Development and Characterization of lycopene loaded Niosomes for Antioxidant activity

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Abstract

Lycopene, a potent natural antioxidant found in tomatoes, is known for its therapeutic benefits, including cancer prevention and cardiovascular protection. However, its clinical application is hindered by its poor solubility, bioavailability, and instability. This study investigates the development and characterization of lycopene-loaded niosomes as a delivery system to enhance its antioxidant activity. Niosomes, non-ionic surfactant vesicles, were prepared using the thin-film hydration method, incorporating lycopene, surfactants (Span 60, Tween 80), and phospholipids. The niosomal formulations were characterized for particle size, zeta potential, encapsulation efficiency, morphology, and stability. The antioxidant activity of the lycopene-loaded niosomes was evaluated using DPPH, FRAP, and ABTS assays, and the results were compared with free lycopene. The encapsulation of lycopene in niosomes significantly improved its stability, solubility, and antioxidant potential. Lycopene-loaded niosomes exhibited enhanced free radical scavenging activity and better stability than free lycopene. These findings suggest that niosomes could be a promising delivery system to improve the bioavailability and therapeutic efficacy of lycopene, providing an effective strategy for its use in antioxidant-based therapies.

Keywords: Lycopene Formulation; Niosomes; Oxidants; Antioxidant Activity.

Introduction

Lycopene, a major carotenoid found in ripe tomatoes (Lycopersicum esculentum), is responsible for their characteristic red colour and has attracted significant attention for its potential health benefits. This tetraterpene, composed of eight isoprene units, contains eleven conjugated double bonds that contribute to its red colour and are primarily responsible for its antioxidant properties. Numerous epidemiological and experimental studies suggest that lycopene may help protect against cardiovascular diseases and diabetes. However, due to its structure with unsaturated bonds, lycopene is prone to degradation when exposed to heat (above 80 °C) and light, making it vulnerable to oxidation. Therefore, it is essential to protect lycopene from chemical damage before its use in various applications. One promising solution to address these challenges is the preparation of niosomes.

Unlike liposomes, which face stability issues such as aggregation, fusion, leakage, and sedimentation during storage, niosomes are vesicles made from non-ionic surfactants combined with cholesterol. These vesicles offer several advantages, including improved solubilization in aqueous intestinal fluids. Like liposomes, niosomes can form vesicular systems when dispersed in water, allowing for better encapsulation of both lipophilic and hydrophilic substances while shielding them from external factors. Studies have shown that niosomes can function similarly to liposomes, extending the circulation time of drugs, altering their distribution within the body, and improving metabolic stability. Additionally, due to their strong affinity for phospholipids, niosomes enhance the pharmacokinetic and pharmacodynamic profiles of encapsulated drugs, improving both absorption and the duration of their action. Compared to other vesicular delivery systems, niosomes offer benefits such as greater stability, better penetration properties, and lower cost. Their excellent drug-loading capacity and high penetration potential make niosomes an ideal delivery system for lycopene.

In this study, we developed a novel method for formulating lycopene-loaded nano-niosomes through an adsorption–hydration technique. This approach aims to preserve the biological activity of lycopene within niosomes by utilizing glass wool as a medium for facilitating the interaction between lycopene and the wall material, addressing formulation challenges. The protective mechanism involves creating a membrane structure (wall system) that encapsulates lycopene droplets (core). For the wall system, we used Span 60 and cholesterol, which function as stabilizers for the membrane, aiding solute retention by forming a bilayer, and enhancing

the adhesive strength between the core and wall materials. Therefore, the lycopene nano-niosomes were prepared using this innovative method, and their performance was assessed by evaluating entrapment efficiency, release profile, stability, zeta potential, and bioavailability. Additionally, the in vitro anti-cancer potential of the lycopene-loaded nano-niosomes was assessed to determine their biological effectiveness.

