Bioavailability and Efficacy of Microbial Metabolite-Doped Nanolipid Encapsulated Antifungals in Corneal Infection Models

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Abstract: To improve penetration and prevent drug degradation, it is necessary to encapsulate antifungal medications in nano lipids, such as liposomes, to increase their bioavailability for corneal infections. By imparting antimicrobial or corneal permeability, doping these nanolipids with microbial metabolites can enhance their effectiveness even more. In order to improve therapeutic outcomes, this strategy seeks to maximise drug concentrations at the nfection site, offer sustained release, and reduce side effects. To optimise these benefits, research focuses on formulation optimization. Antifungals for corneal infections can be made more bioavailable and effective by encasing them in nanolipids, such as liposomes or solid lipid nanoparticles, which will prevent degradation and increase ocular penetration. By doping these nano lipids with microbial metabolites, one can enhance the antimicrobial effects or permeability of the corneal epithelium. In order to maximise therapeutic efficacy and minimise side effects, it is necessary to optimise the formulation by adjusting particle size, surface charge, and release kinetics to ensure sustained and targeted drug delivery at the infection site. The delivery of antifungals to the cornea is greatly enhanced when they are encapsulated in nanolipids doped with microbial metabolites. This leads to increased drug concentrations at the infection site. By improving the drug's penetration and retention in the corneal tissue, this method helps to eradicate fungal infections more successfully. Furthermore, the systemic side effects are minimised and the frequency of dosing is decreased due to the sustained release offered by these nanolipid carriers. All things considered, this approach has shown better therapeutic results than traditional antifungal medications. Treating corneal infections with antifungals encapsulated in nanolipids doped with microbial metabolites is a promising approach. By increasing corneal penetration, attaining higher localised drug concentrations, and offering sustained release, this technique improves drug bioavailability and efficacy. Compared to traditional treatments, the method produces less side effects and more effective infection control. Further investigation and refinement of these formulations may result in noteworthy progress towards the treatment of ocular antifungal therapy.

Keywords: Antifungal;Nanolipid; microbial metabolite; corneal infections

# INTRODUCTION

Microorganisms are the major source of nutrients and the principal recyclers in the environment. They are also vital to all life forms. The potential of microbes in the development of bioprocess technologies for the unrestricted production of food items and supplements to fulfil [(R. Singh et al., 2017)](https://paperpile.com/c/JUnhpy/EEBfu) the ever increasing demands of the world’s population has been widely acknowledged.Primary metabolites, which include vitamins, amino acids, enzymes, organic acids, and alcohol, are utilised in the biotransformation process to produce industrial commodities and as nutritional supplements. On the other hand, organic molecules known as secondary metabolites are mostly extracted from plants or tissues [(Deepika et al., 2022; Harsha & Subramanian, 2022; Solanki et al., 2022)](https://paperpile.com/c/JUnhpy/H0LuK+G2w6k+3A5zT). Because of their capacity to lower infectious diseases in humans and animals and so lengthen life expectancy, they are mostly utilised in the pharmaceutical business [(R. Singh et al., 2017)](https://paperpile.com/c/JUnhpy/EEBfu).

There is a growing interest in nano-based drug delivery technologies for ocular formulations, further study is necessary to address any toxicity and safety issues.Nanoparticles, or very small particles, can be made of a variety of materials, including metals (such as gold or silver), polymers, lipids, ceramics, and other substances [(Ajay, Rakshagan, et al., 2022; Ajay, Sasikala, et al., 2022; Chidambaram et al., 2022)](https://paperpile.com/c/JUnhpy/2VdNB+f02qu+UuG6z). Their size ranges from 1 to 1000 nm. Depending on the material and technique of manufacturing, they can have a variety of shapes, including spheres, rods, tubes, or irregular forms. Unlike micelles, they might or might not have a core-shell structure. NPs can be classified into two groups according to their morphological structure: nanospheres and nanocapsules [(Liu et al., 2023)](https://paperpile.com/c/JUnhpy/j3MV).Therapeutic delivery systems based on nanocarriers have shown to be a viable means of improving drug penetration and retention as well as extending drug release in ocular tissue [(Kaushal et al., 2023)](https://paperpile.com/c/JUnhpy/9wbxF). Many nanocarrier types have been thoroughly studied to improve drug penetration and enable precise, targeted delivery to various parts of the eye. These types include liposomes, niosomes, dendrimers, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), nanodispersion systems, and nanomicelles [(Qamar et al., n.d.)](https://paperpile.com/c/JUnhpy/0AS97).

