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Formulation & Evaluation of Naringin Nanoethosome by Cold Method

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Naringin is a flavonoid which shows various pharmacological effects, such as, anti-inflammatory and antioxidant,cholesterol lowering activity, free radical scavenging activity. Although naringin is easily found in citrus fruits but has lower bioavailability, biodistribution and undergoes biotransformation to naringenin. To overcome this, the main objective of this work is to formulate nanoethosome formulation containing naringin. The use of nanoethosomes as vesicle drug carrier having ability to increase solubility, improve biodistribution, slows the biotransformation which improves the activity of naringin for treating neurological disorder. The ethosomes were formulated by varying the variables such as concentrations of soya lecithine, polyethylene glycol, and ethanol. The formulations were evaluated with entrapment efficiency, and particle size. Results specify that prepared nanoethosomes of naringin shows decreased particle size, better entrapment efficiency as compared to rigid ethosomes. The F4 was selected as optimized formulation which was further characterized for vesicle size determination. The F4 shows vesicles size of 145.9 nm having 83.9% entrapment efficiency. The nanoethosomes were proved to be significantly superior in

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terms of amount of drug permeated into the skin, with an enhancement ratio of 3.77 when compared to rigid ethosomes. Our results suggests that nanoethosomes are an efficient carrier for improved naringin permeation & stability.

Keywords: Naringin; nanoethosome; drug transport; cold method; spectroscopy.

1. INTRODUCTION

Naringin, chemically flavonoid which shows various pharmacological effects, such as, anti-inflammatory and antioxidant,cholesterol lowering activity, free radical scavenging activity. Although naringin is easily found in citrus fruits but has lower bioavailability, biodistribution and undergoes biotransformation to naringenin. By considering bioavailabilty issues there is need to formulate novel drug delivery system for such herbal ingridients. For pretty some time natural drug treatments had been now no longer taken into consideration for development as novel formula because of absence of medical justification and processing difficulty, for example, standardization, extraction, isolation, and identity of person components in complicated polyherbal structures [1,2]. Not with standing, cutting-edge phytopharmaceutical studies can solve the medical necessities of natural drug treatments to be included in novel drug transport, for example, liposomes, strong lipid nanoparticles, microsphere, ethosomes, transferosomes, micro bubbles, carbon nanotubes, hydrogel, nanoparticles, micro emulsions, matrix structures, strong dispersions, and copolymer micelles.

Ethosome are novel lipid vesicles containing high concentration of phospholipids alcohols in water. This vesicular system containing ethanol has been firstly developed by Touitou. As per report Ethosomes are improving the skin delivery of various drugs. Ethosomes are also prepared by using penetration enhancers such as propylene glycol and showed improved penetration efficacy. The presence of age- activator agents (i.e. ethanol and sodium cholate) in lipid bilayers efficiently improves permeation through the stratum corneum, allowing an improving local and systemic delivery of both hydrophobic and hydrophilic drugs. Ethosomes are good delivery carrier in transdermal field and its enhancement effect has been widely acknowledge. Composition of ethosmoes are phospholipids, polyglycol, alcohol, cholesterol, dye and vehicle [2-4]. As compared to liposome, ethosomes shows higher penetration effect through the skin and its used widely as compared to liposomes. Ethosomes are slighty modified version of well established drug liposomal system. Liposomes are only known for the drug delivery to the outer layers of skin,but in ethosome enhances permeation of drug through the stratum corneum which is major barrier of skin. The main reason responsible for deeper distribution and penetration of ethosomes in the skin was might be due to the synergistic effects of combination of phospholipids and high concentration of ethanol in ethosomes [5,6]. Synthetic drugs shows adverse or toxic effects where as herbal drugs are always safe since ancient times [7]. In herbal medicines and phytochemicals may have limitations like instability in acidic pH, pre-systemic liver metabolism, solubility and absorption,which affects drug level below therapeutic range in the plasma, less or no therapeutic effects. Also, most of the plant having active constituents such as glycosides, tannins, flavonoids, etc.which are polar in nature and are absorbed poorly due to large molecular size which decresed the absorption trough passive diffusion, and poor lipid solubility, which severely decreased their ability to cross the biological lipiodal membranes [7,8]. These disadvatage lead to lower bioavailability and low therapeutic index. Fusion of phytoconstituents in novel drug delivery technology were minimizes the presystemic metabolism, degradation of drug in the gastrointestinal tract, drug distribution / accumulation in the non targeted tissues and organs, and hence minimize the side effects and inhance the therapeutic efficacy and patient compliance [8-10]. Although naringin is easily found in citrus fruits but has lower bioavailability, biodistribution and undergoes biotransformation to naringenin. To overcome this, the main objective of this work is to formulate nanoethosome formulation containing naringin.

2. MATERIALS AND METHODS

2.1 Material

Naringin were purchased from Yarrow Chem, Mumbai. Soya lecithine & ethanol, sodium hydroxide, potassium dihydrogen phosphate, were purchased from modern lab, Nashik. Other

chemicals and solvents were pharmaceutical grade and used as received.

