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Research Article

Resveratrol Loaded Nanostructured Lipid Carrier for Enhanced Ocular Delivery: Formulation, *In vitro* and *Ex vivo* Evaluation

Short Running Title: Nano-Lipid Carrier of resveratrol for ocular use.

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Abstract:

Resveratrol, a natural polyphenol with antioxidant, anti-inflammatory and neuroprotective properties, has limited clinical applications due to its poor aqueous solubility and low bioavailability through conventional administration routes. The current work aimed to develop and evaluate resveratrol-loaded nanostructured lipid carriers (RS-NLCs) to improve resveratrol's ocular delivery. RS-NLCs were manufactured by melt emulsification and ultrasonication, optimized for particle size (104.47 ± 13.32 nm), polydispersity index (0.394 ± 0.070), and high entrapment efficiency (85.88 ± 0.32%). *In vitro* release studies demonstrated an initial burst release followed by sustained release of resveratrol from RS-NLCs over 24 hours. Transmission electron microscopy revealed smooth, spherical RS-NLC morphology with a size of 205.88 nm. *Ex vivo* trans-corneal permeation investigation using isolated goat corneas showed significantly higher resveratrol flux (4.03 ± 0.20 μg/cm^2/h) and permeability coefficient (40.37 ± 2.02 x 10-4 cm/h) for RS-NLCs compared to resveratrol suspension. The nanostructured lipid formulation enhanced resveratrol's corneal penetration by 11 folds, suggesting RS-NLCs as a promising carrier for improved ocular delivery of resveratrol and treatment of ocular diseases associated with oxidative stress and inflammation.

Keywords: Ocular delivery, corneal permeability, antioxidant, NLC.

Introduction

Ocular drug delivery presents a significant challenge due to the complex anatomy and physiology of the eye, as well as the need for effective and targeted treatment of various ocular diseases [1–3]. Conventional eye drops often suffer from low bioavailability and rapid clearance, leading to frequent dosing and potential side effects [4]. Therefore, there is a growing interest in developing novel drug delivery systems that can improve the therapeutic efficacy of drugs administered to the eye [1,5].

Resveratrol, a natural polyphenol found in various plants such as grapes and berries, has gained considerable attention in recent years due to its antioxidant, anti-inflammatory, and neuroprotective properties [6,7]. However, its clinical application is limited by its poor aqueous solubility and low bioavailability, particularly when administered through conventional routes. Nanostructured lipid carriers (NLCs) have emerged as promising drug delivery systems for overcoming the limitations of conventional formulations [8,9]. NLCs offer advantages such as high drug loading capacity, improved stability, controlled release, and enhanced permeation across biological barriers [10–12]. These properties make NLCs particularly suitable for ocular drug delivery, where efficient penetration through the ocular barriers is crucial for therapeutic efficacy.

This study aims to develop and evaluate a resveratrol-loaded nanostructured lipid carrier (RS-NLC) for enhanced ocular delivery. The formulation will be optimized to achieve maximum drug loading efficiency and stability. *In vitro* evaluations will include assessing the release profile of resveratrol from the NLCs. Furthermore, *ex vivo* evaluation using animal eye tissue will be conducted to investigate the penetration and distribution of RS-NLCs within the ocular tissues.

The findings of this research are expected to provide valuable insights into the feasibility and potential benefits of utilizing NLCs as a carrier for enhancing the ocular delivery of resveratrol. Ultimately, such an approach holds promise for the development of more effective treatments for ocular diseases, including those associated with oxidative stress, inflammation, and aging.

Materials and Methods

Solid lipid material, glyceryl monostearate was procured from Delhi-based firm Central Drug House Ltd. Liquid Lipid, Soya oil supplied by Shiv Sales Corporation, Delhi. Resveratrol was procured from GLR Innovation, G.L.R. Scientific Co. Surfactants (Poloxamer-407 and Tween 80) were obtained from BASF (Mumbai) and CDH, respectively, New Delhi. Sodium Chloride, Calcium Chloride, potassium chloride, and sodium bicarbonate were received from Mumbai-based Lobachemie Pvt Ltd. Methanol was procured from Merck Millipore. All other solvents of GR grade were used for evaluations.