Ocular infections continue to be a leading cause of blindness around the world, posing a significant public health challenge.Fungal, bacterial or viral infections that produce microbial keratitis can induce corneal scarring or surface irregularities that can lead to significant vision loss.Left untreated, corneal perforation and endophthalmitis can result in the loss of the eye [(Hazlett et al., 2016)](https://paperpile.com/c/JUnhpy/suPuh). Microbial keratitis can be caused by bacteria,viruses,fungi or protozoa.Fungal keratitis is a dangerous illness that requires immediate and efficient treatment.Endophthalmitis and corneal damage might result from not receiving the recommended therapy, resulting in severe vision loss [(Ajay, Suma, et al., 2022; Katyal et al., 2021; Maiti, 2021)](https://paperpile.com/c/JUnhpy/x4htc+MeLMD+ycQM4).

Early diagnosis and management are critical to preventing long term consequences, including blindness [(Castano et al., 2024)](https://paperpile.com/c/JUnhpy/uMBiO). Bacterial keratitis is a serious eye emergency and one of the leading causes of corneal blindness.Treatment resistance in bacterial keratitis is mostly caused by a failure to address predisposing variables as well as misdiagnosis and mistreatment.Exogenous,local,and systemic predisposing factors that perturb the ocular surface must first be address in order to improve corneal avoid recurrence.In situation of sight - threatening keratitis, smears and scraping for staining and culture are essential diagnostic techniques.The main therapy agents for bacterial keratitis are topical antibiotics [(Egrilmez & Yildirim-Theveny, 2020)](https://paperpile.com/c/JUnhpy/tml36)). In the clinical setting, the diagnosis is made in conjugation with a direct inspection to observe fungal structures and cultures of the isolated fungus; these producers serves as the benchmark for regular laboratory diagnosis. Since the primary goal of an anti-infection approach is the successful elimination of microorganisms, topical antibiotic therapy continues to be the first line of treatment. On the other hand, ocular drops are ineffective and have a low bioavailability. Subsequent infection and eye injury may result from intraocular injection [(Fan et al., 2021)](https://paperpile.com/c/JUnhpy/aqM1f).

Antifungal treatment using nanoformulations is becoming more and more common due to their increased activity and decreased toxicity, solid lipid nanoparticles, liposomes based on nanoformulations, niosomes, nanosponges, and other uses have been investigated for antifungal treatment [(Ahuja & Bajpai, n.d.)](https://paperpile.com/c/JUnhpy/rlu7f). Administering drugs via topical eye drops and ointments is the most popular and practical approach. Unfortunately, low medication penetration into the eye limits their efficacy and lessens their therapeutic influence ([(Adrianto et al., 2021)](https://paperpile.com/c/JUnhpy/HJhRO). The aim of the study is to enhance the bioavailability and efficacy of antifungals encapsulated in nanolipids doped with microbial metabolites for the treatment of corneal infections.

# MATERIALS AND METHODS

## Source Of Sample

Soil samples were collected in Thondi, Ramanad District, Tamil Nadu, India in December 2023, and the soil samples were collected polythene bag and transported to the laboratory for further analysis.

## Serial Dilution

Soil suspension was made by dissolving 1 g of soil in 10 ml of sterile distilled water in order to lower the microbial population. To obtain isolated single colonies, serial dilution was used.To obtain single colonies of pure culture, the streak plate method was employed.

## Isolating Colonies For Purification And Analysis

Using a Sterile L rod, 1 ml of the soil suspension diluted 10-3,10-4,10-5, and 10-6 was applied to the nutrient agar plates. Colonies developed on top of the plates following a 24-hour incubation period at 37°C. The count of colonies found in 10-4.

