



## Formulation and Evaluation of Naringin Loaded Phytosomes for Improving Bioavailability

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### ABSTRACT

The objective of the present study was to prepare phytosomal formulation loaded with phytoconstituent naringin. Phytosomes of naringin were prepared by solvent evaporation method using lecithin as the lipid molecule. The particle size of the phytosomes was from 651 nm to 2235 nm in size with a polydispersity index varying between 0.357 - 0.629. The phytosomes were visible as rigid, spherical vesicles in SEM image. The surface of the phytosome vesicles was found to be regular and smooth. Sharp and distinct endothermic peaks in DSC revealed the formulation of stable phytosomes due to molecular interactions between the extract and lecithin. The effect of drug concentration was evident in release. The naringin release could be controlled up to 24h from the phytosomes. NP1 released 54.06% naringin while NP6 released the maximum 87.96% naringin in 24h. It was evident that increasing the drug concentration led to a higher release of drug. The phytosomes were found to be stable under the storage conditions. The best formulation with respect to particle size and drug release was NP3 that contained 1:1.5 ratio of lecithin: naringin.

**Keywords:** Phytosome, naringin, lecithin, drug delivery, bioavailability

### Introduction

Naringin is a disaccharide derivative that is (S)-naringenin substituted by a 2-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyranosyl moiety at position 7 via a glycosidic linkage. It has a role as a metabolite, an antineoplastic agent and an anti-inflammatory agent. It is a disaccharide derivative, a dihydroxyflavanone, a member of 4'-hydroxyflavanones, a (2S)-flavan-4-one and a neohesperidoside. It derives from a (S)-naringenin.<sup>1</sup> Phytosomes are known contain the bioactive phytoconstituents of herb extract bounded by lipids and are developed by incorporating standardized plant extract or water soluble bioactive plant constituent into phospholipids to make lipid compatible molecular complex called phytosomes and so progress their absorption and bioavailability.

Over the last few years an increasing interest has been gaining momentum among the pharmaceutical scientists to incorporate the herbal extracts or phytoconstituents in phytosomes for improving the bioavailability as well as patient compliance. Every year more than 15 research articles for phytosomal formulation of ingredients like diosgenin, curcumin, quercetin, chrysin, *Brassica nigra* extract, Diospyros kaki extract etc have been published in peer review journals. The following year 2020 witnessed an increase in the research in phytosomes with several modified formulations containing phytosomes. Some noteworthy work included Icaria phytosomes with improved anticancer action, nanoformulations containing vasaka phytosomes for improved bioavailability and Centella asiatica phytosomes with improved cognitive performance. In 2021, the trend continued and surface modification of the phytosomes was also witnessed in research work. Snake venom functionalized quercetin loaded phytosomes was highlight of the most prominent research work on phytosomes.<sup>2-12</sup>

Hence it was envisioned to formulate phytosomes loaded with naringin in an attempt to improve its bioavailability.

### Material and methods

#### Preformulation Studies<sup>13</sup>

Preformulation studies provide the necessary information of the drug for ascertaining its utilization with excipients for developing a particular formulation. They also fulfill the purpose of authenticating the drug using certain parameters.

#### Standard Curve of Naringin<sup>14</sup>

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.

#### Preparation of Phytosomes by solvent evaporation method<sup>15</sup>

The specific amount of Naringin and soya lecithin (Table 1) were taken into a 100 mL round bottom flask and refluxed with 20 mL of acetone at a temperature 40 – 50°C for 2 h. The mixture is concentrated to 5-10 ml to obtain the precipitate which was filtered and collected. The dried precipitate phytosomes complex was placed in amber colored glass bottle and stored in refrigerator.

**Table 1 Batch formula for phytosome preparation**

Formulation Code	Ratio of Lecithin : Naringin	Acetone (mL)
NP 1	1:0.5	20
NP 2	1:1	20
NP 3	1:1.5	20
NP 4	1:2	20
NP 5	1:2.5	20
NP 6	1:3	20

#### Evaluation of phytosomes<sup>16</sup>

##### Visualization

Visualization of phytosomes was accomplished by utilizing scanning electron microscopy. Scanning electron microscopy has been utilized to decide particle size estimate appropriation and surface morphology of the complex. The samples were sputter-covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive SEM (Hitachi-S 3400N) and watched for morphology at voltage of 15.0 kV.

##### Particle size and size distribution

The particle size (z-average) and size distribution of the prepared phytosomes was calculated from the auto correlation function of the intensity of light scattered from the particles expecting a circular type of particles using Malvern Zeta sizer.