Methods

Preparation of Resveratrol loaded Nanostructured lipid carriers.

The RS-NLC prepared by melt emulsification and ultrasonication method as per the previously reported method [13]. Heat glyceryl monostearate (250 mg) and soybean oil (107 mg) in a separate vessel at 87 ºC. Add 100 mg of resveratrol (RS) and mix under magnetic stirring at 200 rpm for 5 minutes. We refer to this mixture as the oil phase. In another container, take Millipore water and heat it. Add surfactants, Tween 80 (250 mg) and Poloxamer 407 (150 mg) and mix for 10 minutes with a magnetic stirrer at 200 rpm. We refer

to this mixture as the water phase. Mix the water phase and oil phase at 700 rpm to form a primary emulsion. Subject the primary emulsion to sonication using a probe sonicator for 10 minutes with an amplitude of 70% and a 10 sec on – 10 sec off cycle. Place the resultant nanodispersion in a cold environment to convert lipid globules into lipidic nanoparticles. We call the resulting resveratrol-loaded nanoparticles RS-NLC and adjust the volume to 10.0 mL for further evaluation.

Evaluation of RS-NLC

Prepared RS NLC evaluated for particle size and PDI.

Particle size and PDI of RS-NLC

The size of the RS-NLC particles was measured using a Malvern Zetasizer, which uses Differential Light Scattering [14]. The Polydispersity Index (PDI) was also measured using the same instrument. To take measurements, 1 mL of the RS-NLC sample was poured into a disposable polystyrene cuvette, and the particle size and PDI were measured at 25°C. The measurements were conducted three times for accuracy.

Entrapment efficiency of resveratrol in RS-NLC

RS-NLCS's ability to entangle in NLC was evaluated, and its entrapment efficiency was estimated using the indirect method [15,16]. To perform this, 1.0 mL of RS-NLC sample was placed in a microcentrifuge tube and rotated at a cooling centrifuge with a temperature of 4°C and speed of 14500 rpm. After 30 minutes of centrifugation, the clear supernatant was separated and used for the subsequent procedure. A measured volume of 1.0 mL of the supernatant portion, along with 9.0 mL of methanol, was placed in another microcentrifuge tube and centrifuged again under the same conditions. The clear portion was removed, and resveratrol was estimated by the UV method at 306 nm. The entrapment of resveratrol was calculated using Equation 1.

$$\% \text{ Entrapment efficiency} = \frac{\text{Total Concentration of RS} - \text{Concentration RS in Supernatant}}{\text{Total concentration of RS in NLC}} \times 100 \quad \textbf{...(1).}$$

Transmission electron microscopy of RS-NLC

The morphology of RS-NLC was analyzed through Transmission Electron Microscopy (TEM) using negative staining [17]. In this process, a copper grid coated with carbon was used to drop the RS-NLC sample slowly. The sample was then air-dried for about 6 minutes and negative staining was performed by using 1% phosphotungstic acid. The sample was allowed to air-dry for another 4 minutes and then visualized under a transmission electron microscope.

***In vitro* release study form RS-NLC**

An *in vitro* release study of RS was conducted using the Franz diffusion cell assembly and cellulose membrane [18]. Three Franz diffusion cells, each with a capacity of 10 mL, were used for the study. Simulated tear fluid and methanol (in equal parts) were used to perform the study. Continuous warm water circulation at 38 ºC was maintained through the jacket of the Franz diffusion cells to maintain body temperature. Magnetic stirring at 200 rpm was applied to ensure uniform release and distribution of RS. After maintaining all the standard sets of conditions, RS-NLC samples were placed in the donor compartment, and samples were withdrawn after a specific duration from the sample port. Approximately 1 mL of samples were withdrawn from the sampling port, and the amount of RS was calculated using the UV- method at 306 nm. 1 mL of STF-methanol media was added to compensate for the

volume in the diffusion cell. The cumulative release of RS was calculated and plotted against time. The same experiment was performed for RS dispersion for a comparative study with RS-NLC.