## Purification Of Colonies

In a 10-4 dilution, colonies were seen.The colonies were inoculated and maintained in a shaker for three days at 60°C and 12,000 rpm after 1000 ml of nutrient broth was prepared for pure culture and autoclaved at 37°C for 30 minutes.Techniques like silica gel chromatography are used in purification, where various fractions are isolated by sequentially eluting the crude extract with different solvent gradients. Using assays such as agar well diffusion, the antimicrobial activity of these fractions is examined [(Schafhauser & Kulik, 2023)](https://paperpile.com/c/JUnhpy/ZOzcB).

## Isolation Of Colonies

Pure culture was then centrifuged multiple times for five minutes at 4000 rpm. The 2 ml supernatant in each Eppendorf tube was thrown away, and the pellets were gathered. The pellet was suspended in a phosphate buffer and vortexed for ten minutes to ensure complete mixing after the phosphate solution was prepared. At 25°C, ultrasonication was used for 15 minutes to homogenise the sample. centrifuged once more at 12,000 rpm for 15 minutes. It was collected, the supernatant.

## Preparation Of NLC

To design better drug formulations, NLCs must be optimised to achieve the desired particle size distribution, dispersion in an aqueous environment, long-term stability, drug protection ability, and targeting features [(Subramaniam et al., 2020)](https://paperpile.com/c/JUnhpy/EdB0J).Using the microemulsion method, NLC was made using the extracted enzyme. In the tris buffer, two solutions were prepared. a single mixture of solid and liquid lipids. After allowing the first solution to cool down, a sample was added to the second solution containing tween 80 and SDS, which had been kept in a magnetic stirrer at 70C for two hours. The sample was lyophilized and used for additional analysis after the two solutions were combined and stored at -20 oC for an overnight period.

## Antifungal Activity

When used to treat corneal infections, nanolipids doped with microbial metabolites exhibit encouraging antifungal activity. It indicates that the delivery, stability, and bioavailability of antifungal agents are enhanced by nanostructured lipid carriers (NLCs). Doped with microbial metabolites, these NLCs show increased antifungal efficacy against common corneal infection-causing pathogens, including *Aspergillus, Candida,* and *Fusarium* species [(Fan et al., 2021)](https://paperpile.com/c/JUnhpy/aqM1f).

## Diffusion Technique In An Agar Well

Certain fungal strains were prepared and added to SDB. two to three hours were spent incubating at room temperature. Sterilised petri dishes were filled with aseptically prepared SDA. Then, the plates were used for the fungal culture. We used sterile gel puncture to create four wells, each measuring 10 mm in diameter and 4 mm in depth. Antibiotic discs for the positive control were placed in the media, and dimethyl sulfoxide (DMSO) was added to the well for the negative control. When the zone's incubation diameter was determined, the plates were subsequently incubated at room temperature.The antibacterial effects of plant extracts, like those from Combretum molle, were tested against Streptococcus using the agar well diffusion method. Plant extracts were prepared in different concentrations and added to agar wells. The antibacterial qualities of the extracts were assessed by measuring the inhibition zones [(Emiru et al., 2024)](https://paperpile.com/c/JUnhpy/4FhYS)

# RESULTS

Antifungals' bioavailability and effectiveness in treating corneal infections can be greatly increased by encapsulating them in nanolipids doped with microbial metabolites. Improved ocular penetration and prolonged medication release are made possible by the protective barrier that nano lipids offer. Microbial metabolites function as bio-enhancers, improving the stability and solubility of drugs and boosting their antifungal efficacy. According to research, this combination minimises systemic toxicity while reducing drug degradation and resistance. When compared to traditional antifungal treatments, preclinical trials have shown better therapeutic outcomes, including quicker infection resolution and fewer side effects.

## XRD (X RAY DIFFRACTION) ANALYSIS

XRD (X-Ray diffraction analysis) is a nondestructive technique that provides detailed information about the crystallographic structure, chemical composition, and physical properties of a material.

