

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF NIOSOMES CONTAINING PUNICALAGIN FROM PEELS OF *PUNICA GRANATUM**Priya Hanu¹, Singh Harmanpreet²¹Department of Pharmaceutics, Lovely Professional University, Phagwara, Punjab, India²Department of Pharmaceutics, Lovely Professional University, Phagwara, Punjab, IndiaCorresponding Author's Email: hanupriya.yadav@gmail.com

Received 13 Oct 2012; Review Completed 25 Oct 2012; Accepted 01 Nov 2012, Available online 15 Nov 2012

ABSTRACT

Plant based formulations have been used since ancient times and playing a role as a remedial against various human and animal diseases. The interest in traditional medicines has increased in various parts of world. A well known ancient fruit named as *Punica granatum* (family-Punicaceae) which is commonly known as Pomegranate, Anar or Dalim in North India whose therapeutic qualities have rebounded and echoed throughout the millennia and regarded as an agent of resurrection for Babylonians; symbol of invincibility on the battle fields for the Persians, while for the ancient Chinese, symbolized for longevity and immortality. Punicalagin is chemically named as 2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose and belongs to a category of hydrolysable tannin. In this vein, present investigation was an endeavor to formulate the vesicular formulation of Punicalagin. For this, the Punicalagin was extracted, isolated and purified from peels of *Punica granatum*. Thus, to protect its hydrolysis, it is formulated into a nanocarrier system known as niosomes which is based on the preparation of niosomes by using a non-ionic surfactant in varying amounts and keeping the amount of cholesterol constant. The formulations were evaluated on the basis of evaluation parameters and thus optimized for the best formulation.

Keywords: *Punica granatum*, Punicalagin, Novel Drug Delivery System, Niosomes, Vesicular system.**Abbreviations used-** EA- Ellagic acid, NSV- Non-ionic surfactant vesicles, TEM- transmission electron microscopy, rpm- revolutions per minute, v/v- volume by volume

INTRODUCTION

Plants itself offer an immense and prodigious, but not impossible challenge for anyone trying to work out in detail the products of their chemical machinery. Tannins are one of the most widely occurring groups of substances in different families of higher plants. They are high molecular weight plant polyphenols¹ and the secondary metabolites which are present in solution form in cell sap and also in distinct vacuoles. Chemically, tannins contain the mixture of complex organic substances in which polyphenols are present, generally with o-dihydroxy or o-trihydroxy groups on phenyl ring and they are devoid of nitrogen².

The pomegranate (*L. Punica granatum*) belongs to family Punicaceae is commonly known as Anar or Dalim in North India³. It is an ancient fruit which has been widely consumed in various cultures for thousands and thousands of years. The Babylonians regarded pomegranate seeds as an agent of resurrection; the Persians believed the seeds conferred invincibility on the battle fields, while for the ancient Chinese, the seeds symbolized longevity and immortality⁴. Pomegranate husk is a traditional Chinese medicine used as antibacterial, anti-inflammatory and hemostasis agent which is rich in phenolic compounds. Among these polyphenols, the most abundant compound is Punicalagin. Punicalagin levels in husk depend upon the area for fruit growth, processing conditions as well as storage conditions⁵. Punicalagin is a potent antioxidant whose bioactivity can be explained by its ability to hydrolyze into Ellagic acid (EA) *in vivo* and across the mitochondrial membrane *in vitro*.

For the better performance of pharmaceutical formulations with respect to controlled release, bioavailability, storage stability and lesser side effects constitute the main motivation for research of novel drug delivery systems⁶. Novel drug delivery systems are used to improve the drug

potency, control drug release to give sustained therapeutic effect, provide safety and reduces toxic effects. It may target/delivery of drugs specifically to tissue, organ or location in the body. There are various novel drug delivery strategies like liposomes, niosomes, aquasomes, microsponges, microemulsions, and solid lipid nanoparticles to enhance the topical delivery of agents. Among different carriers, liposomes and niosomes are well documented for dermal drug delivery⁷. Vesicles formed on hydration of mixture of cholesterol and single alkyl-chain non-ionic surfactants were first introduced by Handjani-Vila. Initially reported as a feature of cosmetic industry, they are now extensively used as drug delivery systems⁶.

Niosomes may be defined as a unilamellar or multilamellar vesicles in which the aqueous solution is enclosed in highly ordered bilayers made up of non-ionic surfactants with or without cholesterol and dicetylphosphate and exhibit behaviour similar to liposomes *in-vivo*⁷. They are capable of entrapping both hydrophilic and hydrophobic drugs as shown in figure 1.

Surfactants play an important role in the development of such formulations. A number of non-ionic surfactants have been used to prepare vesicles viz. polyglycerol alkyl ethers, glucosyl dialkyl ethers, crown ethers, ester linked surfactants, polyoxyethylene alkyl ether, brij, and series of spans and tweens. They are made up of biocompatible, non-toxic, non-immunogenic, and non-carcinogenic agents. NSV's are highly resistant to hydrolytic degradation. NSVs result from the self assembly of hydrated surfactant monomers. The surfactant molecules self-assemble in aqueous media in such a fashion that the hydrophobic tails face each other to minimize the high energy interactions between the solvent and tails⁶.

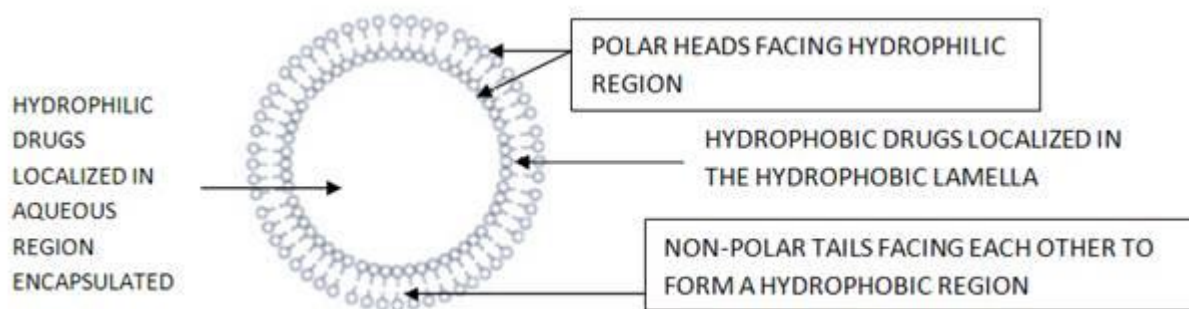


Figure 1: Microscopic structure of a niosome

Advantages of niosomes are: accommodate hydrophilic, lipophilic as well as amphiphilic drug moieties; niosomes exhibit flexibility in their structural characteristics (composition, fluidity and size) and can be designed according to the desired situation; improve the therapeutic performance of the drug, protect from the biological environment, restricts its effect to target cells, thereby reducing the clearance of drug; act as depot to release the drug slowly and offer a controlled/sustained release; increase oral bioavailability of drug; increase the stability of entrapped drug; enhance the skin penetration of drugs^{8,9}. They can be made to reach the site of action by oral, parenteral as well as topical routes; surfactants used are biodegradable, biocompatible and non-immunogenic; handling and storage of surfactants do not require any special conditions; the vesicle suspension being water based offers greater patient compliance over oily dosage forms^{10,11}.

