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Designing Antihyperlipidemic Oral Nano Formulations using Quality by Design Principles: (A-Review of Recent Advances)

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ABSTRACT

This review highlights recent advancements in the design of oral nano-formulations for antihyperlipidemic drugs using Quality by Design (QbD) principles, emphasizing their pivotal role in managing hyperlipidaemia, a major risk factor for cardiovascular diseases. Conventional therapies often face challenges such as poor bioavailability, first-pass metabolism, and low patient compliance due to the limitations of traditional oral dosage forms. By applying QbD methodologies, including systematic evaluation of Critical Quality Attributes (CQAs) and optimization of formulation parameters, researchers have developed innovative nano-drug delivery systems such as solid lipid nanoparticles (SLNs) and self-nanoemulsifying drug delivery systems (SNEDDS). These advancements significantly enhance drug solubility, stability, and therapeutic efficacy. This review underscores the importance of continuous refinement in formulation processes and adherence to regulatory standards to ensure the safety, stability, and clinical effectiveness of these next-generation antihyperlipidemic therapies, ultimately improving patient outcomes.

Keywords: Nano-formulation, Hyperlipidaemia, Quality by design, Bioavailability, Antihyperlipidemic therapies.

INTRODUCTION

Hyperlipidemia, characterized by elevated levels of low-density lipoprotein (LDL) cholesterol (>190 mg/dL), reduced high-density lipoprotein (HDL) cholesterol (<40 mg/dL), and high triglyceride levels (>200 mg/dL)[1], is a significant cause of atherosclerosis and heart diseases, which are the biggest causes of death globally.[2] In summary, there are two primary methods of therapies for hyperlipidaemia: pharmacological therapy and non-pharmacological therapy. Pharmacological therapy primarily includes the use of medications, which are typically categorized into the following types: medications that inhibit lipolysis and triglyceride synthesis, such as nicotinic acid and omega-3 fatty acids; bile acid sequestrants (resins); 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins); and fibrates, which activate lipoprotein lipase. Non-pharmacological therapy includes lifestyle modifications, exercise, and dietary changes.[1]

Hyperlipidaemia can be caused by congenital abnormalities in lipid metabolism, as well as by medications, medical conditions, or dietary factors that lead to elevated cholesterol levels.[3] Even so, just a minor proportion of patients exhibit identifiable genetic variations, the cause of hyperlipidaemia can be categorized into primary and secondary types. Primary hyperlipidaemia is predominantly of genetic origin. Secondary hyperlipidaemia, on the other hand, can result from chronic conditions such as diabetes, liver disease, kidney disorders, thyroid dysfunction, Cushing's syndrome, obesity, estrogen therapy, alcohol consumption, and other drug-induced changes in lipid metabolism.[4]

Numerous conventional formulations of widely used antihyperlipidemic medications have been authorized by the United States Food and Drug Administration (FDA). In a nutshell, it is evident that these medications, while essential, are associated with several significant and common pharmacological adverse effects. Additionally, the pharmacokinetic and biopharmaceutical properties of antihyperlipidemic drugs present certain limitations that warrant consideration. Despite these challenges, the approval of these formulations underscores their therapeutic importance in managing hyperlipidaemia.[5]

Conventional Therapy for Hyperlipidaemia

The most common methods of administering antihyperlipidemic medications is the non-invasive oral route, where the dosage form is taken by mouth. However, oral approach has several limitations. Frequent administration is often required for drugs with short half-lives, increasing the risk of missed doses and leading to poor patient compliance. Additionally, fluctuations in drug concentrations make it difficult to maintain a steady-state condition. A major drawback of oral administration is the first-pass metabolism, which significantly reduces the bioavailability of many important drugs. Other factors, such as, variations caused by food intake and physiological conditions further contribute to inconsistent drug absorption and effectiveness. In summary, the limitations of conventional drug delivery systems include poor site specificity, low bioavailability and biodistribution, insufficient therapeutic response even at high doses, and an increased risk of toxicity and adverse effects. To overcome these challenges, novel drug delivery systems (NDDS) are essential. These approaches involve strategies such as drug modification (chemical or physical), encapsulation of drugsinside polymeric or lipidic vesicles, or reducing the size of particle of drug. NDDS not only addressing the drawbacks of conventional delivery systems but also help mitigate the pharmacological and biological limitations of existing medications, enhancing their therapeutic efficacy and safety.[5]

Novel Anti-Hyper lipidemic Drug Delivery Methods

In order to overcome the shortcomings of different drugs and their traditional dose forms, scientists have worked hard over the last ten years to create and assess innovative drug delivery methods. As a result, several innovative drug delivery methods have emerged.[5] Patients undergoing long-term treatment for hyperlipidaemia predominantly rely on oral dosage forms. However, approximately 60% of the drugs used to treat hyperlipidaemia exhibit poor water solubility, particularly those classified as Biopharmaceutical Classification System (BCS) Class II drugs. such medications are defined by high permeability but low solubility, with their absorption being solubility-dependent. While most of these drugs are absorbed in the digestive system of ileum, a limited absorption window of the oral route poses a significant challenge in designing effective pharmaceutical formulations for such poorly water-soluble drugs.[6] Enhancing the solubility of antihyperlipidemic medications remains a critical challenge for pharmaceutical scientists, as poor aqueous solubility often results in reduced oral bioavailability (BA). Various advanced techniques have been developed to address these challenges:

Solid Dispersions (SDs)

Solid Dispersion (SD) is defined as a mixture of a poorly water-soluble hydrophobic drug dispersed within one or more hydrophilic carriers in a solid state. This system enhances the drug's surface area, wettability, solubility, and dissolution rate, thereby improving bioavailability and absorption. Unlike liquid formulations, SD offers the advantage of a solid oral dosage form, which improves patient compliance and stability.SD has demonstrated superior performance compared to other solubility-enhancing techniques such as co-crystallization, salt formation, and micronization. Key benefits include: Prevention of drug agglomeration, Release in a fully

saturated state for optimal absorption, Enhanced wettability and surface area of the drugSDs are typically prepared by combining a hydrophobic drug with water-soluble polymers or carriers using various techniques, including: Solvent evaporation, melt extrusion, Co-precipitation, Spray drying, Freeze drying, Electrospinning, Supercritical fluid technology, Kneading method. These methods facilitate the molecular dispersion of the drug within the carrier, further improving its dissolution properties.[7]

Inclusion Complexes

The entities known as inclusion complexes are made up of two or more molecules, where one of the molecules is the "host" molecule and the other is a "guest" molecule. The host cavity contains molecules or portions of molecules that are hydrophobic and can fit inside it when water is present. In general, the cyclodextrin (CD) complexes exhibit positive modifications to the guest molecule's properties, including enhanced solubility, stability, fewer adverse effects, regulation of the release profiles of lipophilic drugs, and also, an overall increase in bioavailability. By absorbing the drug molecule or its lipophilic component, the CD can create inclusion complexes with the drugs in aqueous solutions. core cavity where water molecules in an intrinsically unflavoured state occupy the polar cyclodextrin cavity. These molecules can easily be changed by a suitable guest molecule, which is less polar compared to water. Two key components determine a cyclodextrin's capacity to form an inclusion complex with a guest molecule. Stearic, the first type, is dependent on the cyclodextrin's size with the guest molecule's size or specific important functional groups inside the guest. The guest will not fit into the cyclodextrin cavity appropriately if it is the incorrect size. The thermodynamic interactions among the system's various constituents (cyclodextrin, guest, and solvent) constitute the second crucial element. A positive net energetic driving force that draws the guest inside the cyclodextrin is necessary for a complex to develop. Cyclodextrin complexes are formed using a variety of methods like grinding, the neutralization method, slurry complexation, extrusion, kneading, melting, precipitation, and freeze-drying. Characterization of these complexes includes guest content determination, thermos analytical techniques, IR spectroscopy, X-ray powder diffraction, and SEM.[8]

