



Development and Characterization of *Terminalia arjuna* Phospholipid Complex and Its Tablet Formulation by Qbd Approach

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Abstract: Phytosomes are a newly introduced novel drug delivery system and novel botanical formulation to induce lipophilic molecular complexes to enhance absorption and bioavailability of phytoconstituents. *Terminalia arjuna* phospholipid complex and its tablet formulations targeted for cardiovascular systems was prepared. Our study aims is for improving the cardioprotective activity by formulating *Terminalia arjuna* phospholipid complex tablet by using solvent evaporation method. and characterized by various parameters like solubility studies, particle size determination, infrared absorption (FTIR), Scanning electron microscopy (SEM), entrapment efficiency etc. as well as by applying QbD approach various general characteristics such as entrapment efficiency etc were also done. A QbD- based approach using a Box-Behnken design was done to obtain a response surface design expert software 9.0.5 to systematically study the combined influence of the formulation and process variables such as the phospholipids-drug ratio(X1, w/w), the reaction temperature(X2, °C), and the reaction time (X3, hrs) on a critical quality attributes (CQAs) of the product i.e., the entrapment efficiency. Using this design, the experimental trials were carried out at all 15 possible combinations. The preliminary investigation of the influence of factors revealed that all the tested variables, i.e., the phospholipids to drug ratio, the reaction temperature and the reaction time had a significant influence on the entrapment efficiency of the prepared phytosomes. The study revealed that the entrapment efficiency of *Terminalia arjuna* Phytosomes was found to be 83.0-97.9 %w/w.

Keywords: QbD approach, *Terminalia arjuna* extract, Cardioprotective, *Terminalia arjuna* phytosome complex, Tablet formulation.

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I. INTRODUCTION

Our study was aimed at improving the drug release characteristics of *Terminalia arjuna* bark extract by formulating *Terminalia arjunaphytosomal* tablets targeted for cardiac problems by implementing a QbD approach. Cardiovascular diseases are the number one cause of death worldwide. The *arjuna* bark has been used in the treatment of cardiovascular disorders by ayurvedic physicians.¹ Phytosomes are more bioavailable as compared to herbal extract, it helps to enhance its capacity to cross the lipid rich biomembranes and finally reaches to the systemic circulation.² The lipophilic substances that are used to make phytoconstituents lipid compatible are phospholipids from soya, mainly phosphatidylcholine. Phosphatidylcholine is the main molecular building block of cell membrane, is miscible both in water as well as in oil and is well absorbed orally. Phospholipids are small lipid molecules in which the glycerol is bonded only to two fatty acids, instead of three as in triglycerides, with the remaining site occupied by a phosphate group.³ Most of the biologically active constituents of plants are polar or water-soluble. However, water-soluble phytoconstituents like flavonoids, tannins, glycosidal aglycones etc. are poorly absorbed either due to their large molecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability.⁴ Phytosomes is a newly introduced patented technology developed to incorporate the standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, which improves their absorption and bioavailability.^{5,6,7} Phytosome technology has been effectively used to enhance the bioavailability of many popular herbal extracts including milk thistle, ginkgo biloba, grape seed, green tea, hawthorn, ginseng etc and can be utilized for various therapeutic uses or for administration of dietary supplements. The flavonoid and terpenoids components of these herbal extracts lend themselves quite well for the direct binding to phosphatidylcholine. Lipid insoluble herbal extracts can be redesigned into lipid compatible therapeutic candidate by chemically assimilating herbal extracts into phospholipids in specific ratio.⁸ The medicinal importance of *Terminalia arjuna* (Family: Combretaceae) is well documented in Indian pharmacopeia, according to this its stem, bark and leaves possess glycosides, large quantities of flavonoids, tannins and minerals. *Arjunais* a deciduous tree of height above 60 feet, found abundantly in the Indian subcontinent. It is a medicinal plant, and has been used for ayurvedic medicinal preparations for over a thousand years. The plain part of the tree used for medicinal purposes is the bark of the stem. The use of the bark of *arjuna* in the management of hypercholesterolemia has also been widely reported.^{9,10,11} It has been reported that the bark may have blood vessel relaxing properties. The bark has shown promise in the treatment of angina, a

condition in which blood vessels in the heart cannot carry adequate oxygen to the heart muscle.^{12,13} Many useful phytoconstituents have been isolated from *Terminalia arjuna* which included triterpenoids for cardiovascular properties, tannins and flavonoids for its anticancer, antimicrobial properties and so on.¹⁴

2. MATERIALS AND METHODS

2.1 Materials

Terminalia arjuna extract was obtained as a gift sample from Sunpurepvt Ltd, Mumbai (India), *Terminalia arjunawas* identified and authenticated by Late BabruvanVithalrao Kale (Manjara) Ayurved Medical College and Hospital, Latur. 50 % Soya phosphatidylcholine was purchased from LipidomeLifesciences, Kheda (Gujarat, India). All the other chemicals and reagents used in this study were of analytical grade.

