequently, it becomes essential to apply appropriate methodologies to evaluate surface characteristics and protein interactions in order to gain insights into in vivo behavior. Techniques such as hydrophobic interaction chromatography can be employed to measure surface hydrophobicity, and the quantitative and qualitative assessment of protein adsorption following the intravenous injection of drug nanosuspensions in animals can be conducted using methods like 2-D PAGE.[4,17,52]

Topical Applications of Nanosuspensions

Nanosuspensions have been widely studied in preparation of topical applications, either for ocular, pulmonary, or dermal uses, as summarized below:

Nanosuspension for Ocular Drug Delivery

Topical ocular medication delivery is the most widely adopted method for addressing both external and internal ocular conditions.[53–55] The choice of approach depends on whether drugs are required to be retained at the cornea and/or conjunctiva (eg, for conditions such as conjunctivitis, blepharitis, or keratitis sicca) or whether they need to traverse these barriers to access the internal eye tissues (eg, for conditions like glaucoma or uveitis), based on the specific target sites for various ocular diseases (Table 1).

Despite the numerous biological processes and physical barriers within the eye, it is well-documented that the ocular bioavailability of administered drugs is low, at approximately 5%.[60–69] This is due to the constant drainage of fluids, the blink reflex, and the activity of metabolic enzymes, all of which can rapidly degrade drug molecules. Consequently, after application, the drug remains in the precorneal region for only a brief duration. For drugs intended to reach the inner eye,

Table 1 Examples of Ocular Drugs Nanosuspension with Their Methods of Preparation

Preparation Method	Drug	Pharmacological Uses	Type of Study	Results	References
Wet milling	Brinzolamide	Ocular hypertension	In vivo	The nanosuspensions (NSs) exhibited homogeneity and stability. In vitro, they rapidly dissolved and led to a substantial reduction in intraocular pressure values.	[56]
	Loteprednol Etabonate	Anti-inflammatory	In vivo	An elevated concentration of loteprednol etabonate (LE) was noted in ocular tissues and fluids, along with an enhanced pharmacokinetic profile, exemplified by a threefold increase in the maximum concentration (Cmax) in rabbit ocular tissues, when compared to the Lotemax 0.5% suspension.	[57]
	Ciclosporin A	Keratoconjunctivitis	In vivo	The utilization of nanosuspension with a PVA stabilizer resulted in reduced eye irritation in comparison to the commercial product Restasis.	[58]
High pressure homogenization (HPH)	Dexamethasone, Hydrocortisone and prednisolone	Conjunctiva	In vivo	Nanosuspensions (NSs) displayed an enhanced drug action intensity and greater drug absorption extent.	[71]

they must traverse two primary barriers that envelop the eyeball: the cornea and the conjunctiva. The choice of a specific approach depends on the physicochemical properties of the drug, the desired release profile, and the safety considerations for ocular administration. It is essential to carefully evaluate the trade-offs between improved drug delivery and potential adverse effects to select the most appropriate strategy for a given therapeutic agent.[66,70,71]

Topical Pulmonary Applications of Nanosuspensions

Pulmonary medication delivery, both locally and systemically, provides a non-invasive option for lung treatment (Table 2). Inhalers and nebulizers produce aerosols that can be directly administered to the lungs in individuals.[72] The local application of therapeutic drugs to the lungs is the predominant approach for managing various respiratory disorders. It offers the advantages of greater selectivity and higher local drug concentrations compared to other routes of administration.[17,73]

In contrast, the pulmonary route has gained increasing attention as a potential method for systemic drug delivery due to the extensive alveolar surface area, the delicate epithelial barrier, and the significant vascularization conducive to drug absorption.[75–78] Furthermore, drugs administered via the pulmonary route bypass the first-pass metabolism of the gastrointestinal tract, enter the systemic circulation, and may eliminate barriers associated with patient compliance.[5,19,79] However, the effectiveness of drug delivery through this route depends significantly on factors such as aerosol particle size, particle shape and geometry, surface adhesive properties, and the mechanism and rate of removal from the respiratory system.[80–86]

One critical feature of the drug formulation is the aerodynamic diameter of the aerosol, which characterizes its aerodynamic behavior, taking into account size, density, and shape. Optimizing this parameter is essential for effective pulmonary administration. It's important to note that the success of nanosuspensions in pulmonary delivery depends on various factors, including the specific drug properties, patient characteristics, and the intended therapeutic outcomes. Each approach has its advantages and limitations, and the choice of strategy should be tailored to the unique requirements of the drug being delivered.[16,87,88]

Table 2 Examples of Pulmonary Drugs Nanosuspension with Their Methods of Preparations

Preparation Method	Drug	Pharmacological Uses	Type of Study	Result	References
Wet milling	Beclomethasone Dipropionate and curcumin	Bronchial asthma	In vitro	A notable enhancement of approximately 54-fold in the apparent solubility of curcumin over its raw material was observed. The utilization of a multicomponent nanosuspension, coupled with optimized dimensional properties and aerodynamic parameters, suggests that the formulated curcumin should be administered with precision and effectiveness to reach deeper lung regions.	[73]
High pressure homogenization (HPH)	Budesonide	Asthma	In vitro	The results indicate that either a portable inhaler system or a traditional nebulizer may be employed in conjunction with a long-term stable pulmonary budesonide nanosuspension.	[74]
Wet milling combined with high pressure homogenization (Smart Crystal)	Fluticasone propionate	Asthma	In vitro and in vivo	The local anti-inflammatory impacts of fluticasone are primarily influenced by its dissolution profile. When administered intratracheally, nanosuspensions considerably extended the local anti-inflammatory effectiveness of fluticasone. This was achieved by diminishing mucociliary clearance, prolonging the pulmonary absorption duration, and enhancing local retention.	[75]

Topical Dermal Applications of Nanosuspensions

Typically, drug nanocrystals are considered a safe and well-tolerated dosage form for various administration routes.[17,22,89] Nanocrystalline formulations are more effective in oral drug delivery due to two key mechanisms: enhanced solubility and dissolution rates and increased bioadhesion to the intestinal wall. Consequently, taking a drug in the form of nanocrystals orally results in a large concentration gradient between the gastrointestinal tract and blood vessels, leading to increased absorption and higher bioavailability.[90,91] In addition to marketed products, there is significant ongoing research focused on developing new formulations to enhance oral bioavailability using nanosuspension technology. In ocular administration, carrier-free nanocrystals consisting of 100% pure medication may offer advantages by reducing eye irritation, which, in turn, reduces tearing and drainage of the implanted dosage.[5,36,90]

Several researchers are currently exploring the use of anti-inflammatory medications in the form of nanocrystals for ocular applications. Drug nanocrystals can also be found in the aqueous phase of pharmaceutical skin preparations, such as anti-inflammatory creams or gels, as well as in cosmetic skin preparations, including sunscreen and anti-aging treatments.[17,20,92]

