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Formulation Optimization Of Nanostructured Lipid Carrier Loaded With Plant Phenolic Naringenin For Improved Bioavailability

Tanveer Khan[1*], Ashish Jain[2]
[1]*Tanveer Khan, LNCT University, Sarvadharam, Bhopal, Madhya Pradesh, 462042 - India
[2]Ashish Jain, LNCT University, Sarvadharam, Bhopal, Madhya Pradesh, 462042 - India

INTRODUCTION

Nanotechnology has become a powerful tool in recent years for tackling the limitations of traditional drug delivery methods [1]. Nanocarriers are a type of particulate system having a size ranging from 10 to 1000 nm [2]. The encapsulating moieties of nanocarriers can be altered to improve their pharmacokinetic and biodistribution characteristics, decrease toxicity, regulate release, improve solubility and stability, and deliver their payload to targeted sites [3]. The use of solid lipid nanoparticles (SLNs) has been intended for controlled as well as targeted delivery of drugs but its use has been limited owing to drug expulsion on storage due to their rigid structure, unpredictable gelation tendency, particle growth, and unexpected polymeric transitions [4-6]. To overcome the drawbacks associated with the solid lipid nanoparticles, nanostructured lipid carriers (NLCs) were developed. The solid lipids in the SLNs were replaced by the blend of liquid and solid lipids varying in a ratio of 70:30 up to a ratio of 99.9:0.1 [7]. Regardless of the presence of liquid lipids in a high proportion, the NLCs are solid at room temperature. The blend of solid and liquid lipids gives rise to an unstructured matrix with more imperfections that holds a greater number of drug molecules than SLN and thus has high entrapment efficiency.

Naringenin (Nrg) belongs to the flavanone class of flavonoids and is abundantly present in citrus fruits. It has therapeutic interventions as antioxidant, anti-inflammatory, antidiabetic and anticancer agent [8-11]. Poor solubility, fast metabolism and inadequate bioavailability hinder the application of flavonoids, which can be addressed through increased absorption, solubility and stability [12-15]. So far, several types of nanocarriers have been fabricated for NRG delivery that enhance its solubility in water, biocompatibility, bioavailability, and therapeutic efficiency, which is also translated into dose reductions [16-20]. NLCs are known to overcome several of the drawbacks associated with the common polymeric nanocarriers, SLNs, dendrimers, etc. [21]

In the current investigation, an attempt was made to optimize the formulation parameter for NLCs loaded with Nrg in order to improve its bioavailability.

MATERIAL AND METHODS

Naringenin (Nrg) was purchased from Yucca Enterprises, Mumbai; Oleic acid, PEG600, Tween 20, Tween 80 and Paraffin oil were purchased from CDH.

Preformulation studies

The procured sample of Nrg was observed for its organoleptic properties, qualitative solubility, melting point, partition coefficient and loss on drying (LOD) as per reported method [21, 22]. The calibration curve of Nrg was prepared in methanol using UV spectrophotometer at concentrations of 10-50µg/mL.

Solubility determination in various liquid lipids

To determine the solubility of Nrg in liquid lipids and surfactants, an excessive amount of Nrg (10 mg) was added to a 2.0 mL eppendorf tube and 1 mL of the vehicle (lipid) was added to it. The mixture was shaken for 72 h using shaker at 25°C to attain equilibrium. The mixtures were then centrifuged at 5000 rpm for 10 min and the supernatant was filtered through 0.45 µm syringe filter to remove the undissolved drug. The drug in the filtrates was determined by measuring the absorbance by UV spectrophotometer after appropriate dilution with methanol [23].

Preparation of NLC

Stearic acid was used as the solid lipid for preparation of NLCs. Oleic acid was used as the liquid lipid and Tween 80 as the surfactant. For preparation of NLC, weighed quantity of stearic acid was melted at 80°C in a clean beaker. Separately, Oleic acid was dissolved in ethanol and Tween 80 was dissolved in deionized water (Table 1). To the molten stearic acid was added oleic acid and Nrg (5 mg). Finally, solution of Tween 80 maintained at 80°C was added drop-wise to the lipid phase and stirred for 10 min. The mixture was then sonicated at 25°C using probe sonicator at pulse of 2 sec on and 3 sec off for 5 min [24,25].