The objective of the present study is to develop and validate a simple, precise, accurate, and economical analytical method for the estimation of Punicalagin extracted from peels of *Punica granatum*. To perform the compatibility study of drug, Punicalagin with the excipients used in formulating niosomes. To develop a vesicular system like niosomes which act as carriers and hence will help in penetration of drug through skin and provide a prolong release.

MATERIALS

Punicalagin was extracted from pomegranates which were purchased from local market in Bhiwani, Haryana (India). Peels were authenticated from National Institute of Science Communication and Information Resources, New Delhi, India. The common excipients like methanol, ethanol, acetone, potassium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were obtained from Loba Chemie Pvt. Ltd., Mumbai. Diethyl ether was obtained from Central Drug House Pvt. Ltd., New Delhi. Span 60 and Cholesterol were obtained from S.D. Fine Chemicals Ltd., Mumbai, India.

METHODS

1. Extraction, Isolation and Purification of Punicalagin from peels of *Punica granatum*

1.1 Extraction and Isolation of Punicalagin from peels of *Punica granatum*

Fruits were washed, separated from seeds and juice and cleaned to yield husks/peels. Peels of the *Punica granatum* were dried in shade and powdered them with

the help of blender. Powdered husk was macerated with water and methanol for 7 days with occasional stirring. After maceration process was completed, thick husk puree was squeezed by hand and filtered through muslin cloth to yield dark brown aqueous extract. The column having Sephadex LH-20 used as stationary phase and prepared for chromatography by pre-washing in methanol and pre-equilibrated in water for 12 hr. The aqueous extract was divided into portions and adsorbed onto the Sephadex LH-20. Each column was eluted with excess amount of distilled water until sugary pale yellow elute was clear in colour. The adsorbed tannins were eluted with methanol to yield dark brown solution. The collected fractions were then passed through C18 cartridges (Waters Sep-Pak[®] Vac 20cc) and eluted with methanol to obtain the yellowish brown solution which contained pure compound, Punicalagin. The methanol was removed by Rota-evaporator in vacuo at low temp. (37°C) and obtained the dark brown powder as TPT (total pomegranate tannins)¹.

1.2 Purification of Punicalagin from TPT Sephadex LH-20 resin column was used to isolate the pure compound from TPT. TPT obtained was adsorbed onto a Sephadex LH-20 column that was pre-equilibrated with water: methanol (8:2 v/v) and eluted with increasing amount of methanol. The fraction was evaporated in vacuo and then re-chromatography was done by pre-equilibrating column with ethanol. Elution was done with increasing amounts of water and acetone then to ethanol: water: acetone (6:3:1 v/v/v) and finally with ethanol: acetone (1:1 v/v). The fraction was collected and then evaporated in vacuo to obtain yellowish brown powder as Punicalagin¹.

2. Preformulation Studies of Punicalagin

2.1 Organoleptic Properties The organoleptic properties include physical state, colour and odour was done by visual inspection.

2.2 TLC Identification Test

i) Preparation of sample- The powdered drug (0.5g) was macerated in methanol for 4 days. The extract was filtered and process was repeated thrice. The filtered extract was pooled, evaporated to dryness under reduced pressure and residue was dissolved in methanol.

ii) Preparation of Solvent system- Chloroform: Ethyl acetate: Formic acid: Methanol in a ratio of 4: 5.2: 0.6: 0.2 was mixed to prepare solvent system. Spotting of the sample was done with the help of capillary on TLC plate. The plate was then placed in the solvent system till the saturation point was reached.

iii) Visualisation- TLC plate was dipped in a solution consisting of anisaldehyde (0.5ml), glacial acetic acid (9.5 ml), methanol (85ml) and conc. sulphuric acid (5ml) for a few seconds. It was then heated at 110°C in hot air oven till coloured band appeared and R_f value was then calculated as in equation (1).

$$R_f \text{ value} = \frac{\text{Distance travelled by solute from}}{\text{Distance travelled by solvent from}} \quad \text{--- (1)}$$

2.3 Chemical identification test- To identify the drug chemically, a pinch of drug was taken in a test tube and 2 ml of ferric chloride solution was added to it. Presence of a colour shows the presence of tannin.

2.4 Characterisation of Punicalagin

2.4.1 UV spectroscopy Punicalagin was dissolved in phosphate buffer saline pH 7.4 and the sample was scanned at wavelength ranging from 200-400nm by UV spectrophotometer. The absorption maxima were compared with that available in the literature¹².

2.4.2 IR spectroscopy IR spectroscopy of Punicalagin was performed using FTIR 8400S (Shimadzu). KBr pellets of Punicalagin were prepared by applying a pressure of 8 tons in a hydraulic press. The pellets were scanned over a wave number range of 4000–400 cm^{-1} . The spectrum obtained was interpreted by the literature¹³.

3. Standard UV Plots

3.1 Determination of absorbance maxima (λ_{max}) Punicalagin (10mg) was accurately weighed and transferred to a 100 ml volumetric flask. To this, pH 7.4 PBS was added to dissolve the drug. From this solution, 1 ml of solution was pipetted out in 10 ml volumetric flask and volume was made upto 10 ml with distilled water. The sample was scanned on a double beam UV-visible spectrophotometer. An absorbance maximum of Punicalagin was determined in pH 7.4 PBS.