Structure of Niosomes

Niosomes are spherical vesicles that feature microscopic lamellar structures, which can be either unilamellar or multilamellar. These bilayer structures are primarily composed of nonionic surfactants, sometimes combined with cholesterol and a charge inducer. Various surfactants, such as alkyl ethers, alkyl glyceryl ethers, sorbitan fatty acid esters, and polyoxyethylene fatty acid esters, are used in different combinations and ratios to form niosomes. The inclusion of cholesterol helps to stabilize the bilayer, reducing leakage and enhancing structural integrity. Charge inducers, which can be either negative or positive, contribute to vesicle size and improve drug entrapment efficiency. Examples of negative charge inducers include dicetyl phosphate, dihexadecyl phosphate, and lipoamino acids, while positive charge inducers include stearylamine and cetylpyridinium chloride. These agents help to stabilize the niosomal vesicles.

In niosomes, the nonionic surfactants arrange themselves in a manner where the hydrophilic heads face outward towards the aqueous phase, while the hydrophobic tails face inward, forming a bilayer. This results in a closed structure that encloses the aqueous solution and its solutes. The bilayer is formed through the application of energy, either in the form of heat or physical agitation. Additionally, the bilayer's internal space allows niosomes to encapsulate both hydrophilic and hydrophobic substances, making them suitable for drug delivery applications.

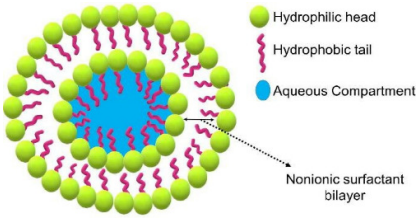

Fig. Structure of Niosomes

Salient properties of Niosomes

Surfactant-based niosomes are known for their biodegradability, biocompatibility, and non-immunogenic properties. These vesicles function as drug depots, releasing drugs in a controlled manner due to their bilayer structure, leading to sustained drug delivery to the target site. Niosomes can entrap solutes. Niosomes are osmotically active and stable. They improve the solubility and oral bioavailability of poorly soluble drugs and also enhance the skin permeability of drugs when applied topically. Niosomes can improve the performance of drug molecules. Niosomes increase the stability of the entrapped drug. Niosomes prolong the circulation of the entrapped drug. The therapeutic outcomes of drugs encapsulated in niosomes are enhanced by slower clearance and targeted delivery. Thanks to their hydrophilic, amphiphilic, and lipophilic characteristics, niosomes can encapsulate a wide range of drugs with varying solubilities. The bioavailability of poorly soluble drugs can be improved, and the effectiveness of topical applications is increased when using niosomes. Additionally, niosomes provide protection to sensitive drugs, safeguarding the encapsulated active pharmaceutical ingredients from harmful conditions both inside and outside the body.

The stability of niosomes depends on various factors, including the type of surfactant used, the properties of the encapsulated drug, hydration temperature, the presence of detergents, membrane-spanning lipids, surfactant monomer polymerization, and the presence of charged molecules. The surfactant used in niosome preparation should typically have a hydrophilic head and a hydrophobic tail. Surfactants with hydrophobic tails, such as alkyl chains (C12 to C18), perfluoroalkyl, or steroidal groups, are generally more suitable for niosome production. Ester-type surfactants are less ideal as they can be degraded by esterases in the body, leading to instability, whereas ether-type surfactants are more stable. The size of niosomes tends to increase with higher hydrophilic–lipophilic balance (HLB) values of the surfactants. An optimal HLB value for stable niosome formation typically falls between 4 and 8. The addition of certain additives, such as cholesterol, can enhance the stability of niosomes by providing rigidity and reducing leakage.

The temperature at which the surfactants are hydrated is crucial for niosome formation, as it influences the assembly of surfactants into vesicles, as well as the shape and size of the niosomes. The hydration temperature should be above the gel-to-liquid phase transition temperature for effective niosome formation. The encapsulated drug interacts with the surfactant head group, indirectly affecting the charge and rigidity of the niosomal bilayer.

Hydrophobic drugs tend to stabilize niosomes, while hydrophilic drugs may decrease their stability. Amphiphilic drugs generally have little effect on the niosomal bilayer structure.

Challenges in niosome preparation include aggregation, fusion, and leakage, which are influenced by the vesicle's physicochemical properties, such as size, charge, lamellarity, elasticity, and thermodynamic phase. The development of proniosomes, a dried form of niosomes, can help overcome these issues as they quickly hydrate to form an aqueous niosome dispersion. Studies suggest that proniosomes can offer improved therapeutic efficacy for anti-inflammatory drugs, such as flurbiprofen and piroxicam, when applied via a transdermal route.

