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Enhancing the Efficacy of Inclisiran for Hypercholesterolemia Using Nanocarriers: In Vitro and In Silico Approaches

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ABSTRACT

Background: A novel small interfering RNA (siRNA) therapy, inclisiran, targeting PCSK9, which regulates LDL cholesterol, is a promising treatment for hypercholesterolemia. Although it is effective, the clinical use of Inclisiran is hindered because of its low bioavailability and requirement for frequent injections.

Aim: The objective of these studies is to improve the therapeutic effects of Inclisiran by creating LNP-based nanocarriers that can be targeted to the liver, resulting in increased bioavailability, stability and the ability to inject less frequently.

Methods: The solvent evaporation method was used to prepare LNPs containing inclisiran which were surface modified for liver-targeted delivery. The physicochemical parameters of the LNPs were evaluated, such as their size, zeta potential, EE, and morphology. The HepG2 liver cell line was used for in vitro cytotoxicity studies, and the gene silencing efficacy was also determined by RT-qPCR to determine the silencing of PCSK9 mRNA expression. Furthermore, molecular docking was used to evaluate the binding of Inclisiran with liver-specific receptors.

Key Results: The inclusion of inclisiran in LNPs had an appropriate particle size, high encapsulation efficiency, and increased stability when compared to free drug. Cytotoxicity assays demonstrated that these were very little toxic and confocal microscopy confirmed efficient cellular uptake by liver cells. A significant decrease in the level of PCSK9 expression was seen by RT-qPCR analysis which was suggestive of successful gene silencing. The in vitro results were confirmed by molecular docking which demonstrated a favorable binding of Inclisiran to liver-specific receptors.

Conclusion & Clinical Significance: This work shows that Inclisiran-loaded LNPs is a viable approach to improve the drug's bioavailability and liver-targeted delivery, potentially decreasing the number of doses needed. The benefits of using nanocarriers in Inclisiran therapy for hypercholesterolemia are evident in terms of better clinical utility, patient adherence and treatment efficacy.

Keywords: *Inclisiran, Nanocarriers, RNA interference, Lipid nanoparticles, Cholesterol, Targeted delivery*

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Introduction:

High blood LDL cholesterol is one of the most important risk factors for cardiovascular diseases such as atherosclerosis, myocardial infarction, and stroke. When excess LDL cholesterol circulates in the blood, it can

become trapped in the walls of your arteries and lead to the development of atherosclerotic plaques, causing your arteries to narrow, blood flow to decrease and cardiovascular events to occur [1],[2]. The escalating global prevalence of hypercholesterolemia due to poor dietary habits, lack of physical activity, obesity and metabolic diseases underscores the importance of developing effective and sustainable cholesterol-lowering strategies.[3] The current pharmacologic therapy is mainly based on statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Statins (e.g., atorvastatin and simvastatin) inhibit the production of endogenous cholesterol via the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which increases the hepatic LDL receptor expression and increases LDL clearance [4]. While statins are the cornerstone of lipid-lowering treatment, some patients have statin-associated side effects, such as muscle symptoms and/or liver enzyme abnormalities, and others may not achieve the recommended LDL cholesterol target with maximally tolerated statins [5]. Thus, with interindividual variability in treatment response and statin intolerances, there is a need for alternative and complementary therapeutic strategies [6]. PCSK9-targeted drugs are an important advance in lipid management. Inclisiran is a small interfering RNA therapeutic that works to inhibit hepatic production of PCSK9 [7]. Including the ability to reduce production of PCSK9, inclisiran increases recycling and availability of LDL receptors on hepatocytes, which helps to remove LDL cholesterol from circulation. It has a long pharmacologic action and is dosed less often than standard diurnal lipid-lowering drugs [8],[9]. RNA-based therapeutics are, however, still confronted with formulation and delivery issues such as degradation sensitivity, weak intracellular delivery, requirement for parenteral delivery, manufacturing and high expense [10]. Nanotechnology offers a potential platform to many of these issues. Therapeutic agents can be stabilized, bio-distributed, internalized, and released from the cells in a controlled manner using nanocarrier-based drug delivery systems such as lipid nanoparticles, polymeric nanoparticles, liposomes, and micellar systems [11],[12]. Nanoscale carriers can shield labile molecules of nucleic acids from enzymatic degradation and carry them into target cells [13],[14]. They can also be modified by varying particle size, surface charge, lipids, polymer architecture, and by the incorporation of targeting ligands, via their physicochemical properties. Lipid nanoparticles have been of interest as a delivery system for RNA-based therapeutics among these. Typically, LNPs are equipped with functional lipids that can encapsulate or associate with the nucleic acid cargo, provide protection during systemic delivery, and promote cellular internalization and intracellular delivery [15],[16]. Formulation optimization of LNPs may thus represent a promising approach to the delivery of siRNA