Lipid-Based Drug Delivery Systems

To address the formulation challenges of drugs in the biopharmaceutics classification system (BCS)(Fig. 1) class II and IV, various approaches are being used. These include pre-dissolving the compound within an appropriate solvent and filling it into capsules or developing it as a solid solution via water-soluble polymers. These techniques may partially address the issue of drug dissolving in the GIT's aqueous condition. However, major restrictions such as drug precipitation or crystallization in the polymer matrix may remain unaddressed. The adoption of lipid-based drug delivery systems has successfully addressed these issues. Lipid-based drug delivery systems (DDS) enhance the solubilization of water-insoluble drugs, thereby improving their absorption. When a drug is dissolved within the lipid matrix, its bioavailability tends to increase. Even when the drug is suspended rather than fully dissolved, its absorption is still superior to that of conventional solid dosage forms. This improvement is attributed to the lipid matrix facilitating the wetting of hydrophobic drug particles, a process further enhanced by the presence of surfactants. Additionally, lipidic matrices may promote drug entrapment within micelles, contributing to improved solubilization. For poorly water-soluble drugs, where dissolution in water is the rate-limiting step in oral absorption, ingested lipids and their lipolytic products play a crucial role in enhancing dissolution. Recent advances in novel lipid-based drug delivery systems like liposomes, vesosomes, transferosomes, methosomes, ethosomes, ufasomes, discosomes, niosomes, virosomes, cubosomes, nanoparticles, and nanoemulsions.[9]

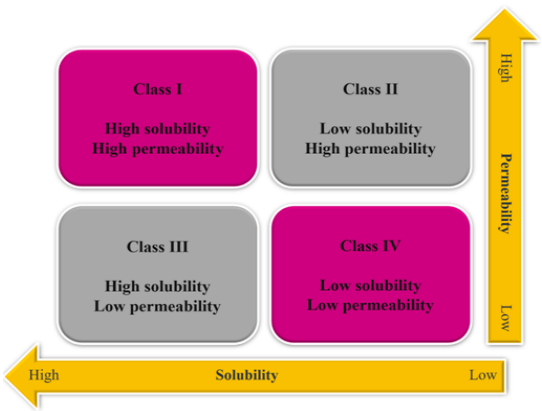

Fig. 1. Biopharmaceutics Classification System (BCS): Solubility and Permeability Classification of Drug Substances

Lipid Formulation Classification System (LFCS) Type III, self-nanoemulsifying drug delivery systems (SNEDDS) significantly enhanced the oral bioavailability of poorly soluble fenofibrate. The use of mixed glycerides facilitated drug solubility and the formation of nanosized droplets, leading to improved dissolution and potentially lymphatic transport. This resulted in substantial increases in Cmax (78%) and AUC0–t (67%), indicating a 1.7-fold improvement in bioavailability. These findings highlight LFCS Type III SNEDDS as a promising strategy for achieving more effective and consistent fenofibrate delivery.[10]

To enhance the dissolution rate and oral bioavailability of simvastatin (SIM) and glimepiride (GLM), nanosuspensions and solid self-nanoemulsifying drug delivery systems (S-SNEDDS) were developed. Nanosuspensions, prepared via liquid anti-solvent precipitation, demonstrated a 6.4-fold and 4.45-fold increase in the dissolution rate of GLM and SIM, respectively. Similarly, S-SNEDDS, formulated with Capmul MCM, Labrafil M1944CS, Tween-80, and Transcutol P followed by spray drying, also improved drug dissolution.For SIM, the enhancements were 1.76-fold for nanosuspension and 2.68-fold for S-SNEDDS, compared to unprocessed drugs.[11]

Atorvastatin nanocrystals, stabilized with poloxamer-188 and prepared by high-pressure homogenization followed by lyophilization, were developed to address its poor solubility and low bioavailability. 1 Optimization of formulation parameters yielded uniform nanocrystals (225.43 nm) with enhanced gastric solubility (~40-fold) and dissolution rate. 2 Solid-state characterization confirmed reduced drug crystallinity without incompatibility. 3 Pharmacokinetic studies in rats demonstrated a significant 2.66-fold increase in oral bioavailability. Notably, the nanocrystal formulation exhibited comparable lipid-lowering efficacy at a 50% lower dose with an improved safety profile. This study highlights atorvastatin nanocrystals as a safe, efficacious, and scalable approach for improved hyperlipidimia treatment.[12]

To improve the poor aqueous solubility and bioavailability of bezafibrate (BCS class II), formulations using PVP K30 and Cremophor ELP were prepared via solvent evaporation and electrospraying. The optimized formulation (bezafibrate/PVP K30/Cremophor ELP: 1/12/1.5, w/w/w) exhibited a 14-fold increase in solubility and a significantly faster dissolution rate (85.48% in 10 min vs. 4.06% for plain drug). Solid-state characterization confirmed the amorphous state of bezafibrate in the formulation without drug-excipient interactions. *in vivo* studies in rats demonstrated a 5.5-fold enhancement in oral bioavailability. Furthermore, the optimized formulation showed improved antihyperlipidemic effects in Wistar rats. This research suggests that the developed formulation is a promising approach for enhancing the oral delivery and therapeutic efficacy of bezafibrate.[13]

Liposomal formulations, especially those with sodium deoxycholate (SDC), were explored to enhance oral fenofibrate bioavailability. Despite limited *in vitro* release, *in vivo* studies showed significantly improved absorption and a 5.13-fold increase in bioavailability with SDC-liposomes, suggesting alternative absorption mechanisms. SDC-liposomes outperformed conventional liposomes, highlighting the benefit of bile salts in delivering poorly soluble drugs. This work emphasizes the critical role of *in vivo* evaluations for assessing novel drug delivery systems. The findings support the potential of bile salt-containing liposomes for improving the oral delivery of lipophilic drugs like fenofibrate.[14]

Other Techniques

Additional methods include complexation, salt formation, crystal engineering, and particle size reduction. Solid dispersions (SD's) in particular, has garnered significant attention due to its ability to enhance solubility and oral bioavailability. This is evidenced by the numerous studies conducted and the approval of several USFDA-approved commercial products over the past decade.[7]

Development of QbD

Drug manufacturers traditionally rely on

standard methods to develop pharmaceutical products, making it challenging to consistently achieve desired quality and characteristics in the final product. To address these challenges, regulatory bodies such as the USFDA, EMEA, and MHRA have emphasized the adoption of the Quality by Design (QbD) approach, that ensures the improvement of products with high level quality with greater reliability and a deeper understanding of the process. The core principle of QbD is that Quality cannot be tested into products; it must be built-in or designed. A key statistical tool in the QbD framework is the Design of Experiment (DoE), which facilitates the planning of experiments involving multiple variables. DoE helps identify design spaces and corresponding response-surface models with minimal experimental runs. For instance, a central composite rotatable design (CCRD) is commonly employed to explore the interactions between critical process parameters (CPPs) and critical material attributes (CMAs) with their impact on critical quality attributes (CQAs).[15] Recently, the systematic approach of 'Formulation by Design' (FbD), grounded in the principles of DoE and QbD, has emerged as a logical framework for understand-ding potential interactions among variables. FbD enables finding the most effective formulation with reduced time, effort, and development costs. The FbD approach includes several critical steps:

- Developing the Target Product Profile for Quality (QTPP).
- Recognizing CPPs, CMAs, and CQAs through screening as well as evaluation of risk.
- Optimizing evaluation of data by employing DoE.
- Modelling followed by identifying optimal solutions using response surface methodology (RSM).

