World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/
WJBPHS

(Research Article)

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Formulation and *In vitro* characterization of the sustained release liposphere containing flavonoid Naringin

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World Journal of Biology Pharmacy and Health Sciences, 2023, 15(03), 166–177

Publication history: Received on 08 August 2023; revised on 22 September 2023; accepted on 25 September 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.15.3.0396

Abstract

The purpose of the study was to formulate sustained release liposphere of naringin. The aim was to increase the solubility and improve the loading of drug as well as drug rlease. The liposphere was formulated using melt dispersion method and then sonicated to achieve the desired size. The formulated liposphere were then evaluated for drug loading, entrapment of drug, solubility and *In vitro* drug release studies. The highest percentages of drug loading and drug entrapment are seen observed to be 16.233±0.208% and 97.395±0.189%. The manufactured liposphere of naringin showed sustained release to be greater than 96.397±0.124% within 12 hours.

Keywords: Liposphere; Naringin; Novel drug delivery system; Sustained release; Flavonoid; Encapsulation

1. Introduction

Lipospheres were first described as a dispersion of solid spherical particles into solid hydrophobic fat core (fatty acid derivatives or triglycerides), stabilized by a phospholipid monolayer of particle size diameter between 0.01 to 100 m. The major components for the composition of lipospheres are;

- Lipid core
- External coat
- Stabilizer.

Such fat encapsulated systems were meant for topical and parenteral delivery of bioactive molecules.

Techniques such as melt dispersion, solvent emulsification evaporation, hot and cold homogenization, ultrasonication and high-pressure homogenization are used for the production of lipospheres or nanolipospheres. Several benefits of liposphere drug delivery system include improved drug stability and bioavailability, prevention of hydrolysis of drugs, possibility for controlled drug release and controlled particle size with high drug loading capacity.

Naringin is a flavanone-7-O-glycoside between the flavanone naringenin and the disaccharide neohesperidose. The flavonoid naringin occurs naturally in citrus fruits, especially in grapefruit, where naringin is responsible for the fruit's bitter taste. In commercial grapefruit juice production, the enzyme naringinase can be used to remove the bitterness created by naringin. In humans naringin is metabolized to the aglycone naringenin (not bitter) by naringinase present in the gut.

Naringenin is endowed with broad biological effects on human health, which includes a decrease in lipid peroxidation biomarkers and protein carbonylation, promotes carbohydrate metabolism, increases antioxidant defenses, scavenging

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reactive oxygen species, modulates immune system activity, and also exerts anti-atherogenic and anti-inflammatory effects.

Stearic acid is widely used in oral and topical pharmaceutical formulations. It is a hard, white or faintly yellow-colored, somewhat glossy, crystalline solid or a white or yellowish white powder. It has a slight odor and taste suggesting tallow. It is mainly used in oral formulations as a tablet and capsule lubricant, although it may also be used as a binder or in combination with shellac as a tablet coating. It has also been suggested that stearic acid may be used as a sustained-release drug carrier. In topical formulations, stearic acid is used as an emulsifying and solubilizing agent. When partially neutralized with alkalis or triethanolamine, stearic acid is used in the preparation of creams.

2. Material and methods

Table 1 Active Pharmaceutical Ingredients (API)

S.no.	Active Pharmaceutical Ingredients (API)	Source/Manufacturer
1	Naringin	Jigs chemicals

Table 2 Chemicals used during the Experimental work

S. No.	Name	Manufacturer
1	Stearic acid	S.D. Fine chemicals Ltd
2	Methanol	Finar chemicals
3	Sodium Hydroxide	S.D. Fine chemicals Ltd.
4	Chloroform	S.D. Fine chemicals Ltd
5	n-hexane	S.D. Fine chemicals Ltd
6	n-Octanol	S.D. Fine chemicals Ltd
7	Dichloromethane	Loba Chemie, Mumbai
8	Ethanol	Changshu Yangyuan Chemical, China
9	Potassium dihydrogen phosphate	S.D. Fine chemicals Ltd
10	Di-sodium hydrogen phosphate	S.D. Fine chemicals Ltd

Table 3 List of Equipment Used

Instruments	Manufacturer
UV/VIS Spectrophotometer	LABINDIA
Weighing balance	Zeal International
Melting point apparatus	Bio Techno Lab
Water bath	Bio Techno Lab
Water shaker	Bio Techno Lab
Vortex mixer	Bio Techno Lab
Hot air oven	Frontline Electronics and Machinery Pvt Ltd.