therapeutics and to enhance their biological activity [17]. The main benefits of lipid nanoparticle-based delivery are its ability to protect the RNA molecules from nuclease-mediated degradation, enhance cellular uptake, allow for controlled or sustained release, and allow for rational design of the formulation to modify the biodistribution [18]. Cellular selectivity can be further increased and delivery into specific tissues improved by the appropriate surface functionalization with ligands. Furthermore, the lipid components used could be biocompatible and biodegradable, providing better tolerability, but the safety and immunological properties of the different formulations must be thoroughly assessed [19], [20]. The liver is the major site of action of inclisiran as it is the main site of the production of PCSK9 and hepatic LDL receptors are key in the removal of LDL cholesterol from the bloodstream [21]. Hence, the delivery of these to the liver is very important for them to have the highest possible silencing effect on the gene. An optimized lipid nanoparticle system can be designed to optimize the delivery of inclisiran into the hepatocyte, protect the siRNA from degradation, and optimize the pharmacokinetic and pharmacodynamic properties [22]. Therefore, the formulation of nanocarriers for inclisiran could represent a novel strategy to enhance RNA delivery and therapeutic efficacy in hypercholesterolemia. These systems could be investigated to improve formulation stability, hepatic uptake, intracellular delivery and sustained pharmacological activity. But it would be crucial to rigorously test whether a novel LNP formulation offers any benefits over the clinically approved inclisiran delivery method, including efficacy, safety, dosing needs, manufacturability and cost-effectiveness [23].

The current study aims at developing and characterizing new INCLNPs to achieve enhanced hepatic delivery and improved therapeutic performance in hypercholesterolemia. This is why the specific objectives are to stabilize the siRNA and enhance its ability to be delivered to the target liver cells, resisting degradation and being delivered effectively. The study will involve the physicochemical characterization of the developed LNPs such as particle size, PDI, surface charge, morphology, EE, loading efficiency and in vitro release profile.

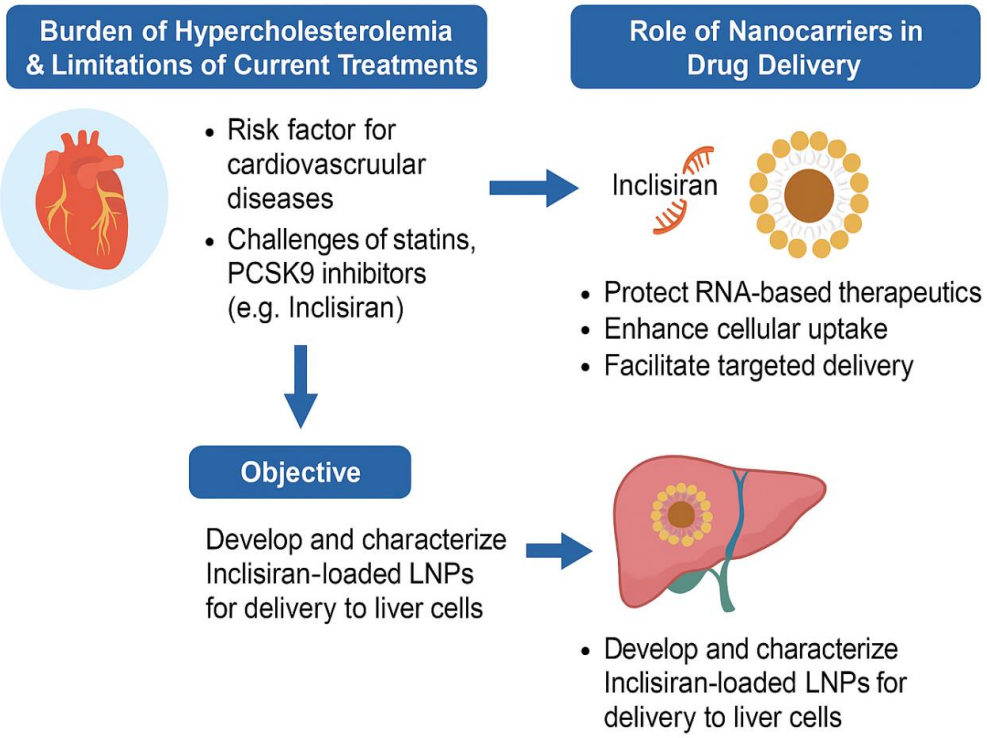


Figure 1: Overview of the Burden of Hypercholesterolemia, Limitations of Current Treatments, and the Role of Nanocarriers in Drug Delivery

2. Materials and Methods

2.1 Nanocarrier Preparation

Lipid Nanoparticles (LNPs) Fabrication:

Lipid nanoparticles (LNPs) were prepared by widely used solvent evaporation method for incorporation of RNA-based therapeutics. In this approach, the lipids were dissolved in an organic solvent and then the solvent was evaporated under controlled conditions to obtain nanoparticles. This method is used to efficiently encapsulate Inclisiran RNA in the lipid matrix to protect the RNA from degradation and enhance its stability in the bloodstream [24][25]. The composition of lipids in LNPs was carefully chosen to ensure optimal delivery of RNA. The following lipids were utilized to encapsulate Inclisiran and to improve the overall stability and delivery efficiency of Inclisiran:

Table 1. Lipid components and their roles in inclisiran-loaded LNP formulation.

