

Formulation and evaluation of solid lipid nanoparticles of naringin to enhance its bioavailability

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Abstract: The objective of the present work was to formulate solid lipid nanoparticles loaded with naringin for improving the bioavailability of naringin. The SLNs loaded with naringin were prepared by using different ratio of palmitic and stearic acid as the lipids and Tween 80 as the surfactant. The method nanoprecipitation was used for preparation of the SLNs. The surfactant (Tween 80) helps in steric stabilization of the SLNs. The size range of SLNs obtained on sonicating for 7 was found to be 429 ± 76 nm when stearic acid was used and 365 ± 28 nm when palmitic acid was used for the preparation of SLNs. The zeta potential of all the SLNs ranged from -16 to -27 mV suggesting a stable formulation. The percentage of drug incorporated during nanoparticle preparation was determined by centrifuging the drug loaded nanoparticles at 15,000 rpm for 15 min and separating the supernatant. The highest encapsulation of naringin (71.7 ± 8.6 %) was obtained when the concentration of palmitic acid was 0.14 mmol. The *in vitro* release of naringin from the SLNs was measured by dialysis method using simulated gastric fluid as the dissolution medium. *In vitro* release kinetics studies for naringin loaded SLNs exhibited a sustained release pattern. Sustained release was observed over a period of 3 days. The stability of NSLN-7 was studied by storing at 4 ± 1 °C for 30 days. The particle size remained stable at the end of the study with drug entrapment of 71.5 %. This suggests that the SLNs prepared are stable on storage.

Keywords: Naringin, solid lipid nanoparticles, palmitic acid, nanoprecipitation, release

1. Introduction

Naringin is a disaccharide derivative, derived from Grapefruit and possesses low intrinsic toxicity. Several reviews related to naringin explain the wide therapeutic potential of naringin and its precursor naringenin¹⁻³. It has been reported to possess a variety of pharmacologic effects, including antibacterial, anti-inflammatory, antioxidant, and antitumor properties. However, naringin is hydrophobic and this leads to the instability and poor bioavailability. Despite of its pharmacological potential, naringin could not be marketed as a drug owing to its poor systemic bioavailability as well as stability issues. Several reports have been made that describe that the formulation of nanoparticles could be a viable method to improve the bioavailability of drugs.

Over the last few years several nanoformulations of naringin have been reported each claiming to improve the bioavailability of naringin⁴⁻⁶. It has also been reported that use of polymeric nanomaterials improves the encapsulation of naringin and eventually improve the bioavailability of the drug. These systems although suffer from several drawbacks, such as poor physical stability, drug leakage, and the potential toxicity of the excipients.

Solid lipid nanoparticles (SLNs) have recently been under consideration for drug delivery because they offer the possibility of modulating drug release and provide both stability and compatibility while avoiding the shortcomings of liposomes, including undesired stability problems and the potential toxicity of the materials such as polymeric nanoparticles^{7,8}. A few investigations have also been reported related to formulation of solid lipid nanoparticles using biocompatible and biodegradable lipid substances. Apart from physicochemical features, the intestinal permeability of a drug is another crucial factor for oral bioavailability.

It was there envisioned to use solid lipid nanoparticle approach as the carrier to improve the permeability of naringin in the cells and hence the oral bioavailability. The lipids used to encapsulate naringin into SLNs are likely to improve the aqueous dispersibility and stability of naringin, prolonging its efficacy and cellular uptake and enhancing its bioavailability.

2. Material and methods

Naringin was purchased from Yucca enterprises; palmitic and stearic acid were purchased from CDH. Any other reagent used was of analytical grade and procured from various sources.

Drug excipient compatibility Study

IR spectra of drug and a physical mixture of drug and lipids were obtained using FT-IR spectrophotometer. The spectra were observed for physical and chemical incompatibility amongst the drug and the lipids under study.