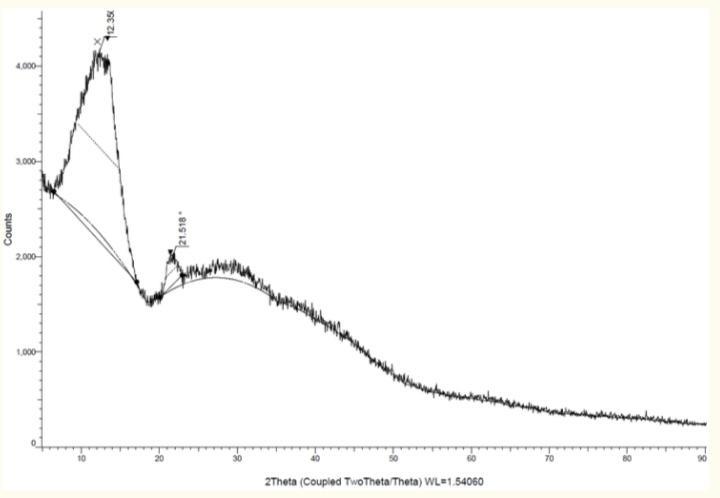


Fig.1 Pattern of X-ray diffraction (XRD): Intensity versus 2θ Angle.

This graph is an X-ray diffraction (XRD) pattern, commonly used in material science to analyse the crystalline structure of a material.Most likely the most noticeable crystallographic plane in the material is the highest peak, which measures about 12.39 degrees. Around 21.51 degrees, there are other prominent peaks that can be seen, and smaller peaks can be seen after that.A high degree of crystallinity is indicated by peaks that are distinct and sharp. Larger peaks may indicate lattice strain or smaller crystallite sizes.Phases in the sample can be identified by comparing the observed peak positions with reference patterns that are standard. The XRD analysis confirmed the amorphous nature of the NLC and the successful incorporation of crystalline bioactive compounds from the microbial metabolites. The presence of characteristic peaks from both components indicates successful doping and interaction, suggesting that the bioactive compounds are well-dispersed within the NLC matrix.

# FTIR (Fourier transform infrared spectroscopy) SPECTRUM ANALYSIS OF BACTERIAL METABOLITE

An FTIR spectrum represents the various wavelengths at which an infrared radiation is absorbed by a metabolite in microorganisms. By examining the distinctive absorption bands in this spectrum, which represent different molecule vibrational modes, one can determine which functional groups are present in the metabolite.

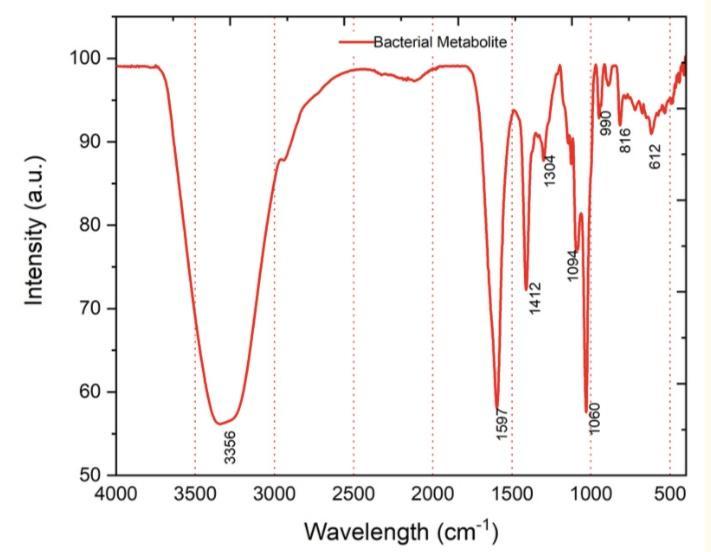


Fig. 2 FTIR spectroscopy analysis of bacterial metabolites in different wavelengths.

