RESEARCH PAPER

Formulation and Characterization of Lycopene-Based Nanostructured Lipid Carriers Prepared by Melt Emulsification Technique

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ABSTRACT

Lycopene is a highly potent antioxidant carotenoid widely recognized for its therapeutic potential in preventing oxidative stress-related diseases. However, its poor aqueous solubility, low stability, and limited oral bioavailability restrict its pharmaceutical application. Nanostructured lipid carriers (NLCs) are advanced lipid-based nanocarriers that enhance drug solubility, stability, and bioavailability. The present study aimed to develop lycopene-loaded NLCs using the melt emulsification technique and evaluate their physicochemical characteristics. Nine formulations (F1–F9) were prepared by varying the ratio of solid lipid (glyceryl monostearate) and liquid lipid (flaxseed oil) at 70:30, 65:35, and 75:25 while applying different rotation speeds (800, 1000, and 1200 rpm). The prepared NLCs were evaluated for particle size, polydispersity index, zeta potential, drug content, entrapment efficiency, and in-vitro drug release. The results demonstrated that lipid composition and rotation speed significantly influenced particle size and in vitro drug release. Among all formulations, the optimized formulation F5 showed nanoscale particle size 100 nm± 2.14, high entrapment efficiency 82.71%, and sustained drug release behaviour upto 24 hours. The findings suggest that lycopene-loaded NLCs prepared through melt emulsification can be a promising approach to enhance the stability and bioavailability of lycopene for oral delivery.

Keywords: Lycopene, Nano lipid carriers, Bioavailability, melt-emulsification.

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1.Introduction

Lycopene is a naturally occurring carotenoid responsible for the red coloration of tomatoes, watermelon, and other fruits (Moia et al., 2020). It possesses strong antioxidant activity and has been associated with the prevention of several chronic diseases including cardiovascular disorders, cancer, and inflammatory conditions (Okonogi & Riangjanapatee, 2015). Despite its beneficial biological activities, lycopene exhibits poor aqueous solubility and is highly susceptible to degradation by heat, light, and oxygen (Kusdemir et al., 2023). These limitations significantly reduce its bioavailability when administered orally (Shirodkar et al., 2019; Viegas et al., 2023a). Nanotechnology-based drug delivery systems have gained considerable attention for improving the pharmacokinetic profile of poorly soluble compounds (Ma et al., 2022). Among these systems, nanostructured lipid carriers (NLCs) represent a second-generation lipid nanoparticle system composed of a mixture of solid and liquid lipids (Naseri et al., 2015; Shidhaye et al., 2008). The incorporation of liquid lipid into the solid lipid matrix creates structural imperfections, which improve drug loading capacity and prevent drug expulsion during storage (Singh et al., 2017). NLCs offer several advantages including improved drug stability, enhanced bioavailability, controlled drug release, and reduced toxicity (Das et al., 2012; Li et al., 2017). The lipid components are generally biocompatible and biodegradable, making them suitable for pharmaceutical applications (Gomaa et al., 2022; Selvamuthukumar & Velmurugan, 2012). Furthermore, the nanoscale size of NLCs facilitates improved absorption and interaction with biological membranes (Krambeck et al., 2021; Poonia et al., 2016). The melt emulsification technique is widely used for the preparation of NLCs due to its simplicity, scalability, and avoidance of organic

solvents (Apostolou et al., 2021; kaur et al., 2015). In this method, the lipid phase is melted and dispersed into a heated aqueous surfactant solution under mechanical stirring to form nano-sized lipid particles upon cooling (Joshi & Patravale, 2008).

In the present study, lycopene-loaded NLCs were prepared using glyceryl monostearate as the solid lipid and flaxseed oil as the liquid lipid (Jain et al., 2017; Viegas et al., 2023b). The formulation variables included different lipid ratios and rotation speeds (Abdel-Salam et al., 2017; Almousallam et al., 2015). The prepared formulations were characterized to determine the effect of formulation parameters on particle size, entrapment efficiency, drug content, and drug release behaviour (Khosa et al., 2018; Parashar & Kanoujia, 2023).