2.1. Preformulation studies

2.1.1. Melting point

Determining the purity of a drug is the primary objective for determining the melting point. To ascertain the melting range, the sample had previously been dried. This determination was made using a digital capillary melting point instrument. A capillary was taken, brought close to the burner flame, and then had one end shut. A little plug of powder was gathered in the open end of the capillary tube after its open end was inserted into a small pile of the medication. The tube was then gently tapped to help the drug in the plug settle down. After that, the capillary tube was inserted into the device used to determine the melting point, and the temperature at which the sample's state changed from solid to liquid was noted.

2.1.2. Solubility

5 mL of each liquid received about 500 mg of naringin. In a glass culture tube, add water, methanol, 0.1NHcl, and phosphate buffer 6.8 pH. After that, the combinations were shaken in a water bath for 24 hours. Sample temperatures were 25.0 ± 0.1 °C. To separate the insolubilized drug after 24 hours, the sample was centrifuged at 10,000 rpm for 10 minutes. After that, the supernatant solutions were filtered to remove any remaining particulates in preparation for sampling. After suitable dilution and interpolation from previously created calibration curves, UV absorbance at 285 nm was measured to estimate the solution concentrations. At least three trials were performed in each solubility experiment.

2.1.3. Partition coefficient studies

In a glass culture tube, 5 mL of a 1:1 combination of water and n-octanol received more naringin. After that, the combinations were shaken in a water bath for 24 hours. Sample temperatures were 25.0 ± 0.1 °C. The saturated solution was transferred to the separating funnel to separate the water and n-octanol layers after 24 hours. To separate the insolubilized drug, both layers were centrifuged at 10,000 rpm for 10 minutes. After that, the supernatant solutions were filtered to remove any remaining particulates in preparation for sampling. After suitable dilution and interpolation from previously created calibration curves, UV absorbance at 285 nm was measured to estimate the solution concentrations. Partition coefficient was determined as ratio of concentration of drug in octanol to the concentration of drug in water and the value were reported as log P.

$$\text{Log p} = \frac{concentration\ of\ drug\ in\ non\ aqueous\ phase}{concentration\ of\ drug\ in\ aqueous\ phase}$$

2.1.4. Determination of Absorption Maxima (λmax) of Drug by UV- Spectrophotometer

UV Spectrophotometer was used to estimate the drug's maximum rate of absorption (max).

Preparation of standard plot of naringin in methanol

10 mg of the naringin were dissolved in 100 ml of methanol in a volumetric flask, a stock solution containing 100µg/ml was prepared. Appropriate dilutions were made from this working standard solution to yield 2–20µg/ml. A UV-visible spectrophotometer was used to measure the absorbance of each sample solution in triplicate using quartz cells with a 10 mm path length and methanol as a blank at a wavelength of 285 nm. Plotting the absorbances versus concentration allowed for the calibration curve to be created.

2.1.5. FT-IR Analysis

Infrared Fourier transform to identify that specific compound, spectroscopies of other substances were run. Using KBr pellets, FT-IR spectroscopy of the final, improved formulation of the pure medication naringin was carried out. Several peaks in the FT-IR spectrum were interpreted to identify various groups in the pure drug's formulation-specific structure. The investigation and forecasting of any physicochemical interactions between various components can also be done using FT-IR spectroscopy.

2.2. Preparation of Liposphere using melt dispersion method

By using the melt dispersion (homogenization) approach, naringin-loaded lipospheres were created. Naringin was used to prepare the lipid phase that contained stearic lipid. At a temperature of 70 °C, the lipid phase was melted before naringin was added. A separate solution of the aqueous phase was made by dissolving surfactant Poloxamer 188 in 50 ml of hot, distilled water, which was above the melting point of the lipid phase at 80 °C. After that, a hot external aqueous phase that was kept at 80 °C and contained a suitable surfactant was added to emulsify the lipid phase. The emulsion

was homogenized for 10 minutes in a speed homogenizer while being kept at 80 °C. The emulsion formulation was then quickly chilled to around 10 °C by submerging it in an ice bath while maintaining agitation to produce homogeneous dispersion of lipospheres. The obtained lipospheres was then washed with water and isolated by filtration through a whattman filter paper.