2.2 Method

2.2.1 Experimental design

A 3-factor, 3-level Box–Behnken design was used to explore the quadratic response surfaces and for constructing second order polynomial models using Design Expert (Stat-Ease Inc). A design matrix comprising of 17 experimental runs was constructed, for which the non-linear computer generated quadratic model is defined as;

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X^2_3$$

where Y is the measured response associated with each factor level combination; b_0 is constant; b_1, b_2, b_3 are linear coefficients, b_{12}, b_{13}, b_{23} are interaction coefficients between the three factors, b_{11}, b_{22}, b_{33} are quadratic coefficients computed from the observed experimental values of Y from experimental runs; and X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_1 X_2 and X_2^1 represent the interaction and quadratic terms, respectively [11-13]. The independent variables selected were the amount of the soya Phospholipid (X_1), polyethylene glycol (X_2), and Ethanol (X_3). The dependent variables were entrapment efficiency (Y_1), vesicles size (Y_2), shown in Table 1 with constraints applied to the formulation of nanoethosome.

2.2.2 Formulation of naringin nanoethosomal dispersion using cold method

Naringin loaded nanoethosome were prepared by cold method as shown in Table 2. Precisely, soya phosphatidylcholine (PC), and drug (naringin) were taken in a clean dry round bottom flask. The lipid mixture and naringin was dissolved in an ethanol in covered vessel at room temperature by vigorous stirring with the use of magnetic stirrer at 700 rpm, propylene glycol in water is added during stirring. This mixture is heated to 30˚C in a water bath, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using probe sonication method at 50 % amplitude for 10 min 3 cycles (A break of 5 min) To remove any un-entrapped drug from the dispersion obtained by both the methods, the dispersion was centrifuged at 4 °C and 10,000 rpm for 60min and pellet obtained was separated from supernatant. The pellet was dispersed in phosphate buffer pH 7.4 which was then used for further studies. Finally, the formulation is stored under refrigeration [3,4,9,10].

2.3 Characterization

2.3.1 Preformulation studies [14-16]

Preformulation is that the initiative in designing or development of rational dosage form of drug. Preformulation studies were perform for determination of physicochemical properties of the new compound which will affect the event of stability, dosage form safety and efficacy.

2.3.2 Organoleptic properties [14-16]

The drug samples of Naringin were studied for appearance color and odour by using visual method drug sample was evaluated for color and texture.

2.3.3 Melting point [17-20]

Melting point of naringin was determined by taking a small amount of sample in sealed capillary tube closed at one end,attached with thermometer with a rubber band, tube were immersed in the theiles tube. Heating is commenced, and therefore the temperature ranges at which the sample melts can then be observed. During heating, the purpose at which melting is observed and therefore the temperature constant is that the freezing point of the sample.

2.3.4 Solubility [21-22]

The Solubility of Drug in water, ethanol and 7.4 buffer solutions was determined by Shake Flask Method, the examined compound was dissolved in solid excess in 1-10 ml respective solvent. The solutions were stirred for 48 hours within the magnetic stirrer under thermo stated circumstances until the solubility equilibrium. To separate phases the solutions were left to sediment under thermo state circustances. The solution was filtered; aliquots were taken from clear a part of the solution. the aliquots were diluted the absorption was measured with UV-Spectrophotometer Shimadzu, UV-2450. The concentrations of the Aliquots were calculated.

2.3.5 Ultraviolet -visible spectroscopy [21-23]

Preparation of calibration curve by U.V. Visible Spectrophotometric method:

A. Determination of λmax in Ethanol

The UV spectrum of Naringin was obtained using UV Shimadzu 2450, Japan. Accurately weighed 10 mg of the drug was dissolved in sufficient quantity of ethanol and volume made up to 10 ml. The stock solution was diluted to get a concentration of 100 µg/ml. The 1 ml of aliquot was withdrawn and volume was made up to 10 ml using ethanol to obtain the concentration of 10 µg/ml. The resultant solution was scanned from 200 to 400 nm and therefore the spectrum was recorded to get reading of maximum wavelength. Results are shown in Table 3 and spectrum of naringin in ethanol shown in Fig. 2

Construction of Beers-Lambert's plot in ethanol

The stock solution of 100µg/ml was prepared in ethanol, used to prepare different dilutions in the range of 5-25 µg/ml. The absorbance of resulting solutions ware measured at 283 nm by UV-visible spectrophotometer.The results are shown in Fig. 4

B.Determination of λmax in pH 7.4 phosphate buffer

Preparation of 7.4 pH phosphate buffer:

0.2M of potassium dihydrogen phosphate (KH_2PO_4) solution was prepared by dissolving 2.722 gm KH_2PO_4 of in 100 ml distil water in another 100 ml of volumetric flask, solution of 0.2 M sodium hydroxide (NaOH) was prepared by dissolving 0.8 gm NaOH in 100 ml distil water. 50 ml of 0.2M KH_2PO_4solution was taken in another beaker and specified volume of 0.2N NaOH solution (22.4 ml) was added in it and the volume was adjusted with distil water to 200 ml.