Calibration curve of Naringin⁹

The maximum absorption of Naringin in ethanol was observed at 295 nm. The calibration curve was obtained using different concentrations of the drug at the above wave length. The stock solution was freshly prepared by dissolving 5 mg of Naringin in 50 ml of ethanol in a 10 ml volumetric flask and then made up the solution upto the mark using the same buffer for obtaining the solution of strength 100 µg/mL (stock I). 5 mL stock solution was taken and volume made up to 50 mL by using ethanol to obtain 10 µg/ml. From this solution with draw 2, 4, 6, 8, 10 ml of solution in to the 10 ml volumetric flask and volume made up to 10 ml by using ethanol to get the solutions of 2, 4, 6, 8, 10 µg/ml. The absorbance of each dilution was observed at 295 nm using UV spectrophotometer employing ethanol as the reference blank and a calibration curve was plotted.

Formulation of SLNs

Nano precipitation method was used for the preparation of the solid lipid nanoparticles. Various concentrations of lipids were used for formulation the SLNs (Table 1).

Table 1:Formula for preparation of SLNs

	NSLN1	NSLN2	NSLN3	NSLN4	NSLN5	NSLN6	NSLN7	NSLN8
Naringin (mg)	100	100	100	100	100	100	100	100
Stearic acid (mmol)	0.1	0.12	0.14	0.16	-	-	-	-
Palmitic acid (mmol)	-	-	-	-	0.1	0.12	0.14	0.16
Tween 80 (%)	5	5	5	5	5	5	5	5

The organic phase was prepared by dissolving the lipid in a mix of 18 mL ethyl acetate and 2 mL ethanol. Naringin was added to the organic phase and dissolved with stirring. A 5% solution of Tween 80 was prepared in distilled water to obtain the emulsifier solution. The organic phase was drop wise added to the aqueous phase with stirring (700 rpm) at room temperature. The resultant turbid suspension containing the nanoparticles was stirred for a time of 5–10 min. The organic solvents were removed by vacuum evaporation and the dispersion was cooled to room temperature. The pH of the dispersion was adjusted to 1.2 by addition of 0.1 M hydrochloric acid solution to the precipitated SLNs, and the precipitate was then collected by centrifuging at 12,000 rpm. The precipitate was re-dispersed in distilled water under sonication (13 mm probe, 65% amplitude and 1 min cycles) for 7 minutes¹⁰.

Characterization of SLNs

Particle size and zeta potential determination¹¹

Particle size was determined using a particle size analyzer while the zeta potential was determine using a zeta sizer. 1 mg/ml of nanoparticulate naringin solution was prepared in double distilled water and sonicated for 30 seconds in an ice bath. 1 mL of this solution was diluted to 100 mL with deionized water and the particle size determination was done.

Entrapment Efficiency¹²

The percentage of drug incorporated during nanoparticle preparation was determined by centrifuging the drug loaded nanoparticles at 15,000 rpm for 15 min and separating the supernatant. The pellet obtained was washed twice with water and dissolved in acetonitrile followed by estimation of the drug by measuring the absorbance at 295 nm using UV-visible spectrophotometer.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Initial amount of drug taken}} \times 100$$

In vitro drug release¹³

The *in vitro* release of naringin from the SLNs was measured by dialysis method. 1 mL of NSLN was placed into a dialysis bag, sealed at both the ends using clamps. The dissolution medium contained simulated gastric fluid (phosphate buffer saline adjusted to pH 2.0 with HCl). The dialysis bag was lowered in the dissolution medium (200 mL) in a beaker acted as the donor compartment. The medium was stirred at 100 rpm and was maintained at 37°C. At predetermined time intervals, 1 mL sample was pipetted out and the medium was replenished with the same quantity of medium in order to maintain the sink conditions. The samples were centrifuged (10000 rpm for 5 min) and the supernatant was appropriately diluted and the absorbance was measured at 295 nm using UV-visible spectrophotometer for determining the concentration of naringin.

Stability Study¹²

The stability of SLNs was studied by storing at 4 ± 1 °C for 30 days. The particle size was observed to assess the physical stability of the SLNs while the drug concentration in SLNs was determined at the end of the study by spectrophotometry.