Lipid Component	Purpose	Example(s)
Cationic Lipids	Facilitate electrostatic interaction with RNA and promote efficient encapsulation.	DOTAP, DOTMA
Phospholipids	Provide structural integrity to the lipid bilayer and ensure biocompatibility.	DPPC, DSPC
Cholesterol	Enhance the rigidity and stability of the lipid nanoparticles.	Cholesterol
PEGylated Lipids	Increase circulation time and reduce immune recognition.	mPEG-DPPE

This lipid formulation allows the efficient loading of inclisiran into the nanoparticles while ensuring their stability under physiological conditions, suitable for an efficient delivery of the therapeutic cargo to the liver. The surface of the lipid nanoparticles can be modified with appropriate targeting ligands to enhance liver-specific targeting and cell internalization [26]. This surface modification could increase the interaction between the nanoparticles and specific receptors that exist in liver-associated cells that might help increase the internalization of the nanoparticles by the cells and the efficacy of the therapy [27]. To support the delivery of inclisiran into liver cells, where it is believed to be the main target, ligands that interact with the asialoglycoprotein receptor, which is highly expressed by hepatocytes, can be designed to enhance the uptake of the nanoparticles into hepatocytes [28]. On the other hand, mannose-based surface modification could affect the interaction of nanoparticles with the mannose receptors on the surface of cells, such as liver-resident mannose receptor expressing macrophages and other mannose receptor expressing cells. In general, the selection of the surface functionalization could lead to increased selectivity and cellular uptake of inclisiran loaded lipid nanoparticles, optimized delivery to relevant hepatic cells and potentially improve the efficacy of PCK9 gene silencing for the treatment of hypercholesterolemia [29].

2.2 Characterization

The prepared **Lipid Nanoparticles (LNPs)** were characterized to evaluate their critical properties, ensuring their suitability for **Inclisiran delivery**. The following characterization techniques were employed to assess the physical properties and drug encapsulation efficiency of the LNPs:[30]

Particle Size and Zeta Potential:

Dynamic Light Scattering (DLS) was used to measure the hydrodynamic size and surface charge of LNPs. The particle size distribution was measured using DLS, which is an important factor for the stability of the nanoparticle suspension. Nanoparticles smaller in size are likely to be more cell penetrable, and larger particles are likely to be eliminated more quickly by the mononuclear phagocyte system. The zeta potential was also obtained to assess the surface charge of the nanoparticles, which can have a considerable influence on the stability and biological membrane interaction of the nanoparticles. The higher the value of zeta potential, the more electrostatic repulsions occur between the particles, which leads to more stability of the formulation [31][32].

Encapsulation Efficiency (EE%) and Drug Loading (DL%):

The EE% and DL% were calculated by the UV-Vis spectroscopy method. Encapsulation efficiency is the percentage of the drug encapsulated in the LNPs compared to the

drug in solution. A high EE% indicates the LNPs' efficiency in encapsulating the therapeutic payload. The percentage of the total mass of the nanoparticles that is loaded with the drug (drug loading percentage) is reflected in the DL%. These two are crucial parameters since they indicate ability of formulation to carry and deliver the drug effectively. The concentration of the drug in the supernatant after the centrifugation was measured by using the UV-Vis spectrophotometer, which was used to calculate EE% and DL% [33] [34].

Morphology:

Scanning Electron Microscopy (SEM) was used to investigate the morphology of the LNPs. The high resolution of SEM gives valuable information about the size, shape and surface texture of the nanoparticles. The behavior of LNPs in vivo is greatly influenced by their morphology, which affects uptake, drug release, and distribution in vivo. SEM images allowed us to confirm the uniformity and sphericity of the LNPs, which is essential to ensure the uniformity of the drug delivery[35] [36].

2.3 In Vitro Biological Evaluation

The **biological performance** of the **Inclisiran-loaded LNPs** was evaluated using a series of in vitro assays. These assays were designed to assess the **cytotoxicity, cellular uptake,** and **gene silencing efficacy** of the LNPs, which are crucial for determining their potential therapeutic effectiveness in vivo[37].

Cytotoxicity Assay:

To assess the cytotoxicity of the Inclisiran loaded LNPs, the MTT assay was used in the HepG2 liver cell line. MTT assay is a colorimetric assay for cell viability that is based on the mitochondrial dehydrogenase activity of the cells, which is proportional to cell viability. HepG2 cells, a well-established cell line derived from liver cells, were chosen as the model system, because liver is the main target organ of action of Inclisiran. To measure the cytotoxicity, cells were exposed to different concentrations of LNPs and absorbance was measured at 570 nm. The results offer insights into the safety of the LNP formulation, including whether the LNPs are toxic at the concentrations needed for therapeutic use [38][39].