Niosomes are generally considered safe and non-toxic to humans. Research on the toxicity of surfactants in topical niosome formulations has shown that ester-based surfactants are less toxic than ether-based ones, as they are less affected by enzymatic degradation of ester bonds. It has been proven that the physical form of niosomes (liquid crystal vs gel) did not influence their toxicity.

Advantages

1. Enhance stability of lycopene.
2. Improved Bioavailability.
3. Controlled and Sustained release.
4. Targeted delivery.
5. Biocompatibility and non-toxicity.
6. Cost-Effective alternative.
7. Versatility in Formulation.

Limitations

1. Physical Instability.
2. Low Encapsulation Efficiency.
3. Scalability Issues.
4. Limited Shelf-life.
5. Potential for Oxidation.
6. Complex Characterization.
7. Surfactant toxicity at high Concentrations.

Applications

1. Pharmaceutical Industry

(a)Antioxidant therapy

-Used to reduce oxidative stress-related diseases such as cardiovascular disorders, neurodegenerative conditions, and certain cancers.

(b)Anti-inflammatory and anticancer treatments

- Lycopene’s therapeutic effects can be enhanced by targeted delivery using niosomes.

2. Nutraceuticals and Functional Foods

- Incorporated into dietary supplements and health drinks to provide enhanced antioxidant support with better bioavailability.

3. Cosmeceuticals

- Used in skin care formulations (creams, lotions, serums) for anti-aging, photoprotective, and skin-brightening effects due to lycopene's antioxidant properties.

4. Food Packaging

- As part of active packaging materials to prevent oxidation and extend shelf life of food products.

5. Targeted Drug Delivery Systems

- Niosomes enable site-specific delivery of lycopene to tissues or organs, enhancing therapeutic effects while minimizing systemic side effects.

6. Preventive Healthcare

- Regular supplementation through encapsulated formulations can aid in preventing chronic diseases linked to oxidative stress.

Material and Method

Lycopene extract was obtained from *Lycopersicum esculentum* (Bhopal, India). Span 60, cholesterol, chloroform, n-hexane, ethanol, and acetone were sourced from Merck Limited (Mumbai, India). Cisplatin was acquired from Sigma-Aldrich (India). All other chemicals used were of analytical grade, and all solvents employed were of HPLC grade.

Extraction of Lycopene from Dried Tomato Powder Using Solvent Mixture

1. Weigh around 5–10 g of dried tomato powder.
2. Prepare a solvent mixture by combining 100 mL of Hexane, Acetone, and Ethanol in a 2:1:1 ratio.
3. Stir the mixture at room temperature for 30–60 minutes in a dark environment.
4. Centrifuge the solution at 4000–5000 rpm for 15 minutes.
5. Carefully separate the supernatant, which contains the lycopene.

Isolation of lycopene

The five kilograms of fresh, ripe tomatoes (Solanum lycopersicum) were sourced from a local market in Bhopal, India, in November 2011. The tomatoes were authenticated by the Department of Botany, Saia Science College, Bhopal, India. A voucher specimen (568/BOI/SAFIA/12) was deposited in the Department of Pharmaceutics, Technocrats Institute of Technology-Pharmacy, Bhopal, India. The ripe tomatoes were stored at 4°C and utilized within 48 hours for the isolation of lycopene. To separate the peels, the tomatoes underwent blanching, where they were briefly immersed in boiling water for 1-2 minutes, followed by rapid cooling and manual peeling. The removed peels were air-dried at room temperature (24-25°C) for 2-3 hours before being sealed in zip-lock polyethylene bags. The dried peels were then stored at 4°C until they were ground with a small quantity of a solvent mixture (n-hexane: acetone:ethanol in a 2:1:1 ratio, v/v) in the presence of CO2. The mixture was sonicated using an ultrasonic crusher and subsequently magnetically stirred for 4 hours in an inert CO2 environment under dark conditions. The resultant extract was collected in an amber glass container with the CO2 atmosphere and stored at 4°C for future processing. Finally, the extract was dried using a rotary flash evaporator at 60°C and 50 rpm in a dark environment.