Preparation of stock solution

10 mg of Drug was accurately weighed and was diluted to 100 ml using phosphate buffer pH 7.4 to get a final solution of conc. 100 µg/ml. This solution was used as stock solution. From this stock solution, 2.5ml of Aliquot was withdrawn and volume made upto 10 ml with phosphate buffer pH 7.4 to obtain the solution with concentration 25µg/ml. The UV Spectrum was recorded in the wavelength range 200-400nm. Wavelength of maximum absorbance was determined. Results are shown in the Table 3 and spectrum of Naringin in phosphate pH7.4 shown in Fig.3.

Construction of Beers-Lambert's plot in pH 7.4 phosphate buffer

The stock solution of 100µg/ml was prepared in pH 7.4 phosphate buffer, used to prepare different dilutions in the range of 5-25 µg/ml. The absorbance of resulting solutions ware measured at 291 nm by UV-visible spectrophotometer. The results are shown in Table 3 and Fig. 5.

2.3.6Fourier transforms infrared spectroscopic (FTIR) studies [24]

The infrared absorption spectrum of Naringin was recorded with KBr. The dried sample of drug was mixed with KBr in the ratio of 1:9, the sample triturate and finally placed in sample holder. The spectrum was run over the wave number 4000 to 650 cm-1 using Fourier Transform Infrared Spectrophotometer Shimadzu, 8400S, Japan. The spectral analysis was done, by standard absorbance of the functional groups. Peaks observed are shown in the spectrum Fig. 6.

2.3.7Differential scanning calorimetric (DSC) studies [13]

DSC analysis was performed using DSC instrument Shimadzu, DSC 60 by taking samples (2to5) samples were hermetically seals in aluminium pan and with nitrogen flow rate 10ml/min conducted over a temperature range of 30-2000c.DSC analysis was also performed to ascertain the purity and identification of drug. The instrument was calibrated with indium standard. Accurately weighed samples were placed in open, flat bottom, aluminum sample pans. Thermograms were obtained by heating the sample at a constant rate of 10^0C/minute. A dry purge of nitrogen gas (20ml/min) was used for all runs. Samples were heated from 35^0C – 250^0C. Scans were obtained from the samples. The melting point and the peak maxima were observed in the DSC graphs. DSC thermogram is shown in Fig. 7.

2.3.8 Evaluation of nanoethosomes

Drug Entrapment efficiency [4-9]

The Encapsulation efficiency (EE%) of nanoethosomes was determined using the

dialysis technique for separating the non-entrapped naringin, 1 ml of naringin-loaded nanoethosome dispersions were dropped into a dialysis bag (molecular weight cut off: 10 kDa) immersed in 50 ml of methanol solution and shook at 50 rpm. 1 ml of ethanol solution was taken out after 2 h and make a appropriate dilution and then analyzed by UV to account the quantify the unentraped drug (Wf). Then 1 ml of naringin -loaded nanoethosome dispersions were demulsified with 25 ml methanol and the obtained solution was detected as total drug in dispersion (Wt). The encapsulation efficiency (EE%) could be counted by the following equations. results are shown in Table 5.

$$\text{Drug entrapment efficiecy} = \frac{\text{Actual drug content in nanoethosome}}{\text{Theoretical drug content}} \times 100$$

Actual drug content [13,18]

Precisely weighed equivalent quantity (10 mg) of nanoethosomes containing drug was kept in 100 ml of phosphate buffer pH 7.4 solutions for an hour with continuous stirring. Filtered samples were further analysed at 283 nm next to blank using UV-visible spectrophotometer (Shimadzu, UV-2600)

Actual Drug content (%) = (Nact / Nms) * 100

Where Nact = actual naringin content in weighed quantity of nanoethosomes,
Nms = weighed quantity of nanoethosomes and
Nthe = theoretical naringin content in nanoethosomes

Particle size and zeta potential determination [25-26]

1 ml of nanoethosomal suspension was diluted by 10 ml of double distilled water and then vesicle size and zeta potential were determined by a dynamic light scattering particle size analyzer Malvern Zetasizer, (Malvern Instrument Ltd.).Mean vesicle size and zeta potential graphical representation given in Fig. 8 and 9.

Polydispersibility index [27]

The Polydispersity index (PDI) is an index of width or spread or variation within the Particle size distribution. PDI can be determined by dynamic light scattering instrument. Lower PDI values are observed in monodisperse sample, whereas higher PDI value indicates particle size distribution and the polydisperse nature of the sample.

PDI can be calculated by using following equation

PDI = ΔD/Dave

Where, D is distribution donated by SD and Dave is the average particle size.

Vesicle morphology [27-28]

Morphology of the vesicles was visualized by scanning electron microscope (SEM). A drop of nanoethosome dispersion was placed on a carbon coated grid to leave a thin film, before the film got dried on the grid, it was negatively stained with 1% phosphotungstic acid. A drop of staining solution was added on the film, and the excess of the solution was drained off with a filter paper. The grid was allowed to air dry, and sample was viewed by SEM at 80 kV. Morphology of vesicle are shown in Fig. 10.