Dermal nanocrystals are available for use in cosmetic products, primarily when conventional formulation methods prove less effective. The use of medication nanocrystals results in an enhanced concentration gradient between the formulation and the skin. "Supersaturated" formulations, with higher saturation solubility, enhance medication absorption through the skin.[7,13] Polymers with a positive charge can serve as stabilizing agents for drug nanocrystals, thereby increasing their stability. The stratum corneum, a layer of the skin, has a negative charge, which further enhances the attraction of drug particles to the skin.[18,93]

Nanocrystals present a relatively new and highly intriguing approach within the various methods aimed at improving cutaneous (skin) application. Despite the increasing availability of various nanosuspension products over the past decade, primarily designed for oral administration.[43,93] Only a limited number of studies have explored skin permeability and the accumulation of medications in the form of nanosuspensions (Table 3). However, the few published findings have unequivocally demonstrated the potential benefits of this approach for enhancing the cutaneous bioavailability of active ingredients with low to medium solubility.[48,51] In fact, nanocrystals not only exhibit improved saturation solubility and dissolution rates but also display increased adhesiveness to the skin, thereby enhancing cutaneous distribution.

Interest in this technique for dermal application has grown since the introduction of the first skin-protective and anti-aging cosmetic products based on nanosuspensions containing poorly soluble antioxidants like rutin and hesperidin.[4,6,44,101] The aglycone of hesperidin, known for its antioxidative and anti-inflammatory properties and considered a highly effective anti-aging compound, was initially developed. The method for manufacturing nanocrystals for oral, intravenous, or cutaneous use remains consistent, with the only difference lying in the choice of stabilizers.[27,30,43]

Therefore, four specific stabilizers suitable for cutaneous use, including Poloxamer 188, Inutec SP1, Tween 80, and Plantacare 2000, are employed to create nanosuspensions of hesperetin using high-pressure homogenization. It's worth noting that nanosuspensions stabilized by Inutec and Plantac are stable and show no changes in mean diameter size, as anticipated from the zeta potential data.[44,46] On the other hand, the slight size increases observed in nanosuspensions stabilized by Poloxamer and Tween are not believed to hinder their use in cutaneous formulations.

Resveratrol, recognized for its antioxidant properties, has been judiciously chosen as the preferred candidate for incorporation into nanocrystals intended for use in creams, lotions, and gel formulations within the realm of cutaneous cosmetics.[45,60] The production of resveratrol nanosuspensions involves the application of a high-pressure homogenization technique. An essential aspect of this process revolves around the meticulous monitoring of how preservatives influence the physical stability of these nanosuspensions. This scrutiny is vital due to the pivotal role that preservation plays in the context of cutaneous preparations.[2,102]

Concurrently, lutein nanosuspensions are prepared through high-pressure homogenization to heighten their dissolution rate and saturation solubility. These two factors are instrumental in determining the extent of oral bioavailability and the potential of these compounds to penetrate the skin. Lutein is widely recognized within the cosmetic and nutraceutical industries for its antioxidant properties and its capacity to counter free radicals. Furthermore, it exhibits potential utility in pharmaceutical applications as a supplementary antioxidant.[46,102]

Comparatively, when lutein is employed in the form of nanocrystals, it manifests a notable improvement in drug saturation solubility in contrast to its coarser powder counterpart. This improvement not only augments its solubility but also facilitates more effective penetration through cellulose nitrate membranes. Additionally, empirical observations have unveiled the impermeability of pig ears, often utilized as a model for skin permeation studies. This observation lends credence to the hypothesis that lutein possesses the inherent capability to traverse the skin barrier and fulfill its role as an antioxidant in the cutaneous environment.[32,51,103]

The utilization of nanosuspension technology for topical pharmaceutical formulations marked a significant advancement with the introduction of diclofenac sodium solid in oil nanosuspension. This innovation addressed the challenge of delivering medicinal drugs with poor solubility. An example of this approach involved ibuprofen, a non-steroidal anti-inflammatory drug used in the treatment of acute and chronic arthritic conditions. Ibuprofen, due to its limited solubility, was considered for enhancing skin permeability through nanosuspension gel formulations.[103,104]

Topical application of ibuprofen offers advantages by minimizing adverse reactions, particularly gastrointestinal issues, which can result from oral administration, potentially harming the gastric mucosa and leading to ulceration. Research has demonstrated that the choice of solubilizer and the particle size of drug crystals play a significant role in improving skin permeability.[17,44,101] These factors lead to the development of a supersaturated solution around the drug crystals, creating a substantial concentration gradient between the crystals and the skin surface. Consequently, the formulation of effective dermal formulations for poorly soluble substances must consider various factors, including drug crystal size, carrier surface properties, and drug-stabilizer interactions.[14,28,104]

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Table 3 Examples of Nanosuspension Formulations for Topical Application and Their Preparation Methods

Preparation Method	Drug	Pharmacological uses	Type of Study	Results	References
Wet milling	Cyclosporin A	Antioxidant	In vivo	The nanosuspension formulation has achieved enhanced skin penetration while maintaining higher stability.	[94]
	Diclofenac sodium	NSAIDs Anti-inflammatory	In vitro	The application of nanosuspensions presents a dual effect: the drug's saturation solubility remains constant, leading to drug accumulation, with no significant alteration in its permeation.	[95]
	Nitrofurazone	Antioxidant and anti-inflammatory agent	In vitro	Upon comparing the drug nanoformulation with drugs prepared using alternative methods available in the market, it was observed that the nanoformulation exhibited enhanced drug dissolution rates and improved drug permeability through the skin membrane.	[96]
	Flurbiprofen	Anti-inflammatory and analgesic	In vitro	Through a comparison between the drug nanogel and conventional gel formulations, pharmacokinetic studies revealed that the drug exhibited superior permeability and achieved higher drug concentrations in rats.	[44]
	Ibuprofen	Anti-inflammatory	In vitro	The results clearly established a correlation between the particle size of nanosuspensions and the choice of stabilizer (Vitamin E TPGS), influencing the drug's transdermal transport.	[97]
	Etodolac	Anti-inflammatory and analgesic	In vitro and in vivo	Nanosuspension-based hydroxypropyl methylcellulose (HPMC) or hydroxyethyl cellulose (HEC) gels demonstrated superiority in enhancing drug penetration, as evidenced by enhanced saturation solubility in both in vitro and ex vivo permeation experiments. Moreover, when compared to the control and physical mixture, the nanosuspension HEC gels exhibited enhanced anti-inflammatory and analgesic activities.	[92]
High pressure homogenization (HPH)	Flurbiprofen	Anti-inflammatory and analgesic	In vitro	The use of drug nanosuspensions resulted in a 5.3-fold increase in drug saturation solubility. In rat skin, the drug nanosuspension exhibited greater permeability compared to the Flurbiprofen solution. The Design of Experiments (DoE) technique proved to be a valuable tool for the preparation of Flurbiprofen nanosuspensions. Plantacare® 2000 UP effectively preserved the stability and crystalline state of the drug nanosuspension. In achieving smaller particle sizes and enhanced nanosuspension stability, Plantacare® 2000 UP emerged as a more efficient stabilizer. When compared to other stabilizers, Plantacare® 2000 UP and PVP displayed superior morphology.	[98]

