Formulation and characterization of solid lipid nanoparticles of naringenin

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Abstract

Naringenin is a natural antioxidant isolated for *Grapefruit* and is known to possess a large number of pharmacological actions. The half-life of the molecule is though low (2.6-3.0h) which limits the use of the molecule in therapy. The objective of the present work was to formulate solid lipid nanoparticles loaded with naringenin for improving bioavailability of naringenin. The SLNs loaded with naringenin were prepared by using different ratio of palmitic and stearic acid as the lipids and Tween 80 as the surfactant using nanoprecipitation technique. The size range of SLNs obtained on sonicating for 25 was found to be 163.6 nm when stearic acid was used and 142.2 nm when palmitic acid was used for the preparation of SLNs. The zeta potential of all the SLNs ranged from -2.79 to -25.9 mV suggesting most stable formulation NSLN-7. The highest encapsulation of naringenin (79.3%) was obtained when the concentration of palmitic acid was 1.6 mmol. The *in vitro* release of naringenin from the SLNs was measured by dialysis method using phosphate buffer as the dissolution medium. *In vitro* release kinetics studies for naringenin 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 67.4%.

Keywords

Naringenin, sustained release, half-life, solid lipid nanoparticles, nanoprecipitation

Introduction

Naringenin is a natural antioxidant isolated for *Grapefruit* and is known to possess a large number of pharmacological actions.[1] The antioxidant potential of the molecule is mainly responsible for almost all of its effect on the human body. The half-life of the molecule is though low (2.6-3.0h) which limits the use of the molecule in therapy.[2] Over the last few years many formulations of naringenin have been reported each claiming to improve its bioavailability. It has also been reported that use of polymeric nanomaterials improves the encapsulation 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.[3,4]

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. A few investigations have also been reported related to formulation of solid lipid nanoparticles using biocompatible and biodegradable lipid substances.[5-8]

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

Material and Methods

Naringenin was purchased from Yucca Enterprises, Mumbai; Palmitic acid and stearic acid were procured from CDH, New Delhi. All the reagents and chemical used in the present study were procured from various sources and used without any drying or purification.

Preformulation Studies

The preformulation studies were carried in order to confirm the purity and identity of the procured naringenin and also to study any possible interaction with the polymeric carrier to be used in the investigation.[9]

Calibration curve of naringenin

Stock solutions of naringenin containing 100 µg/mL were prepared in phosphate buffer pH 7.4 and its aliquots were transferred in a series of 10 mL volumetric flasks in varying fractions and their volumes were made with phosphate buffer pH 7.4 to prepare different standard dilutions (1-6 µg/mL). The solution was scanned using UV-Visible spectrophotometer from 1100 to 200 nm and the absorption maximum (λmax) was obtained to be 295 nm.[10] The absorption of the standard dilutions was recorded at 295 nm to construct a calibration curve of concentration against absorbance. The linearity equation ($y = mx + c$) was generated and was used to calculate the concentration of naringenin in formulations.

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

	NSLN 1	**NSLN 2**	**NSLN 3**	**NSLN 4**	**NSLN 5**	**NSLN 6**	**NSLN 7**	**NSLN 8**
Naringenin (mg)	200	200	200	200	200	200	200	200
Stearic acid (mmol)	1.0	1.2	1.4	1.6	-	-	-	-
Palmitic acid (mmol)	-	-	-	-	1.0	1.2	1.4	1.6
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. Naringenin 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, 25% amplitude and 1 min cycles) for 25 minutes.

Characterization of SLNs

Particle size and zeta potential determination

Particle size was determined using a particle size analyzer while the zeta potential was determine using a zeta sizer. The surface morphology was studied by observing the photomicrograph of the particles by scanning electron microscopy.[11]

Entrapment Efficiency

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.[12]

$$\text{Entrapment Efficiency (\%)} = \frac{Amount\ of\ drug\ in\ nanoparticles}{Initial\ amount\ of\ drug\ taken} x100$$

***In vitro* drug release**

The *in vitro* release of naringenin 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 phosphate buffer ph 7.4. 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.[13] 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 naringenin.