Table 4 Composition of naringin loaded liposphere

S.No.	Formulation code	Naringin (mg)	Stearic acid (mg)	Poloxamer 188 (mg)	Homogenization (rpm)
1	NL1	500	500	500	10000
2	NL2	500	1000	500	10000
3	NL3	500	1500	500	10000
4	NL4	500	2000	500	10000
5	NL5	500	1500	250	10000
6	NL6	500	1500	1000	10000
7	NL7	500	1500	1000	5000
8	NL8	500	1500	1000	12500
9	NL9	500	1500	1000	15000

2.3. Characterization and evaluation of liposphere of naringin

2.3.1. Physical appearance

The presence of aggregation, phase separation, and drug particles were visually checked in every prepared naringin loaded liposphere formulation.

2.3.2. Percentage (%) yield

The prepared liposphere were collected and weighed. The measured weight was divided by the total weight of all the excipients and drug. The % yield was calculated using following formula:

$$\text{Percentage Yield} = \frac{\text{Total formulation weight}}{\text{Total weight of excipient + drug}} \times 100$$

2.3.3. Particle size analysis

Optical microscopy was used to measure the liposphere's particle size. By utilising a calibrated optical microscope, 100 lipospheres were counted in order to determine the particle size.

$$\text{Particle Size} = \frac{\sum nd}{\sum n} * \text{Least count}$$

Here, $\sum n$ = no. of particles
$\sum nd$ = average diameter

2.3.4. Percentage Drug entrapment and percentage drug loading

Each batch of precisely weighed dry lipospheres containing 500 mg of naringin was mixed with 1 ml of chloroform and 9 ml of methanol. A clear solution was produced by sonicating the obtained solution for five minutes. To determine the drug concentration of naringin in methanol, sediment was filtered using Whatman filter paper 42. Following dilutions in methanol solution, spectrophotometry at a wavelength of 285 nm was performed. Using the use of a straight-line equation, the drug content was determined:

$$Percentage\ drug\ entrapment = \frac{Total\ amount\ of\ drug}{Theoritical\ amount\ of\ drug} \times 100$$

$$Percentage\ drug\ loading = \frac{Total\ amount\ of\ drug}{Amount\ of\ liposphere} \times 100$$

2.3.5. In vitro drug release studies

The USP Basket method by dissolution was used to investigate the release of naringin from the lipospheres. In one capsule, there were enough lipospheres to make up 500 mg of naringin. To simulate the physiological fluid conditions of simulated gastric fluid and simulated intestinal fluid without enzyme, *In vitro* drug release was carried out using USP type I basket dissolution apparatus maintained at 37 °C± 0.5 °C, under stirring at 100 rpm. 900 ml of acidic buffer 0.1 N HCl (pH-1.2) for 2hr was followed by phosphate buffer pH 6.8. Each time a sample was taken, it was changed right away with fresh medium in the same volume (30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, and 24 hours). Filtered sample solutions were diluted with diluent and measure absorbance at 285 nm on UV- Visible spectrophotometer. The similar experiment was performed for the pure drug suspension.

3. Results and discussion

3.1. Preformulation study

3.1.1. Organoleptic properties

Organoleptic properties of drug Naringin found to be as per I.P. monograph.

Table 5 Organoleptic property of naringin

S.No.	Test	Specification	Observation
1.	Colour	White powder to light yellow powder	White powder to light yellow powder
2.	Odour	Odourless	Odorless

3.1.2. Melting point

Melting point of drug was determined by capillary fusion method.

The melting point of drug was found to be range 161±1.00 to 167.67±±1.528, hence drug sample was free from any type of impurities.

3.1.3. Absorption maxima of Naringin

Absorption maxima of Naringin in methanol were found to be 285nm at 10µg/ml concentration.

Preparation of Standard Calibration curve of Naringin in methanol

Table 6 Calibration curve of Naringin in methanol

Concentration (µg/ml)	Absorbance
2	0.092±0.002
4	0.186±0.004
6	0.282±0.008
8	0.369±0.003
10	0.454±0.002
12	0.547±0.007
14	0.635±0.006
16	0.725±0.010
18	0.819±0.005
20	0.915±0.001

The naringin solution in methanol, 2 to 20µg/ml, was used to generate the calibration curve for naringin. At 285 nm, the absorbance value was determined. The regression equation Y = 0.0453x + 0.0044 and R^2 value of 0.999, which exhibits strong linearity, are represented by the calibration curve of Naringin as shown in the graph.