3. RESULTS AND DISCUSSION

3.1 Preformulation Studies

Organoleptic properties

The sample of drug received was studied for its organoleptic characters which shows light yellow colour, odourless in nature and amorphous in nature.

Melting Point

Melting point of pure Naringin is found in 167-171˚c which is found to be nearly to standard melting range of naringin. That indicates the purchased samples obtained were of pure quality.

Solubility

The drug naringin was found practically insoluble in distilled water, while the solubility of pure Naringin in ethanol, water and in phosphate buffer pH 7.4 was found to be 2.7, 11.8 and 11.2 mg/ml respectively. It indicates that the drug is soluble in ethanol, methanol as well as sparingly soluble in phosphate buffer ph 7.4.

3.2 Ultraviolet-Visible Spectroscopy Study

8.1.4.1: Determination of λ_{max} of Naringin in ethanol and 7.4 phosphate buffer

The UV spectrum of Naringin solution in ethanol and phosphate buffer pH 7.4 exhibited wavelength of absorbance maximum at 283.73 nm, 291.09 respectively. This is near to the reported value. However, keeping in mind the probable concentrations likely to be encountered while carrying out *In-vitro* release studies and considering the predicted theoretical λ_{max} involved, the working λ_{max} was decided as 283.73nm in ethanol and 291.09 in phosphate buffer pH7.4. The spectrum of Naringin is shown in (Fig.2 and 3).

3.3 Construction of Beers-Lambert's Plot

The calibration curve (Fig. 4 and Fig. 5) was found to be linear in the concentration range of 2 to 25µg/ml (Table 3) having coefficient of regression value R^2 =0.998 and Slop y = 0.053x + 0.002 in ethanol and in pH 7.4 phosphate buffer having coefficient of regression value R^2 =0.994 and y = 0.0108x + 0.008.

3.4 FITR Spectroscopy of Naringin

The powdered mixture of Naringin and KBr was taken in a sampler and the spectrum was recorded by scanning in the wavelength region of 4000- 700 cm^{-1} using FTIR spectrophotometer. The FTIR spectrum of naringin was shown in (Fig. 6) and principle peaks obtained at wave number 1818 cm^{-1} for aromatic C=O, 3416 cm^{-1} for O-H stretching, 1361 cm^{-1} indicates that presence phenol ring, 2957-2859 cm^{-1} for aromatic & aliphatic C-H stretching. The naringin ethosome shows peaks at 3416 cm^{-1},1361 cm^{-1},1818 cm^{-1}, and 2957 cm^{-1} which confirmed that no changes in drug peak at both spectra. Absorption bands shown by Naringin and Naringin nanoethosomes shows all characteristics of the groups present in its molecular structure. The presence of absorption bands corresponding to the functional groups present in the structure of Naringin confirms the identification and purity of purchased Naringin sample.

3.5 Differential Scanning Calorimetric (DSC) Studies

DSC thermogram for Naringin is shown in the Fig. 7. DSC studies indicated a sharp endothermic peak at 167^0c corresponding to the melting of the pure drug and naringin nanoethosome showed DSC thermogram at range of 159.69 which shows that slightly shifting of drug in peak which is due to amorphization of naringin in nanoethosomal matrix. Dsc result indicating that there is absence of incompatibility between drug & excipients.

4. EVALUATION OF NARINGIN NANOETHOSOMES

Evaluations of prepared nanoethosomes of Naringin were carried out for Actual Drug Content, entrapment efficiency and vesicle size and morphology study.

4.1 Actual Drug Content

The uniform dispersion of drug in the nanoethosome can be determined by drug content analysis. It was observed that around 59.6 to 83.9 % drug can be incorporated in the nanoethosome F4 batch showing uniform dispersion of drug in the nanoparticle. Drug content analysis are shown in Table 4.

4.2 Entrapment Efficiency

After preparing nanoethosomal dispersion, unentrapped drug is separated by dialysis method described above and the drug remained entrapped in nanoethosome is determined by Spectrophotometric method.

> % Entrapment efficiency (% EF) = (Amount of drug entrapped/ total amount of drug) x 100

Nanoethosome consisting lipid & surfactants displayed diverse % EE. Maximum %EE (83.48%) was obtained from nanoethosome prepared with SPC (F4). The results could be explicated as that the entrapment of lipophilic drug into lipid vesicle was facilitated by drug distribution coefficient between lipid phase and aqueous solution. From results obtained, the affinity of surfactant to lipid is decrease. Based on affinity of surfactant with lipid and strong lipophilic of a drug (logP = 3.31), naringin would be more prevalent dispersing in double layer lipid established by PEG and soya lecithin in ratio of 100:500 (F4).The entrapment efficiency was shown in Table.5

4.3 Vesicle Size and Zeta Potential Determination

Vesicle Size

Particle size analysis was done by Malvern Zetasizer particle analyzer.The vesicle size of nanoethosome varies with different ratio of lipid use. One from each batches are subjected for vesicle size determination, depending on % entrapment efficiency.There were significant differences in particle size between Nanoethosome consisting different concentration of SPC. Nanoethosome containing SPC and PEG expressed a mean particle size of 145.5 nm. In general, particle size increased with drug lipid ratio increased from 1:5, 1:7 and 1:2. and Graphical representation of size determination given in Fig. 8.