3.2 Standard plot of Punicalagin in pH 7.4 Phosphate buffer The standard plot of Punicalagin was prepared in pH 7.4 PBS. 10 mg of drug was weighed accurately and dissolved in 100 ml of pH 7.4 PBS. Appropriate dilutions were made with buffer to obtain test solutions ranging from 5 $\mu\text{g/ml}$ to 35 $\mu\text{g/ml}$. The absorbance of the drug in the buffer was measured on a double beam UV-visible spectrophotometer at λ_{max} of 253.6nm against the respective blank.

4. Compatibility Study

4.1 Physical characterisation of drug excipient mixture Drug and each excipient were separately passed through sieve # 20. Drug and each excipient were weighed in the ratio of 1:1 and mixed properly with 15 ml of water for injection (WFI) as shown in table 1. Different drug excipient mixtures were introduced into glass vials containing 15 ml of water covered with rubber caps which were followed by labelling. Then the vials were kept under three different conditions, one at $5 \pm 3^\circ\text{C}$ (refrigerated temperature), $25 \pm 2^\circ\text{C}$ (room temperature) and $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$. Observations were taken on 0th, 7th, 14th, 21st and 28th day for physical compatibility.

4.2 Chemical characterisation of drug excipient mixture

For chemical compatibility, the study was carried out by taking the different drug excipient mixtures after 28th day and analyzed spectrophotometrically by UV.

Table 1: Composition for compatibility study of Punicalagin with excipients

S.No.	Drug+ Excipient+ WFI	Ratio of drug and excipients
1.	Drug + WFI	1:1
2.	Drug+ Span 60+ WFI	1:1
3.	Drug+ Cholesterol+ WFI	1:1
4.	All excipients+ WFI	1:1
5.	Drug+ all excipients+ WFI	1:1

5. Preparation and Purification of Niosomes

5.1 Preparation of niosomes by ether injection method

Niosomes were prepared using Ether injection method¹⁴. Drug, surfactant and cholesterol were used in the ratios as indicated in Table 2. Mixture of surfactant (Span 60) and cholesterol dissolved in a mixture of diethyl ether and chloroform was slowly introduced through 20-gauge needle into warm aqueous solution of Punicalagin maintained at 20°C. Evaporation of ether leads to formation of single layered vesicles.

5.2 Purification of niosomes by centrifugation method

The prepared niosomes were separated from untrapped drug by centrifugation method¹⁵. Niosome suspension was centrifuged at 40,000 rpm (1,37,088 G) for 30 minutes. Clear supernatant was removed by pipetting and remaining sediment i.e. niosomes containing only entrapped drug were obtained for further evaluation.

Table 2: Composition of niosomal formulations of Punicalagin

S.No.	Formulation	Surfactant used	Surfactant: Cholesterol: Drug
1.	F1	Span 60	1:1:1
2.	F2	Span 60	2:1:1
3.	F3	Span 60	3:1:1
4.	F4	Span 60	4:1:1
5.	F5	Span 60	5:1:1
6.	F6	Span 60	6:1:1
7.	F7	Span 60	7:1:1
8.	F8	Span 60	8:1:1
9.	F9	Span 60	9:1:1
10.	F10	Span 60	10:1:1

6. Evaluation of niosomes

6.1 Optical microscopy The morphology of prepared niosomes was done by optical microscopy. The photomicrographs of the preparations were obtained with the help of photomicroscope at 1000 X.

6.2 Micromeritics studies For the micromeritics study, the niosomes were probe sonicated and then the vesicle size and size distribution profile were determined using dynamic light scattering (DLS) method (Malvern Instruments Ltd, Worcestershire, UK). Particle size analysis was done by using particle size analyser.

6.3 Entrapment efficiency Ultracentrifugation technique was adopted for the removal of the untrapped drug. The centrifuged niosomes were lysed with 0.1% v/v Triton X-100 and left for 1 hour and filtered to separate drug from vesicles. Thereafter dilutions were made and solution was analyzed spectrophotometrically employing ultraviolet-visible spectrophotometer. The entrapment efficiency was calculated by using following equation:

$$\text{Entrapment efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100$$

6.4 Morphology and structure of vesicles The prepared and optimized formulations were characterized for morphology (i.e. shape and lamellarity) employing Hitachi TEM analyzer.

6.5 Zeta potential analysis Zeta potential of niosomal preparation is related to the stability of niosomes. Zeta potential indicates the degree of repulsion between adjacent similarly charged particles for small molecules and particles, a high value of zeta potential confers stability, i.e. the solution or dispersion will resist aggregation. Zeta potential for niosomal formulation was performed using Zeta sizer Beckman coulter instrument.

6.6 Stability studies of Punicalagin containing niosomes Physical stability studies were carried out to investigate the leaching of drug from niosomes (in a suspension form) during storage. The optimized niosomal formulation (F7) composed of Span 60 and cholesterol in 7:1 molar ratio were sealed in 20 ml glass vials and stored at refrigeration temperature ($5 \pm 3^\circ\text{C}$) for a period of 2 months. Samples were withdrawn at definite time interval of 15 days and the percentage entrapment of the drug was determined as described previously after separation from untrapped drug.

RESULTS

1. Preformulation studies

1.1 Organoleptic properties



Figure 2: Punicalagin

1.2 TLC Identification test Purple pink colour was appeared and R_f value was observed 0.53 (whereas reported value of R_f is 0.58 in ICMR) as shown in figure 3.



Figure 3: TLC showing detection of Punicalagin

1.3 Chemical identification test On addition of ferric chloride to small amount of drug, a blue colour was appeared that indicated the presence of hydrolysable tannin.

1.4 Characterisation of Punicalagin

1.4.1 UV spectroscopy the absorbance maximum (λ_{max}) of Punicalagin in PBS pH7.4 was determined which are shown in figure 4. The λ_{max} of Punicalagin in phosphate buffer pH 7.4 was found to be 253.6 nm.