3. Results and Discussion

The FT-IR spectrum of naringin (figure 1), and a physical mixture of naringin, palmitic acid and stearic acid (figure 2) were obtained and observed for any deletion of the peaks of the pure drug. The FTIR spectrum of Naringin exhibited the stretching and bending vibrations due to OH (3340.46 cm^{-1}), C=O (1699.89 cm^{-1}), C=C (1603.53 cm^{-1}) and C-O-C (1003.43 cm^{-1}). All the peaks were present in the physical mixture indicating a compatibility between the both the components.

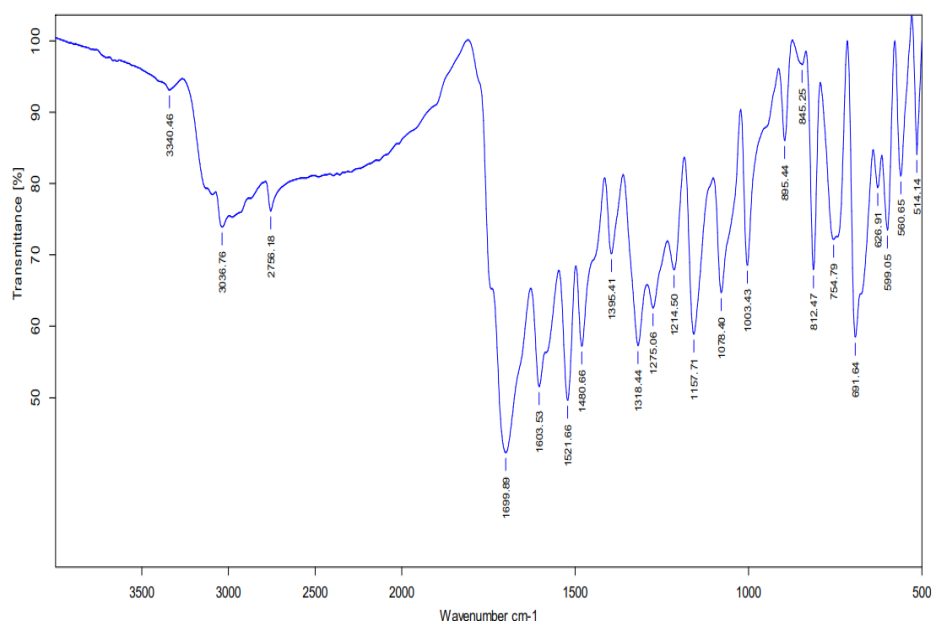


Fig 1: FT-IR spectrum of naringin

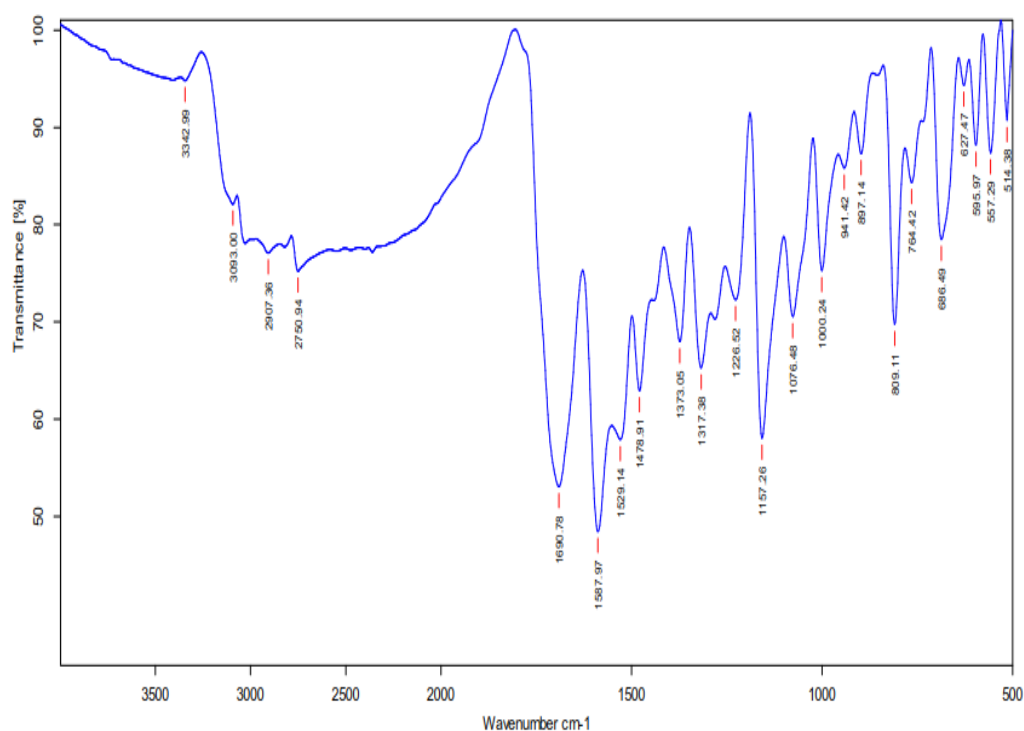


Fig 2: FT-IR spectrum of physical mixture of palmitic acid, stearic acid and curcumin

Calibration curve of Naringin