***Ex vivo* study for RS-NLC**

For an *ex vivo* study, a goat corneal membrane was isolated and used. The membrane was isolated from goat eyes that were obtained from a goat eye at Khanpur Market, New Delhi. A mixture of methanol and STF was loaded into the receptor compartment, and the corneal membrane was placed between the donor and receiver compartments of the Franz diffusion cell. Similar conditions were maintained as described earlier, and the *ex vivo* study was conducted for 24 hours. At specific time intervals, 1 mL of sample was taken out from the sampling port and replaced with fresh media (methanol-STF). The amount of RS was estimated by UV at 306 nm, and the concentrations per unit area (µg/ml.cm^2) were computed and plotted (y-axis) against time (x-axis). A straight-line equation was obtained at the steady state of the plotted graph. The steady-state slope value was termed the flux (J_{ss}). The permeability coefficient was further calculated from J_{ss} by dividing it with the initial RS concentration. The *ex vivo* study was also conducted on resveratrol dispersion, and similar parameters were estimated by replicating the same process [19].

Result and Discussion

Particle Size, PDI and Entrapment efficiency for RS-NLC

Table 1 shows the characterization values of RS-NLC. The mean particle size of RS-NLC falls within the nano range, which is good for corneal permeation, ranging from 88 nm to 121 nm. After performing three measurements, the mean particle size was found to be 104.47 ± 13.32, and the PDI mean value was 0.394 ± 0.070. The nano range particle size distribution is shown in **Figure 1**, which confirms its physical stability and monodisperse nature. The PDI values for RS-NLC below 0.5 indicate that the particles are monodispersed in nature. The entrapment efficiency values for RS-NLC were above 85%, indicating that the majority of RS is entrapped in the lipid matrix of NLC.

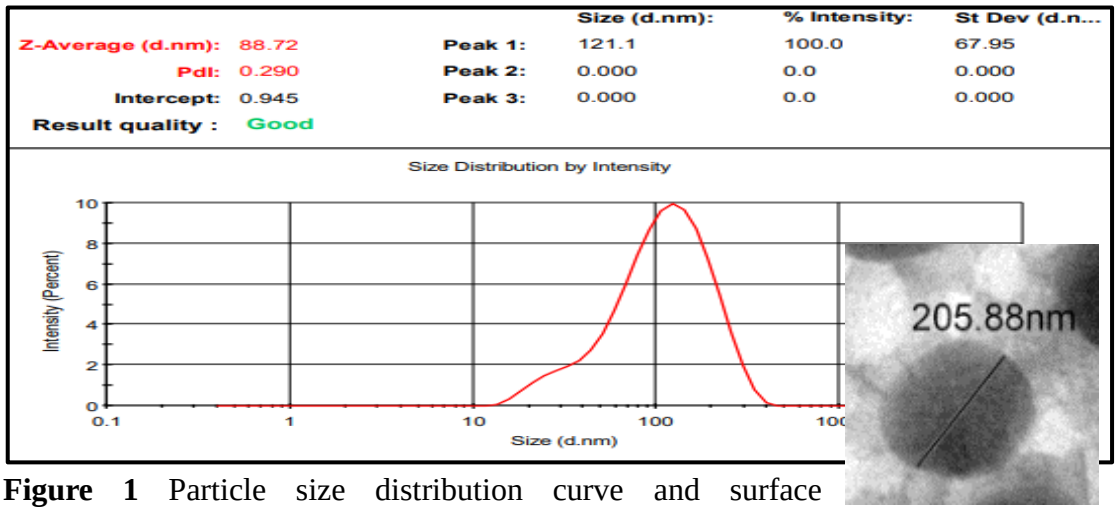

Figure 1 Particle size distribution curve and surface morphology (TEM) OF RS-NLC

TEM analysis of RS-NLC showed a particle size of 205.88 nm (**Figure 1**), which is slightly larger than that measured by the zetasizer. The increase in particle size could be due to the growth of particle size. TEM analysis suggests smooth morphology with a spherical shape.