Wet milling combined with high pressure homogenization (Smart Crystal)	Curcumin	Anti-acne	In vitro	The drug concentration within nanosuspensions can vary from 0.02% for poorly soluble medications to 0.2% for economically feasible pharmaceuticals. The low viscosity of dermal formulations facilitates enhanced skin penetration and targeted accumulation within hair follicles.	[99]
Anti-solvent precipitation combined with high pressure homogenization (Nanoedge)	Glabridin	Psoriasis	In vitro and in vivo	Nanosuspension significantly enhanced the drug penetration flux through rat skin, exhibiting no lag phase in both in vitro and in vivo experiments when compared to the coarse suspension and physical mixture. Following three months of storage at room temperature, the Glabridin nanosuspension exhibited no noticeable aggregates and experienced a minimal Glabridin loss of 5.46%.	[113]

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To enhance the cutaneous targeting and photostability of tretinoin, a poorly water-soluble and unstable compound used in commercial creams and gels for treating acne vulgaris, nanosuspensions were produced using a straightforward precipitation approach.[105,106] The permeation and deposition of drugs were studied through skin diffusion tests on newborn pigs, while UV light exposure was used to assess the photostability of tretinoin (TRA). Additionally, an oil-in-water (O/W) nanoemulsion was created and evaluated as a suitable comparator. Nanoemulsions have proven particularly valuable as carriers for cutaneous and transdermal delivery of hydrophobic substances in pharmaceutical, cosmetic, and chemical industries.[8,89] The nanosuspension effectively localized the drug within pig skin throughout numerous transcutaneous studies, resulting in limited transdermal drug delivery (a factor contributing to systemic adverse effects). In contrast, the nanoemulsion significantly improved drug permeation.[4,6,17,104]

This investigation underscores the substantial potential of nanosuspensions in cutaneous drug delivery. It is noteworthy that nanosuspensions primarily consist of drug nanoparticles and minute amounts of safe and biocompatible surfactants, such as soy lecithin in this study. While there is limited toxicological data available at present, topically applied nanocrystals are not associated with known or anticipated adverse effects.[39,107]

Indeed, as proposed by several authors, it is noteworthy that each solid macro/microparticle applied to the skin undergoes a transformation into nanocrystals during its breakdown process, which is related to the concept of mechanical or physical breakdown, often associated with processes like milling or grinding. Importantly, there have been no documented reports of intolerance to this phenomenon thus far. As mentioned earlier, the authors of this study have provided additional support for the notion of improved drug delivery through the skin, emphasizing the establishment of an increased concentration gradient between the dermal formulation and the skin.[11,41,43]

Subsequent research has indicated that the enhanced dissolution rate of tretinoin nanocrystals can be attributed to their finely fragmented and uniformly dispersed nature. This characteristic promotes quicker dissolution due to the enlarged surface area and improved saturation solubility. Furthermore, in comparison to the control nanoemulsion, the utilization of tretinoin nanocrystals via topical administration yields the advantage of enhancing the drug's photostability.[105–108]

Moreover, over the past two years, nanocrystal technology has been extended to include medium-soluble chemicals. This expansion signifies an important development, introducing a new mechanism by which nanocrystals can enhance cutaneous drug delivery. This mechanism involves the active participation of hair follicles, opening up a second pathway for improved drug delivery.[18,22,107]

These specialized shunts can aggregate nanocrystals of the appropriate size, approximately 700 nm, which function as a reservoir from which the medication can gradually diffuse into the neighboring cells, ensuring an extended release. The aim of augmenting the skin penetration of moderately soluble active substances, such as caffeine, has been a recent focus of various researchers. In pursuit of this objective, they developed a specific manufacturing method involving low-energy milling in dispersion media with low dielectric constants and the application of a chosen stabilizer. This approach was designed to counteract crystal growth and fiber formation, which are common outcomes of supersaturation and recrystallization effects observed in moderately soluble molecules.[109–111]

The innovative strategy they employed revolved around creating nanocrystals from active ingredients with moderate solubility. These nanocrystals were subsequently incorporated into a dermal formulation, serving as rapidly dissolving depots that enhanced saturation solubility and accumulated within hair follicles to further enhance skin penetration.[16,110] For instance, caffeine nanocrystals were generated using a pear milling process in a mixture of ethanol and propylene glycol (3:7) with the addition of 2% carbopol. These nanocrystals exhibited a size range from 660 nm (ideal for hair follicle accumulation) to 250 nm (ideal for rapid dissolution).[112,113]

Challenges and Limitations

Addressing the limitations of nanosuspensions for topical application involves a combination of thoughtful formulation design, the selection of appropriate excipients, and the optimization of manufacturing processes. Formulation scientists should consider the specific requirements of the drug, the intended application, and the desired therapeutic outcomes when developing nanosuspension formulations for topical use.

Toxicity Hazards

Nanosuspension formulations are widely regarded as highly efficient excipients suitable for topical application in various contexts, including cutaneous, pulmonary, or ocular applications. Their effectiveness for these purposes can be attributed to several key advantages.

To begin, nanosuspensions exhibit remarkable physical stability. The reduced particle size of the drug in the formulation facilitates superior dispersion while preventing sedimentation or aggregation. This, in turn, ensures uniform distribution and consistent drug delivery, ultimately leading to enhanced therapeutic efficacy.

Additionally, nanosuspensions possess a notable surface area-to-volume ratio. This characteristic promotes improved drug dissolution and bioavailability, as the augmented surface area enables quicker and more efficient absorption into the target tissues or cells.

Furthermore, nanosuspensions exhibit a favorable toxicological profile. The excipients utilized in these formulations are generally recognized as safe and well-tolerated by the body. This reduces the likelihood of adverse effects or toxicity associated with the excipient itself.

In summary, nanosuspension formulations offer exceptional physical stability, enhanced drug delivery, and a low risk of toxicity. These attributes make them highly effective excipients for topical applications in cutaneous, pulmonary, or ocular treatments.[24,44,105]

Regulatory Considerations

In the realm of pharmaceutical development, the allocation of resources often hinges on the commercial context and the correlation between the developmental costs of a product intended for a particular market and its potential annual sales. Consequently, considerable innovation efforts have traditionally been channeled toward the oral and parenteral/intravenous routes. Nevertheless, there exists a noteworthy avenue for innovation in the application of drug nanocrystals for alternative routes such as cutaneous (topical), pulmonary (inhalation), and ophthalmic (eye) administration, offering opportunities for enhancing drug delivery.