3.1.4. Solubility studies of drug

The solubility of drug in different solvent as given below:

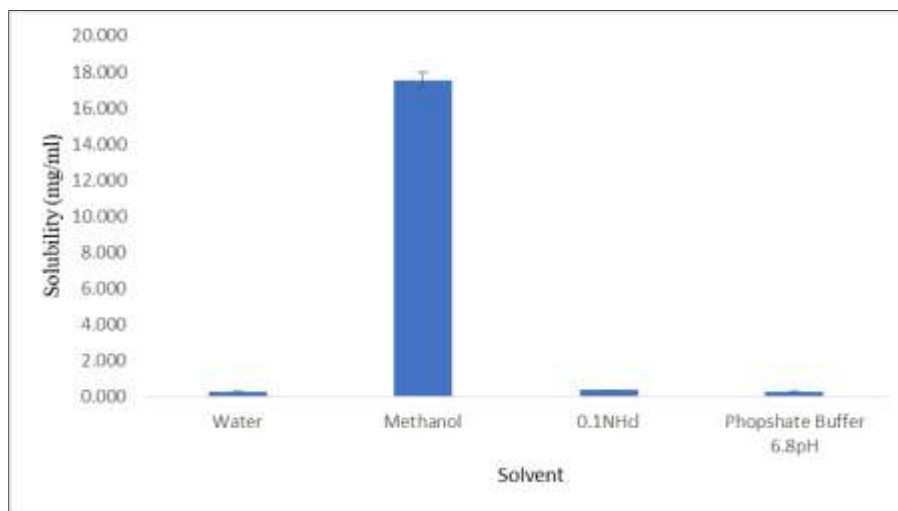

Figure 1 Solubility of Naringin in different solvents

Figure 1 showed that the concentration of naringin in alcohol was the highest. In distilled water, it dissolves very poorly.

3.1.5. Partition coefficient of drug

Naringin was discovered to have a partition coefficient of 0.77±0.41 in n- Octanol: Water. This shows that the substance is lipophilic by nature and that its lipophilicity value is near to 0.37 in the reference literature.