The Calibration curve of Naringin was constructed by plotting absorbance versus concentration ($\mu\text{g/ml}$) at 295 nm. The regression equation was used to calculate the concentration of Naringin in the formulation as well as in the release study.

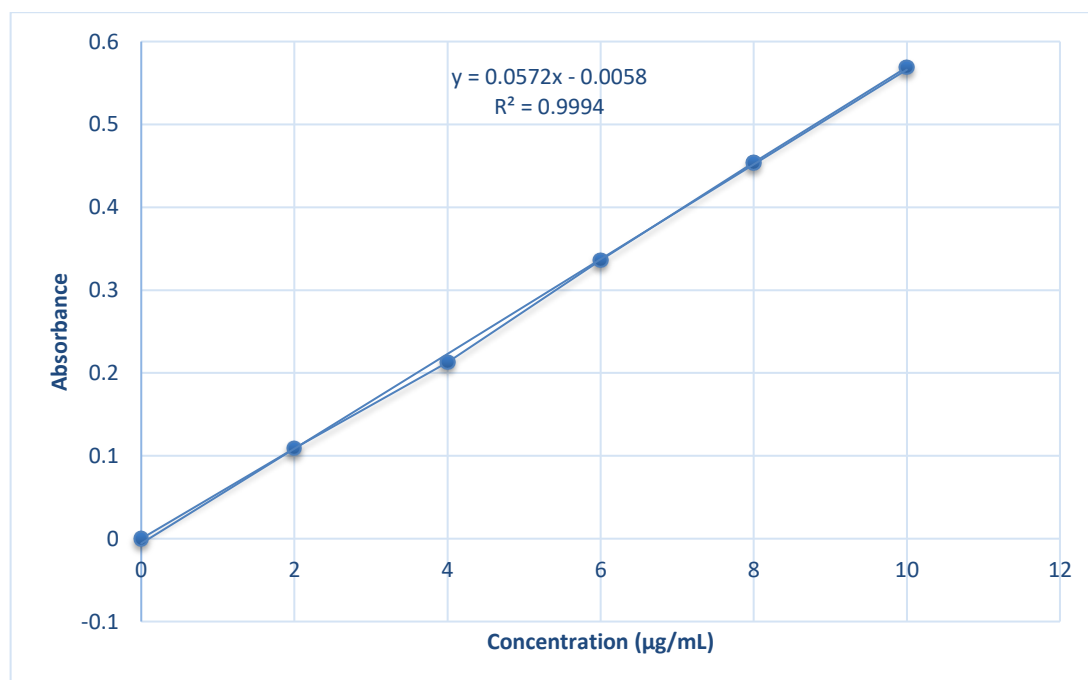


Fig 3: Calibration curve of Naringin in ethanol

Preparation of SLNs

The SLNs loaded with naringin were prepared by using different ratio of palmitic and stearic acid as the lipids and Tween 80 as the surfactant. The method nanoprecipitation was used for preparation of the SLNs. Ethyl acetate was used as the major solvent to dissolve the lipids while the small portion of ethanol facilitates the solubilization of curcumin in the organic phase. The surfactant (Tween 80) helps in steric stabilization of the SLNs. Systems that are sterically stabilized tend to remain well dispersed even at high salt concentrations or under conditions where the zeta potentials of the surfaces are reduced to near zero.