In elucidating this point, it is crucial to acknowledge that the primary focus of innovations in drug delivery systems has been predominantly directed towards oral and parenteral routes. However, there has also been a growing interest in harnessing the potential of drug nanocrystals for alternative modes of administration, including cutaneous, pulmonary, and ophthalmic routes. Each of these routes presents distinct advantages and challenges in the context of drug delivery, and the integration of drug nanocrystals holds the promise of enhancing the effectiveness and safety of these administrations.

For instance, in the domain of cutaneous application, drug nanocrystals have the capacity to facilitate the penetration of drugs through the skin, ultimately enhancing their absorption and therapeutic outcomes. In pulmonary delivery, drug nanocrystals can significantly enhance the deposition of drugs in the lung, thus enabling targeted therapy for respiratory conditions. In the sphere of ophthalmic administration, nanocrystals offer the potential to augment drug permeability across ocular barriers, resulting in improved biodistribution and bioavailability.

Collectively, the integration of drug nanocrystals into these alternative routes of drug administration represents a promising avenue for optimizing drug delivery, diversifying treatment options, and ultimately improving patient outcomes.[39,52]

Future Perspectives and Research Directions

To date, numerous research teams have explored a variety of formulations using nanosuspension technology. However, only a limited number of these formulations have successfully reached the market, particularly for oral delivery. The underlying reasons for this situation extend beyond technical challenges, as the high market entry costs make it difficult to replace a well-established product with a nanosuspension. Additionally, established and proven administration methods are often preferred when introducing a novel chemical. In summary, nanosuspension technology provides a versatile framework for the development of safe and effective formulations for active compounds with poor solubility.[112–114]

In recent years, there has been a growing trend in the utilization of surface-modified nanosuspensions for the creation of tailored formulations. The use of drug nanocrystals holds promise for facilitating targeted drug delivery to specific diseased tissues, such as infected macrophages, tumors, and the brain, thus potentially enhancing pharmacological efficacy. Ongoing research is dedicated to exploring the application of nanosuspensions for the purpose of delivering nanoparticles to specific cells and enhancing their uptake within the cellular environment. Therefore, nanocrystals represent a viable approach for addressing challenges associated with the current dosage formulations.[3,19,46,115]

Conclusion

Nanosuspensions present a promising and economically viable strategy for addressing the challenges associated with delivering hydrophobic drugs, especially those characterized by limited solubility in both aqueous and organic solvents. These challenges predominantly pertain to enhancing drug absorption and bioavailability in the context of poorly water-soluble drugs. The latest nanosuspension manufacturing process can be established using wet milling, high pressure homogenization (HPH), smart crystal, and nanoedge methods. Furthermore, the integration of drug nanoparticles into water-free ointments and creams holds the potential to augment their saturation solubility, thus facilitating improved drug absorption through the skin. The ongoing advancements in mucosal formulations and topical administration are expected to maintain interest in nanosuspension drug delivery, characterized by simplified formulation techniques and a broad spectrum of potential applications.

Abbreviations

API, active pharmaceutical ingredient; DoE, Design of Experiments; GmbH, company with limited liability; HEC, Hydroxyethyl cellulose; HPH, high-pressure homogenization; HPMC, Hydroxypropyl Methylcellulose; IV, intravenous; LD, laser diffraction; MPa, megapascal; NSAIDs, Nonsteroidal Anti-Inflammatory Drugs; NSs, nanosuspensions; PCS, photon correlation spectroscopy; PS, particle size; PVA, polyvinyl alcohol; PVP, Polyvinylpyrrolidone; SC, stratum corneum; TPGS, tocopherol polyethylene glycol succinate; TRA, to assess the photostability of tretinoin; UV, Ultraviolet.

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References

1. Pınar SG, Oktay AN, Karaküçük AE, Çelebi N. Formulation Strategies of Nanosuspensions for Various Administration Routes. *Pharmaceutics*. 2023;15(5):1520. doi:10.3390/pharmaceutics15051520
2. Suhandi C, Alfathonah SS, Hasanah AN. Potency of Xanthone Derivatives from Garcinia mangostana L. for COVID-19 Treatment through Angiotensin-Converting Enzyme 2 and Main Protease Blockade: a Computational Study. *Molecules*. 2023;28(13):5187. doi:10.3390/molecules28135187
3. Suharyani I, Suhandi C, Rizkiyan Y, et al. Molecular docking in prediction of α-mangostin/cyclodextrin inclusion complex formation. In: *AIP Conference Proceedings*. Vol 2706; 2023. doi:10.1063/5.0120782.
4. Suhandi C, Wilar G, Lesmana R, et al. Propolis-Based Nanostructured Lipid Carriers for α-Mangostin Delivery: formulation, Characterization, and In Vitro Antioxidant Activity Evaluation. *Molecules*. 2023;28(16):6057. doi:10.3390/molecules28166057
5. Jacob S, Nair AB, Shah J. Emerging role of nanosuspensions in drug delivery systems. *Biomater Res*. 2020;24(1):1–16. doi:10.1186/s40824-020-0184-8
6. Ahmadi Tehrani A, Omranpoor MM, Vatanara A, Seyedabadi M, Ramezani V. Formation of nanosuspensions in bottom-up approach: theories and optimization. *DARU J Pharm Sci*. 2019;27(1):451–473. doi:10.1007/s40199-018-00235-2
7. Guan W, Ma Y, Ding S, et al. The technology for improving stability of nanosuspensions in drug delivery. *J Nanopart Res*. 2022;24(1):14. doi:10.1007/s11051-022-05403-9