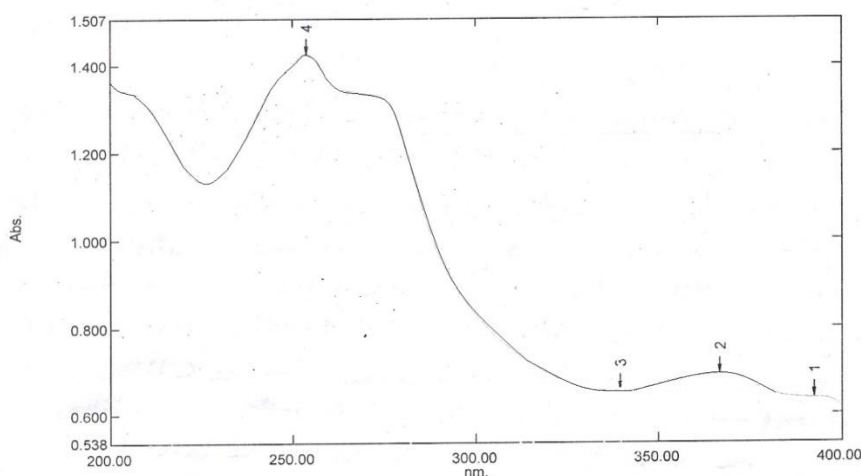


Figure 4: UV absorption spectra of Punicalagin in phosphate buffer pH 7.4

1.4.2 IR spectroscopy

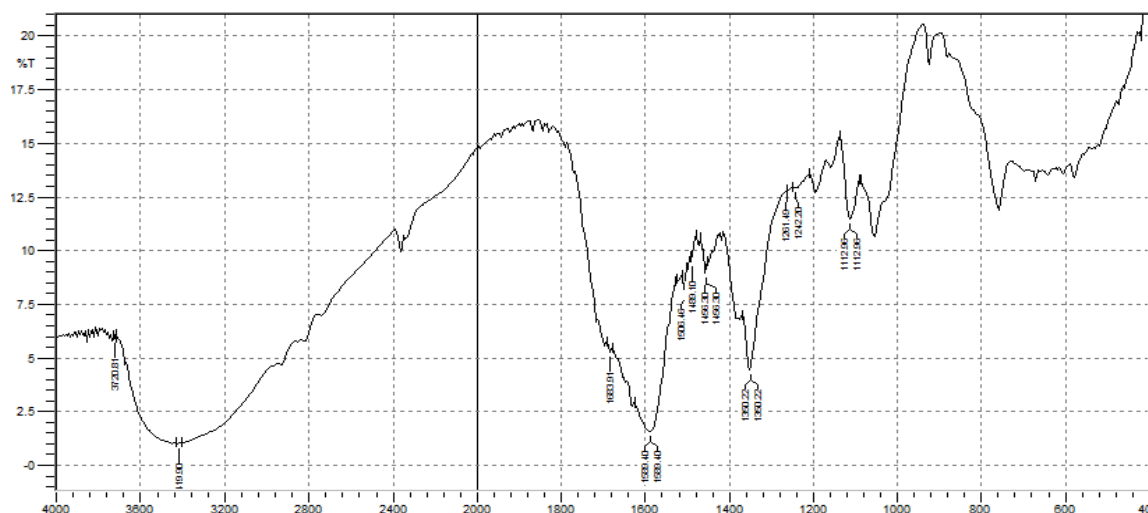


Figure 5: IR spectra of Punicalagin

2. Standard UV plot of Punicalagin in pH 7.4 Phosphate buffer Standard plot of punicalagin was found to be linear with $R^2 = 0.9978$; showing proportional increase in the absorbance with concentration which is shown in figure 6 and data is represented in table 5.

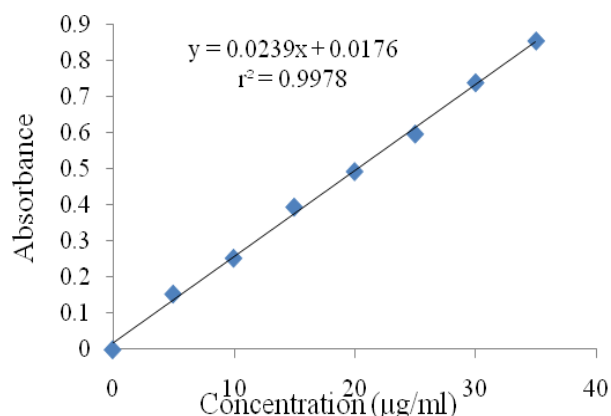


Figure 6: Standard plot data of Punicalagin in PBS pH 7.4
Absorbance range of Punicalagin was found to be 0.154 to 0.855 nm.

3. Compatibility Study

3.1 Physical characterisation of drug excipient mixture

The compatibility study of Punicalagin with various excipients showed that there was no colour change as well as there was no microbial growth occurred in the solution as well as in powder form as shown in table 6. This showed that drug is physically compatible with the excipients.

3.2 Chemical characterisation of drug excipient mixture

For chemical compatibility studies, different drug-excipient mixtures were analyzed by UV spectroscopy that showed that there is no change in λ_{max} i.e. 253.6 nm which are shown in figure 7.

4. Evaluation of niosomes

4.1 Optical microscopy The photomicrographs of prepared niosomes are shown below in figure 8. Single

unilamellar niosomes were observed at 1000X by optical microscopy. The niosomes were found to be spherical in shape.

4.2 Micromeritics studies Particle size analysis and size distribution of the sonicated niosomes was measured by Dynamic Light Scattering phenomenon using a Malvern Zeta Sizer instrument. The average particle size and PDI values are given in table 7. The average particle sizes of the sonicated niosomes were found to be in nanometer range which lies in range of 240.6nm – 625.2nm. The PDI value which characterizes the uniformity of vesicles in suspension that lies in range of 0.226 - 0.548.

4. Evaluation of niosomes

4.1 Optical microscopy The photomicrographs of prepared niosomes are shown below in figure 8. Single unilamellar niosomes were observed at 1000X by optical microscopy. The niosomes were found to be spherical in shape.

4.3 Entrapment efficiency According to the entrapment efficiency calculated, it was found that F5, F6 and F7 has greater entrapment efficiency as compared to other formulations whereas F7 has maximum entrapment efficiency of 65.93% when compared with F5 and F6 as shown in table 8 and figure 10. Thus F7 was selected for formulating gel.

4.4 Morphology and structure of vesicles The transmission electron micrographs of unilamellar niosomes of optimised formulation (F7) composed of span 60 and cholesterol in 7:1 molar ratio are shown in figure 11 (a) and (b). They reveal the presence of well identified and nearly perfect spheres. In figure 11 (a), the size of niosomes lies in range of 37.6 nm to 43.6 nm whereas in 11(b), size of niosomes lies in range of 60.3nm to 358 nm.