4.5 Poly Dispersibility Index: (PDI)

PDI is an index of width or spread or variation within the particle size distribution. Monodisperse sample have lower PDI value, whereas PDI of higher value indicates a wider particle size distribution and polydisperse nature of sample. PDI can be calculated by the following equation $PDI=\Delta d/d_{avg.}$ Where, Δ dis the width of distribution denoted by SD and $d_{avg.}$ Is the average particle size denoted by MV (nm) in particle size data.

$$PDI= \Delta d/davg = 0.4$$

As depicted in Fig. 8 the nature of nanoethosome formulation for the optimized batch shows Mid-8Range monodisperse. The polydispersity indices of nanoethosomes were shown at 0.354 Therefore it can be stated that the polymer based nanoparticle prepared by cold method have exhibited a homogeneous size distribution.

4.6 Zeta Potential Analysis

The zeta potential of optimized batch was found to be -9.31 mV which is in good agreement with literature due to the net charge of the lipid composition in the formulations. PC is a zwitter ionic compound with an isoelectric point [pI] between 6 and 7. Under experimental conditions of pH 7.4, where the pH was higher than its pI, PC carried a net negative charge. The edge activator used was anionic edge activator, and the anion form of naringin was also the predominant form at pH that pH. Therefore, a negative charge in all formulations was observed. Also the negatively charged nanoethosome formulations may improve skin permeation of drugs in transdermal delivery. The skin also has slight negative charge. Therefore, the negative zeta potential of the optimized nanoethosome containing naringin might influence improved naringin permeation through porcine skin due to electrostatic repulsion between the same charge of the skin surface and the optimized formulation. Peak of zeta potential is displayed in Fig. 9

4.7 Vesicle Morphology

The morphology of naringin nanoethosomal dispersion (F4) was as shown in Fig. 10 From the micrograph, it could be observed that unilamellar vesicles with an intact bilayer membrane were formed. The particle size of the vesicles was about 145.9 nm.