Table 1 Particle size, PDI of RS-NLC (N=3, Mean ± SD)

Particle Size (nm)	PDI	% EE (%)	ZP (mV)
104.47 ± 13.32	0.394 ± 0.070	85.88 ± 0.32	-1.63 ± 0.29

The resultant RS-NLC has a negative ZP (-1.63 ± 0.29), which is due to the deposition of the carboxylic acid of GMS. Neutral surfactants, Tween 80 and Poloxamer 407 blend were used, resulting in a negative ZP value for RS-NLC. **Figure 2** shows a sharp peak of ZP for RS-NLC.

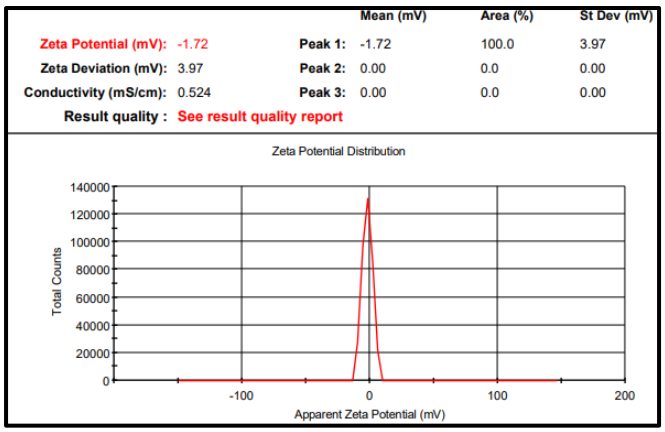

Figure 2 ZP Potential curve for RS-NLC.

***In vitro* RS release study**

Figure 3 represents *in vitro* release study for RS-NLC. The RS release study revealed an initial burst release of resveratrol from RS-NLCs. This rapid release phase is commonly observed in nanoparticulate drug delivery systems and can be attributed to the drug molecules adsorbed or loosely bound to the surface of the carriers. Following the initial burst release, a sustained release of resveratrol was observed over the experimental period.

The release mechanisms of resveratrol from RS-NLCs can be attributed to diffusion, erosion, and degradation of the lipid matrix. Diffusion-controlled release occurs as resveratrol molecules diffuse through the lipid matrix or the aqueous channels within the nanoparticles. Erosion and degradation of the lipid matrix may also contribute to the release as the carriers undergo structural changes over time.