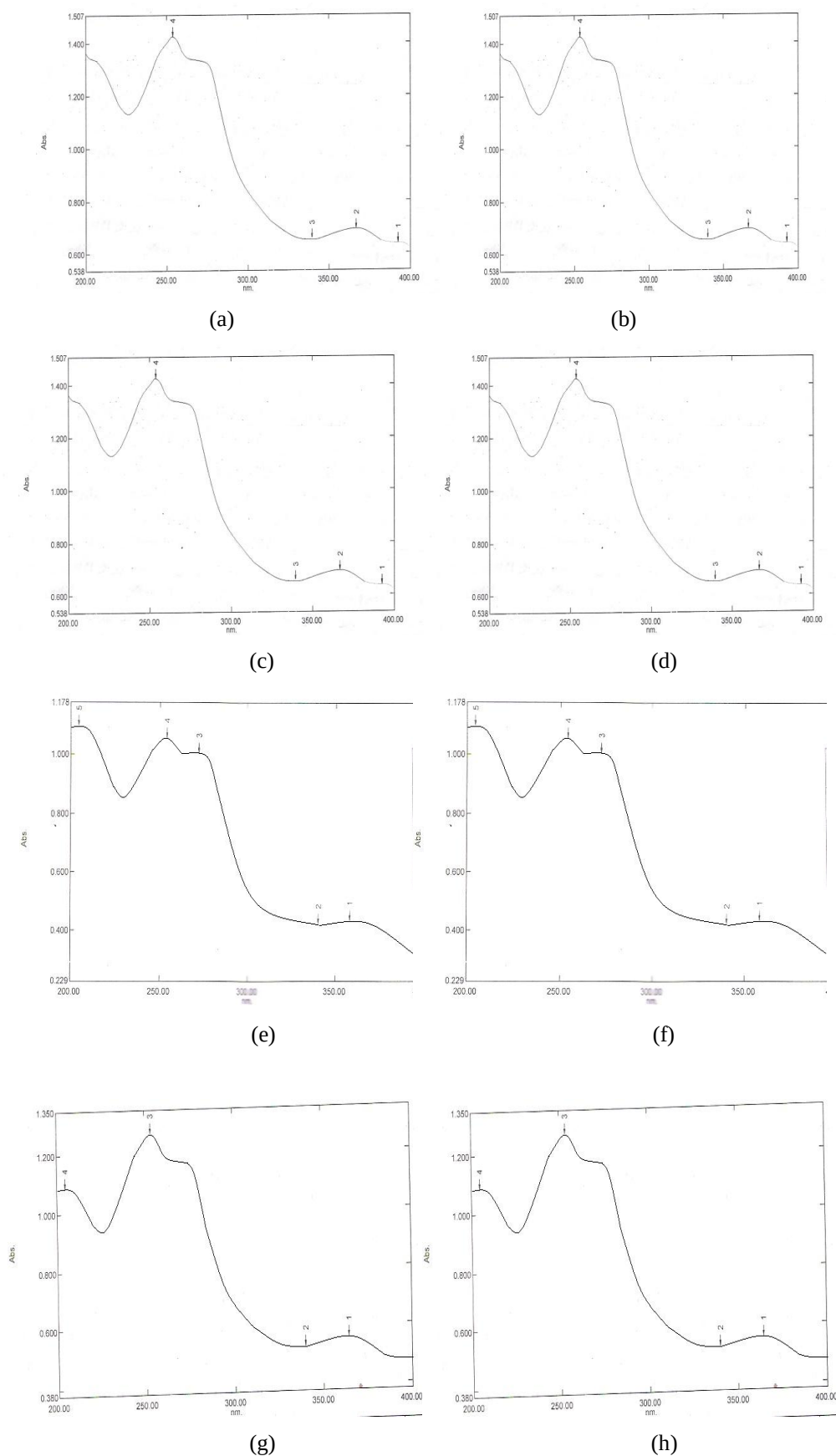


Figure 7: Compatibility study data of Punicalagin with different excipients done and by UV spectroscopy (a) Drug at 0th day (b) Drug at 28th day (c) Drug+ Span60 at 0th day (d) Drug+ Span60 at 28th day (e) Drug+ Cholesterol at 0th day (f) Drug+ Cholesterol at 28th day (g) Drug+ Mixture of all excipients at 0th day (h) Drug+ Mixture of all excipients at 28th day.