Previous study has indicated that a low concentration of surfactant leads to high instability among the particles and a 5% concentration is the most optimum for having stable SLNs that do not undergo extreme coalescence or flocculation and hence result in smaller particles.¹⁴

Characterization of SLNs

Particle size and zeta potential

Table 2 presents the average size of naringin-loaded SLNs prepared using different lipids. All readings were taken as average \pm standard deviation. The final sonication of 7 min was able to reduce the size of the particles to nano range. The lowest size of SLNs obtained on sonicating for 7 min was found to be 429 ± 76 nm when stearic acid was used and 365 ± 28 nm when palmitic acid was used for the preparation of SLNs (figure 3 & 4).

Table 2: Particle size and zeta potential of SLNs

Formulation Name	Particle Size	Zeta Potential
NSLN1	636 ± 47	-26.1 ± 5.9
NSLN2	541 ± 22	-19.8 ± 1.5
NSLN3	429 ± 76	-25.3 ± 3.1
NSLN4	502 ± 81	-19.4 ± 1.7
NSLN5	549 ± 103	-19.1 ± 2.9
NSLN6	431 ± 53	-20.4 ± 5.9
NSLN7	365 ± 28	-21.7 ± 2.2
NSLN8	636 ± 47	-26.1 ± 5.9

The results of particle size show that 0.14 mmol of lipid in solution resulted in the smallest particles whereas a higher or lower ratio increased the particle size of the SLNs. It was also evident that palmitic acid was able to produce smaller SLNs compared to the SLNs produced with stearic acid. Similar results were obtained in a previous study where they studied three fatty acids and found that stearic acid produced the largest sized particles.¹⁵

The zeta potential value of around ± 30 mV is considered to be having stable particles. The zeta potential of all the SLNs ranged from -16 to -27 mV suggesting a stable formulation (figure 5). All the SLNs with zeta potential higher than 20 mV can be considered optimum for a formulation to be stable enough as a result of enough repulsion among the particles that help in avoiding particle aggregation, making them stable for long term.

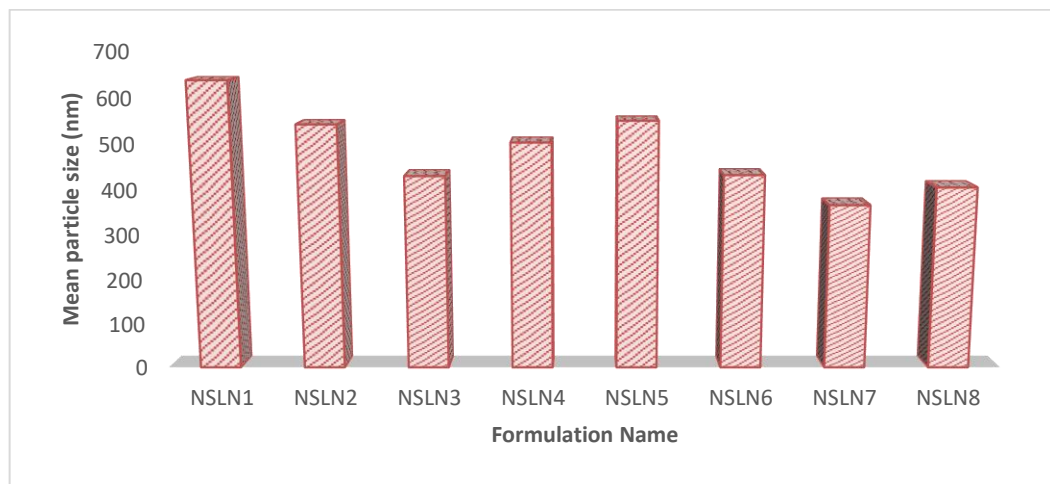


Fig 4: Particle size of SLNs prepared using stearic acid and palmitic acid



Size Distribution Report by Intensity

Sample Details

Sample Name: NSLN-7 Size 1

SOP Name: mansettings.dat

General Notes:

File Name: NSLN- 7 Size.dts	Dispersant Name: Water
Record Number: 1	Dispersant RI: 1.330
Material RI: 1.59	Viscosity (cP): 0.8872
Material Absorption: 0.01	Measurement Date and Time: Saturday, June 03, 2023 12...