3.1.6. FTIR analysis

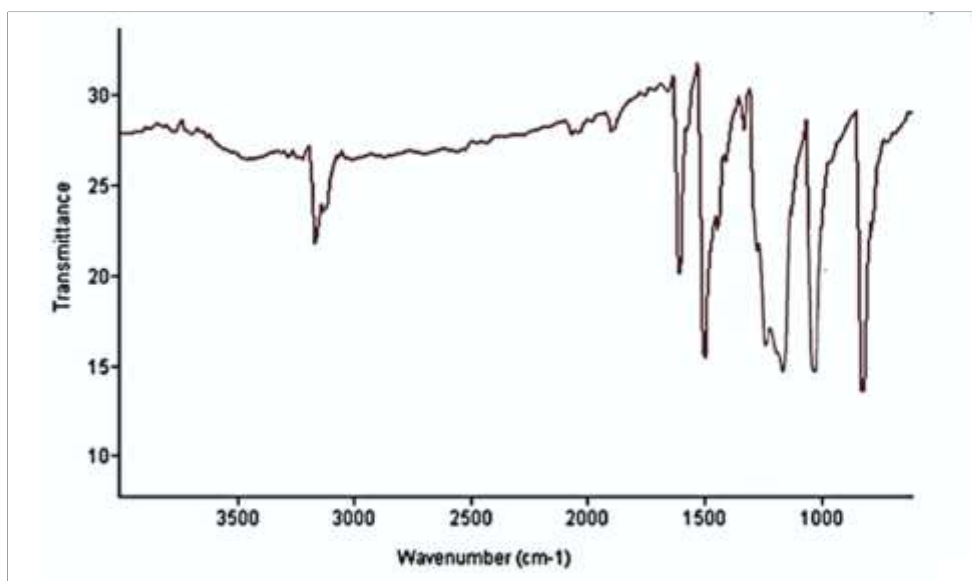

Figure 2 FTIR spectrum of naringin

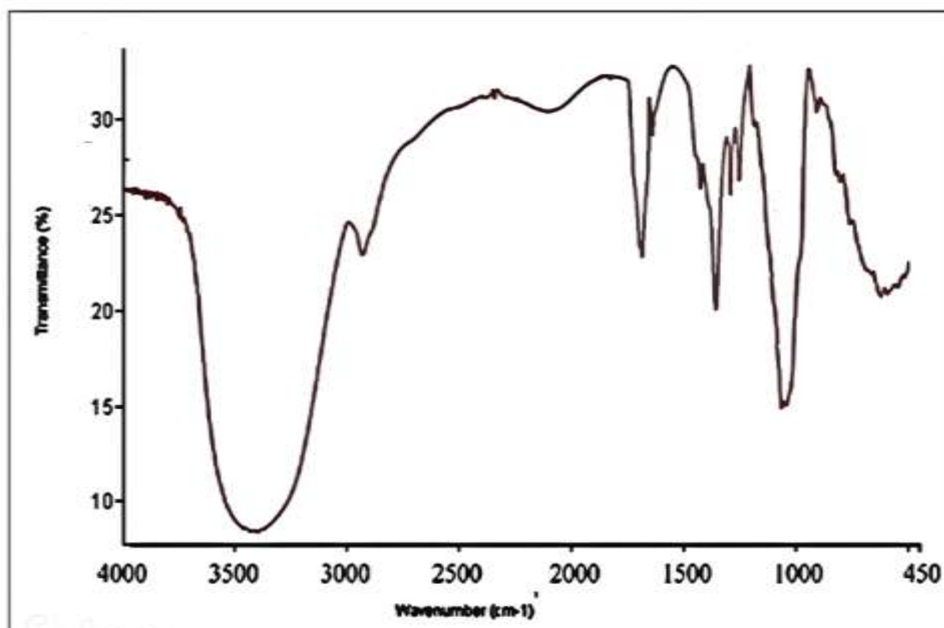

Figure 3 FTIR spectrum of optimized formulation

FTIR spectrum of the naringin demonstrated the characteristic peak as wavenumber 3358.23cm^{-1}, 1639.51cm^{-1}, 1599.36cm^{-1}, 978.36cm^{-1}, 752.32cm^{-1} for the functional group of OH (axial deformation, C=O, C=C, axial deformation of C-O-C and angular deformation C-H. The FTIR spectrum of the optimized formulation displayed the characteristic peaks of the naringin with reduced intensity and absence.

3.2. Formulation of Liposphere

The Melt Method was used to prepare the naringin loaded liposphere.

3.3. Evaluation of naringin containing Liposphere

3.3.1. Appearance and Percentage yield

Appearance and percentage yield of drug containing different liposphere was given below.

Table 7 Appearance of drug containing different liposphere

S.no.	Formulation code	Appearance
1	NL1	Liposphere was not formed
2	NL2	Spherical shape, uniform particle was formed
3	NL3	Spherical shape, uniform particle was formed
4	NL4	Spherical shape, uniform particle was formed
5	NL5	Irregular and spherical shaped particles formed
6	NL6	Liposphere was not formed
7	NL7	Spherical shape, uniform particle was formed
8	NL8	Spherical shape, uniform particle was formed
9	NL9	Spherical shape, uniform particle was formed

Table 7 demonstrated that all lipospheres formulations were spherical in shape and uniform except formulation code NL1.

3.3.2. Percentage yield

Percentage yield of all different liposphere formulations were given below.

Table 8 Percentage yield of different liposphere formulation

S.no.	Formulation code	Percentage yield
1	NL1	94.400±0.353
2	NL2	99.300±0.050
3	NL3	99.560±0.223
4	NL4	99.167±0.186
5	NL5	94.874±0.112
6	NL6	99.433±0.100
7	NL7	96.400±0.145
8	NL8	99.844±0.051
9	NL9	99.500±0.153

Table 8 showed the percent yield of the entire drug-loaded liposphere formulation, which ranged from 94.400±0.353 to 99.844±0.051. Yield also increased with increasing lipid concentration in terms of percentage. Poloxamer employed in lipospheres provides stability to lipid structure, leading to high drug entrapment in lipospheres. As a result, percentage drug entrapment and percentage yield of liposphere formulation were also increased on increasing poloxamer content and stirring.