Significant risk variables for CPPs and CMAs are identified through risk assessment tools like Failure Mode as well as Effect Analysis (FMEA) and also Ishikawa diagrams. This process aids in the development of a design space and the establishment of a robust control strategy approach, promoting continuous improvement and ensuring consistent product quality.[16]

Quality by Design (QbD)

Quality by Design (QbD) is a rigorousmethod of developing and producing pharmaceuticals that places a strong emphasis on comprehending and controlling procedures. Rooted in robust scientific principles and proactive risk management, Quality by Design (QbD) is a strategy endorsed by regulatory authorities such as the FDA and EMA, as well as the International Council for Harmonisation (ICH). QbD aims to guarantee drug quality by establishing manufacturing processes that reliably meet predefined quality attributes.[17] The key elements of QbD are illustrated in Figure 2.

Elements of QbD

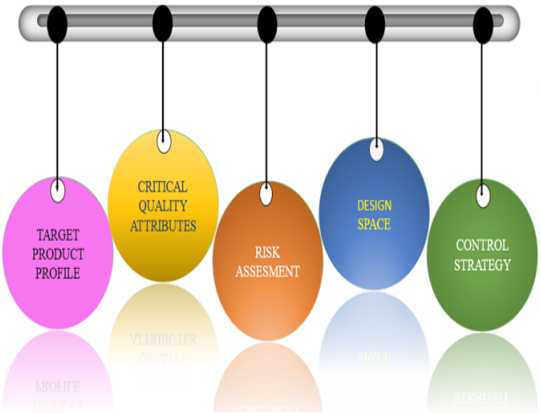

Fig. 2. Key Elements of Quality by Design (QbD) Framework in Pharmaceutical Development

Quality Target Product Profile (QTPP)

Quality Target Product Profile serves as a forward-thinking summary which outlines the expected quality attributes of a medicinal item to assure its stability as well as effectiveness. It addresses issues such as the dose form, route of administration, strength, and release or delivery characteristics.

The QTPP guides the selection of Critical Quality Attributes (CQAs) that are essential for ensuring the stability and efficacy of antihyperlipidemicnano-formulations. These CQAs are evaluated using the following physical and chemical criteria mentioned in the Table 1.

Table 1: Evaluation of CQAs

CQA	Assessment Method	Relevance to Stability/Efficacy
Particle Size	Dynamic Light Scattering (DLS) (e.g., Malvern Zetasizer), Transmission EM, SEM, AFM	Affects drug absorption, release rate, biodistribution, and stability; nano-formulations require specific size ranges for optimal targeting and reduced clearance
Zeta Potential	Electrophoretic Light Scattering (e.g., Malvern Zetasizer)	Indicates particle stability; high zeta potential prevents aggregation and enhances circulation time
Polydispersity Index	DLS	Reflects size uniformity; a narrow PDI ensures consistent drug release and pharmacokinetic behaviour
Drug Release Rate	*In vitro* dissolution studies using various media (e.g., simulated gastric/intestinal fluids)	Determines the rate at which the drug is released from the formulation; controlled release profiles are crucial for sustained therapeutic effect and minimizing side effects
Encapsulation Efficiency	UV-Vis Spectrophotometry, HPLC	Measures the amount of drug incorporated into the nano-formulation; high EE is necessary for dose accuracy and therapeutic efficacy
Bioavailability	Pharmacokinetic studies in animal models or humans (e.g., LC-MS/MS analysis of plasma samples)	Determines the rate and extent of drug absorption and availability at the target site; nano-formulations aim to enhance bioavailability by overcoming barriers like poor solubility and first-pass metabolism
Stability	Stability studies under various conditions (temperature, humidity) with periodic assessment of particle size, drug release, etc.	Evaluates the physical and chemical stability of the formulation during storage and in vivo; ensures that the product maintains its quality and therapeutic effect throughout its shelf life. Aligned with ICH guidelines for stability testing

Critical Quality Attributes (CQAs)

Critical Quality Attributes are physical, chemical, biological, microbiological, etc., characteristics that need to be managed in order to ensure the product's quality. Derived from the QTPP, they are essential for evaluating the level of quality of the finished product.

Risk Management

Risk management is the identification and evaluation of risks associated with the quality of the drug product. It is a risk control strategy to mitigate identified risks. FMEA (Failure Mode and Effects Analysis), Risk priority numbers and fishbone diagrams (represented in Fig. 3) are some of the risk management tools used. An essential part of Quality by Design (QbD) in pharmaceutical development, is risk assessment. It entails locating, evaluating, and reducing possible risks related to the creation and production of pharmaceuticals.

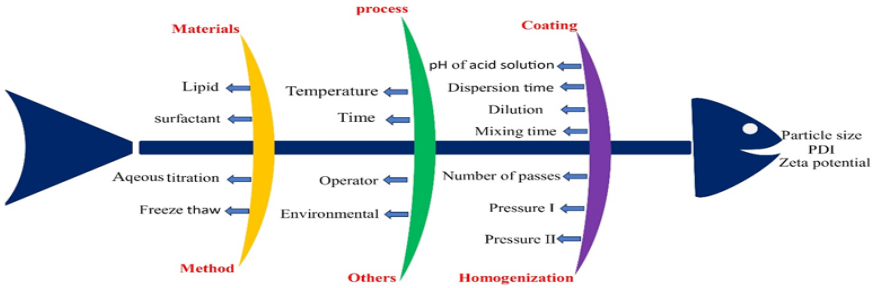

Fig. 3. Ishikawa (Fishbone) Diagram Representing Critical Factors Affecting Particle Size, PDI, and Zeta Potential in Pharmaceutical Formulation

Design space

The term design space refers tothe multifaceted combination and interplay with inputs factors (for example, material characteristics) and procedure variables that have been proved can give quality certification. Operating inside the layout area isn't considered as an alteration; moving outside of the design space is considered a change, whichtypically initiates a regulatory post-approval change process.

Control strategy

A control strategy is a planned set of measures based on recent product and method knowledge that are meant to ensurequality of the product and method performance. It includes controls for raw materials, in-process controls, and end product standards.

Implementation of QbD:

Product Development

Preformulation Studies: This involves characterizing the drug's physical and chemical properties.

Formulation Design: This comprises creating a formulation that delivers the active pharmaceutical ingredient efficiently and consistently.

Process Development: This includes designing and optimizing the production process to assure product quality.