Table 1. Formulation table

Formulation	Stearic acid (mg)	Oleic acid (mg)	Tween (%)	Drug (mg)
NLC1	100	5	20	5
NLC2	100	10	20	5
NLC3	100	15	20	5
NLC4	100	5	5	5
NLC5	100	10	5	5
NLC6	100	15	5	5
NLC7	100	5	10	5
NLC8	100	10	10	5
NLC9	100	15	10	5

Particle size and zeta potential

The particle size and zeta potential of the NLCs was determined by dynamic light scattering using Malvern zeta sizer. The samples were directly added in a quartz cuvette, and all measurements were carried out at 25°C.

Entrapment Efficiency

The entrapment efficiency was determined by measuring the amount of unentrapped drug. In an eppendorf tube 1mL of NLC was taken and centrifuged at 5000 rpm for 20 min. The supernatant was separated and diluted with methanol and the absorbance was measured by UV spectrophotometry. The entrapped drug concentration was calculated by subtracting the unentrapped drug from total drug used [26].

***In vitro* Drug Release**

The release of Nrg from NLCs was determined by Franz-diffusion cell. Dialysis membrane was placed between the donor and receptor compartments and the receptor compartment was filled with phosphate buffer pH 7.4. Optimized NLC formulation equivalent to 1 mg of Nrg was placed in the donor compartment. The cell was incubated at 37°C with continual stirring at 100 rpm. Samples were withdrawn at predetermined time intervals and analyzed by UV spectrophotometery to calculate the amount of drug released [27].

RESULTS AND DISCUSSION

The procured sample of Nrg was white, crystalline powder with no odor, with a melting temperature of 250-252°C. It was highly lipophilic with solubility in ethanol and methanol and the partition coefficient was found to be 1.49. The calibration curve was prepared in methanol by UV spectrophotometry and was used for calculation of concentration of Nrg throughout the study (Figure 1).