System

Temperature (°C): 25.0	Duration Used (s): 100
Count Rate (kcps): 110.4	Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette	Attenuator: 6

Results

	Diam. (nm)	% Intensity	Width (nm)
Z-Average (µm): 365.1	Peak 1: 108.2	74.5	54.78
Pdl: 0.497	Peak 2: 425.4	23.5	137.0
Intercept: 0.972	Peak 3: 5451	2.0	265.4
Result quality Good			

Size Distribution by Intensity

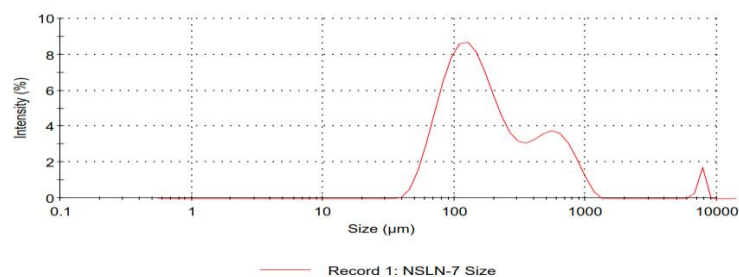


Fig 5: Particle size and distribution of NSLN-7



Zeta Potential Report

Sample Details

Sample Name: NSLN-7 Zeta 1
SOP Name: mansettings.dat
General Notes:

File Name: NSLN-7 Zeta.dts
Dispersant Name: Water
Record Number: 1
Dispersant RI: 1.330
Date and Time: Saturday, Jun 03, 2023 12:53...
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Zeta Runs: 12
Count Rate (kcps): 37.4
Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell
Attenuator: 7

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -21.7	Peak 1: -21.7	100.0	3.36
Zeta Deviation (mV): 3.36	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0891	Peak 3: 0.00	0.0	0.00

Result quality Good

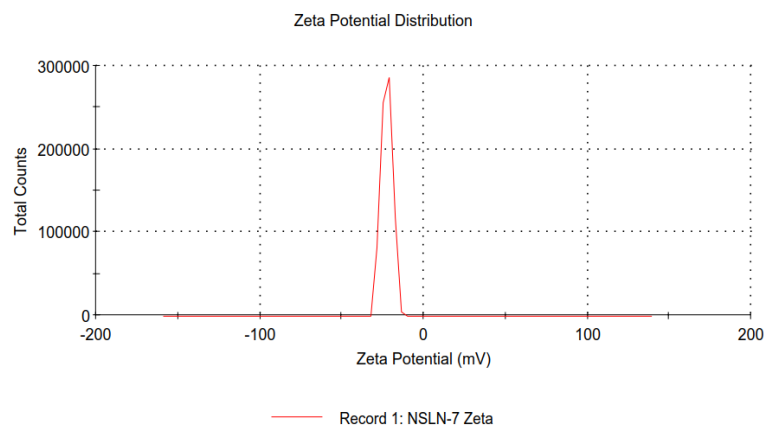


Fig 6: Zeta potential of NSLN-7

Entrapment efficiency

The entrapment efficiency was found to increase initially on increasing the concentration of the lipid but declined when the concentration of lipid was 0.16 mmol. The highest encapsulation of naringin (71.7%) was obtained when the concentration of palmitic acid was 0.14 mmol. The entrapment efficiency of the prepared SLNs is presented in table 3.

Table 3:Entrapment of naringin in SLNs

Formulation Name	Entrapment Efficiency
NSLN1	44.1 ± 9.0
NSLN2	54.5 ± 3.7
NSLN3	66.7 ± 1.1
NSLN4	61.1 ± 2.1
NSLN5	52.6 ± 3.2
NSLN6	58.9 ± 9.1
NSLN7	71.7 ± 8.6
NSLN8	67.6 ± 9.1

In vitro release from SLNs

In vitro release kinetics studies for naringin loaded SLNs exhibited a sustained release pattern (figure 6). Sustained release was observed over a period of 3 days. Initial burst release can be attributed to dissociation of surface absorbed naringin into lipid matrix while sustained released over a period of 3 days can be attributed to release of naringin from nanoparticles.