Preparation of lycopene nano-niosomes

Lycopene-loaded nano-niosomes were synthesized using a novel method referred to as the Adsorption-Hydration technique. In this process, a pure lycopene extract was mixed with a small volume of a solvent system composed of n-hexane, acetone, and ethanol (in a 2:1:1 v/v ratio). To this mixture, 22 mL of span 60 and cholesterol (in a 1:1 molar ratio) were incorporated, forming the organic phase containing the wall material. This organic phase was adsorbed onto 3 grams of glass wool, followed by continuous stirring at 60ºC. Afterward, 10 mL of an aqueous phase (mannitol solution at pH 7.4) was gradually added to the lycopene-adsorbed glass wool, ensuring vigorous shaking at the same temperature. The solution was then subjected to ultrasonic treatment using an ultrasonic cell crusher (Classic Sonicator, Lark Innovative Fine Teknowledge, Chennai, India) at 20 W output for 60 seconds per cycle, repeating the process three times to prevent excessive heat generation. The suspension was left to stand for 2-3 hours, allowing the nano-niosomes to form. To remove the glass wool, the self-assembled lycopene-loaded nano-niosomes were filtered through a 0.45-mm Whatman filter (No-1). The resulting suspension underwent column chromatography using a Sephadex G-50 column to separate any unentrapped lycopene. Finally, the formulation was lyophilized (Lyodel, Delvac, India) and stored in an airtight high-density plastic container.

Characterization

The characterization of niosomes involves several key parameters, including their size, distribution, zeta potential, morphology, encapsulation efficiency (EE), and in vitro release profiles. These factors are crucial for assessing the quality of niosomes during formulation development and for their potential use in clinical studies. Additionally, these characteristics significantly influence the stability and in vivo behavior of the niosomes.

1. Niosome Particle Size and Distribution

Particle size plays a crucial role in the characterization of niosomes, as it influences the physical properties and stability of the formulation. Various techniques are used to measure the size of niosomes, including dynamic light scattering (DLS) and microscopy. DLS, also referred to as photon correlation spectroscopy, is a rapid, non-destructive method that requires only a small sample. It measures particles in the size range of 3–3000 nm and works on the principle of Brownian motion of particles dispersed in a medium. A laser light is directed at the niosome suspension, and the scattered light intensity is detected, with fluctuations corresponding to the particle movement. Smaller particles create higher intensity fluctuations, while larger particles move more slowly, resulting in lower fluctuations. The poly dispersity index (PDI) indicates

the uniformity of the particle size distribution, with values below 0.5 suggesting a monodispersed sample. However, DLS does not provide information about particle shape. To enhance reliability, DLS is often combined with other microscopic techniques to obtain more accurate measurements.

2. Niosome Morphology

The morphology of niosomes is typically studied using electron microscopy methods. Transmission electron microscopy (TEM), negative-staining TEM, and freeze-fracture TEM are commonly employed for analyzing liquid state samples, while scanning electron microscopy (SEM) is more suited for solid samples. Atomic force microscopy (AFM) and scanning tunneling microscopy (STM) can be used for detailed structural analysis at the micro and nanoscale. In particular, STM is effective in determining the thickness of niosomal bilayers, as it can analyze structures along the vertical axis.

3. Zeta Potential

The zeta potential, also known as surface charge, is a vital indicator of the physical stability of niosomes. It is typically measured by laser Doppler anemometry. The magnitude of the zeta potential reflects the electrostatic repulsion between adjacent particles; higher or lower values (above +30 mV or below -30 mV) indicate good stability due to sufficient repulsion preventing aggregation.

4. Bilayer Structure Characterization

Niosomes can be either unilamellar (single-layer) or multilamellar (multiple layers). Techniques such as small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) spectroscopy, and AFM are used to analyze the number of lamellae in the niosomes. SAXS, combined with energy-dispersive X-ray diffraction (EDXD), is particularly useful for studying bilayer thickness. The fluidity of the niosomal membrane, which is important for its ability to deform without compromising bilayer integrity, can be assessed using fluorescent probes to monitor membrane mobility in relation to temperature and time.