Analytical Methods Development

Method Selection: the selection of appropriate analytical procedures to measure CQAs.

Validation: This is the process of confirming that procedures are appropriate for their intended use and can consistently provide accurate results.

Process Analysis Technology (PAT)

Real-time Monitoring: This entails using tools and procedures to monitor the process in real time, maintaining constant quality throughout production.

Data Analysis: The use of statistical methods to examine data and make sound conclusions.

Benefits of QbD:

- Enhanced Product Understanding
- Improved Efficiency
- Regulatory Flexibility Risk Reduction
- Continuous Improvement

Regulatory Perspective:

FDA Guidance: The FDA issues guidance documents outlining the concepts and expectations for incorporating QbD into drug development and production.

ICH Guidelines: Q8 (Pharmaceutical Development), Q9 (Quality Risk Management), and Q10 (Pharmaceutical Quality System).[18]

Lipid type and content

Lipid-based nanocarriers include liposomes, self-nano and micro emulsifying drug delivery systems, nano emulsions, and nano capsules (Fig.4). One thing unites them all: their formulations may be modified to fit the needs and attributes of the oral route. In fact, the majority of the oils and fats used to make these nanocarriers come from dietary sources, which support oral bioavailability and biological degradation. Additionally, nanocarriers made from lipids may be modified to interact with particular intestinal cell types, enhancing the effectiveness of drug release. But administrating drugs orally using nanocarriers made from lipids is difficult because they must occupy the hostiledigestive region and several chemical and physical obstacles. Among these obstacles are the gastrointestinal fluids, which contain ions and digestive enzymes and have extremely high and fluctuating pH levels.[19]

Challenges in lipid-based nanoparticles

The stomach is higher in acidity (pH level 1-2.5), while the pH of the intestine values of 7 to 8.The stomach exhibits high acidity (pH 1-2.5), whereas the intestine has a more neutral to slightly alkaline pH (7-8). Most nanocarriers have groups that are ionizable upon its surfaces; therefore, pH levels close to their isoelectric point would decrease or even remove their surface charge.Since many nanocarriers possess ionizable surface groups, pH values near their isoelectric point can diminish or eliminate their surface charge.

Additionally, the concentration of salts and minerals in physiological fluids differs, which could adversely effect on the surface charge of nanocarriers. Furthermore, variations in the concentration of salts and minerals throughout the gastrointestinal tract can adversely affect the surface charge of nanocarriers, potentially leading to destabilization and aggregation in physiological fluids, which may result in aggregation in physiological fluids and removed as it was merged into the above point. These challenges are overcome by formulating antilipidemic through QbDwhich was mentioned in Table 2. These challenges can be addressed when formulating antilipidemic drugs using QbD principles.[20]

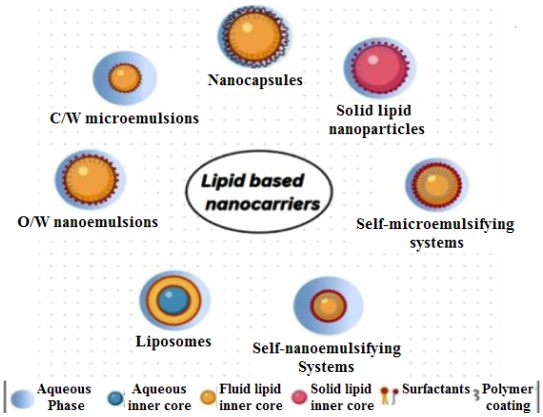

Fig. 4. Types of Lipid-Based Nanocarriers

Table 2: QbD-Based Formulations and Experimental Designs of Anti-Hyperlipidemic Drugs

Drug	Formulation	Polymer/lipids	Method of preparation	Experimental Design	CMAs/CPPs	CQAs	Application	Ref.
Simvastatin	Liposomes	A mixture of DPPC and MPEG-2000-DSPE, CHO	film hydration method	An experimental design with 5 factors at 3 levels and 3 central points	Concentration of cholesterol (mM), proportion of PEG (%), cryoprotectant to phospholipid molar ratio, freezing conditions before lyophilisation, and number of extrusions through 100 nm polycarbonate. membranes	PS, %EE, change in phospholipid transition temperature (◦C), and residual moisture glass transition temperature,	The most important process variable for the quality of the finished product was the quantity of extrusions through polycarbonate membranes., the primary drying time However, out of all the response variables were most impacted by the cholesterol content of the input components	(Porfire *et al.,* 2017)[21]
Pravastatin	Liposomes	DPPC and Na -salt; MPEG-2000-DSPE	freeze-drying process	D-optimal design	Lyoprotectant type, temperature of the shelf and rate of freezing during primary drying and annealing	PS, ZP, residual water content, the macroscopic cake appearance, the glass transition temperature, the primary drying time	The formulation's size and zeta potential were adversely affected by the freezing rate The appearance of the cake and the amount of residual moisture were negatively impactedby the temperature of the product during the beginning of drying The kind of lyoprotectant and the rate of freezing significantly affected the glass transition temperature	(Sylvester *et al.,* 2016)[22]
Fenofibrate	NLC	Compritol® 888 ATO/Precirol® ATO 5	Hot-Melt Extrusion	Plackett-Burman Design (PBD)	Liquid addition area, barrel temperature, screw speed, and drug, lipid, and surfactant	PS,ZP, PDI, %EE	The parameters under study were found to have a significant impact on zeta potential, droplet size, PDI, and trapping efficiency	(Patil *et al.,* 2015)[23]
Simvastatin	Lipid nanocapsule	Labrafaclipophile	phase-inversion method	D-optimal design	concentrations Surfactant, oil, and salted water.	PS,PDI, %DR	The average particle diameter was affectedby the proportion of oil to surfactant. Particle size and the PDI both significantly dropped. when the surfactant concentration was raised. Drug release was significantly impacted by the concentration of the surfactant	(Safwat *et al.,* 2016)[24]
Liposomes	Thermo sensitive liposomes	DPPC, DSPC, and DSPE-PEG3000	thin-film hydration method	two-level 3-factor full factorial design	preparation of the mixture, dissolution of the lipids, hydration medium, and the method of the stabilisation process	zeta potential, particle size, and morphology of the liposomes	Thermosensitive liposomes are vesicles used as drug delivery systems that release the active pharmaceutical ingredient in atargeted way at~40–42 ◦C, i.e., in localized hyperthermia	(Dobó, Dorina Gabriella, *et al.,* 2022)[25]

Manufacturing Process

Preparation methods Several intriguing chemical techniques have been developedin order to produce nanoparticles. Optimizing the energy requirements of the synthesis techniques, along with the process energy limitations, is one of the most important and challenging aspects of selecting a green approach. With minor modifications to established methodologies, several conventional physical techniques have been refined for the controlled production of nanoparticles. Given the inherent tendency of solid lipids to degrade and coalesce, the stability of SLNs (solid lipid nanoparticles) and NLCs (nanostructured lipid carriers), as detailed in Table 3: Overview of Drug Formulation and Methods, is a significant consideration that primarily relies on the physicochemical stability of the solid lipid in the form of their nanoparticles. Vesicle size, zeta potential, drug content, appearance, and viscosity variations can all be monitored to assess the physical stability of SLNs.[25]