8. Huang S, Wu H, Jiang Z, Huang H. Water-based nanosuspensions: formulation, tribological property, lubrication mechanism, and applications. *J Manuf Process*. 2021;71:625–644. doi:10.1016/j.jmapro.2021.10.002
9. Nabavi M, Nazarpour V, Alibak AH, Bagherzadeh A, Alizadeh SM. Smart tracking of the influence of alumina nanoparticles on the thermal coefficient of nanosuspensions: application of LS-SVM methodology. *Appl Nanosci*. 2021;11(7):2113–2128. doi:10.1007/s13204-021-01949-7
10. Ma Y, Cong Z, Gao P, Wang Y. Nanosuspensions technology as a master key for nature products drug delivery and in vivo fate. *Eur J Pharm Sci*. 2023;185:106425. doi:10.1016/j.ejps.2023.106425
11. Guner G, Seetharaman N, Elashri S, Mehaj M, Bilgili E. Analysis of heat generation during the production of drug nanosuspensions in a wet stirred media mill. *Int J Pharm*. 2022;624:122020. doi:10.1016/j.ijpharm.2022.122020
12. Karakucuk A, Celebi N. Investigation of formulation and process parameters of wet media milling to develop etodolac nanosuspensions. *Pharm Res*. 2020;37(6):1–18. doi:10.1007/s11095-020-02815-x
13. Bilgili E, Guner G. Mechanistic modeling of wet stirred media milling for production of drug nanosuspensions. *AAPS Pharm Sci Tech*. 2021;22 (1):1–23. doi:10.1208/s12249-020-01876-w
14. Liu T, Müller RH, Möschwitzer JP. Production of drug nanosuspensions: effect of drug physical properties on nanosizing efficiency. *Drug Dev Ind Pharm*. 2018;44(2):233–242. doi:10.1080/03639045.2017.1386207
15. Du Y, Yuan X. Coupled hybrid nanoparticles for improved dispersion stability of nanosuspensions: a review. *J Nanopart Res*. 2020;22(9):261. doi:10.1007/s11051-020-04991-8
16. Perrin L, Pajor-Swierzy A, Magdassi S, Kamyshny A, Ortega F, Rubio RG. Evaporation of nanosuspensions on substrates with different hydrophobicity. *ACS Appl Mater Interfaces*. 2018;10(3):3082–3093. doi:10.1021/acsami.7b15743
17. Azimullah S, Sudhakar CK, Kumar P, et al. Nanosuspensions as a promising approach to enhance bioavailability of poorly soluble drugs: an update. *J Drug Delivery Ther*. 2019;9(2):574–582. doi:10.22270/jddt.v9i2.2436
18. Wang N, Qi F, He X, et al. Preparation and Pharmacokinetic Characterization of an Anti-Virulence Compound Nanosuspensions. *Pharmaceutics*. 2021;13(10):1586. doi:10.3390/pharmaceutics13101586
19. Khandbahale SV. A review-Nanosuspension technology in drug delivery system. *Asian J Pharm Res*. 2019;9(2):130–138. doi:10.5958/2231-5691.2019.00021.2
20. Dewangan HK. The emerging role of nanosuspensions for drug delivery and stability. *Curr Nanomedicine*. 2021;11(4):213–223.
21. Goel S, Sachdeva M, Agarwal V. Nanosuspension technology: recent patents on drug delivery and their characterizations. *Recent Pat Drug Deliv Formul*. 2019;13(2):91–104. doi:10.2174/1872211313666190614151615
22. Lynnerup JT, Eriksen JB, Bauer-Brandl A, Holsæter AM, Brandl M. Insight into the mechanism behind oral bioavailability-enhancement by nanosuspensions through combined dissolution/permeation studies. *Eur J Pharm Sci*. 2023;184:106417. doi:10.1016/j.ejps.2023.106417
23. Aleandri S, Schönenberger M, Niederquell A, Kuentz M. Temperature-induced surface effects on drug nanosuspensions. *Pharm Res*. 2018;35 (3):1–11. doi:10.1007/s11095-017-2300-6
24. Kovalchuk NM, Johnson D, Sobolev V, Hilal N, Starov V. Interactions between nanoparticles in nanosuspension. *Adv Colloid Interface Sci*. 2019;272:102020. doi:10.1016/j.cis.2019.102020
25. Kirichenko MN, Chaikov LL, V SA, et al. General features of size distributions and internal structure of particles in aqueous nanosuspensions. *Phys Wave Phenom*. 2020;28(2):140–144. doi:10.3103/S1541308X20020077
26. Minakov AV, Pryazhnikov MI, Zhigarev VA, Rudyak VY, Filimonov SA. Numerical study of the mechanisms of enhanced oil recovery using nanosuspensions. *Theor Comput Fluid Dyn*. 2021;35(4):477–493. doi:10.1007/s00162-021-00569-9
27. han WX, Liu Y, ying SC, na ZR, long YH, Yuan H-L. Effect of particle size on in vitro and in vivo behavior of astilbin nanosuspensions. *J Drug Deliv Sci Technol*. 2019;52:778–783. doi:10.1016/j.jddst.2019.05.005
28. Xiang Y, Liang G, Alvaro P, et al. Resonant optical nonlinearity and fluorescence enhancement in electrically tuned plasmonic nanosuspensions. *Adv Photonics Res*. 2021;2(5):2000060. doi:10.1002/adpr.202000060
29. Gaur PK. Nanosuspension of flavonoid-rich fraction from Psidium guajava Linn for improved type 2-diabetes potential. *J Drug Deliv Sci Technol*. 2021;62:102358. doi:10.1016/j.jddst.2021.102358
30. Galinovskiy AL, Htet KM, Provatorov AS. Ultra-Jet as a Tool for Dispersing Nanosuspensions. *Polym Sci Ser D*. 2020;13(2):209–213. doi:10.1134/S1995421220020070
31. Dos Santos AM, Meneguin AB, Fonseca-Santos B, et al. The role of stabilizers and mechanical processes on physico-chemical and anti-inflammatory properties of methotrexate nanosuspensions. *J Drug Deliv Sci Technol*. 2020;57:101638. doi:10.1016/j.jddst.2020.101638
32. Ali AMA, Warsi MH, Abourehab MAS, Ali AA. Preparation and transformation of solid glass solutions of clotrimazole to nanosuspensions with improved physicochemical and antifungal properties. *J Pharm Innov*. 2022;17(4):1420–1433. doi:10.1007/s12247-021-09595-w
33. Tian Y, Wang S, Yu Y, et al. Review of nanosuspension formulation and process analysis in wet media milling using microhydrodynamic model and emerging characterization methods. *Int J Pharm*. 2022;623:121862. doi:10.1016/j.ijpharm.2022.121862
34. Pandey NK, Singh SK, Gulati M, et al. Overcoming the dissolution rate, gastrointestinal permeability and oral bioavailability of glimepiride and simvastatin co-delivered in the form of nanosuspension and solid self-nanoemulsifying drug delivery system: a comparative study. *J Drug Deliv Sci Technol*. 2020;60:102083. doi:10.1016/j.jddst.2020.102083
35. Guner G, Yilmaz D, Bilgili E. Kinetic and microhydrodynamic modeling of fenofibrate nanosuspension production in a wet stirred media mill. *Pharmaceutics*. 2021;13(7):1055. doi:10.3390/pharmaceutics13071055
36. Kathpalia H, Juvekar S, Shidhaye S. Design and in vitro evaluation of atovaquone nanosuspension prepared by pH based and anti-solvent based precipitation method. *Colloid Interface Sci Commun*. 2019;29:26–32. doi:10.1016/j.colcom.2019.01.002
37. Flach F, Breitung-Faes S, Kwade A. Model based process optimization of nanosuspension preparation via wet stirred media milling. *Powder Technol*. 2018;331:146–154. doi:10.1016/j.powtec.2018.03.011
38. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. *Heliyon*. 2022;8(5). doi:10.1016/j.heliyon.2022.e09394
39. Chinthaginjala H, Abdul H, Reddy APG, Kodi K, Manchikanti SP, Pasam D. Nanosuspension as Promising and Potential Drug Delivery: a Review. *Int J Life Sci Pharm Res*. 2020;11(1):P59–66.
40. Jin SM, Lee SN, Kim JE, et al. Overcoming Chemoimmunotherapy-Induced Immunosuppression by Assemblable and Depot Forming Immune Modulating Nanosuspension. *Adv Sci*. 2021;8(19):2102043. doi:10.1002/advs.202102043