3.3.3. Particle size

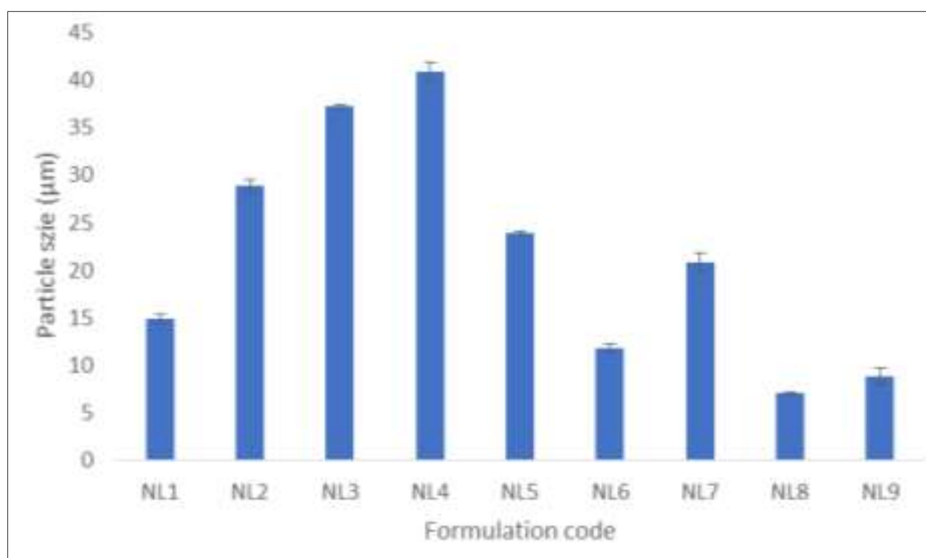

Figure 4 Particle size of different liposphere formulation

Figure 4 showed that the range of particle sizes for all microparticle formulations was between 7.065±0.106µm to 40.885±0.997µm. The mean particle size increased as lipid concentration increased. The emulsion's viscosity increased when the concentration of the core material was increased but the amount of phospholipid remained constant because more lipid was utilized in the preparation. Similar to how particle size of formulations decreased when poloxamer concentration and homogenization speed increased. The formulation with the smallest particle size 7.065±0.106µm is formulation NL8.

3.3.4. Percentage drug loading and Percentage Drug Entrapment

Percentage drug entrapment and percentage drug loading of all different liposphere formulations were given below.

Table 9 Percentage Drug entrapment of different liposphere formulation

S.no.	Formulation code	Percentage Drug entrapment
1	NL1	53.024±0.312
2	NL2	65.166±0.249
3	NL3	91.435±0.937
4	NL4	78.190±0.561
5	NL5	83.709±0.498
6	NL6	90.993±0.312
7	NL7	67.594±0.590
8	NL8	97.395±0.189
9	NL9	94.967±0.446

Table 10 Percentage Drug loading of different liposphere formulation

S.no.	Formulation code	Percentage Drug loading
1	NL1	17.675±0.104
2	NL2	16.291±0.312
3	NL3	18.287±0.187
4	NL4	13.032±0.424
5	NL5	18.798±0.278
6	NL6	15.166±0.052
7	NL7	11.266±0.260
8	NL8	16.233±0.208
9	NL9	15.828±0.115

Table 9-10 showed the percentage of drugs loaded and the percentage of drugs entrapped in the formulation of all drug-laden lipospheres. These percentages were determined to be between 11.266±0.260% to 18.798±0.278 and 53.024±0.312% to 97.395±0.189%, respectively. Percentage drug loading increased together with the concentration of lipid, and percentage drug entrapment increased at the same time. Due to the medication's high solubility in melting lipid, drug entrapment increases as lipid concentration rises. Nevertheless, drug entrapment did not increase with increasing core material concentration at a particular concentration percentage. Poloxamer employed in lipospheres provides stability to lipid structure, leading to high drug entrapment in lipospheres. As a result, percentage drug entrapment and percentage yield of liposphere formulation were also increased on increasing poloxamer content and stirring.