2.2 Determination of λ_{max}

Concentration 100 $\mu\text{g/ml}$ *Terminalia arjuna* extract dissolved in distilled water scanned over a wavelength range of 200-400 nm.

2.3 Preparation of standard calibration curve of *Terminalia arjuna* extract

About 10 mg of *Terminalia arjuna* bark extract accurately weighted by electronic balance and dissolved in 10 ml of distilled water in 100 ml volumetric flask. Content of flask was kept on Sonicator for well mixing of solution for 10 min and transferred in 10 ml volumetric flask. 1000 $\mu\text{g/ml}$ solution was prepared. From prepared standard stock solution 1 ml solution was pipette out and make its volume up to 10 ml by water in 10 ml volumetric flask. 100 $\mu\text{g/ml}$ solution was prepared from this solution pipette out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml and 1.2 ml of solution and make up to 10 ml leads to 2 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$, 6 $\mu\text{g/ml}$, 8 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ and 12 $\mu\text{g/ml}$ concentration solution. This solution was estimated by UV spectrophotometer by using water as blank at 265 nm.¹⁵

2.4 HPTLC analysis of *Terminalia arjuna*

The estimation of quantity for arjunolic acid in *Terminalia arjuna* bark extract was performed using high performance thin layer chromatography technique. The precoated silica gel aluminum plate 60F254 was used for the spotting of different extracts using 10 μl spot volume using a CAMAG Linomat syringe. The CAMAG twin through glass chamber pre-saturated with mobile phase chloroform: methanol (9:1) for 5min at (23 \pm 2 $^{\circ}\text{C}$) and 55 \pm 5 % RH was used for the development of TLC plate [Table I].¹⁶

Table No. 1: Optimized condition for HPTLC analysis of *Terminalia arjuna*

Parameters	Description
Instrument	CAMAG HPTLC
Stationary Phase	Silica gel 60 F254 HPTLC Precoated plates
Sample Applicator	CAMAG LINOMAT V
Application Volume	10 μL
Syringe	CAMAG Linomat Syringe
Mobile Phase	Chloroform: methanol (9:1, v/v)

Development Mode	CAMAG Twin Trough Chamber
Chamber Saturation Time	5 min
Scanning Wavelength	200 nm, 254 nm, 265 nm
Software	WinCATS Version 1.4.6
Lamp	D2 & W
Photo Documentation	CAMAG TLC Visualizer

2.5 Preparation of Terminalia arjuna Phytosomes Complex (Tpc)

Terminalia arjuna phytosomes complex was prepared by using a solvent evaporation method.

2.6 Solvent evaporation method

The specific amount of *Terminalia arjuna* and soya phosphatidyl choline were taken into a 100 ml round bottom flask and refluxed with 10 ml of dichloromethane and 10 ml of n-Hexane at a temperature 40 °C for 1.5 hrs. The mixture is concentrated to 5-10 ml to obtain the precipitate which was filtered and collected. The dried precipitate phytosome complex was placed in an amber colored glass bottle and stored at room temperature. *Terminalia arjuna Phytosomes*

were optimized based on effective concentration of drug and phospholipids ratio.¹⁷

2.7 Design of Experiment

A QbD-based approach using a central composite design to obtain a response surface design expert software 9.0.5 was employed to systematically study the combined influence of the formulation and process variables such as the phospholipids-drugs ratio (X1 w/w), the reaction temperature (X2, °C), and the reaction time (X3, hrs) on a critical quality attributes (CQAs) of the product i.e., the entrapment efficiency. Using this design, the experimental trials were carried out at all 15 possible combinations. A statistical model incorporating interactive and polynomial terms was used to evaluate the response employing the equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3$$

Where Y is the dependent variables, b₀ is the intercept representing arithmetic mean response of the 15 runs, and b₁ to b₉ is the estimated coefficient for the factor (X_i, i=1,2,3). X₁, X₂ and X₃ are the coded levels of independent variables. The interaction terms (X₁X₂, X₂X₃, and X₁X₃) showed how the response changes when all three factors

were simultaneously changed. The polynomial terms (X₁², X₂², and X₃²) were included to investigate nonlinearity. The level values of the central composite design batches are shown in Tables 2 and the resulting entrapment efficiencies are shown in Tables 4.

Table No.2: Coded level and real value for each factor under study.