Stability Study

The stability of NSLNs was studied by storing at 4 ± 1 °C for 30 days. The particle size was observed to assess the physical stability of the NSLNs while the drug concentration in NSLNs was determined at the end of the study by spectrophotometry.[14]

Results and Discussion

Preformulation Studies

The results of organoleptic characterization and melting point are presented in Table 2.

Table 2. Properties of naringenin

Test	Specification	Observation
Color	White- yellow	Pale Yellow
Odor	Characteristic	Characteristic
Taste	Bitter	Bitter

Melting Point	170-171°C	169-171°C
Solubility	Methanol, Ethanol	Soluble in methanol and ethanol

The FT-IR spectrum of naringenin, and a physical mixture of naringenin, palmitic acid and stearic acid were obtained and observed for any deletion of the peaks of the pure drug. The spectrum of naringenin exhibited peaks at 3341 cm^{-1} (OH stretching), 3056 cm^{-1} (CH aromatic stretching), 2923 cm^{-1} (CH_2 stretching), 1647 cm^{-1} (C=O stretching), 1574 cm^{-1} (C=C aromatic stretching), 1441 cm^{-1} (CH_2 bending), 1146 cm^{-1} (C-O stretching). All the peaks were present in the physical mixture indicating a compatibility between the both the components.

The calibration curve of naringenin was prepared in phosphate buffer pH 7.4 using UV-Visible spectrophotometer at 295 nm by plotting the absorbance against concentration (Figure 1).

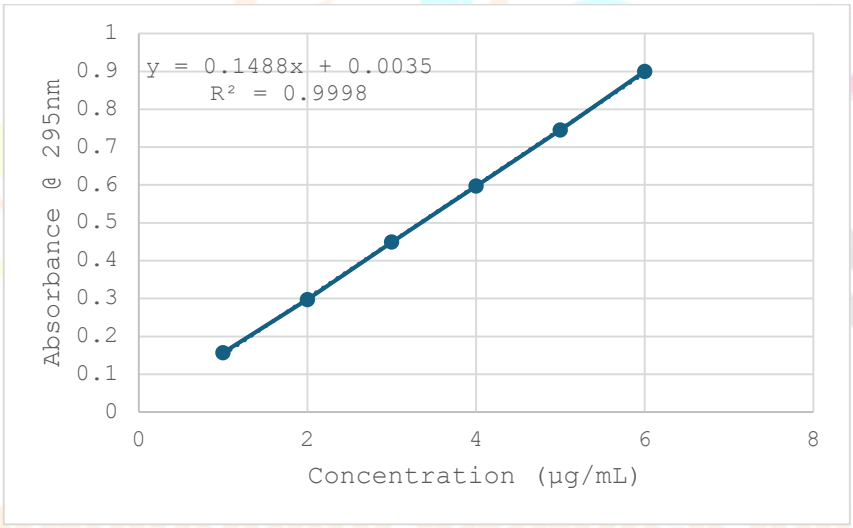

Figure 1. Calibration curve of naringenin

Preparation of SLNs

The SLNs loaded with naringenin 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 narngenin 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.[15]

Characterization of SLNs

Particle size and zeta potential

Table 3 presents the average size of naringenin-loaded SLNs prepared using different lipids. The final sonication of 25 min was able to reduce the size of the particles to the required nano range for effective permeation through the membrane and facilitate oral delivery of naringenin. The size range of SLNs obtained on sonicating for 25 min was found to be 163.6 nm when stearic acid was used and 142.2 nm when palmitic acid was used for the preparation of SLNs (Figure 2).

Table 3. Particle size and zeta potential of SLNs

Formulation Name	Particle Size	Zeta Potential	% Entrapment
NSLN1	247.2	-2.79	44.1
NSLN2	176.1	-17.9	55.4
NSLN3	163.6	-16	62.3
NSLN4	193.4	-13.8	76.0
NSLN5	186.5	-17.3	52.6
NSLN6	173.1	-13	58.9
NSLN7	142.2	-25.9	67.4
NSLN8	161.8	-18.2	79.3

The results of particle size show that 1.4 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.

The zeta potential value of around ±30 mV is considered to be having stable particles. The zeta potential of all the SLNs ranged from -2.79 to -25.9 mV suggesting a lower stability in few formulations (Figure 3). 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. The highest zeta potential and lowest particle size make NSLN7 the most optimum formulation of naringenin.