The highest percentages of drug loading and drug entrapment are seen observed to be 16.233±0.208% and 97.395±0.189% in formulation NL8.

On the basis of above *In vitro* characterization of all liposphere formulation NL8 formulation was selected for further analysis.

3.3.5. In-Vitro Drug Release Studies

Percentage in-vitro drug release study NL8 formulation was given below.

Table 11 Percentage Drug Release of NL8 Formulation

Time(min.)	Percentage Drug release of pure drug suspension	Percentage Drug release of NL8
0	0.000±0.00	0.000±0.00
0.5	7.891±0.112	8.408±0.056
1	8.845±0.169	12.620±0.112
2	10.236±0.175	21.977±0.028
4	11.567±0.084	35.408±0.084
6	12.322±0.028	50.901±0.405
8	13.752±0.140	70.371±0.843
10	14.229±0.095	77.921±0.281
12	14.388±0.197	96.397±0.124
24	14.766±0.112	94.013±0.562

In comparison to pure drug's in-vitro drug release of 14.388±0.197%, the manufactured liposphere of naringin showed sustained release to be greater than 96.397±0.124% within 12 hours.

4. Conclusion

The melting point of drug was found to be range 161±1.00 to 167.67±±1.528, hence drug sample was free from any type of impurities. Absorption maxima of Naringin in methanol were found to be 285nm at 10μg/ml concentration. The naringin solution in methanol, 2 to 20μg/ml, was used to generate the calibration curve for naringin. At 285 nm, the absorbance value was determined. The regression equation Y = 0.0453x + 0.0044 and R2 value of 0.999, which exhibits strong linearity, are represented by the calibration curve of Naringin as shown in the graph. Concentration of naringin in alcohol was the highest. In distilled water, it dissolves very poorly. Naringin was discovered to have a partition coefficient of 0.77±0.41 in n- Octanol: Water. This shows that the substance is lipophilic by nature and that its lipophilicity value is near to 0.37 in the reference literature. The Melt Method was used to prepare the naringin loaded liposphere. All prepared lipospheres formulations were spherical in shape and uniform except formulation code NL1. The percent yield of the entire drug-loaded liposphere formulation, which ranged from 94.400±0.353 to 99.844±0.051. Yield also increased with increasing lipid concentration in terms of percentage. Poloxamer employed in lipospheres provides stability to lipid structure, leading to high drug entrapment in lipospheres. As a result, percentage drug entrapment and percentage yield of liposphere formulation were also increased on increasing poloxamer content and stirring. The range of particle sizes for all microparticle formulations was between 7.065±0.106μm to 40.885±0.997μm. The mean particle size increased as lipid concentration increased. The emulsion's viscosity increased when the concentration of the core material was increased but the amount of phospholipid remained constant because more lipid was utilized in the preparation. Similar to how particle size of formulations decreased when poloxamer concentration and homogenization speed increased. The formulation with the smallest particle size 7.065±0.106μm is formulation NL8. Percentage of drugs loaded and the percentage of drugs entrapped in the formulation of all drug-laden lipospheres. These percentages were determined to be between 11.266±0.260% to 18.798±0.278 and 53.024±0.312% to 97.395±0.189%, respectively. Percentage drug loading increased together with the concentration of lipid, and percentage drug entrapment increased at the same time. Due to the medication's high solubility in melting lipid, drug entrapment increases as lipid concentration rises. Nevertheless, drug entrapment did not increase with increasing core material concentration at a particular concentration percentage. Poloxamer employed in lipospheres provides stability to lipid structure, leading to high drug entrapment in lipospheres. As a result, percentage drug entrapment and percentage yield of liposphere formulation were also increased on increasing poloxamer content and stirring. The highest percentages of drug loading and drug entrapment are seen observed to be 16.233±0.208% and 97.395±0.189% in formulation NL8. On the basis of above *In vitro* characterization of all liposphere formulation NL8 formulation was selected for further analysis. In comparison to pure drug's in-vitro drug release of 14.388±0.197%, the manufactured

liposphere of naringin showed sustained release to be greater than 96.397±0.124% within 12 hours. NL8 formulation adheres to the Higuchi model for drug release.

Compliance with ethical standards

Acknowledgments

I would like to thank my guide and co-guide who helped me to do this research work. I would also like to thank my director for support and help throughout my research work.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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