Table 3: Overview of Drug Formulations and Methods

Formulation	Drug	Method	Mechanism of action	Outcomes	Refe.
Chitosan	Atorvastatin calcium	Solvent evaporation	polymer dispersion in a volatile solvent and emulsification in an aqueous phase. The solvent is then heated to a high temperature and evaporated	Efficient carrier for a controlled medication delivery system	Bathool *et al.,* (2012)[26]
Nanoparticles	Chitosan	Rotary evaporation and spray drying	spray dryer works by rapidly atomizing a liquid feed into tiny droplets using a nozzle or spinning disk, then exposing these droplets to a hot air stream which causes rapid evaporation of the solvent, leaving behind a dry powder; while a rotary evaporator uses a rotating flask under vacuum to efficiently evaporate solvents from a liquid sample by heating it at a low boiling point, pressure drop	Non-toxic chitosan nanoparticles can help reduce body weight growth. as well as serum cholesterol levels	Zhang *et al.,* (2011)[27]
SLN	Fenofibrate	Hot HPH	severe cavitations as a result of the valve's significant pressure drop		(Hanafy *et al.,* 2007)[28]
	Lovastatin Improved oral bioavailability	Hot homogenization -ultrasonication	Intense cavitations brought on by the significant pressure drop across the valve and the development and implosive collapse of bubbles as a result of cavitation, which is the process by which bubbles form, expand, and implosively collapse in a liquid	Improved bioavailability and first-pass metabolism preventing	(Suresh *et al.,* 2007)[29]
NLC	Lovastatin	Probe sonicator	converting electrical energy into mechanical vibrations using a piezoelectric transducer, which then transmits high-frequency sound waves through a probe directly into a liquid sample	controlled drug release	(Chen *et al.,* 2010)[30]
Simvastatin-loaded lipid nanoparticles	Simvastatin	Emulsification solvent evaporation technology	Precipitation is the result of globule emulsification (or diffusion) followed by evaporation	A promising delivery method to improve simvastatin's oral bioavailability	Zhang *et al.,* (2010)[31]
Lipid nano capsule	Simvastatin	Phase-inversion method	As the temperature rises, the o/w transitional emulsion spontaneously inverts to w/o	Drug release was significantly impacted by the concentration of the surfactant	(Safwat *et al.,* 2016)[24]

Characterization:

Average particle size: The ideal particle size range for oral delivery of antihyperlipidemicnano formulations is often cited as 100-200 nm, although the actual range can vary from 10 nm to 500 nm. Smaller particles (10-50 nm) tend to be absorbed more rapidly, but may also be cleared more quickly by the liver. Larger particles (>200 nm) may have slower release profiles and be more stable, which may lead to long-lasting therapeutic effects. They may also be less susceptible to rapid clearance by the kidneys. The bioavailability of smaller particles is often higher due to their larger surface area and improved drug absorption. However, smaller particles may be more prone to aggregation or instability during gastrointestinal transit or systemic circulation.[32] Larger particles can offer controlled release characteristics and increased resistance to enzymatic breakdown, potentially leading to more sustained therapeutic benefits. Since particle size affects drug absorption, stability, and therapeutic efficacy, it is essential to analyze it when creating antihyperlipidemic oral Nano formulations. For this, a number of analytical methods are frequently used:

Dynamic Light Scattering (DLS): DLS measures the Brownian motion of suspended particles to ascertain their size distribution.Its quick analysis and compatibility with submicron-sized nanoparticles contribute to its popularity.[33]

Electron Microscopy (EM): Techniques like Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) allow for direct examination of particle size and form and provide images with a high resolution. These processes are essential for confirming the shape and size of particles obtained by other means.[34]

Atomic Force Microscopy (AFM): Three-dimensional surfaces profiles of nanoparticles, are provided via AFM offering detailed information on particle size and morphology at the nanometer scale. It's advantageous for analysing particles in their native state without extensive sample preparation.[35]

Nanoparticle Distribution: Nanoparticle dispersion refers to the degree to which the medication is distributed throughout the body after oral delivery. Several factors influence this distribution.

Surface Charge: Nanoparticles with a neutral or slightly negative surface charge tend to circulate more efficiently throughout the body and interact less with blood components, the likelihood of rapid clearance from the liver or spleen. In contrast, nanoparticles with a positive charge may experience faster aggregation or clearance, although they may enhance cellular absorption.[36]

Polydispersity Index (PDI): The particle size distribution of nanoparticles obtained via photon association spectroscopic study is defined by the polydispersity index, a dimensionless number that is extrapolated from the autocorrelation function. It ranges from 0.01 for monodispersed particles to values typically between 0.5 and 0.7, with samples with very broad size distribution having polydispersity index values greater than 0.7.[37]

Pharmacokinetic Behaviour: After being administered, the particles travel through the bloodstream, preferentially reaching the lungs, liver, and spleen, which are responsible for processing and eliminating nanoparticles. If nanoparticles are designed for specific targeting using ligands or surface modifications, they can more effectively reach the intended tissue. The pharmacokinetic behaviour of nano formulations is significantly affected by size of the particle, which also affects the profiles of excretion, metabolism, distribution, and absorption (ADME). Studies show that increased effect of permeability along with retention (EPR) could possibly be used to target tumors with nanoparticles smaller than 600 nm. However, renal filtration may quickly remove particles smaller than 10 nm, shortening their duration in circulation. The biodistribution and molecular absorption of nanoparticles are also impacted by its size. According to studies, smaller particles are more probable to enter the alveolar space, whereas nanoparticles with sizes ranging from 1 and 5 µm often settle within bronchioles. Furthermore,nanoparticle interactions with biological systems are largely determined by their surface characteristics and charge, which are influenced by particle size.[38]

Zeta Potential: The potential of zeta reflects the energy of nanoparticles within a dispersed medium. It measures the suspended particles electrophoretic movement within a colloid. This technique assesses particle velocity in an

electric field by illuminating the sample with a laser. The electrical charge that exists at the double-layer interface is known as the zeta potential.When an electric field is used, particles move due to both Brownian motion and the attractive or repulsive forces acting on them. The formulation becomes more stable if the force that repels outweighs the force of attraction. Through compressing the electric double layer, high valency ions and stronger ions can lower the zeta potential. Several studies have highlighted that the pH level and the amount of hydrogen ions in the liquid solution significantly affect its particle charge. In an acidic environment with lower pH (more hydrogen ions), more positive charge will be added to the particles, and vice versa. Understanding the stability, surface charge, and interactions of Nano formulations in biological systems requires measuring their zeta potential.[39]

For this, a number of sophisticated tools are used:

Malvern Panalytical Zeta sizer Series: These devices measure the zeta potential of nanoparticles using methods like electrophoretic resolution nanoparticle characterization. light scattering. Their accuracy and dependability make them popular.[40]

Tunable Resistive Pulse Sensing (TRPS): allows for single-particle analysis that measures both size and zeta potential, enabling high-resolution nanoparticle characterization. Devices like Izon Science's Exoid are known for their high efficacy in this application.[41]

Scanning electron microscopy of the surface morphology (SEM)

Scanning electron microscopy was used to analyze the surface structure of Pure ZT and the thawed ZT-SLN mixture (SEM S-3700, Hitachi, Japan). Samples were put on a brass rod and lightly covered in gold to make them conductive to electricity. SEM pictures were taken at a constant voltage.[42]

Transmission electron microscopy:

One essential method for examining the morphology of oral Nano formulationsfor examining antihyperlipidemic oral nano formulations is transmission. Electron Microscopy (TEM). To better understand how nanoparticles behave in biological systems, provides high-resolution images that offer crucial information about their size, shape, and surface properties.[43]

TEM Sample Preparation:

Dilution and Deposition: Usually, a dip of the diluted nano formulation is used to a copper grid that has been covered with a carbon film. During imaging, the nanoparticles are supported by this thin coating. Drying: To ensure that the nanoparticles are sufficiently attached to the grid without experiencing severe aggregation, excess material is carefully removed, and the grid is left to air dry.