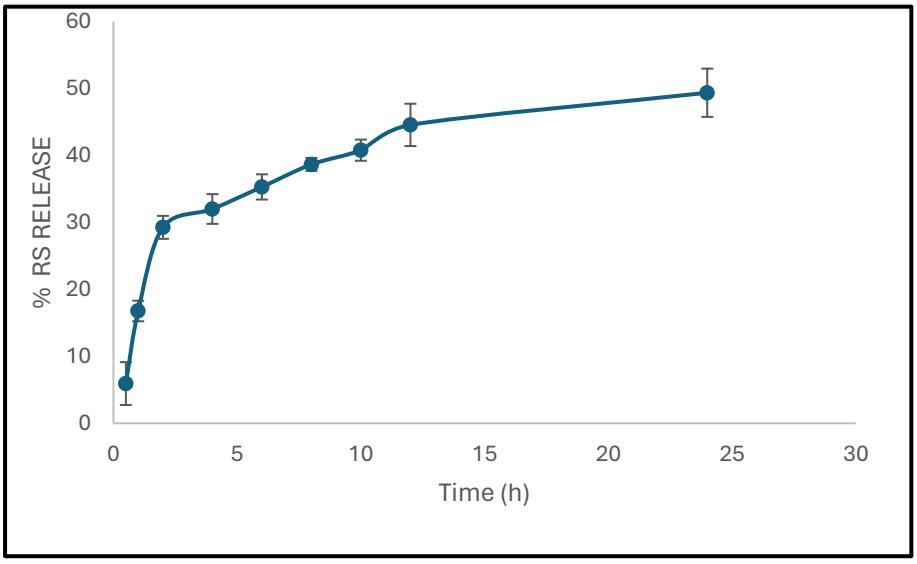

Figure 3 *In vitro* release study for RS-NLC

***Ex vivo* permeability study for RS-NLC**

The study showed that the permeation rate (J_{ss}) of resveratrol from RS-NLCs was significantly higher than that of the RS suspension. The RS-NLC Jss formulation code (µg cm^{-2} h^{-1}) was found to be 4.03 ± 0.20, while in the RS suspension, it was 1.77 ± 0.56 (as indicated in **Table 2**). This indicates that RS-NLCs enhance the transcorneal permeation of resveratrol, likely due to their nanostructured lipid matrix facilitating drug penetration through the corneal epithelium.

Similarly, the corneal permeability coefficient (K_p) of resveratrol from RS-NLCs was significantly higher than that of the RS suspension. The permeability coefficient ($Kp \times 10^4$) for RS-NLC was 40.37 ± 2.02 cm/h, whereas for the RS suspension it was 1.77 ± 0.56. This suggests that RS-NLCs enhance the corneal permeability of resveratrol by several folds compared to the suspension formulation.

The significant improvement in transcorneal permeation and corneal permeability coefficient demonstrated by RS-NLCs indicates their potential as effective carriers for ocular drug delivery. The nanostructured lipid carriers facilitate the sustained release of resveratrol, allowing for prolonged contact time with the cornea and improved absorption.

Table 2 *Ex vivo* transcorneal study parameters i.e. Flux and permeability coefficient for RS-NLC and RS Suspension

Formulation code	**J_{ss}** (µg cm^{-2} h^{-1})	**$Kp \times 10^4$** (cm/h)
RS-NLC	4.03 ± 0.20	40.37 ± 2.02
RS Suspension	1.77 ± 0.56	3.56 ± 1.12

Conclusion

This study successfully developed and evaluated a novel resveratrol-loaded nanostructured lipid carrier (RS-NLC) formulation for enhanced ocular drug delivery. The RS-NLCs exhibited desirable physicochemical properties, including a mean particle size within the nano-range (104.47 ± 13.32 nm), narrow size distribution (PDI 0.394 ± 0.070), high entrapment efficiency (85.88 ± 0.32%), and negative zeta potential (-1.63 ± 0.29 mV).

The *in vitro* release kinetics demonstrated an initial burst release followed by sustained release of resveratrol from the R-NLCs over 24 hours, which can potentially prolong the residence time of resveratrol in the ocular environment. Transmission electron microscopy confirmed the smooth, spherical morphology of the R-NLCs with a particle size of 205.88 nm.

Importantly, corneal permeation investigation using isolated goat corneas revealed significantly enhanced corneal permeation of resveratrol from the RS-NLCs compared to the resveratrol suspension formulation. The resveratrol flux (4.03 ± 0.20 µg/cm^2/h) and permeability coefficient (40.37 ± 2.02 × 10^{-4} cm/h) for R-NLCs were several folds (11 times) higher than those of the suspension, indicating the potential of R-NLCs to improve resveratrol's bioavailability in ocular tissues.

Overall, the results demonstrate the promising potential of R-NLCs as an effective carrier system for enhancing the ocular delivery and therapeutic efficacy of resveratrol. These findings lay the foundation for further in vivo studies and potential clinical applications of R-

NLCs in the treatment of ocular diseases associated with oxidative stress, inflammation, and aging, where resveratrol's antioxidant and anti-inflammatory properties could be beneficial.

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