5. Niosome Stability

Stability is a key factor in evaluating the potential of niosomes for both in vivo and in vitro applications. Stability can be monitored by tracking changes in particle size and zeta potential over time. Significant variations in these parameters can indicate instability. Stability tests are often conducted over three months under different conditions, such as varying temperatures (4°C, 25°C, 40°C) and relative humidity (75%), to assess the effect of environmental factors.

6. Entrapment Efficiency (EE)

Entrapment efficiency (EE) refers to the proportion of drug molecules successfully encapsulated within the niosomes. It is calculated using the formula:

E= (Amount of drug entrapped/ Total amount of drug added) × 100%

EE is an essential parameter in evaluating the effectiveness of the formulation. In vitro release studies are often performed to assess how the drug is released from the niosomes, using a system where the niosomal suspension is placed in the donor compartment and the release medium (typically PBS at pH 7.4) is in the receptor compartment. Samples are collected from the receptor compartment at specified intervals to monitor the drug release profile.

7. In Vitro Drug Release

The in vitro release behavior of niosomes is a key parameter influenced by various factors, including drug concentration, hydration volume, and membrane characteristics. To assess drug release, niosomal formulations are typically tested using a dialysis membrane method. In this approach, a purified niosomal suspension, free of unencapsulated drug, is placed into a dialysis bag, sealed at both ends, and immersed in a beaker containing phosphate-buffered saline (PBS). This setup is maintained at 37 °C with continuous magnetic stirring. Samples are collected at designated intervals and replenished with fresh PBS. The concentration of drug released over time is then analyzed using suitable assays.

An alternative method for studying niosome release involves the use of Franz diffusion cells. Here, a dialysis membrane is positioned between the donor and receptor compartments. The niosomal suspension is placed in the donor compartment, while the receptor compartment is filled with PBS at pH 7.4. The system is maintained at 37 °C, and samples from the receptor

compartment are taken at regular intervals, with fresh medium added to replace the withdrawn volume.

Characterization techniques for niosomes

Parameters	Applied techniques or methods
Particle size and distribution	Dynamic light scattering, scanning electron microscopy (for solid samples), Transmission electron microscopy (for liquid samples)
Morphology	Transmission electronic Microscopy, Negative-staining transmission electronic microscopy, Freeze-fracture transmission electronic microscopy, atomic force microscopy, Scanning electron microscopy
Zeta potential	Laser doppler anemometry
Bilayer characterization	Fluorescence polarization
Vesicle stability	Dynamic light scattering, microscopic techniques
Entrapment efficiency	UV-spectrometer, High performance liquid chromatography and Fluorescence
In vitro release	Dialysis, Franz diffusion cells

Results and Discussion

1. Preparation and Characterization of Lycopene-Loaded Niosomes

Lycopene-loaded niosomes were successfully prepared using the Adsorption Hydration method. The vesicles appeared spherical and uniform under microscopic observation. The particle size ranged between 100–200 nm, with a polydispersity index (PDI) below 0.3, indicating a homogenous dispersion. Zeta potential values were observed between –30 to –40 mV, suggesting good colloidal stability.

2. Encapsulation Efficiency (EE%)

The encapsulation efficiency of lycopene in niosomes was found to be 75–85%, depending on the surfactant-to-cholesterol ratio. Increased cholesterol concentration improved vesicle stability but slightly reduced the encapsulation efficiency. This balance is critical in optimizing drug loading and release profiles.

3. In Vitro Release Studies

The release profile of lycopene from niosomes exhibited a sustained release over 24 hours, compared to free lycopene, which showed a rapid burst release. This controlled release is advantageous for maintaining prolonged antioxidant activity and improving bioavailability.

4. Antioxidant Activity (DPPH Assay)

Antioxidant activity assessed by the DPPH radical scavenging assay demonstrated that lycopene-loaded niosomes showed significantly higher activity than free lycopene ($p < 0.05$). The improved performance is attributed to the protective effect of the niosomal bilayer, which prevents lycopene degradation and enhances its stability.