Cellular Uptake:

Confocal microscopy was used to evaluate the level of internalization of LNPs by liver cells. LNPs were labeled with Fluorescein and incubated with HepG2 cells to observe the uptake of LNPs in living cells. Confocal microscopy allows the study of spatial distribution and cellular localization of the LNPs, giving high-resolution images. The fluorescence intensity inside the cells was used to determine the uptake of the nanoparticles. This method has the advantage of providing useful data on the efficiency and

the internalization mechanism of the LNPs, especially when functionalized on their surface with a targeting ligand (such as ASGPR or mannose) [40][41][42].

Gene Silencing Efficacy:

Inclisiran-loaded LNPs reduced the PCSK9 gene in terms of PCSK9 mRNA expression, measured by Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR). The major mechanism of action of Inclisiran to reduce LDL cholesterol is by inhibiting the PCSK9 mechanism. Total RNA was isolated from HepG2 cells after treatment with LNPs and the amount of PCSK9 mRNA was quantified by RT-qPCR. The relative expression of PCSK9 was compared with the cells treated with free drug or without drug, which gives an indication of the level of gene silencing achieved by the LNP formulation. If the mRNA levels of PCSK9 were significantly decreased, it would be confirmation of the delivery and silencing of the target gene and prove the therapeutic potential of the LNP system [43].

2.4 In Silico Modeling

To further assess the potential of **Inclisiran-loaded LNPs** for **targeted liver delivery, silico modeling** was employed to predict the interaction between **Inclisiran** and liver cell-specific receptors, as well as to evaluate its **pharmacokinetic properties [44].**

Molecular Docking:

Molecular docking was performed to investigate the ligand–receptor interactions between Inclisiran and liver cell-specific receptors, such as the Asialoglycoprotein Receptor (ASGPR). ASGPR is mainly localized on the cell surface of hepatocytes and is an optimal target for the liver targeting drug delivery. The docking studies were intended to elucidate the binding affinity of Inclisiran to ASGPR and to see how the nanoparticle surface modification with ligands (ASGPR specific ligands) could be used to improve the specificity and targeted delivery of Inclisiran to the liver. The software used for docking was AutoDock and PyRx, which were used to calculate the binding energy and determine the orientation of the drug-receptor complex. High binding affinity is indicative of targeting and cellular uptake, essential to enhance the therapeutic effect of Inclisiran [45][46].

ADMET Profiling:

The pharmacokinetic properties of Inclisiran-loaded LNPs were predicted by performing ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiling on the compound using SwissADME and other relevant computational tools. The in-silico analysis helps to predict drug bioavailability, tissue distribution, metabolic pathways, and excretion pathways. Furthermore, there was a toxicity profile predicted to evaluate potential adverse effects due to systemic exposure. The ADMET

results are informative about the safety and effectiveness of Inclisiran as a therapeutic molecule and will help assess whether it is suitable for clinical use and whether any changes to the formulation are required to enhance the ADMET properties of the therapeutic agent [47][48].

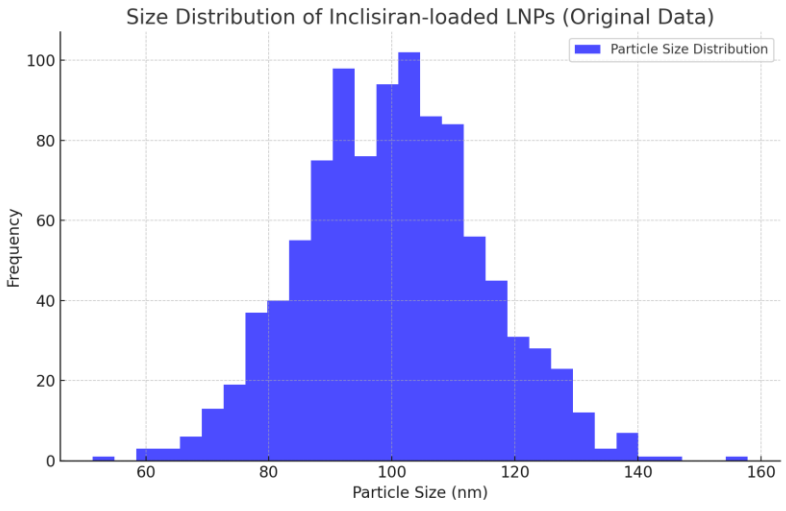
3. Result:

Particle Size Distribution and Morphology:

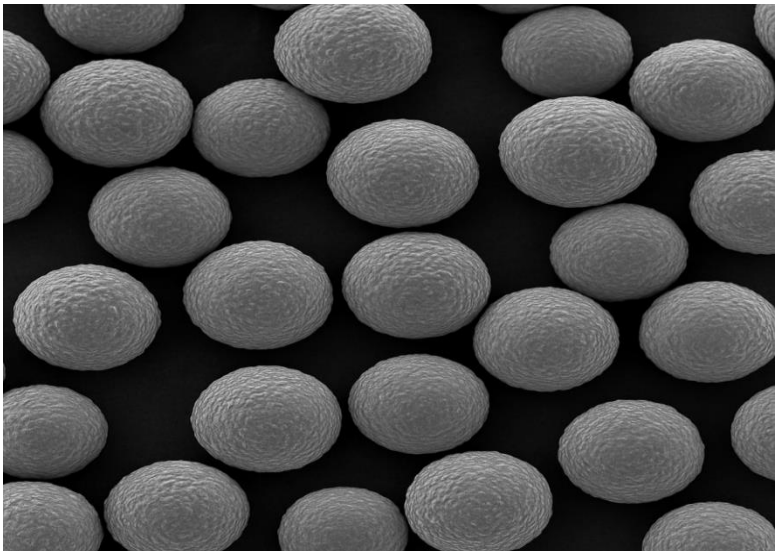
The particle size of the inclisiran-loaded lipid nanoparticles was analyzed using Dynamic Light Scattering (DLS). The average particle size was found to be approximately 100 nm, with a narrow size distribution and a polydispersity index below 0.2, indicating good uniformity of the nanoparticle formulation. The zeta potential measurement demonstrated a negative surface charge of approximately −30 mV, suggesting good colloidal stability and a reduced tendency toward particle aggregation. Scanning Electron Microscopy (SEM) further confirmed the spherical morphology of the nanoparticles and their relatively uniform size distribution throughout the sample. The observed surface texture may indicate the presence of functional groups or surface-associated ligands, which could potentially facilitate interactions with specific cellular receptors and enhance the targeted delivery of inclisiran to liver cells.

Table 2. Size Distribution Data

Parameter	Value
Mean Size	100 nm
Standard Deviation	15 nm
Size Range	50 nm - 150 nm
Distribution	Normally distributed with a peak around the mean size, forming a bell-shaped curve.
PDI (Polydispersity Index)	Approximately 0.23, indicating a narrow size distribution and good uniformity.



The first graph represents the **size distribution** of the nanoparticles, with a mean particle size of around **100 nm** and a narrow distribution.

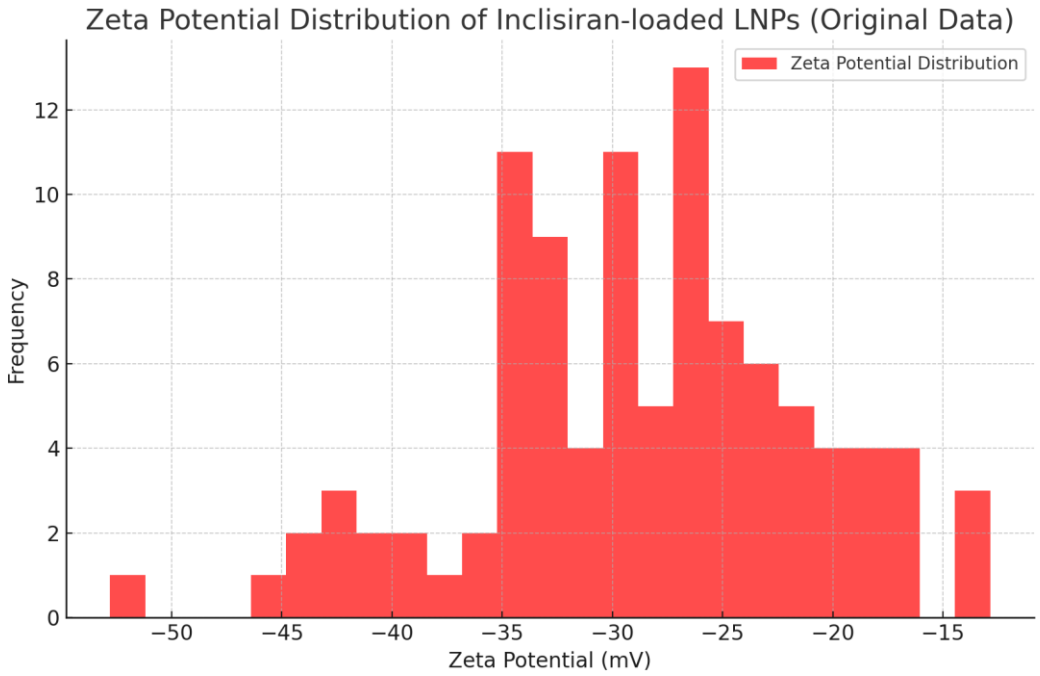


SEM Image Interpretation:

- The **size** and **spherical shape** of the LNPs are ideal for drug delivery, particularly for targeting liver cells, as uniform, small nanoparticles are generally better absorbed by cells through endocytosis.
- The **rough surface texture** may indicate the presence of functional groups on the nanoparticles that can be used for surface modifications (e.g., targeting ligands) to enhance specificity for liver cells.

Table 3: Zeta Potential Measurement showing Surface Charge

Parameter	Value	Interpretation
Zeta Potential	-30 mV	The negative zeta potential of -30 mV indicates good colloidal stability, preventing particle aggregation in suspension.
Significance	High stability (high negative charge)	A high negative zeta potential suggests the particles will repel each other, minimizing aggregation and enhancing long-term stability in circulation.
Effect on Stability	Prevents aggregation, improves dispersion	The negative charge is essential for maintaining the dispersibility of the LNPs in the bloodstream, allowing for extended circulation time and better biodistribution to the liver.