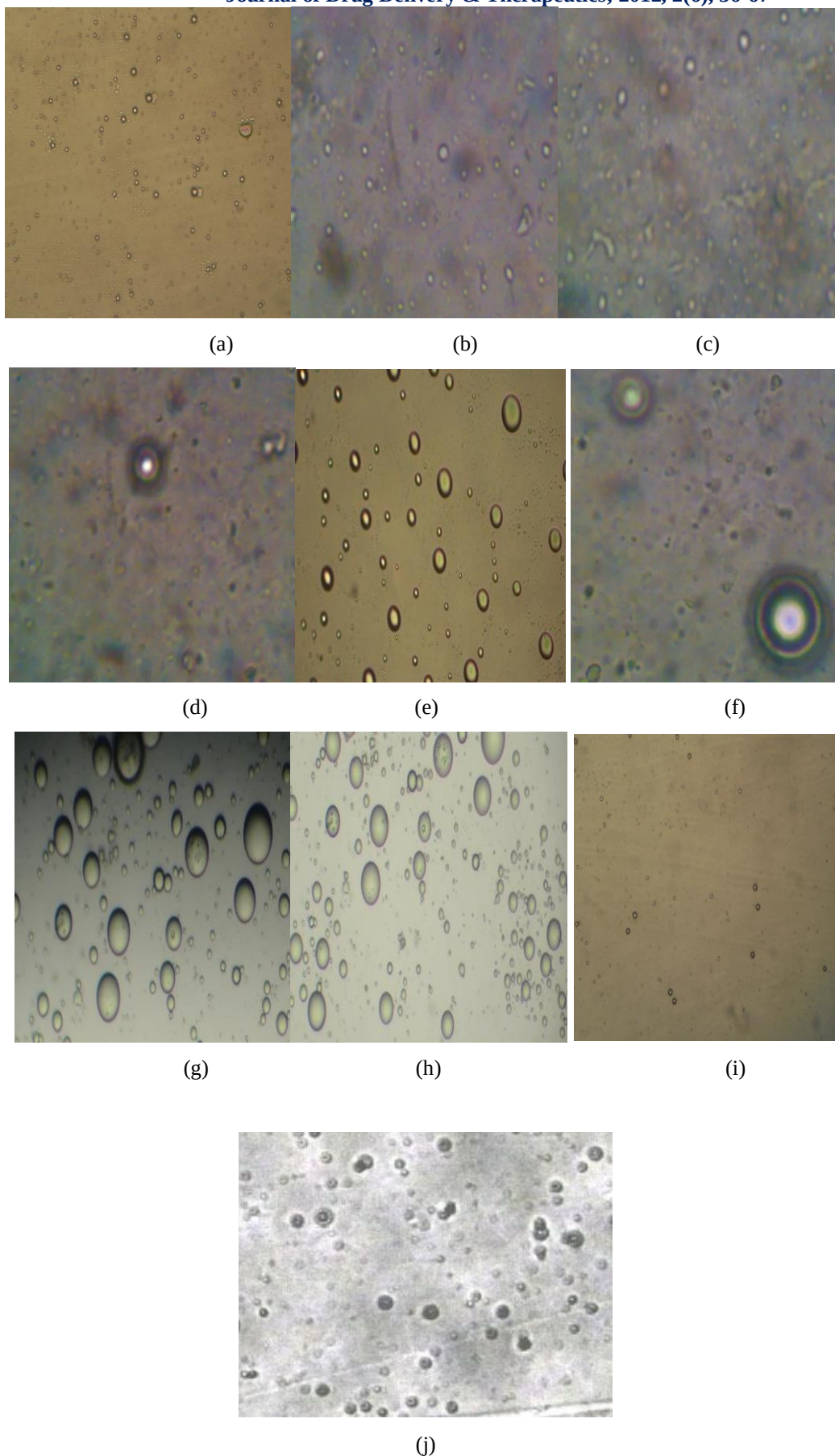


Figure 8: Photomicrographs of different formulations at 1000 X (a) F1 (b) F2 (c) F3 (d) F4 (e) F5 (f) F6 (g) F7 (h) F8 (i) F9 (j) F10

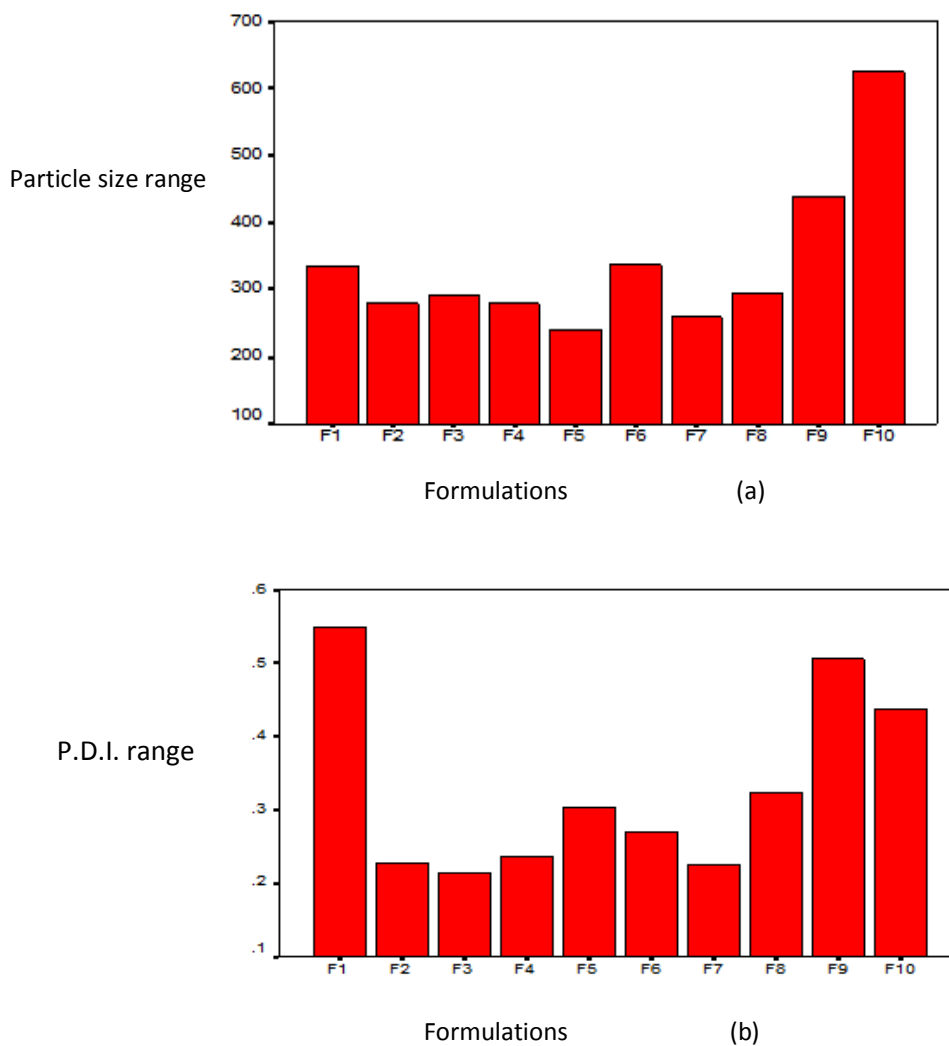


Figure 9: Graphs showing (a) Particle size (b) PDI range for all niosomal formulations