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 142.2	Peak 1:	156.0	100.0	115.1
PdI: 0.430	Peak 2:	0.000	0.0	0.000
Intercept: 0.964	Peak 3:	0.000	0.0	0.000
Result quality Good				

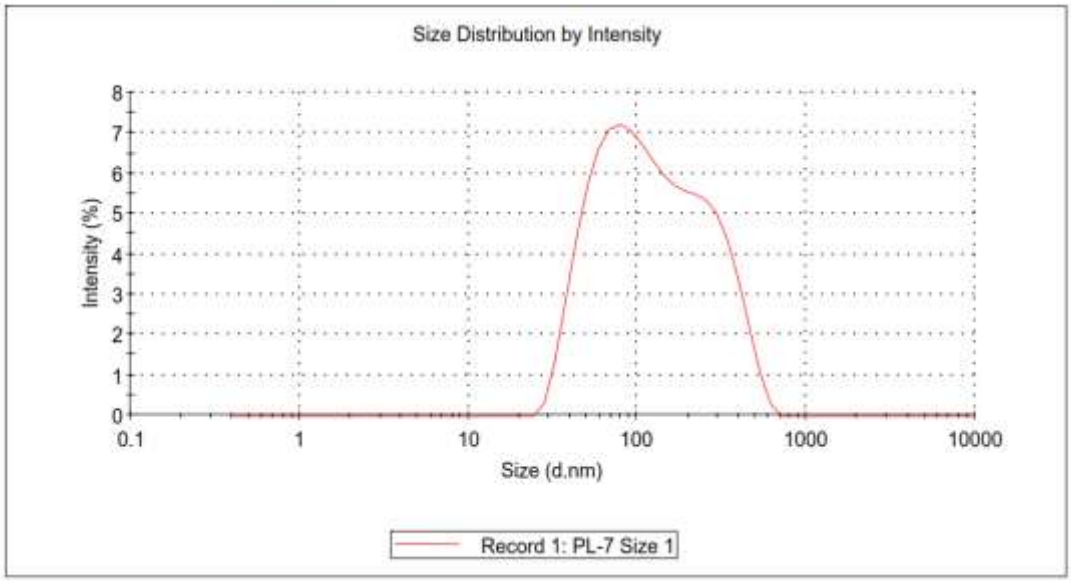

Figure 2. Particle size and distribution of NSLN-7

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -25.9	Peak 1:	-25.9	100.0	4.71
Zeta Deviation (mV): 4.71	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0494	Peak 3:	0.00	0.0	0.00
Result quality Good				