Materials and Methods

2.1 Materials

Standard lycopene was obtained from Panacea biotech ltd. As a gift sample. Glyceryl monostearate and stearic acid were purchased from Merck (Mumbai, India). Polyethylene glycol 4000, Tween 80, Methanol, Hydrogen peroxide, ascorbic acid and carboxy methyl cellulose were purchased from CDH (P) Ltd. New Delhi. DPPH and Flaxseed oil were purchased from SRL Mumbai and Dev Herbes.

2.2 Preparation of Lycopene-Loaded NLCs

Lycopene-loaded nanostructured lipid carriers were prepared using the melt emulsification technique. In this method, the required quantity of glyceryl monostearate was melted at approximately 70–75°C. Lycopene was dissolved in the molten lipid mixture containing flaxseed oil.

The aqueous phase was prepared separately by dissolving Tween 80 in distilled water and heating it to the same temperature as the lipid phase. The hot aqueous phase was then added to the lipid phase under continuous stirring using a mechanical stirrer at different rotational speeds (800, 1000, and 1200 rpm). The hot nanoemulsion was then cooled to room temperature with continuous stirring, resulting in the formation of lycopene-loaded NLCs.

3. Characterization of NLCs

3.1 Particle Size and Polydispersity Index

Particle size and polydispersity index were determined using dynamic light scattering. The NLC dispersion was diluted with distilled water before measurement. The average particle size provides information about the nanoscale dimension of the formulation, while the polydispersity index indicates particle size distribution.

3.2 Zeta Potential

Zeta potential analysis was performed to evaluate the surface charge of the nanoparticles. The measurement provides insight into the stability of the dispersion system. Higher absolute zeta potential values generally indicate better stability due to electrostatic repulsion between particles.

3.3 Drug Content

Drug content analysis was performed to determine the amount of lycopene present in the formulation. A specific volume of NLC dispersion was dissolved in a suitable solvent and analysed using UV-visible spectroscopy at the characteristic wavelength of lycopene. The percentage drug content was calculated using the following equation:

Drug Content (%) = (Actual amount of drug present / Theoretical amount of drug) × 100

3.4 Entrapment Efficiency

Entrapment efficiency indicates the percentage of lycopene successfully encapsulated within the lipid matrix. The formulation was centrifuged to separate free drug from the nanoparticle dispersion. The supernatant containing free drug was analyzed spectrophotometrically.

Entrapment Efficiency (%) = [(Total drug – Free drug) / Total drug] × 100

3.5 In-Vitro Drug Release

The drug release study was performed using a dialysis membrane method in phosphate buffer (Ph 7.4). A measured quantity of NLC dispersion was placed in a dialysis bag and immersed in the release medium maintained at 37°C under continuous stirring. At predetermined intervals, samples were withdrawn and replaced with fresh medium. The amount of drug released was determined spectrophotometrically.

3.6 Percentage drug release of F5 and Lycopene

The in-vitro drug release profiles of formulation F5 and lycopene suspension (prepared in 0.25% CMC solution) were evaluated using a dissolution apparatus. The study was conducted in phosphate buffer solution (Ph 7.4) maintained at 37 ± 0.5 °C with continuous agitation at 100 rpm for a total duration of 24 hours. At predetermined time intervals, 5 ml of the dissolution medium was withdrawn and replaced with an equal volume of fresh buffer to maintain constant sink conditions. The collected samples were analysed using UV–visible spectrophotometry to determine the amount of lycopene released from the formulation. The

percentage drug release was subsequently calculated based on the measured absorbance values.

3.7 Drug Release kinetic

The release data obtained from the optimized formulation were fitted to various kinetic models, including zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas models, to understand the mechanism of drug release. The correlation coefficient (R^2) values were calculated for each model. The model exhibiting the highest R^2 value was considered the best fit for describing the release behavior of the formulation.