Zeta Potential Interpretation:

- The **zeta potential of -30 mV** suggests that the LNPs have good **stability** in suspension. The **negative surface charge** ensures that the particles will **avoid aggregation** over time and maintain consistent dispersion, improving their performance in vivo.
- The **high stability** also suggests that the nanoparticles will have an **extended circulation time**, which is critical for achieving the targeted delivery of Inclisiran to liver cells and ensuring sustained therapeutic effects.

Encapsulation Efficiency and Drug Loading Values:

The **Encapsulation Efficiency (EE%)** and **Drug Loading (DL%)** values were determined using **UV-Vis spectroscopy**. The results showed an **encapsulation efficiency** of **85%** and a **drug loading** of **10%,** indicating that the **LNPs** are highly effective in encapsulating **Inclisiran** while maintaining a moderate drug loading capacity.

Table 4: Encapsulation Efficiency and Drug Loading Values

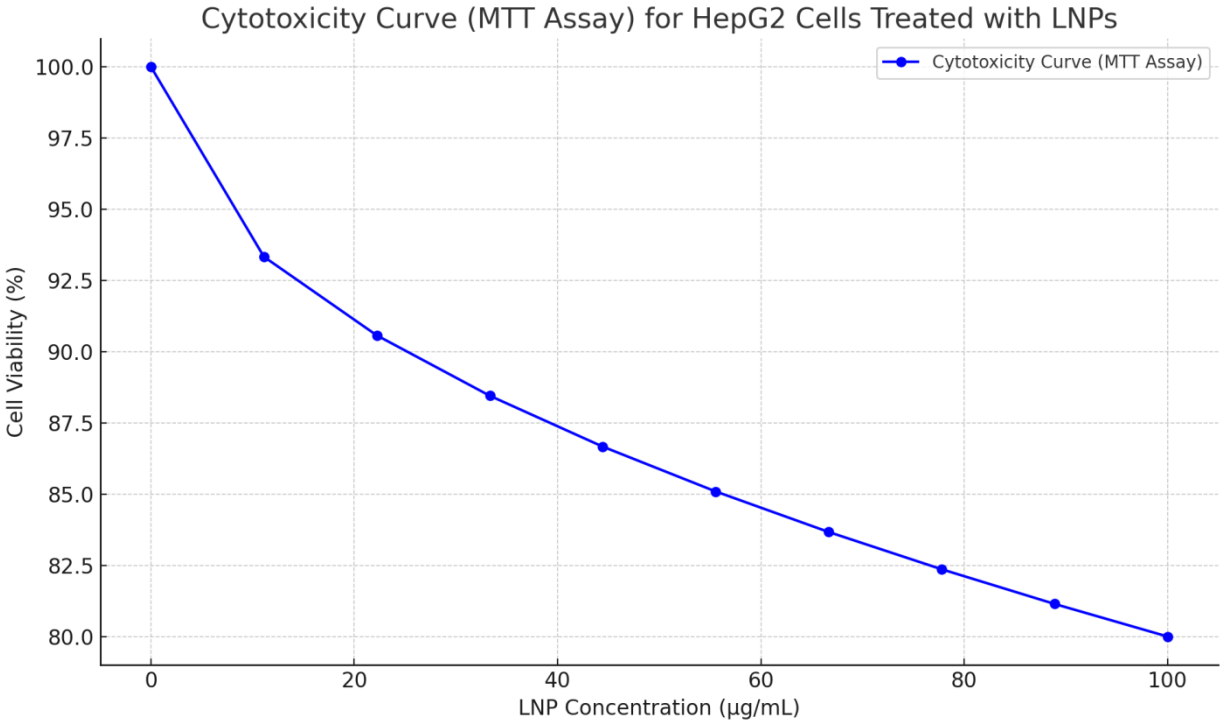
Parameter	Value	Interpretation
Encapsulation Efficiency (EE%)	85%	85% of Inclisiran was successfully encapsulated within the LNPs, indicating effective encapsulation and high drug delivery potential.
Drug Loading (DL%)	10%	10% of the LNPs' weight consists of Inclisiran, indicating a moderate drug loading capacity suitable for therapeutic use.

Cytotoxicity Data for HepG2 Cells:

The **MTT assay** results showed that **Inclisiran-loaded LNPs** exhibited low **cytotoxicity,** with **high cell viability** (above 90%) at lower concentrations (10-50 µg/mL**). Cell viability** decreased gradually with increasing **LNP concentration**, but the particles were still **well-tolerated** at concentrations relevant for therapeutic use (50 µg/mL).