41. Wan KY, Weng J, Wong SN, Kwok PCL, Chow SF, Chow AHL. Converting nanosuspension into inhalable and redispersible nanoparticles by combined in-situ thermal gelation and spray drying. *Eur J Pharm Biopharm*. 2020;149:238–247. doi:10.1016/j.ejpb.2020.02.010
42. Petersen R Nanocrystals for use in topical cosmetic formulations and method of production thereof; 2015.
43. Shen C, Shen B, Liu X, Yuan H. Nanosuspensions based gel as delivery system of nitrofurazone for enhanced dermal bioavailability. *J Drug Deliv Sci Technol*. 2018;43:1–11. doi:10.1016/j.jddst.2017.09.012
44. Oktay AN, Ilbasmis-Tamer S, Uludag O, Celebi N. Enhanced dermal delivery of flurbiprofen nanosuspension based gel: development and ex vivo permeation, pharmacokinetic evaluations. *Pharm Res*. 2021;38(6):991–1009. doi:10.1007/s11095-021-03060-6
45. Pireddu R, Schlich M, Marceddu S, et al. Nanosuspensions and microneedles roller as a combined approach to enhance diclofenac topical bioavailability. *Pharmaceutics*. 2020;12(12):1140. doi:10.3390/pharmaceutics12121140
46. Pireddu R, Sinico C, Ennas G, et al. The effect of diethylene glycol monoethyl ether on skin penetration ability of diclofenac acid nanosuspensions. *Colloids Surf B Biointerfaces*. 2018;162:8–15. doi:10.1016/j.colsurfb.2017.11.012
47. Zuo W, Qu W, Li N, et al. Fabrication of multicomponent amorphous bufadienolides nanosuspension with wet milling improves dissolution and stability. *Cells Nanomed Biotechnol*. 2018;46(7):1513–1522. doi:10.1080/21691401.2017.1375938
48. Stahr PL, Keck CM. Preservation of rutin nanosuspensions without the use of preservatives. *Beilstein J Nanotechnol*. 2019;10(1):1902–1913. doi:10.3762/bjnano.10.185
49. Arora D, Khurana B, Rath G, Nanda S, Goyal AK. Recent advances in nanosuspension technology for drug delivery. *Curr Pharm Des*. 2018;24 (21):2403–2415. doi:10.2174/1381612824666180522100251
50. Zhang T, Li X, Xu J, Shao J, Ding M, Shi S. Preparation, characterization, and evaluation of breviscapine nanosuspension and its freeze-dried powder. *Pharmaceutics*. 2022;14(5):923. doi:10.3390/pharmaceutics14050923
51. Evstropiev SK, Vasilyev VN, Nikonorov NV, Kolobkova EV, Volkova NA, Boltenkov IA. Photoactive ZnO nanosuspension for intensification of organics contaminations decomposition. *Chem Eng Process Intensif*. 2018;134:45–50. doi:10.1016/j.cep.2018.10.020
52. Chakravorty R. Nanosuspension as an emerging Nanotechnology and Techniques for its Development. *Res J Pharm Technol*. 2022;15 (1):489–493. doi:10.52711/0974-360X.2022.00079
53. Shaikh F, Patel M, Patel V, et al. Formulation and optimization of cilnidipine loaded nanosuspension for the enhancement of solubility, dissolution and bioavailability. *J Drug Deliv Sci Technol*. 2022;69:103066. doi:10.1016/j.jddst.2021.103066
54. Kurhe SA, Katkar K, Bakkam A, Mokal S, Mane A, Jain A. Ocular Nanosuspension a Novel Approach–Review. *Res J Pharm Dos Forms Technol*. 2023;15(1):45–50. doi:10.52711/0975-4377.2023.00008
55. Josyula A, Omiadze R, Parikh K, et al. An ion-paired moxifloxacin nanosuspension eye drop provides improved prevention and treatment of ocular infection. *Bioeng Transl Med*. 2021;6(3):e10238. doi:10.1002/btm2.10238
56. Tuomela A, Liu P, Puranen J, et al. Brinzolamide nanocrystal formulations for ophthalmic delivery: reduction of elevated intraocular pressure in vivo. *Int J Pharm*. 2014;467(1–2):34–41. doi:10.1016/j.ijpharm.2014.03.048
57. Schopf L, Enlow E, Popov A, Bourassa J, Chen H. Ocular pharmacokinetics of a novel loteprednol etabonate 0.4% ophthalmic formulation. *Ophthalmol Ther*. 2014;3(1–2):63–72. doi:10.1007/s40123-014-0021-z
58. Kim JH, Jang SW, Han SD, Hwang HD, Choi HG. Development of a novel ophthalmic ciclosporin A-loaded nanosuspension using top-down media milling methods. *Die Pharm Int J Pharm Sci*. 2011;66(7):491–495.
59. Kassem MA, Rahman AAA, Ghorab MM, Ahmed MB, Khalil RM. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. *Int J Pharm*. 2007;340(1–2):126–133. doi:10.1016/j.ijpharm.2007.03.011
60. Popov A. Mucus-penetrating particles and the role of ocular mucus as a barrier to micro-and nanosuspensions. *J Ocul Pharmacol Ther*. 2020;36 (6):366–375. doi:10.1089/jop.2020.0022
61. Wang C, Pang Y. Nano-based eye drop: topical and noninvasive therapy for ocular diseases. *Adv Drug Deliv Rev*. 2023;194:114721. doi:10.1016/j.addr.2023.114721
62. Güven UM, Yenilmez E. Olopatadine hydrochloride loaded Kollidon® SR nanoparticles for ocular delivery: nanosuspension formulation and in vitro–in vivo evaluation. *J Drug Deliv Sci Technol*. 2019;51:506–512. doi:10.1016/j.jddst.2019.03.016
63. Qin T, Dai Z, Xu X, et al. Nanosuspension as an efficient carrier for improved ocular permeation of voriconazole. *Curr Pharm Biotechnol*. 2021;22(2):245–253. doi:10.2174/1389201021999200820154918
64. MADHAN G, KUPPUSWAMY G. Safe Use of Ocular Nanosuspension. *Int J Pharm Res*. 2021;13(1):65.
65. Jaiswal P, Mishra A, Kesharwani D, Das Paul S. Overview on Ocular Drug Delivery through Colloidal Nano-Suspension. *Res J Pharm Technol*. 2023;16(3):1533–1539. doi:10.52711/0974-360X.2023.00251
66. Hanagandi V, Patil AS, Masareddy RS, Dandagi PM, Bolmal UB. Development and Evaluation of Nanosuspension Incorporated in situ gel of Brimonidine Tartarate for Ocular Drug Delivery. *Indian J Pharm Educ Res*. 2022;56(1):94–102. doi:10.5530/ijper.56.1.12
67. Mansuk AG, Pachpute TS. Comprehensive Assessment of Transcorneal Permeation, Antimicrobial, and Antifungal Activities of Andrographolide-Loaded Nanosuspension: in vitro and In vivo Studies. *J Drug Delivery Ther*. 2023;13(6):35–42. doi:10.22270/jddt.v13i6.5847
68. Peter S, Mathews MM, Saju F, Development PS. Optimization and In Vitro Characterization of Eudragit-Ganciclovir Nanosuspension or Treating Herpes Simplex Keratitis. *J Pharm Innov*. 2023;1–10.
69. Agrahari V, Singh ON. Ocular Suspension and Nanosuspension Products: formulation Development Considerations. In: *Ophthalmic Product Development: From Bench to Bedside*. Springer; 2022:317–347.
70. Alaimo A, Pérez OE. Chitosan-based nanosuspensions for ocular diagnosis and therapy. In: *Advanced Nanoformulations*. Elsevier; 2023:13–41.
71. Qamar Z, Qizilbash FF, Iqubal MK, et al. Nano-based drug delivery system: recent strategies for the treatment of ocular disease and future perspective. *Recent Pat Drug Deliv Formul*. 2019;13(4):246–254. doi:10.2174/1872211314666191224115211
72. Pramanik S, Mohanto S, Manne R, et al. Nanoparticle-based drug delivery system: the magic bullet for the treatment of chronic pulmonary diseases. *Mol Pharm*. 2021;18(10):3671–3718. doi:10.1021/acs.molpharmaceut.1c00491
73. Casula L, Lai F, Pini E, et al. Pulmonary delivery of curcumin and beclomethasone dipropionate in a multicomponent nanosuspension for the treatment of bronchial asthma. *Pharmaceutics*. 2021;13(8):1300. doi:10.3390/pharmaceutics13081300
74. Jacobs C, Müller RH. Production and characterization of a budesonide nanosuspension for pulmonary administration. *Pharm Res*. 2002;19 (2):189–194. doi:10.1023/A:1014276917363