3.8 Morphological study

The morphology of LYC loaded NLCs was analysed by transmission electron microscope (TEM). Sample was diluted and a drop of that sample as stained negatively by 2 % solution of phosphotungstic acid. The sample was dried after application of carbon coated grid on the slide. After this the slide was subjected in TEM for the morphological evaluation of NLCs

4. Result and Discussion

4.1 Particle Size and Polydispersity Index

The lycopene-loaded NLCs prepared by the melt emulsification technique were successfully obtained with nanoscale particle sizes ranges from 100 nm± 2.14 to 582.5 ±2.22. The formulation parameters, including lipid ratio and homogenization speed, had a significant influence on the physicochemical properties of the NLCs. An increase in rotation speed resulted in a reduction in particle size due to improved dispersion of the lipid droplets. On the other hand, the polydispersibility index was less than 1 which indicates the homogeneity of suspended particles. The obtained results were shown in table no 1.

Table 1: Particle size, PDI of LYC- NLCs

Formulation	Size (nm) ±sd	PDI±sd
F1	375.7±8.21	0.51±0.16
F2	261.4 ±3.72	0.74±0.12
F3	582.5 ±2.22	0.83±0.07
F4	519.57 ±3.25	0.95±0.04
F5	100 nm± 2.14	0.194±0.03
F6	559.2 ±2.21	0.86±0.07
F7	302.2±4.22	0.47±0.08
F8	225.4±3.2	0.44±0.07
F9	472.7 ±4.25	0.60±0.60

4.2 Zeta Potential

The zeta potential of the prepared lycopene-loaded nanostructured lipid carriers ranged approximately between –20 Mv to –35 Mv, indicating moderate to good stability of the dispersion system. The negative surface charge observed in the formulations may be attributed to the presence of fatty acids from the lipid components, particularly glyceryl monostearate and flaxseed oil. Among the prepared formulations, formulation F5 exhibited the most desirable zeta potential value, indicating improved dispersion stability.

Table 2: Zeta potential of LYC- NLCs

Formulation	Zeta potential (mV) ±sd
F1	-30.25±0.08
F2	-20.37±0.12
F3	-27.99±0.07
F4	-31.94±0.04
F5	-32.9±0.04
F6	-49.89±0.07
F7	-11.74±0.08
F8	-41.94±0.07
F9	-42.57±0.10

4.3 % Drug content

Drug content of lycopene loaded nano lipid carriers were found to be in between 65.34 to 78.73 %. From the obtained data it was observed that the formulation F5 having greater percentage of drug content in comparative to the others.

Table 3: % Drug Content of LYC- NLCs

Formulation	% Drug Content ±sd
F1	65.34±0.08
F2	72.57±0.01
F3	70.60±0.09
F4	74.87±0.04
F5	78.73±0.01
F6	69.86±0.04
F7	75.70±0.07
F8	76.69±0.01
F9	73.42±0.04

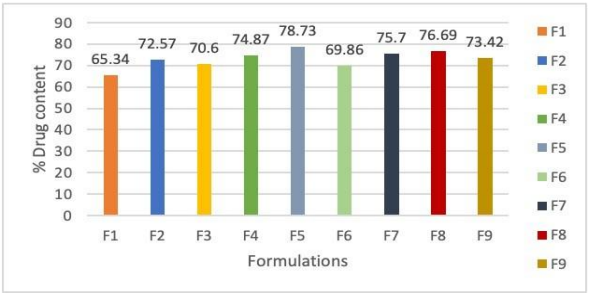


Figure 1: % Drug Content of Lycopene Loaded NLCs

4.4 % Entrapment Efficiency

Variations in the ratio of solid lipid and liquid lipid influenced drug loading and entrapment efficiency. Formulations containing a balanced mixture of solid and liquid lipids showed improved drug entrapment due to the formation of a less ordered lipid matrix. Among the prepared formulations, the formulation F5 with a moderate lipid ratio and optimal stirring speed exhibited the most desirable characteristics, including high entrapment efficiency 82.71 %.