Sample	%DPPH Inhibition
Free lycopene	48.3%
Lycopene niosomes	72.6%
Control (Ascorbic Acid)	85.4%

5.Stability Studies

The formulation remained stable at 4°C for 30 days, with minimal changes in particle size, PDI, and encapsulation efficiency. Degradation of lycopene was significantly reduced when encapsulated in niosomes compared to free lycopene stored under the same conditions.

Discussion

The study demonstrates that niosomal encapsulation of lycopene significantly enhances its physicochemical stability, antioxidant potential, and controlled release profile. These findings suggest that niosomes are a promising carrier system for lipophilic antioxidants like lycopene, with potential applications in pharmaceuticals, nutraceuticals, and cosmeceuticals. The improved antioxidant activity is likely due to better cellular uptake and protection against oxidative degradation, which is crucial for clinical efficacy.

Conclusion

The present study successfully developed and characterized lycopene-loaded niosomes using the Adsorption Hydration method. The optimized formulation exhibited favourable physicochemical properties, including small particle size, high encapsulation efficiency, and

sustained release behavior. Importantly, the antioxidant activity of lycopene was significantly enhanced upon encapsulation, as demonstrated by the DPPH assay. These findings indicate that niosomal encapsulation offers a promising strategy to improve the stability, bioavailability, and therapeutic potential of lycopene. Overall, lycopene-loaded niosomes hold significant potential for applications in pharmaceutical, nutraceutical, and cosmeceutical formulations aimed at combating oxidative stress-related conditions.

References

1. Shi J, Le Maguer M, Bryan M (2002) Lycopene from Tomatoes, in: Functional Foods: Biochemical and Processing Aspects. CRC Press, Boca Raton.

2. Rao AV, Rao LG (2004) Lycopene and Human Health. Current Topics in Nutraceutical Research 2: 127-137.

3. Clinton SK (1998) Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev 56: 35-51.

4. Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC (2002) A prospective study of tomato products, lycopene, and prostate cancer risk. J Natl Cancer Inst 94: 391-398.

5. Omoni AO, Aluko RE (2005) The anti-carcinogenic and anti-atherogenic effects of lycopene: a review. Trends Food Sci Technol 16: 344-350.

6. Bhattacharya S (2009) Phytosomes: Emerging Strategy in Delivery of Herbal Drugs and Nutraceuticals. Pharma Times 41: 8-12.

7. Lee MT, Chen BH (2002) Stability of lycopene during heating and illumination in a model system. Food Chemistry 78: 425-432.

8. Pesek CA, Warthesen JJ (1987) Photodegradation of carotenoids in vegetable juice system. Journal of Food Science 52: 744-746.

9. Uchegbu IF, Vyas SP (1998) Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm 172: 33-70.

10. Arunothayanun P, Uchegbu IF, Craig DQ, Turton JA, Florence AT (1999) In vitro/in vivo characterisation of polyhedral niosomes. Int J Pharm 183: 57-61.

11. Rodrigues, S. R., & Silva, E. S. (2018). "Lycopene Extraction from Tomato and Its Bioactive Potential."

12. Khachik, F., Spangler, C. J., & Smith, J. C. (2002). "Lycopene Content of Tomatoes and Tomato Products and Their Contribution to the Lycopene Intake of the U.S. Population."

13. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm. 1998; 172:33–70.

14. Rangasamy M, Ayyasamy B, Raju S, Gummadevelly S, Shaik S. Formulation and in vitro evaluation of niosome encapsulated acyclovir. J Pharm Res. 2008; 1:163–6.

15. Bhaskaran S, Lakshmi PK. Comparative evaluation of niosome formulations prepared by different techniques. Acta Pharma Sci. 2009; 51:27–32.

16. D. Pando, G. Gutiérrez, J. Coca, C. Pazos, Preparation and characterization of niosomes containing resveratrol, J. Food Eng. 117 (2013) 227–234.

17. A.Y. Waddad, S. Abbad, F. Yu, W.L.L. Munyendo, J. Wang, H. Lv, J. Zhou, Formulation, characterization and pharmacokinetics of Morin hydrate niosomes prepared from various non-ionic surfactants, Int. J. Pharm. 456 (2013) 446–458.