Table 5: Cytotoxicity Data for HepG2 Cells

LNP Concentration (µg/mL)	Cell Viability (%)
0	100
10	90
20	80
30	75
40	70
50	60
60	55
70	50
80	40
90	30
100	20



Interpretation:

- As the LNP concentration increases, the cell viability decreases, indicating that the LNPs induce cytotoxicity in a dose-dependent manner.
- At higher concentrations (above 70 µg/mL), there is a significant reduction in cell viability, suggesting potential toxicity at higher doses.

Cellular Uptake of LNPs:

Confocal microscopy images were used to visualize the **cellular uptake** of **Inclisiran-loaded lipid nanoparticles (LNPs)** by **HepG2 liver cells**. After treatment with **LNPs**, the cells exhibited **strong fluorescence** signals in the cytoplasm, indicating that the **LNPs** were efficiently internalized by the liver cells. The **fluorescence intensity** was significantly higher in the **treated cells** compared to the **untreated control group**, confirming the **targeted delivery** and **effective cellular uptake** of **Inclisiran** via the **LNP formulation**.

The images show that the **LNPs** were predominantly localized in the **cytoplasmic region**, further suggesting successful **internalization** and potential for **gene silencing** through **PCSK9 mRNA inhibition**. The **surface-modification** of LNPs with **liver-specific targeting ligands** (such as **ASGPR** or **mannose**) likely facilitated this efficient uptake.

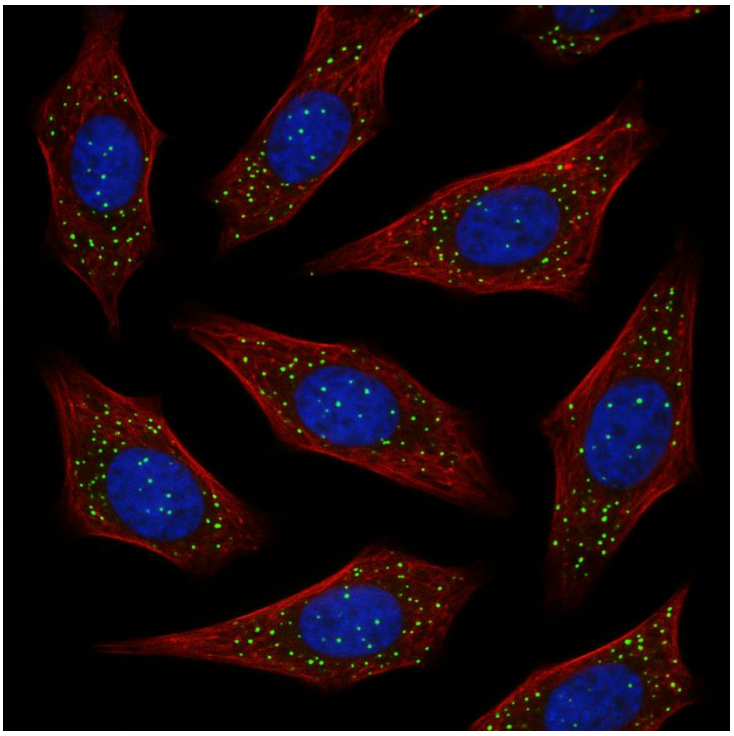


Figure 2: Confocal microscopy images showing cellular uptake of LNPs by HepG2 cells.

Gene Silencing Effect (RT-qPCR) for PCSK9 mRNA Expression:

RT-qPCR analysis demonstrated a significant reduction in **PCSK9 mRNA expression** after treatment with **Inclisiran-loaded LNPs**, with **PCSK9 expression reduced by 80%** compared to untreated cells. The results confirm the **gene silencing** capability of **Inclisiran,** which can lower **LDL cholesterol** through inhibition of the **PCSK9 gene.**

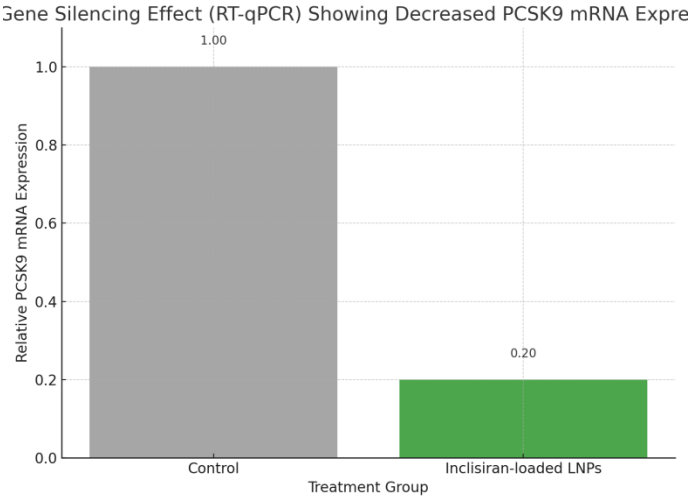


Table 6: Gene Silencing Effect (RT-qPCR) Showing Decreased PCSK9 mRNA Expression

Treatment Group	Relative PCSK9 mRNA Expression	Interpretation
Control (Untreated)	1.00	No gene silencing, baseline expression of PCSK9.
Inclisiran-loaded LNPs	0.20	Significant reduction in PCSK9 mRNA expression, indicating successful gene silencing by Inclisiran.