Imaging Method: After preparation, the specimen is positioned inside the TEM and subjected to an electron beam with excellent resolution. Nanoparticle morphology may be observed at the nanoscale and the comprehensive pictures created by the interaction of the electrons with the material.[44]

Microscopy of Atomic Force

Atomic Force microscopy was utilized to examine the morphology and form of the nanoparticles. After being diluted 500 times, the nanoparticles were sprinkled onto the slide and allowed to air-dried at a room temperature. Next choosing the contact mode,repulsive forces were used to capture images.[35]

Entrapment Efficiency

The entrapment efficiency was determined using a displayed typical curve for varying Qu values in phosphate-buffered saline. The amount of curcumin trapped in the nanoparticles was computed using the formula that follows to measure the encapsulation efficiency (EE) for the produced solid lipid nanoparticles (SLNs):

$$Entrapment\ Efficiency\ (EE) = \frac{Amount\ of\ drug\ in\ nanoparticles}{Total\ amount\ of\ drug\ introduced} \times 100$$

The nanoparticles (NPs) were centrifuged for 30 min at 15,000 rpm to assess drug entrapment efficiency (EE). A UV-1700 Pharma Spec, SHIMADZU UV-Vis absorption spectrometer was then employed to evaluate the supernatant at 275 nm and drug concentration within the supernatant was determined using the Qu standard curve, allowing for the calculation of the EE.[45]

Drug Content of Nanoparticles

One important element influencing the particle size in nano formulations is the drug content, or drug-loading capacity, which in turn impacts the pharmacokinetic behaviour and therapeutic efficacy of antihyperlipidemic drugs. Depending on the formulation technique and materials utilized, research suggests that a higher drug-loading content may affect particle size. To determi1ne the drug content in the nanoparticles, 10 mg of prepared nanoparticles is mixed with 100 mL of a 10% methanol-water solution. One millilitre of this solution is then pipetted into 10 mL of the mixture, and the results are analyzed using UV spectroscopy at 246 nm.[46]

***In vivo* Stability:** *In vivo* stability refers to the ability of antihyperlipidemic oral nano formulations to remain stable, effective, and consistent after being administered to a living organism (such as a human or animal). This stability is crucial to achieving and maintaining the intended therapeutic effects of antihyperlipidemic medications, which are used to manage high cholesterol or lipid levels. The therapeutic efficacy of antihyperlipidemic medications depends on the stability of their Nano formulations *in vivo*. A number of strategies have been investigated to improve this stability;

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs): Are examples of lipid-based nanoparticles (LNPs), which have been employed to increase the stability as well as bioavailability of antihyperlipidemic medications. For instance, NLCs filled with simvastatin showed increased bioavailability, indicating better stability inin vivo. The discovery of the oral small-molecule PCSK9 inhibitor, NYX-PCSK9i, presents a promising avenue for cholesterol lowering. Studies in hyper lipidemic mice demonstrated significant reductions in total cholesterol (up to 57%) through disruption of PCSK9-LDLR interaction. Notably, combination therapy with atorvastatin further enhanced this effect (up to 65%). NYX-PCSK9i exhibited good tolerability in mice, promoting hepatic LDLR levels and fecal cholesterol excretion despite upregulating hepatic PCSK9 transcription. This suggests a post-transcriptional mechanism and positions NYX-PCSK9i as a potentially cost-effective oral alternative to injectable PCSK9 inhibitors for hypercholesterolemia and cardiovascular disease prevention.[47]

Surface Modification: Stabilizers such as Glycol copolymer (PEG) or surfactants can be included in the surface of nanoparticles for improve stability and stop aggregation. Surface stabilization increased the safety and effectiveness of atorvastatin nanocrystals in the treatment of hyperlipidaemia, according to a study on the drug.

Formulation parameter optimization: Modifying elements including drug loading, lipid composition, and particle size can affect the stability of the Nano formulation. According to research, it is important to maintain an ideal particle size since too-large particles could be dangerous because they could clog tiny lung capillaries.[48]

Drug Release Kinetics: The profile of release of the antihyperlipidemic medication of its nanoparticle carrier is difficult for maintaining the drug's therapeutic level in the body. To minimize adverse effects and maximize the drug's efficacy, sustained or controlled release is preferred.[45]

First-Pass Metabolism: The medication may undergo significant degradation during its initial passage through the liver. While stability in the liver and systemic circulation remain essential, Nano formulations may avoid or mitigate the effects of the first-pass metabolism.[49]

Process Design Considerations: The preparation methods for these nanoformulations significantly impact their properties and efficacy. Common techniques include Microemulsions, solvent emulsification/evaporation, and extreme-pressure homogenization. The technique used influences particle size, encapsulation efficiency, and drug release characteristics. For instance, high-pressure homogenization of solid lipid nanoparticles (SLNs) has been shown to produce nanoparticles of suitable sizes and stability for oral distribution.[50]

Solid lipid nanoparticles (SLNs) have shown promise in enhancing the oral bioavailability of statins. Studies with simvastatin (SIM)-loaded SLNs demonstrated significant cholesterol reduction and increased drug absorption[30]. Similarly, lovastatin (LOV) bioavailability was improved using SLNs prepared by ultrasonication and high-temperature homogenization, resulting in nanoparticles of 60-119 nm and a substantial 173% increase in

relative bioavailability, with an even greater 324% rise for its active hydroxyacid form.[51]

Dudhipala, N. *et al.,* in their study aimed to enhance rosuvastatin calcium (RC) bioavailability and efficacy using solid lipid nanoparticles (SLNs) to overcome its poor absorption due to first-pass metabolism. RC-loaded SLNs were prepared via hot homogenization and ultrasonication with various lipid matrices and surfactants. Optimized SLNs, formulated with glyceryltrilaurate, exhibited a small particle size (67.21 nm), good stability, high entrapment efficiency (93.51%), and negative zeta potential. Pharmacokinetic studies demonstrated a 4.6-fold increase in RC oral bioavailability from SLNs compared to suspension. Furthermore, pharmacodynamic evaluation in hyperlipidemic rats showed a prolonged lipid-lowering effect (36 h) with SLNs compared to the suspension (24 h), indicating improved therapeutic potential.[52]

Statins, despite their widespread use, suffer from limitations like poor solubility, low drug loading, high hepatic metabolism, and instability in the stomach, leading to toxicity. To address these issues for rosuvastatin, a Quality by Design (QbD) approach was used to develop cuboidal-shaped mucoadhesive microcrystalline delivery systems (Limicubes). By employing risk assessment and a Plackett-Burman design, the study identified Monoolein, Poloxamer, and stirring speed as critical process variables significantly impacting particle size, entrapment efficiency, and drug loading. Optimized Limicubes, prepared with specific levels of these variables, exhibited desirable characteristics: an average particle size of 1.8 µm, 80.32% entrapment efficiency, and 0.93% drug loading. This QbD-enabled process demonstrates a controlled method to enhance the biopharmaceutical performance of rosuvastatin through Limicube formulation.[53]