75. Fu TT, Cong ZQ, Zhao Y, et al. Fluticasone propionate nanosuspensions for sustained nebulization delivery: an in vitro and in vivo evaluation. *Int J Pharm*. 2019;572:118839. doi:10.1016/j.ijpharm.2019.118839
76. Ma X, Xia K, Xie J, et al. Treatment of Idiopathic Pulmonary Fibrosis by Inhaled Silybin Dry Powder Prepared via the Nanosuspension Spray Drying Technology. *ACS Pharmacol Transl Sci*. 2023;6(6):878–891. doi:10.1021/acsptsci.3c00033
77. Liu Y, Ma Y, Xue L, Guan W, Wang Y. Pulmonary multidrug codelivery of curcumin nanosuspensions and ciprofloxacin with N-acetylcysteine for lung infection therapy. *J Drug Deliv Sci Technol*. 2023;84:104474. doi:10.1016/j.jddst.2023.104474
78. da Silva LH A, Vieira JB, Cabral MR, et al. Development of nintedanib nanosuspension for inhaled treatment of experimental silicosis. *Bioeng Transl Med*. 2023;8(2):e10401. doi:10.1002/btm2.10401
79. Md S, Abdullah ST, Alhakamy NA, et al. Ambroxol hydrochloride loaded gastro-retentive nanosuspension gels potentiate anticancer activity in lung cancer (A549) cells. *Gels*. 2021;7(4):243. doi:10.3390/gels7040243
80. Haghighi DM, Faghihi H, Darabi M, Mirmoeini MS, Vatanara A. Spray freeze drying to solidify Nanosuspension of Cefixime into inhalable microparticles. *DARU J Pharm Sci*. 2022;1–11.
81. Hashem FM, Abd Allah FI, Abdel-Rashid RS, Hassan AAA. Glibenclamide nanosuspension inhaler: development, in vitro and in vivo assessment. *Drug Dev Ind Pharm*. 2020;46(5):762–774. doi:10.1080/03639045.2020.1753062
82. Simkova K Spray-dried nanosuspensions for pulmonary drug targeting and in vitro testing thereof; 2021.
83. Casula L, Sinico C, Valenti D, et al. Delivery of beclomethasone dipropionate nanosuspensions with an electronic cigarette. *Int J Pharm*. 2021;596:120293. doi:10.1016/j.ijpharm.2021.120293
84. Bhattacharjee A, Thomas S, Palit P. Nebulizer spray delivery of phytopharmaceutical nanosuspension via oral and nasal route: a challenging approach to fight against COVID-19. In: *Applications of Multifunctional Nanomaterials*. Elsevier; 2023:437–457.
85. Thiyagarajan D, Huck B, Nothdurft B, et al. Spray-dried lactose-leucine microparticles for pulmonary delivery of antimycobacterial nanopharmaceuticals. *Drug Deliv Transl Res*. 2021;11(4):1766–1778. doi:10.1007/s13346-021-01011-7
86. Aref ZF, Bazeed SEES, Hassan MH, et al. Clinical, biochemical and molecular evaluations of ivermectin mucoadhesive nanosuspension nasal spray in reducing upper respiratory symptoms of mild COVID-19. *Int J Nanomed*. 2021;Volume 16:4063–4072. doi:10.2147/IJN.S313093
87. Garkal A, Bangar P, Mathur K, Parikh D, Mehta T. Nanosuspensions in Treatment of Tuberculosis. In: *Tubercular Drug Delivery Systems: Advances in Treatment of Infectious Diseases*. Springer; 2023:189–205.
88. Mehanna MM, Mohyeldin SM, Elgindy NA. Rifampicin-carbohydrate spray-dried nanocomposite: a futuristic multiparticulate platform for pulmonary delivery. *Int J Nanomed*. 2019;Volume 14:9089–9112. doi:10.2147/IJN.S211182
89. Pelikh O, Stahr PL, Huang J, et al. Nanocrystals for improved dermal drug delivery. *Eur J Pharm Biopharm*. 2018;128:170–178. doi:10.1016/j.ejpb.2018.04.020
90. Oktay AN, Karakucuk A, Ilbasmis-Tamer S, Celebi N. Dermal flurbiprofen nanosuspensions: optimization with design of experiment approach and in vitro evaluation. *Eur J Pharm Sci*. 2018;122:254–263. doi:10.1016/j.ejps.2018.07.009
91. Oktay AN, Ilbasmis-Tamer S, Han S, Uludag O, Celebi N. Preparation and in vitro/in vivo evaluation of flurbiprofen nanosuspension-based gel for dermal application. *Eur J Pharm Sci*. 2020;155:105548. doi:10.1016/j.ejps.2020.105548
92. Karakucuk A, Tort S, Han S, Oktay AN, Celebi N. Etodolac nanosuspension based gel for enhanced dermal delivery: in vitro and in vivo evaluation. *J Microencapsul*. 2021;38(4):218–232. doi:10.1080/02652048.2021.1895344
93. Manca ML, Lai F, Pireddu R, et al. Impact of nanosizing on dermal delivery and antioxidant activity of quercetin nanocrystals. *J Drug Deliv Sci Technol*. 2020;55:101482. doi:10.1016/j.jddst.2019.101482
94. Romero GB, Arntjen A, Keck CM, Müller RH. Amorphous cyclosporin A nanoparticles for enhanced dermal bioavailability. *Int J Pharm*. 2016;498(1–2):217–224. doi:10.1016/j.ijpharm.2015.12.019
95. Pireddu R, Sinico C, Ennas G, et al. Novel nanosized formulations of two diclofenac acid polymorphs to improve topical bioavailability. *Eur J Pharm Sci*. 2015;77:208–215. doi:10.1016/j.ejps.2015.06.006
96. Shen C, Shen B, Liu X, Yuan H. Nanosuspensions based gel as delivery system of nitrofurazone for enhanced dermal bioavailability. *J Drug Deliv Sci Technol*. 2018;43:1–11. doi:10.1016/j.jddst.2017.09.012
97. Ghosh I, Michniak-Kohn B. Influence of critical parameters of nanosuspension formulation on the permeability of a poorly soluble drug through the skin - A case study. *AAPS Pharm Sci Tech*. 2013;14(3):1108–1117. doi:10.1208/s12249-013-9995-4
98. Oktay AN, Karakucuk A, Ilbasmis-Tamer S, Celebi N. Dermal flurbiprofen nanosuspensions: optimization with design of experiment approach and in vitro evaluation. *Eur J Pharm Sci*. 2018;122:254–263. doi:10.1016/j.ejps.2018.07.009
99. Vidlářová L, Romero GB, Hanuš J, Štěpánek F, Müller RH. Nanocrystals for dermal penetration enhancement - Effect of concentration and underlying mechanisms using curcumin as model. *Eur J Pharm Biopharm*. 2016;104:216–225. doi:10.1016/j.ejpb.2016.05.004
100. Wang WP, Hu J, Sui H, Zhao YS, Feng J, Liu C. Glabridin nanosuspension for enhanced skin penetration: formulation optimization, in vitro and in vivo evaluation. *Pharmazie*. 2016;71(5):252–257. doi:10.1691/ph.2016.5152
101. Jassim ZE, Rajab NA. Review on preparation, characterization, and pharmaceutical application of nanosuspension as an approach of solubility and dissolution enhancement. *J Pharm Res*. 2018;12:771–774.
102. Shegokar R, Muller RH, Ismail M, Gohla S. Algal nanosuspensions for dermal and oral delivery. *Curr Nanomedicine*. 2018;8(1):45–57.
103. Abdelghany S, Tekko IA, Vora L, Larrañeta E, Permana AD, Donnelly RF. Nanosuspension-based dissolving microneedle arrays for intradermal delivery of curcumin. *Pharmaceutics*. 2019;11(7):308. doi:10.3390/pharmaceutics11070308
104. Permana AD, McCrudden MTC, Donnelly RF. Enhanced intradermal delivery of nanosuspensions of antifilariasis drugs using dissolving microneedles: a proof of concept study. *Pharmaceutics*. 2019;11(7):346. doi:10.3390/pharmaceutics11070346
105. Eckert RW, Wiemann S, Keck CM. Improved dermal and transdermal delivery of curcumin with smartfilms and nanocrystals. *Molecules*. 2021;26(6):1633. doi:10.3390/molecules26061633
106. Assem M, Khowessah OM, Ghorab D. Nano-crystallization as a tool for the enhancement of beclomethasone dipropionate dermal deposition: formulation, in vitro characterization and ex vivo study. *J Drug Deliv Sci Technol*. 2019;54:101318. doi:10.1016/j.jddst.2019.101318
107. Jin N, Pyo SM, Keck CM, Müller RH. Azithromycin nanocrystals for dermal prevention of tick bite infections. *Die Pharm Int J Pharm Sci*. 2019;74(5):277–285.