##### Differential scanning calorimetry

The thermograms were obtained for the phytosome and lecithin to ensure compatibility. Each sample was heated in the range of temperature 25°C to 300°C at a heating rate of 5°C per minute. The thermograms were observed for enthalpy changes, appearance/vanishing of peaks, and changes to a peaks onset time, shape, and relative area.

##### Stability studies of optimized phytosome formulation

The prepared phytosomes were subjected to stability studies at 40±2°C/75±5% RH and 30±2°C/60±5% RH according to the ICH guidelines for a period of 3 months.

#### Results and Discussion

##### Physical characterization of Naringin

The physical characterization of the drug was performed according to the reported procedure and the results obtained are presented Table 2.

**Table 2 Physical Characteristics of Naringin**

S No	Parameter	Observation
1	Physical appearance	Amorphous powder
2	Color	Pale Yellow
3	Odour	Odorless
4	Taste	Bitter
5	Melting Point	231-240°C
6	Solubility	Soluble in water, methanol and ethanol, poorly soluble in petroleum ether

Previous study has also revealed the solubility order of Naringin to be methanol > ethyl acetate > n-butanol > isopropanol > petroleum ether > hexane.<sup>17</sup>

The compatibility of Naringin with lecithin was studied using FT-IR spectrum of the pure drug as well as the physical mixture. On comparison of the FTIR spectra of the drug and the mixture it was observed that no peak was deleted and only the intensities of the existing peaks changed which might be due to the coupling of absorption frequencies. This provides an evidence of compatibility between the drug and the matrix forming polymers. The FTIR

spectrum of Naringin exhibited the stretching and bending vibrations due to OH ( $3340.56\text{ cm}^{-1}$ ), C=O ( $1699.89\text{ cm}^{-1}$ ), C=C ( $1603.53\text{ cm}^{-1}$ ) and C-O-C ( $1003.43\text{ cm}^{-1}$ ).

### Calibration curve of Naringin

The Calibration curve of Naringin was constructed by plotting absorbance versus concentration ( $\mu\text{g/ml}$ ) at 295 nm (Figure 1).

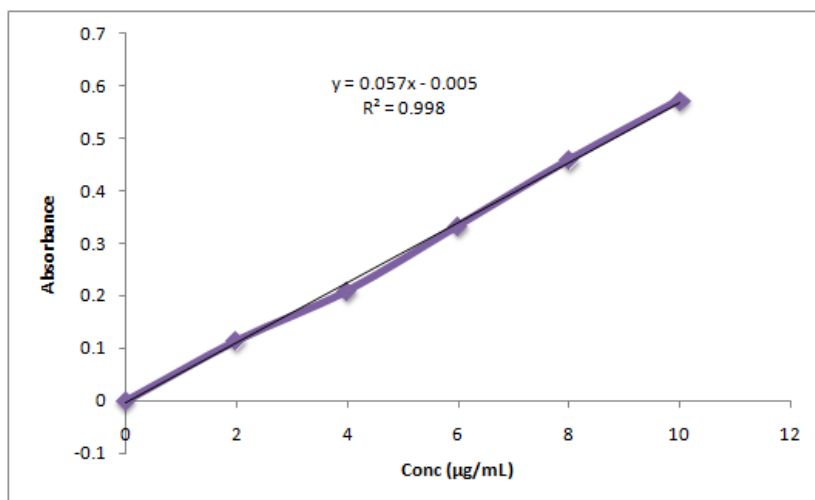


Figure 1. Calibration curve of Naringin in ethanol

The regression equation was used to calculate the concentration of Naringin in the formulation as well as in the release study.

### Preparation of phytosomes

The phytosomes loaded with Naringin were prepared using solvent evaporation method. In this technique, the phytoconstituents or extract and the lipid (lecithin) are kept in a flask containing organic solvent. This reaction mixture is kept at an optimum temperature usually  $40^{\circ}\text{C}$  for specific time period to attain maximum drug entrapment in the phytosomes formed. The organic solvent is then removed using rotary evaporator.

### Particle size and size distribution

The particle size and size distribution for each batch of phytosomes was determined using zeta sizer. The formulations ranged from 651 nm to 2235 nm in size with a polydispersity index varying between 0.357 - 0.629 (Table 3).

Table 3. Particle size and size distribution of various batches of phytosomes

Formulation Code	Particle Size (nm)	Polydispersity Index (PDI)
NP 1	651	0.428
NP 2	1053	0.357
NP 3	1317	0.511
NP 4	1529	0.618
NP 5	1874	0.468
NP 6	2235	0.629

It was evident from the results of the particle size that the amount of lipid and drug had a significant effect on the particle size of the phytosome. The phytosomes prepared with lower concentration of drug were found to be of lower sizes whereas increasing the concentration of drug increased the size of the phytosomes.