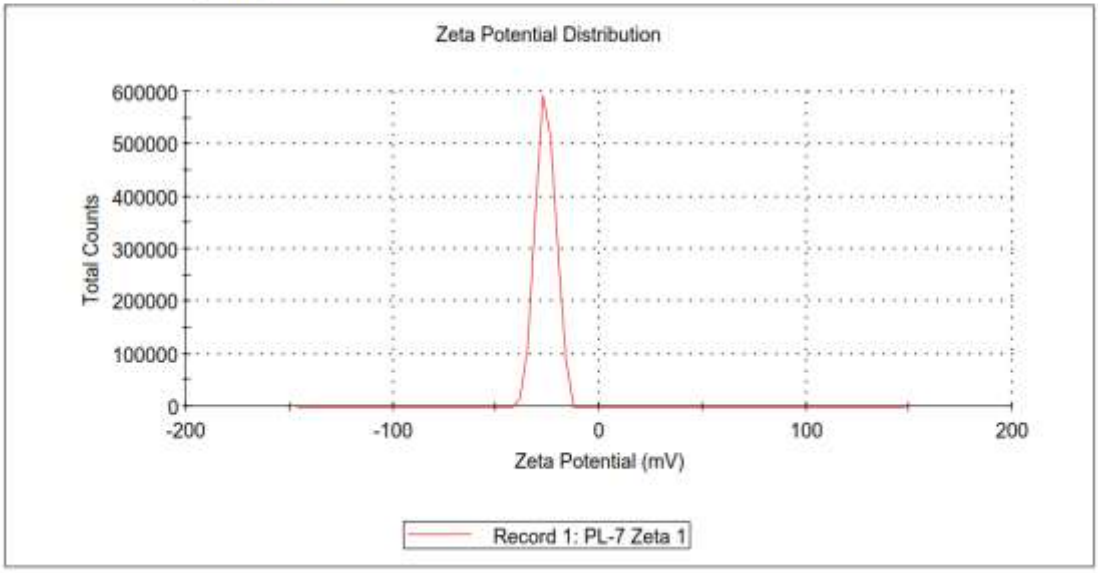

Figure 3. Zeta potential of NSLN-7

Entrapment efficiency

The entrapment efficiency was found to increased on increasing the concentration of the lipid. The highest encapsulation of naringenin (79.3 %) was obtained when the concentration of palmitic acid was 1.6 mmol (Table 3).

***In vitro* release from SLNs**

In vitro release kinetics studies for naringenin loaded SLNs exhibited a sustained release pattern. Sustained release was observed over a period of 3 days. Initial burst release can be attributed to dissociation of surface absorbed naringenin into lipid matrix while sustained released over a period of 3 days can be attributed to release of naringenin from nanoparticles (Figure 4).

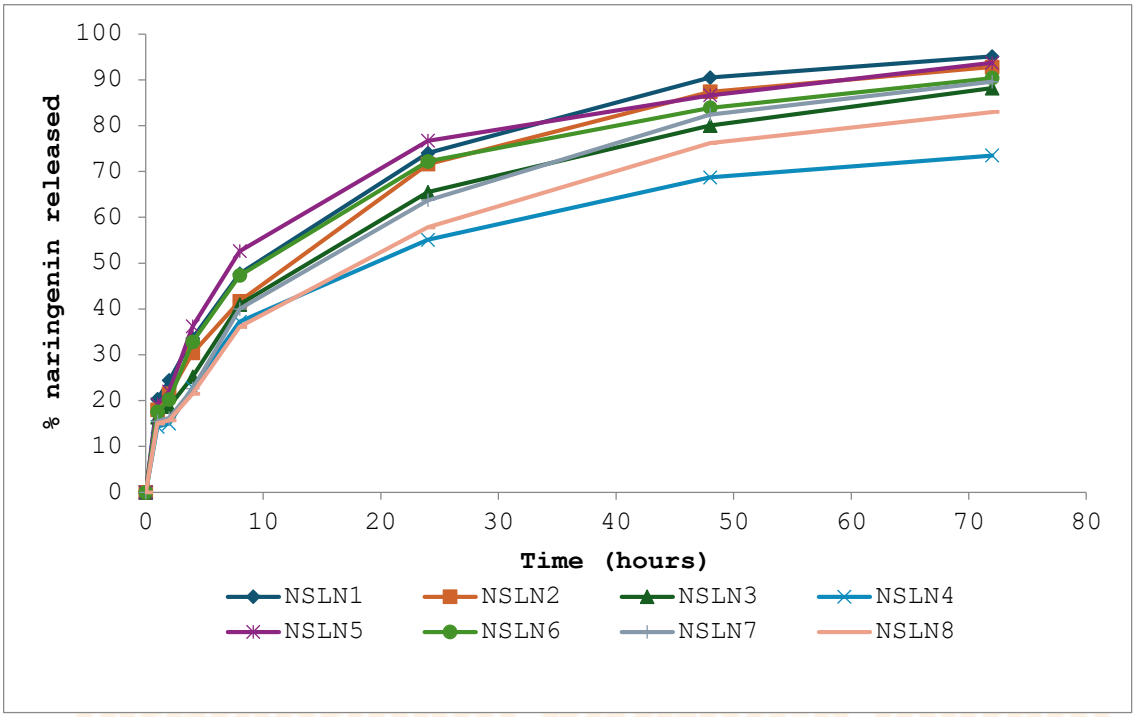

Figure 4. Release of naringenin form SLNs

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 67.4%. This suggests that the SLNs prepared are stable on storage.

Conclusion

The present study was to prepared naringenin -loaded SLN to improve its bioavailability. 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 particles were of higher size when long chain fatty acid (stearic acid) was used as the lipidic matrix for preparation of the SLNs. 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 1.4 mmol of palmitic acid as the lipid component (NSLN-7). It could be concluded from the study that the SLN provide sustained release and are preferred to overcome oral bioavailability problems and suggests that naringenin can be formulated as SLN system.

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