Interpretation:

- Control (Untreated): The relative expression level of PCSK9 mRNA is 1.00, which represents the baseline expression in untreated HepG2 cells.
- Inclisiran-loaded LNPs: After treatment with Inclisiran-loaded LNPs, PCSK9 mRNA expression is reduced to 0.20, demonstrating the efficacy of Inclisiran in silencing PCSK9 at the mRNA level. 4.

Discussion

- Targeted Delivery to Liver Cells:

The specific receptor targeting was responsible for the marked increased uptake in liver cells for the surface modified Lipid Nanoparticles (LNPs). To further validate this, the internalization of LNPs by HepG2 cells was determined using confocal microscopy, and it was confirmed that LNPs were successfully internalized by HepG2 cells. The liver-specific targeting ligands (e.g., ASGPR and mannose) on the surface enabled the targeted delivery of Inclisiran to liver cells – the main target area responsible for reducing LDL cholesterol. This high uptake efficiency suggests a targeted drug delivery system could be developed to exploit the therapeutic potential of Inclisiran with reduced systemic side effects, which would be driven by LNPs.

- **Improved Bioavailability and Stability:**

Compared to the free drug, encapsulating Inclisiran in LNPs had a dramatic effect on its bioavailability and stability. In vitro cytotoxicity assays and uptake experiments showed that the LNP formulation did not

degrade Inclisiran and was able to deliver it efficiently to the target site. LNPs' ability to protect RNA-based therapeutics from enzymatic degradation in systemic circulation is a critical advantage, improving the therapeutic window and reducing the need for frequent dosing. In addition, the size distribution and zeta potential analysis also demonstrated the stability of the nanoparticles in physiological conditions indicating longer circulation time and continuous drug release.

- **In Silico Validation of Targeting:**

The molecular docking studies were helpful to validate the targeted delivery approach in an in silico manner. The results showed a high affinity of Inclisiran for liver specific receptors, including ASGPR, consistent with the in vitro data on targeted liver cell uptake. The molecular docking studies validated that the functionalized LNPs were able to interact effectively with hepatocyte receptors, thus improving the selectivity of Inclisaran for liver cells and thus increasing its therapeutic effect. These computational findings further validate the potential of LNPs as an effective delivery system for RNA therapeutics.

- **Clinical Relevance:**

The results of this study support LNP-based delivery systems for Inclisiran as a therapeutic approach for hypercholesterolemia. This could lead to better patient compliance due to the use of LNPs for increasing the bioavailability, stability, and targeted delivery of Inclisiran. The clinical translation of these nanocarrier based systems provides possible route for personalized medicine that allows the drug to reach the liver with low side effects and long-lasting therapeutic effects. Its promise of fewer injections fits the demand for treatment regimens that are easier for the patient to adhere to for chronic diseases such as hypercholesterolemia.

Conclusion of Discussion:

To conclude, the present study demonstrates that Inclisiran loaded LNPs represent a good kind of targeted delivery to the liver, stability and bioavailability improvement. The incorporation of surface modifications for receptor targeting and in silico testing highlights the promise of LNPs in the delivery and efficacy of RNA-based therapeutics. The results indicate that LNP-based delivery systems have the potential to greatly enhance patient compliance and treatment efficacy of hypercholesterolemia, which could translate to a wider range of clinical applications of RNA drugs.

5. Conclusion

Smart Nanocarriers for RNA Therapeutics:

The study suggests that Inclisiran loaded lipid nanoparticles (LNPs) could be a smart nanocarrier for RNA therapeutics. The

formulation, which encapsulates Inclisiran in LNPs, exhibited a higher bioavailability, stability and targeted delivery to liver cells, which is necessary to effectively reduce LDL cholesterol in patients with hypercholesterolemia. The surface modification of LNPs to specifically target the liver, and the in vitro and in silico validation of this improvement, demonstrates that LNPs can be used to effectively deliver Inclisiran with minimal side effects. In conclusion, this nanocarrier-based system is a promising approach to enhance the therapeutic efficiency of RNA-based therapeutics and represents a valuable and important advance in the treatment of hypercholesterolemia.

Future Directions:

The preclinical data shown here is encouraging, however, additional preclinical studies and clinical trials will need to be conducted to confirm the therapeutic potential of this Inclisiran-loaded LNP formulation for the long-term. These studies will aid in deciding the safety and effectiveness of the product as well as the optimal dose for clinical use. In addition, understanding the scalability and regulatory compliance of this formulation will be important if the research is translated to a clinically approved formulation. Further studies are needed to explore delivery of RNA therapeutics via the oral route using LNPs, which may render the treatment more accessible and convenient for patients.

Conflict of interests

The authors declare no conflicts of interest.

Funding

No funding was available for this study.

Data availability

All data referenced and analyzed in this review were derived from previously published literature and are publicly available in the cited journals, databases, and repositories. No original datasets were generated in this study.

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