108. Pathan IB, Sakhare M, Ambekar W, Setty CM. Dermal Delivery of Meloxicam Nanosuspensions based Gel: optimization with Box Behnken Design Experiment Approach: ex Vivo and In Vivo Study. *Nanosci Nanotechnology-Asia*. 2020;10(6):766–777. doi:10.2174/2210681209666190809103155
109. Shi T, Lv Y, Huang W, et al. Enhanced transdermal delivery of curcumin nanosuspensions: a mechanistic study based on co-localization of particle and drug signals. *Int J Pharm*. 2020;588:119737. doi:10.1016/j.ijpharm.2020.119737
110. Shaikh MS, Kale MA. Formulation and molecular docking simulation study of luliconazole nanosuspension–based nanogel for transdermal drug delivery using modified polymer. *Mater Today Chem*. 2020;18:100364. doi:10.1016/j.mtchem.2020.100364
111. Ahmed IS, Elnahas OS, Assar NH, Gad AM, El Hosary R. Nanocrystals of fusidic acid for dual enhancement of dermal delivery and antibacterial activity: in vitro, ex vivo and in vivo evaluation. *Pharmaceutics*. 2020;12(3):199. doi:10.3390/pharmaceutics12030199
112. Pelikh O, Hartmann SF, Abraham AM, Keck CM. Nanocrystals for dermal application. *Nanocosmetics from Ideas to Prod*. 2019;161–177.
113. Nagpal S, Kumari P, Saini K, Kakkar V. Pain Management with Topical Aceclofenac Nanosuspension In-Vitro/In-Vivo and Proof of Concept Studies. *Curr Drug Ther*. 2022;17(4):289–304. doi:10.2174/1574885517666220518094723
114. Pornpitchanarong C, Rojanarata T, Opanasopit P, Ngawhirunpat T, Patrojanasophon P. Clotrimazole nanosuspensions-loaded hyaluronic acid-catechol/polyvinyl alcohol mucoadhesive films for oral candidiasis treatment. *J Drug Deliv Sci Technol*. 2020;60:101927. doi:10.1016/j.jddst.2020.101927
115. Li J, Ni W, Aisha M, Zhang J, Sun M. A rutin nanocrystal gel as an effective dermal delivery system for enhanced anti-photoaging application. *Drug Dev Ind Pharm*. 2021;47(3):429–439. doi:10.1080/03639045.2021.1890113