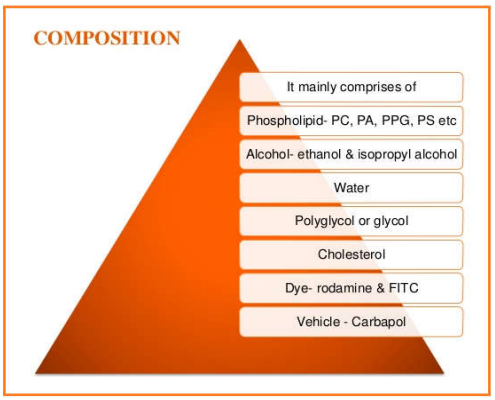

Fig. 1. Component of ethosomal system

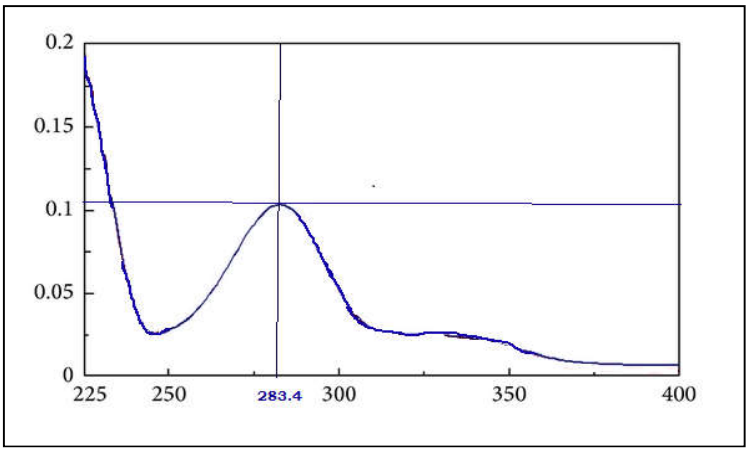

Fig. 2. UV-visible spectrum of Naringin in Ethanol

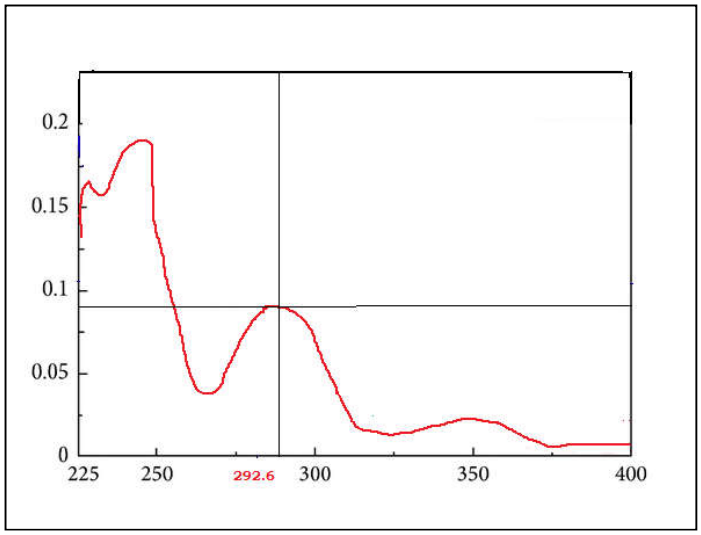

Fig. 3. UV-visible spectrum of Naringin in phosphate buffer PH 7.4

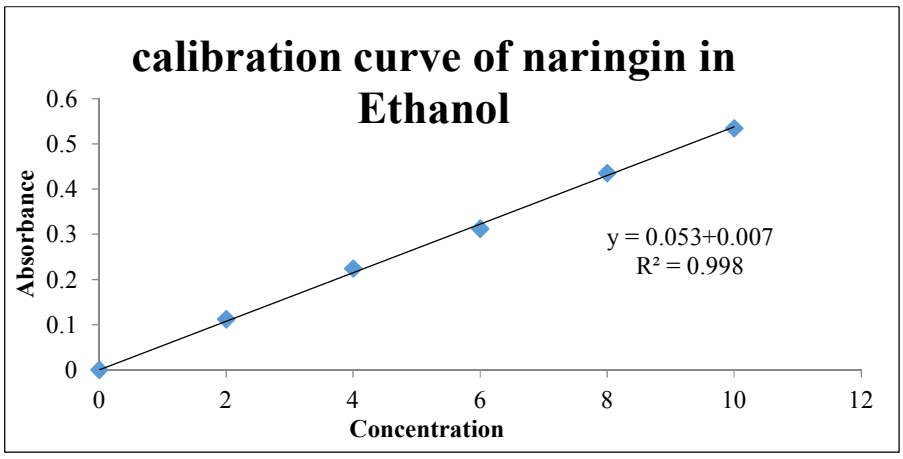

Fig. 4. Calibration curve of naringin in ethanol

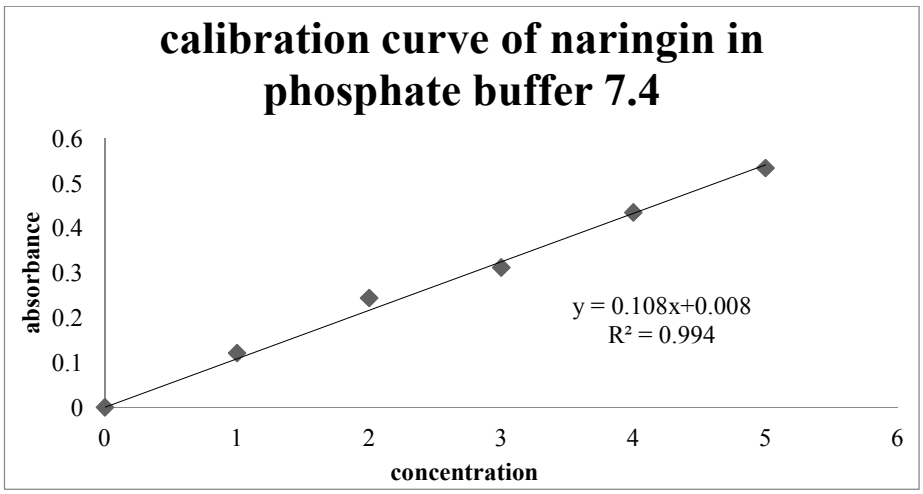

Fig. 5. Calibration curve of naringin in phosphate buffer PH7.4

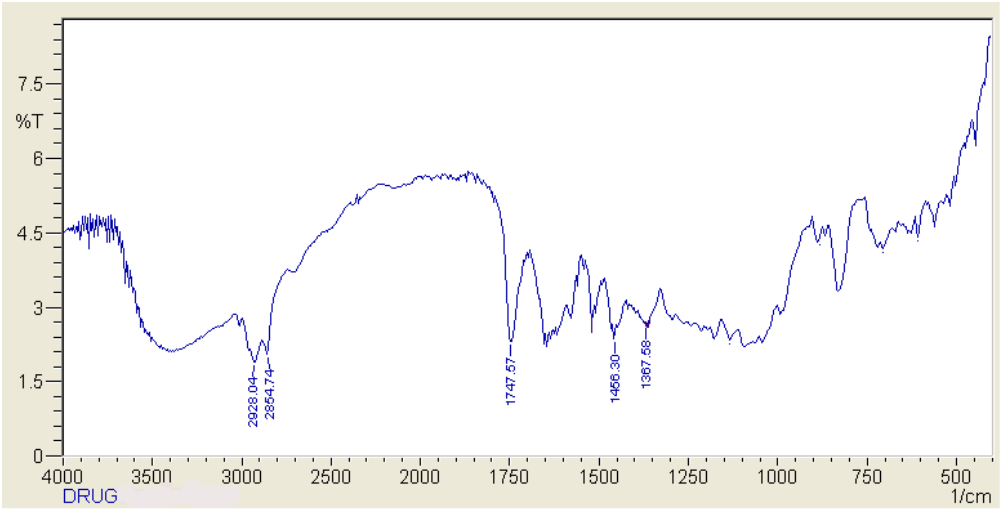

Fig. 6. FTIR spectra of naringin

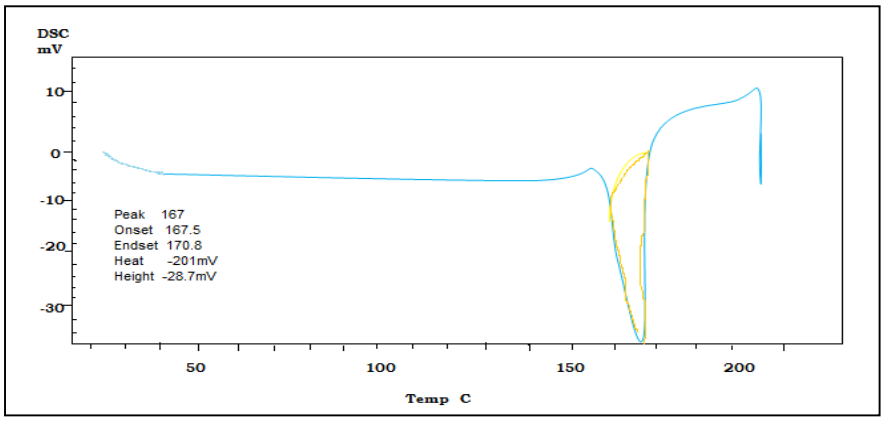

Fig. 7. DSC Thermogram of Naringin

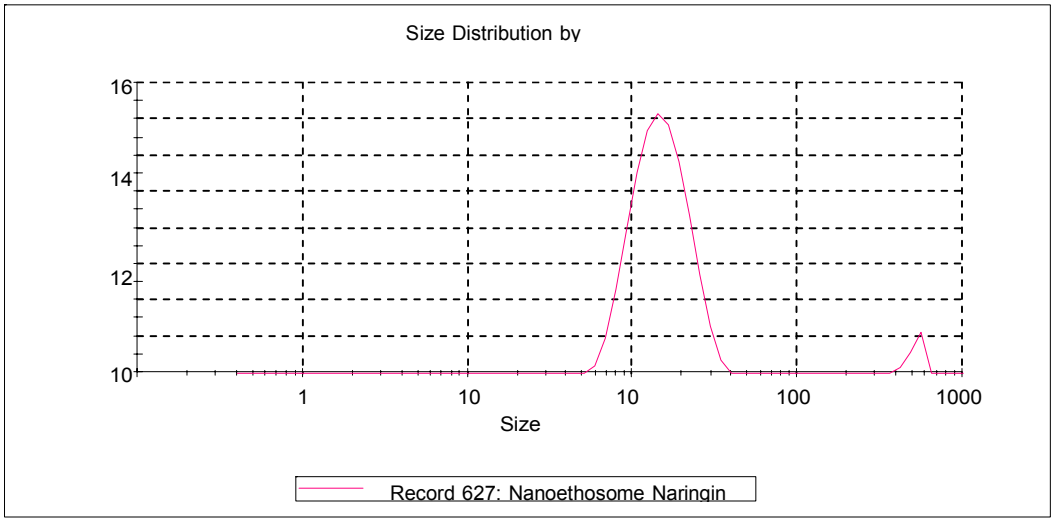

Fig. 8. Particle size distribution of optimized batch

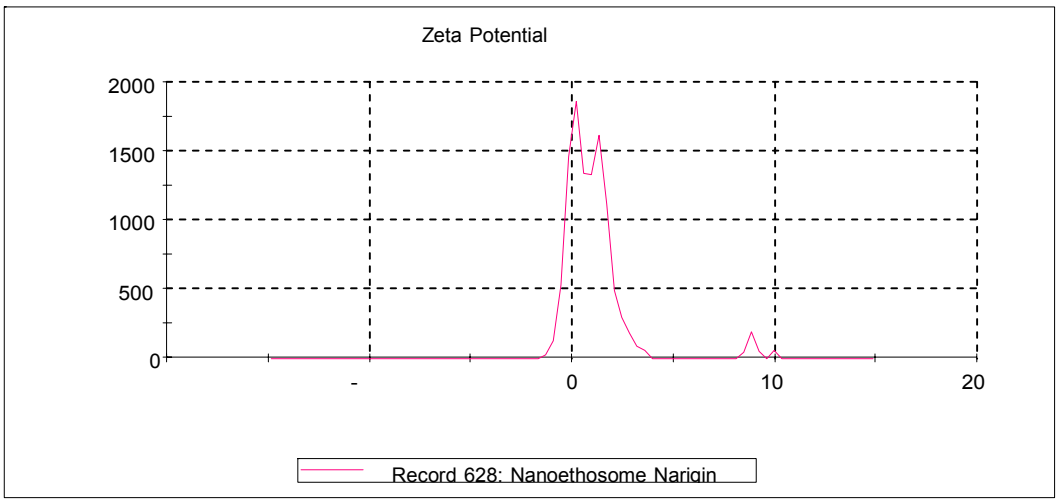

Fig. 9. Zeta potential of optimized batch

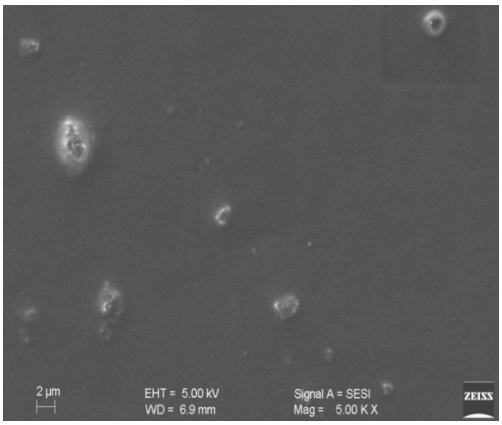

Fig. 10. Vesicle morphology by using scanning electron microscopy

Table 1.Variables in Box-Behnken design for preparation of naringin nanoethosomes

Factor	Level Used,Actual (Coded)		
	Low(-1)	Medium(0)	High(+1)
Independent variables			
X_1= soya phospholipid (gm)	200	500	700
X_2=polyethylene glycol(ml)	4	5	6
X_3=Ethanol(ml)	10	20	30
Dependent variables			
$Y_{1=}$Entrapment efficiency(%)			
$Y_{2=}$ Particle Size (nm)			

Table 2. Formulation of naringin nanoethosome.