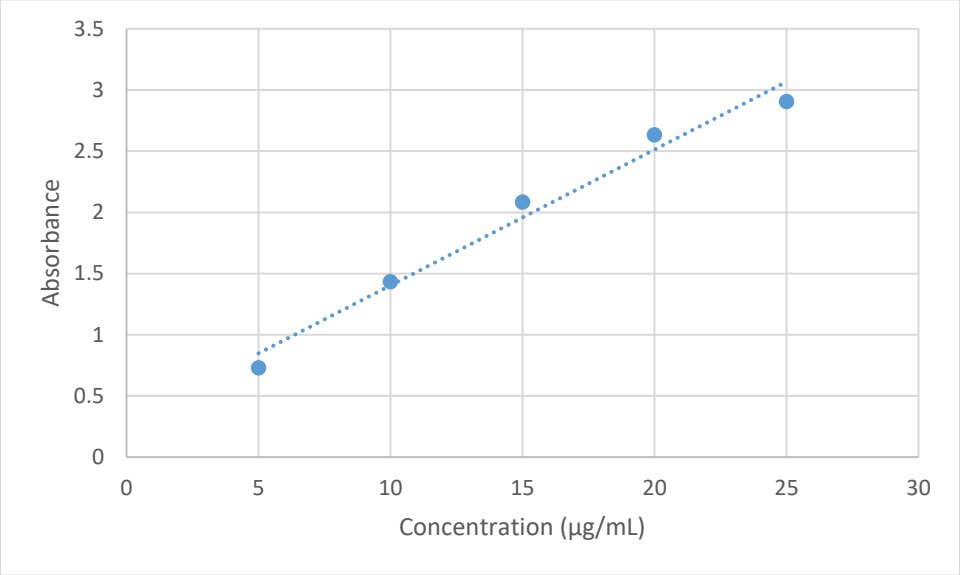

Figure 1. Calibration curve of Nrg

Solubility Study

As Nrg is poorly soluble in water, the selection of a suitable vehicle was one of the most important process for the formulation of the NLCs. Among the liquid lipids tested, Nrg showed the highest solubility in oleic acid (4.09 mg/mL) and poor solubility in paraffin oil (1.03 mg/mL). Tween 80 showed the highest solubility among surfactants of 1.90 mg/mL mg/mL, followed by PEG600, and Tween 20 (Table 2). Thus, oleic acid and Tween 80 were selected for the optimization of the formulation with the solid lipid (i.e., stearic acid) for the preparation of the NLCs.

Table 2. Solubility of Nrg

Carrier	**Solubility (mg/mL)**	
Liquid Lipid	Oleic acid	4.09
	Paraffin oil	1.03
Surfactant	PEG 600	1.89
	Tween 20	1.63
	Tween 80	1.90

Characterization of NLC

The particle size of the NLC varied from 655.4 nm to 10.480 µm. The PDI of the formulations varied from 0.409-1.000. The zeta potential was in range of -10 mV to -31 mV. The negatively charged solid lipid contributed towards the surface charge of the NLC. The higher zeta potential permits for stabilization of the particles.

The release of the best formulation (NLC 7) was studied for a period of 12h was found to be 39.52%. The release of Nrg from the NLC was found to be steadily controlled throughout the duration of 12h of the study (Figure 2). On the other hand maximum Nrg released from solution was at 6th hour (91.42%) and started to decrease thereafter suggesting degradation of the drug.

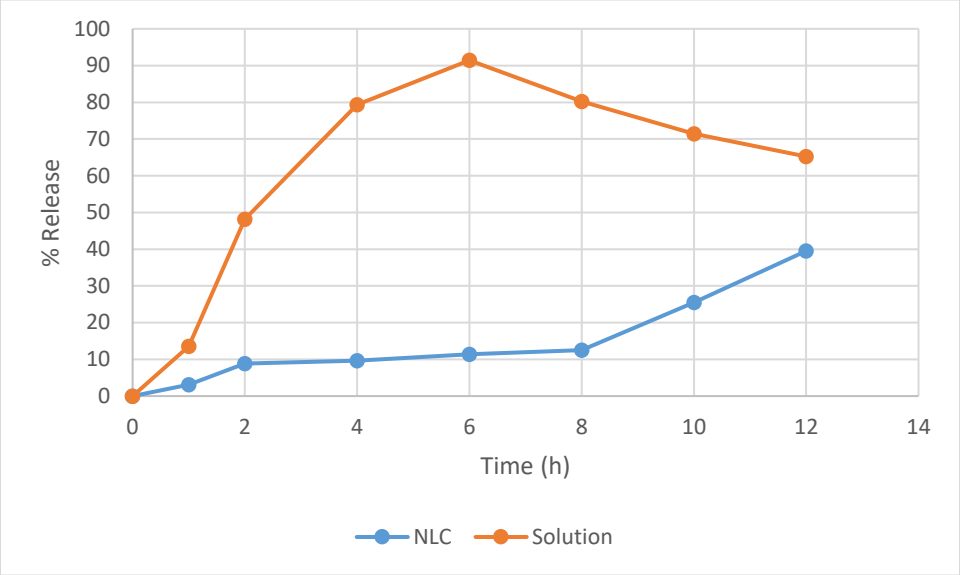

Figure 2. Cumulative release of Nrg from NLC and solution

CONCLUSION

A Nrg-loaded NLC formulation was successfully optimized using the Box-Behnken design. The optimized NLC formulation significantly controlled the release of Nrg compared to that by the Nrg solution. Moreover, as the optimized NLC formulation released Nrg for more than 12 h, it could be easily used for improving the oral bioavailability of Nrg and hopefully other plant phenolics.

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Conflict of Interests

The authors declare no conflict of interests.

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