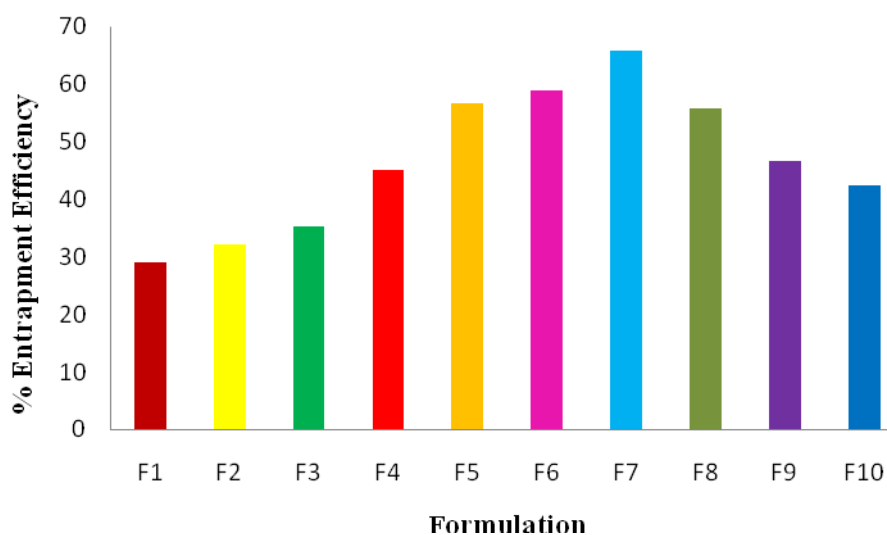


Figure 10: Comparison of entrapment efficiency of all formulations

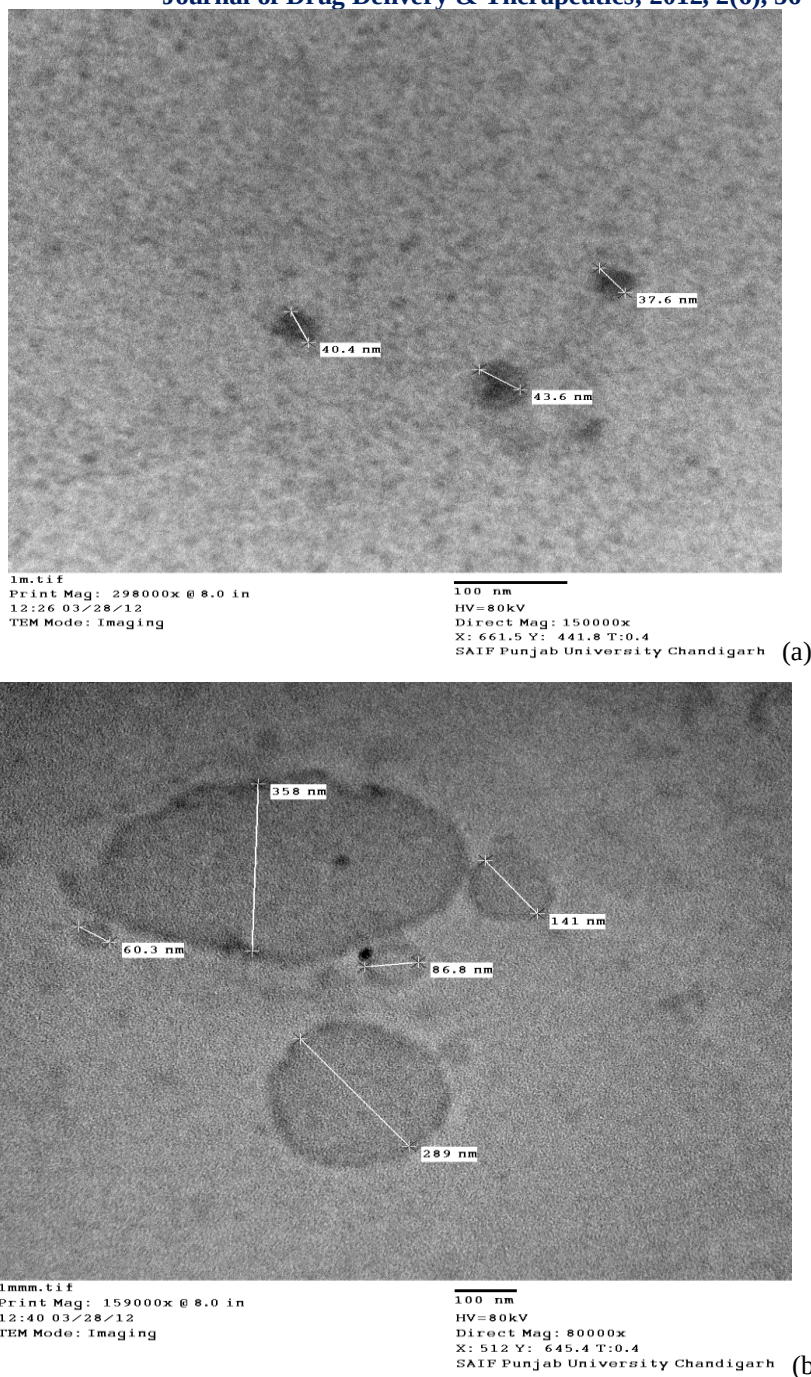


Figure 11: TEM micrographs of optimized niosomes (F7)

4.5 Zeta potential analysis Zeta potential of the optimized formulation (F7) as shown in figure 12 which was found to be -27.2 mV depicts the stability of niosomal formulation.

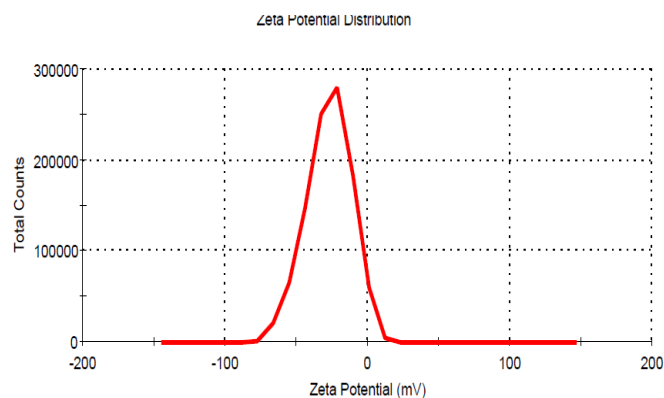


Figure 12: Zeta potential analysis for optimized niosomes (F7)

4.6 Stability studies: Drug leakage study Physical stability studies were carried out to investigate the leaching of drug from the niosomes of optimized formulation (F7) during storage at refrigerator temperature. The percentage of entrapment efficiency of Punicalagin after a period of 2 months got reduced to 25% as shown in table 9.