### Surface morphology (visualization)

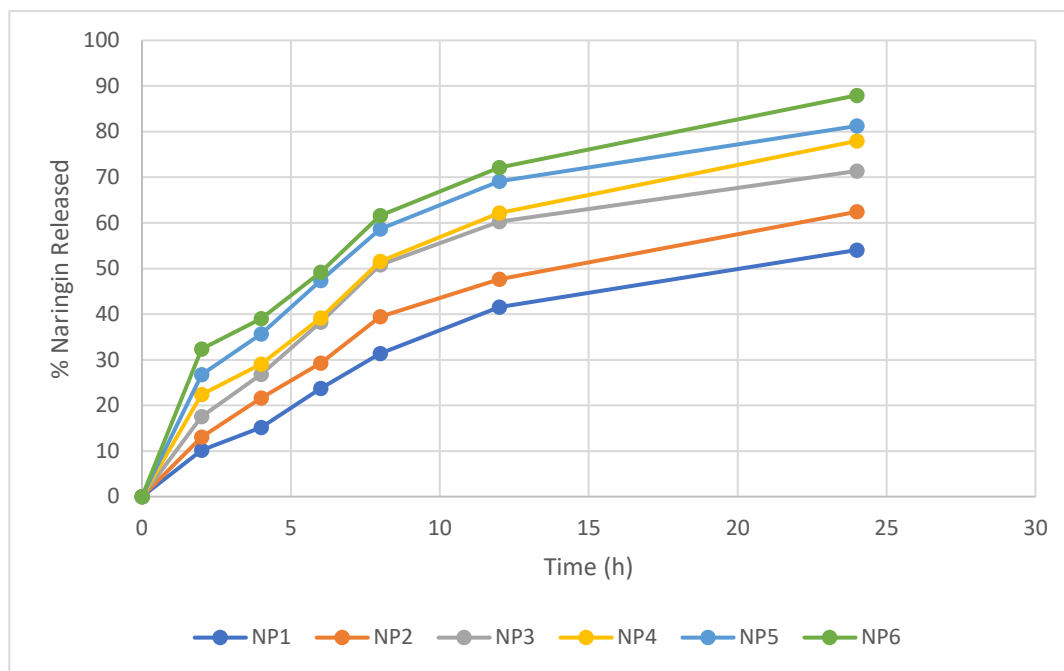
The phytosomes were visible as rigid, spherical vesicles in SEM image. The surface of the phytosome vesicles was found to be regular and smooth.

### Differential Scanning Calorimetry

The thermogram of soya lecithin gives distinct peak at  $57^{\circ}\text{C}$  indicating melting. Sharp endothermic peak was found in the thermogram of the phytosome at  $260^{\circ}\text{C}$ . From the DSC it can be concluded that a stable formulation is formed by some molecular interaction that can be either van der Waals forces or hydrogen bonding between extract and phospholipids that distributed the extract molecularly into phospholipid.

### Release of naringin from phytosomes

The *in vitro* release of naringin from the phytosomes was studied and the concentration of naringin released from the formulation was calculated using the calibration curve (Figure 2).



**Figure 2. Release-Time graph for naringin from phytosome**

The effect of drug concentration was evident in release. It can be seen from the Figure 2 that naringin release could be controlled up to 24h from the phytosomes. NP1 released 54.06% naringin while NP6 released the maximum 87.96% naringin in 24h. It was evident that increasing the drug concentration led to a higher release of drug. This might be partly explained by the fact that the size of particles increased which may have caused increased porosity and hence higher release.

#### Stability study of phytosomes

The formulation NP1 was subjected to stability studies according to ICH guidelines and the phytosomes were evaluated for particles size changes after 3 month. Stability of the formulation must be maintained until it reaches the targeted tissue. Lecithin plays a vital role in maintaining the physical stability by making the lipid bilayer flexible. It was found that at room temperature and accelerated temperature conditions very slight variation occurs in mean particle size. Hence the formulation could be considered to be stable in the storage conditions.

#### Conclusion

The study presented in this thesis reveals the excellent potential of phytosome based drug delivery system for controlling the release of phytoconstituent naringin. We can conclude that phytosome based formulation could be a valuable approach to improve the therapeutic efficacy, to reduce dose and improvement in dosage regimen for phytoconstituents.

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