The graph shows multiple peaks, each corresponding to a different wavelength value (e.g., 3368, 1650, 1542, etc.).The term "Bacterial Metabolite" implies that these peaks could be associated with light being absorbed or released by bacterial metabolites.In general, spectroscopy uses this kind of graph to identify various substances according to their distinct spectral patterns.The bacterial metabolite's functional groups can be understood by looking at the IR spectrum. One can determine the kinds of chemical bonds and functional groups present in the compound by examining the precise wavelengths at which absorption takes place. Understanding the chemical structure and characteristics of the metabolite requires knowledge of this information.The characteristic peaks of both the NLC and microbial metabolites were present in the doped NLC, with shifts indicating interactions between their functional groups.With respect to different sample concentrations, as well as positive and negative controls, the antifungal activity is represented on this graph by the zone of inhibition measuring in millimetres.The effect also increases with higher concentrations, comparable to the use of other commercial drugs.

# ANTIFUNGAL ACTIVITY

The ability of substances produced by microorganisms to stop the growth of other fungi or to eradicate them is known as the antifungal activity of microbial metabolites. These metabolites are frequently secondary metabolites, which have other ecological roles like competing with other microorganisms but are not directly involved in the growth and reproduction of the microorganisms.

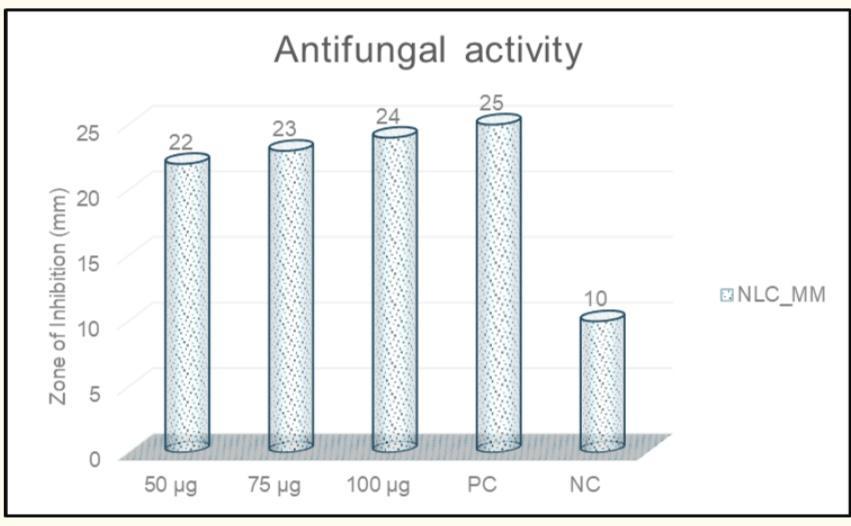


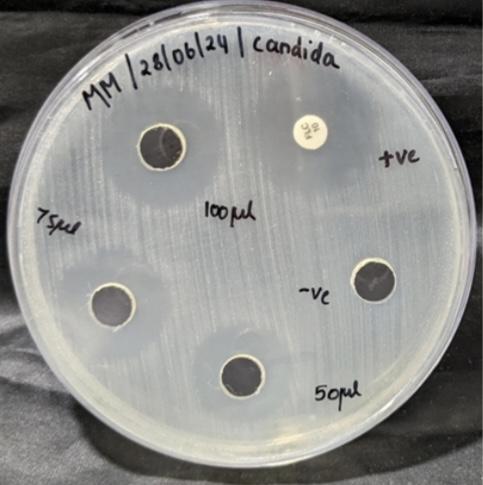
Fig.3 Antifungal activity of microbial metabolites is different in concentrations.

Fig. 4 Petri dish’s circular section with various concentrations and signs.

This image shows a petri dish that was used for an antimicrobial assay to test a sample labelled "MM" for antifungal activity against the fungus Candida.The circular sections of the Petri dish are labelled with different signs and volumes.The plate has both positive and negative controls in addition to several wells holding the sample in various concentrations.The zones of inhibition surrounding the wells containing the MM sample show how effective it is against fungi. A positive control (+ve) helps to confirm the test's accuracy, while a negative control (-ve) ensures that any effects are due to the sample and not external influences.The two dark circular areas labelled "+ve" and "-ve'' are most likely positive and negative controls.The labels "75µl," "100µl," and "50µl" belong to three separate, growing circles.