Table 4: % EE of LYC- NLCs

Formulation	% Entrapment Efficiency ±sd
F1	68.14±0.01
F2	73.72±0.01
F3	72.49±0.01
F4	77.53±0.01
F5	82.71±0.01
F6	76.78±0.01
F7	78.97±0.02
F8	80.41±0.01
F9	75.56±0.02

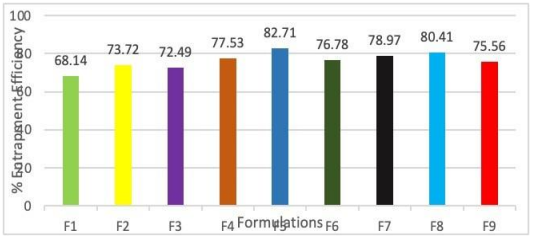


Figure 2: % Drug Entrapment Efficiency of Lycopene Loaded NLCs

4.5 In-Vitro Drug Release

The in-vitro release profile of lycopene from the developed nanostructured lipid carrier (NLC) formulations (F1–F9) was evaluated in phosphate buffer (Ph 7.4) for a period of 24 hours. The cumulative percentage drug release was plotted against time to examine the release behavior of each formulation. Among the tested formulations, F5 showed the highest cumulative drug release, reaching approximately 85–88% within 24 hours, indicating efficient drug diffusion from the lipid matrix and sustained release behaviour of lycopene from the NLC system. The lipid matrix controlled the diffusion of the drug, resulting in gradual release over time. This controlled release pattern is advantageous for improving oral bioavailability and maintaining therapeutic drug levels.

Table 5: % Drug Release at pH 7.4 Phosphate Buffer

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.083	3.91± 0.04	11.91±0.06	13.20±0.03	26.88±0.02	15.82±0.04	10.52±0.13	8.81±0.02	13.51±0.05	9.08±0.03
0.167	33.87±0.05	18.14±0.03	18.54±0.03	33.42±0.04	29.71±0.02	22.22±0.03	14.42±0.02	22.74±0.02	16.94±0.03
0.25	38.29±0.22	26.35±0.07	29.23±0.03	36.45±0.03	39.77±0.02	36.61±0.02	21.74±0.03	46.70±0.03	26.720.02
0.5	40.18±0.34	34.22±0.05	29.92±0.02	40.75±0.02	49.230.01	58.58±0.03	32.18±0.03	51.03±0.04	29.29±0.04
1	49.65±0.22	38.49±0.30	35.09±0.06	44.74±0.06	63.93±0.03	63.17±0.01	35.61±0.03	55.61±0.03	41.81±0.04
2	57.43±0.39	45.05±0.50	42.33±0.02	68.67±0.01	70.57±0.02	67.28±0.03	53.53±0.02	58.39±0.04	52.07±0.04
4	61.01±0.19	56.21±0.49	51.12±0.09	70.43±0.03	76.20±0.02	75.97±0.02	63.50±0.02	61.57±0.02	68.44±0.02
6	65.64±0.06	68.35±0.03	57.84±0.04	77.29±0.01	82.85±0.02	83.56±0.04	70.20±0.03	69.36±0.02	76.57±0.03
12	73.84±0.07	78.52±0.04	68.35±0.04	83.67±0.02	88.68±0.02	83.56±0.02	78.84±0.04	69.22v0.04	81.43±0.03
24	71.53±0.08	76.88±0.03	67.83±0.04	82.71±0.02	89.69±0.01	82.61±0.02	77.52±0.02	68.35±0.03	79.83±0.04

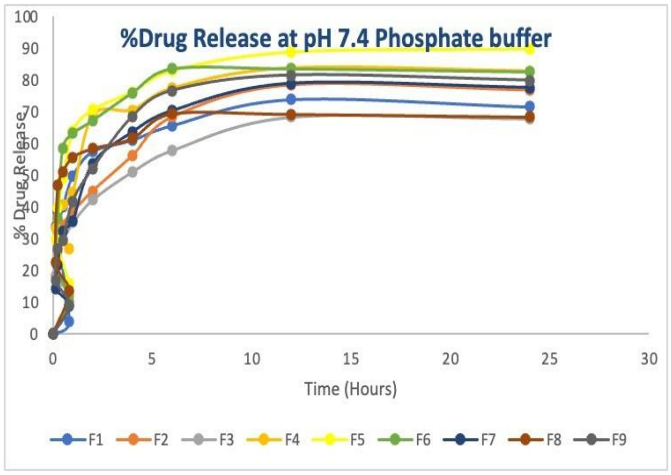


Figure 3: %Drug Release of Lycopene Loaded NLCs at pH 7.4 Phosphate Buffer

4.6 Percentage drug release of F5 and Lycopene (0.25% CMC)

From the comparative study of % drug release of optimized lycopene loaded NLC formulation F5 and conventional suspension it was found that the the NLC having better relase than the suspension at initial state the release of F5 was found to be 15.82 ehich was two times greater than the pure lycopene and after 24 hours the sustained release of F5 formulation was maintained about 89.70 %.