Patel *et al.,* studied explored a combined approach of stirred media milling followed by ultrasonication for producing stable fenofibrate nanoparticles. Pre-milling reduced the drug's resistance to size reduction, enabling subsequent ultrasonication to yield even smaller particles. The resulting nanoparticles exhibited excellent stability, confirmed by particle size, zeta potential, and light scattering analyses. TEM and XRD further characterized the physicochemical properties of these nanoparticles. This combined technique proves effective for generating stable nanoscalefenofibrate with potentially enhanced dissolution. The findings suggest a scalable method for improving the formulation of poorly soluble drugs.[54]

Nano vs. Micro Size

Particle size does have a significant impact on the efficacy of lipid carriers, and nano-sized carriers have a number of clear advantages over micro-sized ones, especially when it comes to antihyperlipidemicmedicationadministration.

The greater surface area to volume ratio of nanoparticles (usually 10–500 nm) results in higher medication absorption and increased solubility and bioavailability. In order to minimize off-target effects, they can also be developed to have certain release patterns, enhanced stability, and the potential for tailored administration to particular cells or tissues. These properties are essential for antihyperlipidemic medications, many of which have low bioavailability and poor water solubility. Although micro-sized particles may provide certain economic and preparatory benefits, they often exhibit worse absorption, restricted targeting efficacy, and significant challenges related to stability and sedimentation.

Preparation Methods and Particle Size

Preparation methods play a key role in determining the resulting particle size. This review highlights methods such as hot-melt extrusion, phase-inversion, and solvent evaporation for producing lipid-based nanocarriers. Achieving the desired nano-scale size, critical for the formulations' therapeutic efficacy, hinges on the optimization of these preparation techniques.

Economic and Time Considerations

Economic savings and preparation time are undoubtedly important factors in pharmaceutical development. However, for poorly soluble drugs such as many antihyperlipidemics, the use of nano-formulations offers significant advantages in terms of enhanced efficacy and reduced toxicity. These benefits frequently outweigh the potentially higher costs and longer preparation times associated with nano-formulations. It's also important to note that the Quality by Design (QbD) approach, as discussed in this review, has the potential to streamline

formulation and manufacturing, leading to greater efficiency and cost-effectiveness over time.

Approved marketed Nano antihyperlipidemics-Fenofibrate-Nanocrystal Tricor (Abbvie)-Hyperlipidemia 2004.[55]

CONCLUSION

The goal of Quality by Design is to create better oral antilipidemicnano formulations that overcome the limitations of conventional lipid-lowering therapies. Pharmacological therapies combined with lifestyle modifications are used to treat hyperlipidaemia and its associated risks of cardiovascular disease. It includes, Low medication exposure, the need for repeated doses, and significant enzymatic breakdown in the body during drug administration are problems with many conventional drug administration techniques. Lipid nanoparticles in solid form (SLNs) and self-nano emulsion administration methods (SNEDDS), and inclusion complexes are examples of nanotechnology-based drug delivery systems that address pharmaceutical challenges by enhancingdrug dissolution and penetration in biological systems. The methodical formulation changes made possible by the QbD technique yields high-quality medications that demonstrate reliable therapeutic efficacy. Stable, efficient formulations that increase drug availability result from the combination of critical process parameters, QbD risk assessment, and essential quality principles. However, several critical issues still affect gastrointestinal stability, aggregation formation, and the need for regulatory compliance" for smoother transition. It is now necessary for research to devote resources to creating novel nano formulation techniques and testing procedures that satisfy all clinical compliance requirements. The management of hyperlipidaemia is becoming more effective because of the emergence of QbD-based nano formulations, leading to improved patient adherence and more efficient medication administration while minimizing adverse effects.

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Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Sreedhar, R.; Kumar, V. S.; BhaskaranPillai, A. K.; Mangalathillam, S., *Adv Pharm Bull.*, **2019**, *9*, 271–280.
2. Dayar, E.; Pechanova, O., *Biomedicines.*, **2022**, *10*, 1090.
3. Rizvi, S. Z. H.; Shah, F. A.; Khan, N.; Muhammad, I.; Ali, K. H.; Ansari, M. M.; Din, F. U.; Qureshi, O. S.; Kim, K.-W.; Choe, Y.-H.; Kim, J.-K.; Zeb, A., *International Journal of Pharmaceutics.*, **2019**, *560*, 136–143.
4. Sharma, K.; Kumar, K.; Mishra, N., *Drug Delivery.,* **2016**, *23*, 684–699.
5. Mathur, M.; Kusum Devi, V., *Journal of Drug Targeting.*, **2016**, *24*, 916–926.
6. Singh, P.; Nanda, A., *Int J App Pharm.*, **2023**, 23–31.
7. Tran, P.; Pyo, Y.-C.; Kim, D.-H.; Lee, S.-E.; Kim, J.-K.; Park, J.-S., *Pharmaceutics.,* **2019**, *11*, 132.
8. Chaudhary, V. B.; Patel, J. K., *Int. J. Pharm. Sci. Res.*, **2013**, *4*, 68–76.
9. Chime, A., *Afr. J. Pharm. Pharmacol.*, **2013**, *7*, 3034–3059.
10. Hussain, M. D.; Mohsin, K.; Alamari, R.; Ahmad, A.; Raish, M.; Alanzi, F., *IJN.*, **2016**, 2829.
11. Pandey, N. K.; Singh, S. K.; Gulati, M.; Kumar, B.; Kapoor, B.; Ghosh, D.; Kumar, R.; Khursheed, R.; Awasthi, A.; Kuppusamy, G.; Wadhwa, S.; Satija, S.; Dureja, H.; Jain, S. K.; Chellappan, D. K.; Anand, K.; Mehta, M.; Dua, K., *Journal of Drug Delivery Science and Technology.*, **2020**, *60*, 102083.
12. Sharma, M.; Mehta, I., *Sci Rep.*, **2019**, *9*(1), 16105.
13. Sun, R.; Shen, C.; Shafique, S.; Mustapha, O.; Hussain, T.; Khan, I. U.; Mehmood, Y.; Anwer, K.; Shahzad, Y.; Yousaf, A. M., *IJN.*, **2020**, *15*, 705–715.