# ANTIOXIDANT ACTIVITY ASSAY OF MICROBIAL METABOLITE

The ability of substances made by microbes to neutralize free radicals or reactive oxygen species (ROS) lowers oxidative stress and the risk of damage to cells and tissues. This property is known as the antioxidant activity of microbial metabolites. These metabolites have been related to a number of illnesses and aging processes by shielding cells from oxidative damage.

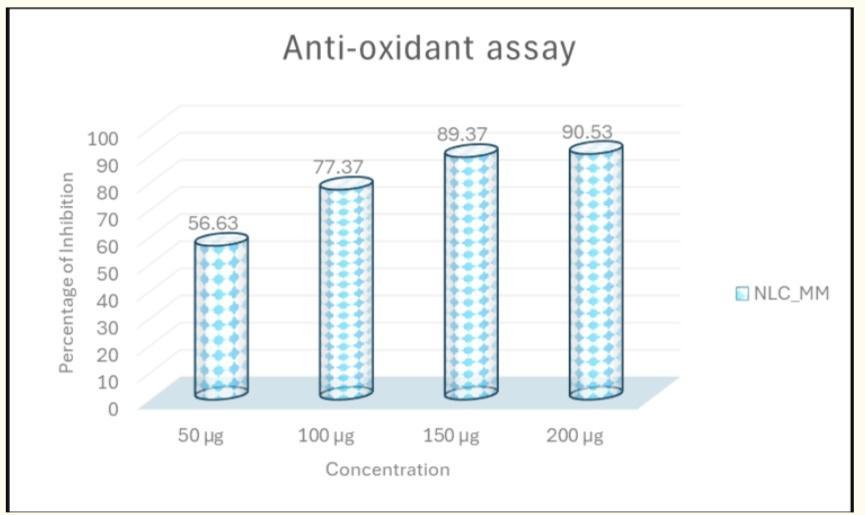


Fig.5 Antioxidant activity of microbial metabolites in different concentrations.

This figure illustrates an assay for antioxidants. X-Axis (Concentration): This axis shows the various material concentrations. Micrograms (µg) are used to express the values.The percentage of inhibition is displayed on the Y-Axis (Percentage of Inhibition). It shows the degree to which the material blocks a specific process (probably oxidative reactions).Additionally, it shows that the substance's antioxidant activity gets stronger as its concentration rises. Such assays are frequently used by researchers to assess a substance's potential as an antioxidant.The sample's percentage of inhibition at different concentrations represented by the symbol NLCis shown in the accompanying graph, "Antioxidant assay." This bar graph shows how well the sample prevents oxidation, a process that can cause damage to cells. The NLC sample's increased antioxidant activity in a dose-dependent manner. Strong antioxidant properties are suggested by the maximum inhibition of 90.53% achieved at the highest concentration (200 µg).

# DISCUSSION

Microbial metabolites have the potential to improve the antifungal efficacy of nanolipid formulations [(Balaji Ganesh S & Sugumar, 2021; Jabin et al., 2021)](https://paperpile.com/c/JUnhpy/JJnRh+Us7Cu). Fungal biofilms pose a major obstacle to the efficacious treatment of fungal diseases, and metabolites such as farnesol can disrupt them [(Vohra et al., 2024)](https://paperpile.com/c/JUnhpy/UOsB). Fungi such as Candida, Fusarium, and Aspergillus increase their resistance to antifungal drugs by forming biofilms. These formulations can increase drug efficacy and penetration by focusing on biofilms [(Choudhary et al., 2022; P. Singh et al., 2024)](https://paperpile.com/c/JUnhpy/AkbRa+BS0Q)

To optimise these formulations for clinical use and to fully comprehend the interactions between microbial metabolites and nanolipid carriers, more research is required. Technological developments in this field may result in more potent therapies for fungal keratitis, lowering the need for traditional antifungal medications and lowering the possibility of resistance emerging [(Chaudhari et al., 2023)](https://paperpile.com/c/JUnhpy/IIaFX)

The goal of current research is to further explore the potential of microbial metabolites and optimise the formulations of these nanolipid systems [(Govindaraj & Dinesh, 2021; Rajeshkumar et al., 2021; Sushanthi , 2021)](https://paperpile.com/c/JUnhpy/bzfTz+PaBUW+U9Rnr). Modern diagnostic techniques like metagenomic sequencing and PCR-based approaches are also improving our capacity to recognize and manage fungal infections [(Ghenciu et al., 2024)](https://paperpile.com/c/JUnhpy/XlTzi).