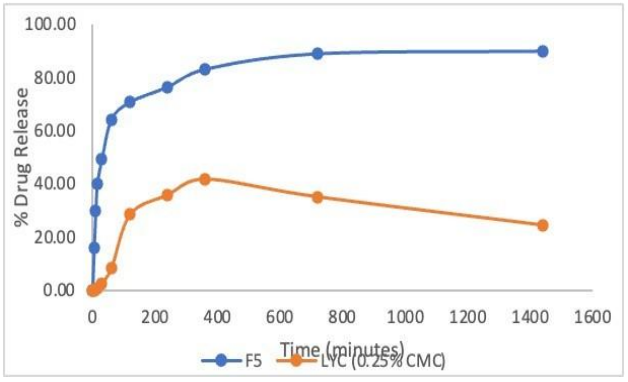


Figure 4: Formulation F5 and Lycopene (0.25% CMC)

4.7 Drug Release kinetics

For the drug release kinetic the in-vitro release data were fitted into different kinetic models including zero-order, first-order, Higuchi, and Korsmeyer– Peppas models. From the obtained results it was found that the lycopene released by diffusion from the lipid matrix and followed non – fickian anomalous diffusion mechanism.

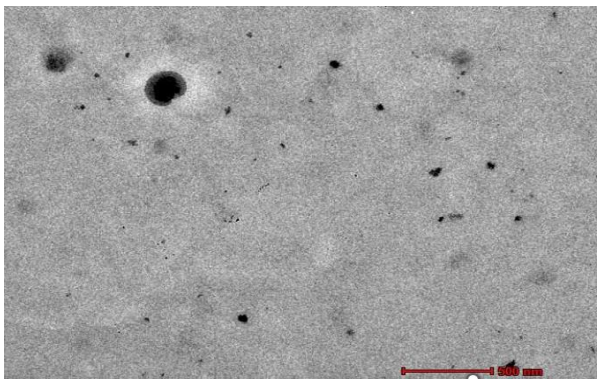
Table 3: Drug Release kinetics of F5 formulation

F5	R^2	K
Zero Order	0.458	3.43
First Order	0.519	-0.09
Higuchi Matrix	0.933	10.29
Peppas	0.845	3.61
Hix. Crow.	0.505	0.02
Parameters for Korsmeyer-Peppas Equation		
N		0.705
K		3.612

4.8 Morphological study

The TEM micrograph revealed that the nanoparticles were generally spherical in shape and well dispersed, indicating successful formation of the nanostructured lipid carrier system. The nanoscale size of the particles can also be confirmed from the scale bar (500 nm), indicating that most of the nanoparticles were within the nanometer size range, which is consistent with typical nanostructured lipid carrier systems.

Figure 5: Transmission Electron Microscopy (TEM) of F5 Formulation



Conclusion

The present study successfully developed lycopene-loaded nanostructured lipid carriers using the melt emulsification technique. Different formulations were prepared by varying the ratio of solid lipid (glyceryl monostearate) and liquid lipid (flaxseed oil) along with homogenization speed. The prepared NLCs exhibited nanoscale particle size, acceptable surface charge, and good dispersion stability. Among the formulations, F5 demonstrated desirable characteristics including suitable zeta potential, high drug encapsulation, and sustained drug release over 24 hours. Drug release kinetic analysis indicated that the release followed the Higuchi diffusion model with non-Fickian transport behaviour. Overall, the developed NLC system shows potential as an effective carrier to improve the stability and controlled delivery of lycopene.

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