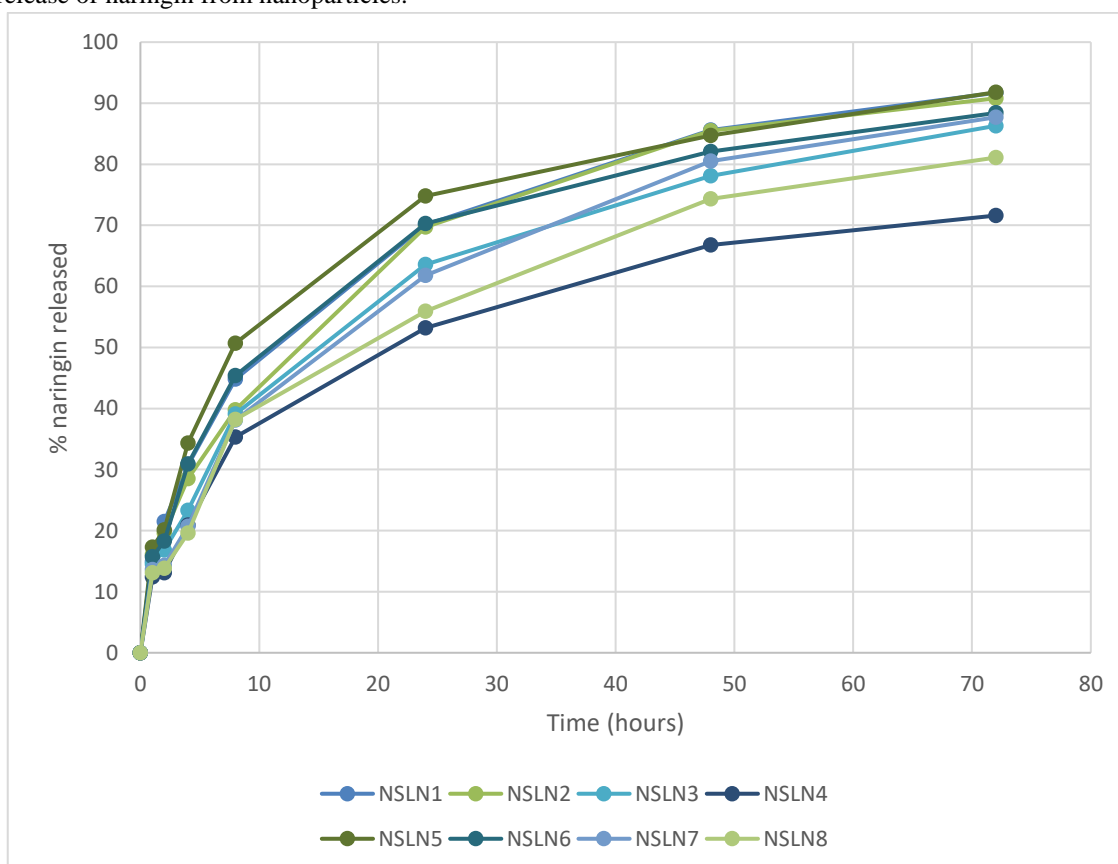


Fig 7: Release of naringin form SLNs

Stability Study

NSLN-7 was observed for change in particle size and percent drug entrapped on storage at 4°C for a period of 30 days. The particle size remained stable at the end of the study with drug entrapment of 71.5 %. This suggests that the SLNs prepared are stable on storage.

4. Conclusion

The present study was to prepared naringin -loaded SLN to improve its bioavailability and drug loading. The results suggest that nanoprecipitation method is a highly feasible method for preparing the SLNs. The SLNs were evaluated for particle size, entrapment efficiency and drug release. The SLNs prepared by palmitic acid were found to be comparatively smaller in size and exhibited better entrapment efficiency. The best formulation was one that was prepared with 0.14mmol of palmitic acid as the lipid component (NSLN-7), considering its lowest particle size and highest entrapment efficiency.

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