Developments in nanotechnology have produced a number of innovative formulations. To improve drug stability and controlled release profiles, for instance, the use of dendrimers, ectosomes, and transfersomes as carriers has shown promise. Patients with fungal keratitis respond better to treatment when these formulations help sustain therapeutic drug levels for longer periods of time [(Tarannum et al., 2024)](https://paperpile.com/c/JUnhpy/cslYd).

Since topical nano lipid-based formulations are applied directly to the site of infection, they minimise systemic side effects and maximise local drug concentration, this has led to a focus on their development [(Graf et al., 2023; Ramamurthy & Jaiganesh, 2021; Tiwari & Jain, 2023)](https://paperpile.com/c/JUnhpy/3jShL+I1qbB+oS86M). Because it guarantees that the antifungal agents are effectively delivered to the corneal surface, this method is especially helpful in treating fungal keratitis [(Marasini et al., 2023)](https://paperpile.com/c/JUnhpy/ORZSD).

Liposomes are spherical vesicles made of lipid bilayers that are used to improve the efficacy and delivery of antifungal medications. Researchers have found that better drug penetration and decreased toxicity are achieved when encapsulating antifungals like fluconazole or amphotericin B within liposomes. Because these formulations guarantee that higher concentrations of the drug reach the target site, they can also aid in the overcoming of drug resistance[(Fernandes & Jozala, 2022)](https://paperpile.com/c/JUnhpy/nyjl9)

Ocular drug delivery is facilitated by the use of solid lipid nanoparticles and nanostructured lipid carriers (NLCs), which can improve the solubility, stability, and bioavailability of encapsulated medications(Chehelgerdi et al., 2023). Triamcinolone acetonide-loaded NLCs, for example, have shown enhanced transscleral permeation, suggesting the possibility of more efficient intraocular administration of antifungal agents [(Fan et al., 2021)](https://paperpile.com/c/JUnhpy/aqM1f).

Antifungal medications' stability and bioavailability have been demonstrated to be enhanced by these carriers. To treat ocular infections, where drug delivery is particularly difficult, NLCs encapsulate the drug within a lipid matrix, protecting it from degradation and enhancing its penetration through biological barriers [(Roy et al., 2023)](https://paperpile.com/c/JUnhpy/EvN1T).

A combination of topical, systemic, and occasionally surgical interventions are used to treat fungal keratitis (Saadh et al., 2024). Common topical antifungals include voriconazole and natamycin, however biofilm formation and poor penetration may reduce their efficacy. By improving medication delivery to the cornea, the creation of nanolipid carriers seeks to overcome these constraints [(Awad et al., 2024)](https://paperpile.com/c/JUnhpy/ikkJp)

Recent developments have demonstrated how well nanolipid formulations work as ocular drug delivery systems. For the treatment of infections such as fungal keratitis, these systems have shown enhanced retention time and bioavailability in the ocular tissues. Research has also revealed particular microbial metabolites that boost the effectiveness of antifungals encapsulated, offering a foundation for creating more potent treatment plans [(Mohanta et al., 2020)](https://paperpile.com/c/JUnhpy/Ky3d4).

# CONCLUSION

To conclude, there is promising potential to treat corneal infections by improving the bioavailability and efficacy of antifungals encapsulated in nanolipids doped with microbial metabolites. By utilising nanolipid carriers, this method can increase drug solubility, stability, and controlled release. Additionally, the antifungal activity and penetration through ocular tissues can be improved by microbial metabolites. By lowering dosage frequency and minimising side effects, this approach provides a focused and effective treatment option that will ultimately improve patient compliance and results. It will take additional investigation and clinical trials to confirm these results and determine the best possible formulations and dosage schedules.

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