14. Chen, Y.; Lu, Y.; Chen, J.; Lai, J.; Sun, J.; Hu, F.; Wu, W., *International Journal of Pharmaceutics*., **2009**, *376*(1–2), 153–160.
15. Gurumukhi, V. C.; Bari, S. B., *Journal of Drug Delivery Science and Technology*., **2020**, *56*, 101545.
16. Beg, S.; Sandhu, P. S.; Batra, R. S.; Khurana, R. K.; Singh, B., *Drug Delivery*., **2015**, *22*(6), 765–784.
17. Cunha, S.; Costa, C. P.; Moreira, J. N.; Sousa Lobo, J. M.; Silva, A. C., *Nanomedicine: Nanotechnology, Biology and Medicine*., **2020**, *28*, 102206.
18. Sangshetti, J. N.; Deshpande, M.; Zaheer, Z.; Shinde, D. B.; Arote, R., *Arabian Journal of Chemistry*., **2017**, *10*, S3412–S3425.
19. Rehman, M.; Tahir, N.; Sohail, M. F.; Qadri, M. U.; Duarte, S. O. D.; Brandão, P.; Esteves, T.; Javed, I.; Fonte, P., *Pharmaceutics*., **2024**, *16*, 1376.
20. Hallan, S. S.; Sguizzato, M.; Esposito, E.; Cortesi, R., *Pharmaceutics*., **2021**, *13*, 549.
21. Porfire, A.; Muntean, D. M.; Rus, L.; Sylvester, B.; Tomu, I., *Saudi Pharmaceutical Journal*., **2017**, *25*, 981–992.
22. Sylvester, B.; Porfire, A.; Achim, M.; Rus, L.; Tomu, I., *Drug Development and Industrial Pharmacy*., **2018**, *44*, 385–397.
23. Patil, H.; Feng, X.; Ye, X.; Majumdar, S.; Repka, M. A., *AAPS J*., **2015**, *17*, 194–205.
24. Safwat, S.; Hathout, R. M.; Ishak, R. A.; Mortada, N. D., *Journal of Liposome Research*., **2017**, *27*, 1–10.
25. Dobó, D. G.; Németh, Z.; Sipos, B.; Cseh, M.; Pallagi, E.; Berkesi, D.; Kozma, G.; Kónya, Z.; Csóka, I., *Molecules*., **2022**, *27*, 1536.
26. Bathool, A.; D. Vishakante, G.; S. Khan, M.; Shivakumar, H. G., *Adv. Mater. Lett.,* **2012**, *3*, 466–470.
27. Zhang, H.; Tao, Y.; Guo, J.; Hu, Y.; Su, Z., *International Immunopharmacology*., **2011**, *11*, 457–461.
28. Hanafy, A.; Spahnlangguth, H.; Vergnault, G.; Grenier, P.; Tubicgrozdanis, M.; Lenhardt, T.; Langguth, P., *Advanced Drug Delivery Reviews*., **2007**, *59*, 419–426.
29. Suresh, G.; Manjunath, K.; Venkateswarlu, V.; Satyanarayana, V., *AAPS PharmSciTech*., **2007**, *8*, E162–E170.
30. Chen, C.-C.; Tsai, T.-H.; Huang, Z.-R.; Fang, J.-Y., *European Journal of Pharmaceutics and Biopharmaceutics*., **2010**, *74*, 474–482.
31. Zhang, Z.; Bu, H.; Gao, Z.; Huang, Y.; Gao, F.; Li, Y., *International Journal of Pharmaceutics*., **2010**, *394*, 147–153.
32. Jamous, Y. F.; Altwaijry, N. A.; Saleem, M. T. S.; Alrayes, A. F.; Albishi, S. M.; Almeshari, M. A., *Processes*., **2023**, *11*, 3039.
33. Hatami, E.; Nagesh, P. K. B.; Chowdhury, P.; Chauhan, S. C.; Jaggi, M.; Samarasinghe, A. E.; Yallapu, M. M., *Pharmaceutics*., **2018**, *10*, 111.
34. Malatesta, M., *IJMS*., **2021**, *22*, 12789.
35. Klapetek, P.; Valtr, M.; Ne◦as, D.; Salyk, O.; Dzik, P., *Nanoscale Res Lett*., **2011**, *6*, 514.
36. Ren, Y.; Qi, C.; Ruan, S.; Cao, G.; Ma, Z.; Zhang, X., *Pharmaceutics*., **2023**, *15*, 821.
37. Abd-Elghany, A. E.; El-Garhy, O.; Fatease, A. A.; Alamri, A. H.; Abdelkader, H., *Pharmaceutics*., **2024**, *16*, 763.
38. Khan, M. A.; Khan, S.; Kazi, M.; Alshehri, S. M.; Shahid, M.; Khan, S. U.; Hussain, Z.; Sohail, M.; Shafique, M.; Hamid, H. A.; Kamran, M.; Elhissi, A.; Wasim, M.; Thu, H. E., *Pharmaceutics*., **2021**, *13*, 1632.
39. Öztürk, K.; Kaplan, M.; Çalı◦, S., *International Journal of Pharmaceutics*., **2024**, *666*, 124799.
40. Ke, W.; Crist, R. M.; Clogston, J. D.; Stern, S. T.; Dobrovolskaia, M. A.; Grodzinski, P.; Jensen, M. A., *Advanced Drug Delivery Reviews*., **2022**, *191*, 114591.
41. Eldridge, J. A.; Willmott, G. R.; Anderson, W.; Vogel, R., *Journal of Colloid and Interface Science*., **2014**, *429*, 45–52.
42. Joy, D. C., *Current Opinion in Solid State and Materials Science*., **1997**, *2*, 465–468.
43. Mast, J.; Verleysen, E.; Hodoroaba, V.-D.; Kaegi, R., *Elsevier*., **2020**, 29–48.
44. Michen, B.; Geers, C.; Vanhecke, D.; Endes, C.; Rothen-Rutishauser, B.; Balog, S.; Petri-Fink, A., *Sci Rep*., **2015**, *5*, 9793.
45. Pandita, D.; Ahuja, A.; Lather, V.; Benjamin, B.; Dutta, T.; Velpandian, T.; Khar, R. K., *AAPS PharmSciTech*., **2011**, *12*, 712–722.

46. Zafar, A., *J. Oleo Sci*., **2020**, *69*, 1389–1401.
47. Suchowerska, A. K.; Stokman, G.; Palmer, J. T.; Coghlan, P. A.; Pieterman, E. J.; Keijzer, N.; Lambert, G.; Chemello, K.; Jaafar, A. K.; Parmar, J.; Yan, L.; Tong, Y.; Mu, L.; Princen, H. M. G.; Bonnar, J.; Evison, B. J., *Journal of Lipid Research*., **2022**, *63*(11), 100293.
48. Rahmanian-Devin, P.; Askari, V. R.; Sanei-Far, Z.; BaradaranRahimi, V.; Kamali, H.; Jaafari, M. R.; Golmohammadzadeh, S., *Biomedicine & Pharmacotherapy*., **2023**, *168*, 115823.
49. Milligan, J. J.; Saha, S., *Cancers*., **2022**, *14*, 1741.
50. Blanco, E.; Shen, H.; Ferrari, M., *Nat Biotechnol*., **2015**, *33*, 941–951.
51. Padhye, S. G.; Nagarsenker, M. S., *Indian J Pharm Sci*., **2013**, *75*(5), 591–598.
52. Dudhipala, N.; Veerabrahma, K., *European Journal of Pharmaceutics and Biopharmaceutics*., **2017**, *110*, 47–57.
53. Javed, M. N.; Kohli, K.; Amin, S., *AAPS PharmSciTech*., **2018**, *19*(3), 1377–1391.
54. Patel, C. M.; Chakraborty, M.; Murthy, Z. V. P., *Ultrasonics Sono Chemistry*., **2014**, *21*(3), 1100–1107.
55. Choi, Y. H.; Han, H.-K., *J. Pharm. Investig*., **2019**, *49*(1), 201.