Formulation code	Factor X 1	Factor X2	Factor X3	Drug entrapment Efficiency (%) Y1	Particle size(nm) Y2
	SPC (mg)	PEG (ml)	Ethanol (ml)		
F1	+1	+1	+1	79.91%	159.1
F2	+1	0	-1	79.83 %	171.3
F3	+1	-1	0	75.83 %	168.9
F4	0	+1	-1	83.60 %	145.7
F5	0	0	0	80.66 %	148.2
F6	0	-1	+1	80.16 %	150.5
F7	-1	+1	0	73.44 %	180.67
F8	-1	0	+1	73.40 %	177.4
F9	-1	-1	-1	74.05 %	160.6

Table 3. Absorbance's of different concentration of Naringin

In Ethanol			pH 7.4 phosphate Buffer	
Sr. No.	Concentration in µg/ml (ppm)	Absorbance	Concentration in µg/ml (ppm)	Absorbance
1	2	0.112	5	0.121
2	4	0.224	10	0.244
3	6	0.312	15	0.389
4	8	0.435	20	0.494
5	10	0.534	25	0.598

Table 4. Actual drug content and Drug Loading of Naringin Nanoethosome

Sr No.	Formulation batches	Actual drug content %	Drug loading %
1	F1	70.3 %	75.7 %
2	F2	68.89 %	75 %
3	F3	71.78 %	73.9 %
4	F4	83.67 %	78.5 %
5	F5	80.4 %	78 %
6	F6	81.2 %	77.77 %
7	F7	63.5 %	63.23 %
8	F8	59.6 %	60 %
9	F9	70.45 %	71.42 %

Table 5. Entrapment Efficiency of Naringin Nanoethosome

Batch Code	Absorbance at 283 nm	Concentration µg/ml	Unentraped drug (%)	% Entrapment In Nanoethosomes
F1	0.412	27.91	27.91	72.08
F2	0.474	34.11	34.11	65.88
F3	0.391	25.91	25.91	74.18
F4	0.298	16.51	16.51	83.48
F5	0.309	17.61	17.61	82.37
F6	0.341	20.81	20.81	79.18
F7	0.388	25.51	25.51	74.38
F8	0.474	25.91	25.91	74.58
F9	0.355	22.21	22.48	77.52

5. CONCLUSION

It has been nearly twenty years since the invention of ethosomes and through this era these nanocarriers have proved their distinctive ability to deliver therapeutic agents. From all above observations and results obtained, it can be concluded that all the formulations show satisfied organoleptic properties. The Preformulation study of drug and excipients shows purity of drug and excipients. .The compatibility study of drug and excipients was performed and it was found that the drug is compatible with excipients used in formulation. Nanoethosomes of Naringin were successfully formulated by cold method and examined for entrapment efficiency, drug content, size and shape of vesicle and morphology. The results obtained from study confirms, maximum entrapment efficiency and drug content were found of F4 batch, hence it was taken as optimized batch. It shows % entrapment efficiency of 83.48%, percent drug content of 95.16%. Also the average mean diameter of optimized batch of nanoethosomes was found to be 145.7 nm. The zeta potential was found to be -9.2 mV. The vesicles were found as unilamellar. Hence it can be concluded that above formulation can be effectively used in nanoethosomal delivery.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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