DISCUSSION

Punicalagin which was extracted, isolated and purified from the peels of the *Punica granatum*, was identified by carrying out chemical identification test, thin layer chromatography (TLC), UV spectrophotometry and FTIR. Compatibility studies of Punicalagin with the excipients used during the work were conducted for 28 days and observed that drug is compatible with the excipients physically and chemically. On the basis of the findings, it

can be stated that the formulation F7 is the best formulation from all the other formulations which were prepared by different compositions of Span 60. The F7 formulation has greater entrapment efficiency as compared to other formulations, as F7 showed 65.93% whereas F5 and F6 showed 56.68% and 59% respectively. Also, F7 has particle size of lowest value as compared to others i.e. 259.5 nm as shown in figure 13. TEM, zeta potential and stability studies were conducted for the best formulation F7. TEM studies showed that niosomes in F7 are nearly perfectly spheres whereas zeta potential study showed the stability of formulation F7. The future perspective of this study is that further experiments can be carried out by incorporating this drug into different vesicular system and their comparison or by using different non-ionic surfactants in different ratios or by changing the amount of cholesterol

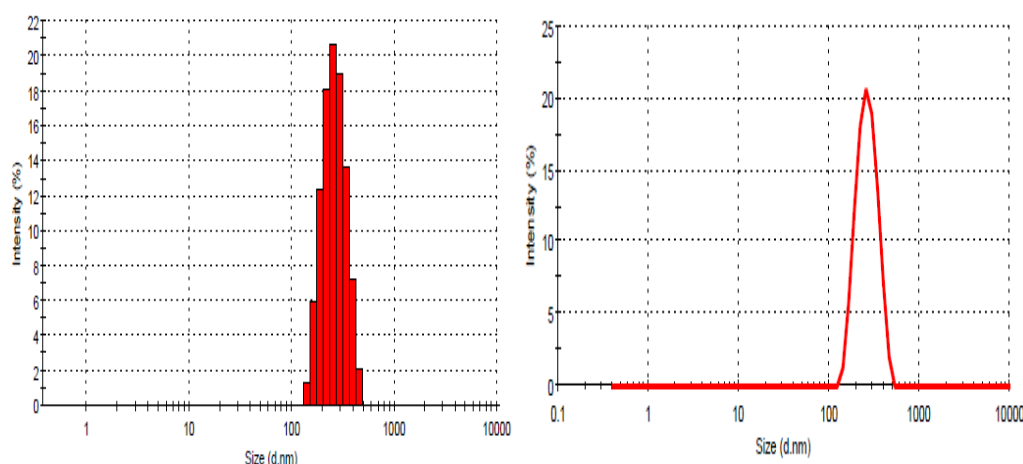


Figure 13: Particle size distribution and Particle size analysis of F7

ACKNOWLEDGEMENTS

I wish to acknowledge and give special thanks to the Assistant Professor Dr. Umarnjan Lal and Ms. Purnima Verma for their help and guidance during the research.

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Table 3: Preformulation parameters and their observation

Preformulation property	Observations
Physical state	Solid as powder
Colour	Yellowish brown (shown in figure 2)
Odour	Pleasant smell

Table 4: IR spectra showing the peaks of functional groups present in Punicalagin

Wave number cm^{-1}		The peak corresponds to
Observed value	*Reference value	
1589	1560	C-O
3419 and 3720	3400-3550	O-H
1683	1680	C=O conjugated with aliphatic C=C
1261-1242	1270 -1230	-O- (Aralkyl ether)
1112 and 1350	1100 -1300	COOR
1456-1589	1450 -1600	Aromatic ring

*Reported peaks in literature³

Table 5: Absorbance data for calibration curve of Punicalagin

Concentration ($\mu\text{g/ml}$)	Absorbance*			Average	$\pm\text{SD}$	% RSD
	Abs 1	Abs 2	Abs 3			
0	0	0	0	0	0	0
5	0.154	0.156	0.153	0.15433	0.00152	0.9897
10	0.253	0.255	0.253	0.25366	0.00115	0.4552
15	0.395	0.396	0.394	0.395	0.001	0.2531
20	0.496	0.493	0.492	0.49366	0.00208	0.4216
25	0.593	0.599	0.601	0.59766	0.00416	0.6965
30	0.736	0.743	0.74	0.73966	0.00351	0.4747
35	0.855	0.857	0.854	0.85533	0.00152	0.1785

*Each value is average of three determination, SD = Standard Deviation, %RSD = Percent Relative Standard Deviation

Table 6: Compatibility study data of Punicalagin with different excipients done at 0th, 7th, 14th and 28th day by physical observation

Physical compatibility study of Punicalagin with different excipients											
Sample (Drug+Excipients)	Ratio	Appearance at 0 th day	Observation at different day and condition								
			A (7 th day); B (14 th day); C (28 th day)								
			5°C $\pm$ 3°C			25°C $\pm$ 2°C			40°C $\pm$ 2°C (75% $\pm$ 5%RH)		
			A	B	C	A	B	C	A	B	C
Drug + WFI	1:1	Yellow colour	√	√	√	√	√	√	√	√	√
Span 60	1	White colour	√	√	√	√	√	√	√	√	√
Cholesterol	1	White colour	√	√	√	√	√	√	√	√	√
Carbopol 934	1	White colour	√	√	√	√	√	√	√	√	√
Drug + Span 60 + WFI	1:1:1	Slight yellow colour	√	√	√	√	√	√	√	√	√
Drug + Cholesterol + WFI	1:1:1	Slight yellow colour	√	√	√	√	√	√	√	√	√
Drug + Carbopol 934 + WFI	1:1:1	Slight yellow colour	√	√	√	√	√	√	√	√	√
Drug+ Span 60 + Cholesterol+ Carbopol 934	11:1:1:1	Slight yellow colour	√	√	√	√	√	√	√	√	√

√ Same as original

Table 7: Values of Average particle size and Polydispersity index (P.D.I) for different formulations

Formulation	Particle size range (in nm)	P.D.I. range
F1	334.8	0.548
F2	279.6	0.227
F3	291.7	0.214
F4	278.7	0.236
F5	240.6	0.304
F6	336.8	0.270
F7	259.5	0.226
F8	294.1	0.324
F9	440.7	0.505
F10	625.2	0.438

Table 8: Entrapment efficiency of niosomal formulations

S. No.	Formulation name	% drug entrapment*
1	F1	28.97 ± 0.06
2	F2	32.18 ± 0.06
3	F3	35.29 ± 0.06
4	F4	44.96 ± 0.04
5	F5	56.68 ± 0.12
6	F6	59.00 ± 0.06
7	F7	65.93 ± 0.06
8	F8	55.70 ± 0.08
9	F9	46.73 ± 0.12
10	F10	42.50 ± 0.13

**Average of three determinants ± S.D.*

Table 9: Stability data of niosomal formulation F7

Days	5±3°C
0 th day	65.66 ± 0.14
15 th day	62.66 ± 0.03
30 th day	60.16 ± 0.04
45 th day	52.20 ± 0.11
60 st day	49.63 ± 0.10

**Average of three determinations ± S.D*