Variables	Levels				
	-1.68	-1	0	1	1.68
X1 (w/w)	0.5	1	1.75	2.5	3
X2 (°C)	35	40	45	50	55
X3 (Hrs)	1	1.5	2	2.5	3

2.8 Characterization of Terminalia arjuna Phytosomes Complex (Tpc)

The consistency of Phospholipids complex depends upon various factors like drug to phospholipids ratio, reaction temperature and reaction time (in solvent evaporation method). All these parameters are optimized statistically through quality by design (QbD) approach.

2.9 Microscopic view

Optical microscopy was used for the characterization of *Terminalia arjuna* phospholipids complex. The complex was suspended in acetone and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 10×10.⁹

2.10 Compatibility studies

The formation of the complex can be confirmed by FTIR spectroscopy, comparing the spectrum of the complex with the spectrum of the individual components and their

mechanical mixtures. The spectral scanning was done in the range between 4000-400 cm⁻¹. The scans were evaluated for presence of principal peaks of drug, shifting and masking of drug peaks, and appearance of new peaks due to excipient interaction. This spectral analysis was employed to check the compatibility of drugs with the excipients used.¹⁸

2.11 Determination of Entrapment efficiency

Entrapment efficiency (EE) was measured using UV- visible spectrophotometer (UV-1601, Shimadzu). A weighed quantity of phyto-phospholipid complex TAP equivalent to 10 mg of extract was added to 50 ml methanol in a 100 ml beaker. The contents were stirred on a magnetic stirrer for 4 h and then allowed to stand for one hour. Clear liquid was decanted and centrifuged at 12000 rpm for 45 min.¹⁹ After centrifugation the supernatant was filtered through 0.45 μ whatman filter paper and after suitable dilution absorbance was measured in UV at 265nm; the concentration of drug was measured. All measurements were performed in triplicate. The EE (%) was calculated using the following formula:²²

$$\text{Entrapment efficiency (\%)} = \frac{T-S}{T} \times 100$$

Where,

T- Weight of total drug,

S- Weight of free drug.

2.12 Stability studies

The stability of phytosomes was carried out as per ICH guidelines. The optimized formulation of phytosomes was placed at room temperature in a humidity chamber for a period of 3 months and studied for drug entrapment.¹⁵

2.13 Differential scanning calorimetry (DSC)

Terminalia arjuna Phytosomes complex was placed in the aluminum crimp cell and heated at 10 °C/min from 0-400 °C in the atmosphere of nitrogen [SETARAM Instrumentation, France, model Themys one plus]. Peak transition onset temperatures were recorded using an analyzer.²⁰

2.14 Scanning electron microscopy (SEM)

Scanning electron microscopy has been used to determine

particle size distribution and surface morphology of the complexes. Samples were studied and the particle size of the formulation was viewed and photographed using scanning electron microscope [Field Electron & Ion Company, USA, model Apreo Lo Vac].²⁰

2.15 Preparation of *Terminalia arjuna* Phytosomal Tablet

Terminalia arjuna phytosomal tablets were prepared by direct compression technique. All the ingredients were thoroughly mixed. Then the powder was passed through sieve mesh 40 # to get the uniform size of the particle. Magnesium stearate was finally added as lubricant respectively. The powders were compressed using the tablet compression machine.²¹

Ingredient(mg)	F1	F2	F3	F4	F5	F6
<i>Terminalia arjuna</i> phytosome	200	200	200	200	200	200
Lactose	60	60	60	60	60	60
HPMC	18	-	18	-	16	-
Microcrystalline cellulose	-	18	-	16	-	16
Croscarmellose sodium	12	12	12	-	-	-
Starch	-	-	-	14	14	14
Talc	6	6	6	6	6	6
Magnesium stearate	4	4	4	4	4	4

2.16 Evaluation of *Terminalia arjuna* Phytosomal Tablets

1) Hardness

The hardness of a tablet is associated with the resistance of the solid specimen towards fracturing and attrition. The hardness of tablets can be determined by using Monsanto hardness tester and measured in terms of kg/cm².²²

2) Thickness

The thickness of individual tablets is measured by using vernier caliper which gives the accurate measurement of

thickness. It provides information on variation of thickness between tablets. Generally the unit for thickness measurement is mm. The limit of the thickness deviation of each tablet is ± 5%.²³

3) Friability

Ten tablets were weighed and placed in a Roche friabilator and the equipment was rotated at 25 rpm for 4min. The tablets were taken out, de-dusted, and reweighed. The percentage friability of the tablets was measured as per the following formula.²⁴

$$\text{Percentage friability} = \frac{\text{Initial wt. of tablets} - \text{Final wt. of tablets}}{\text{Initial wt. of tablets}} \times 100$$

4) Uniformity of weight

Twenty tablets were taken and their weight was determined individually and collectively using single pan electronic balance. The average weight of the tablets was determined from collective weight. From the individual tablet weight, the range and percentage deviation was calculated not more than 2 tablets should deviate from the average weight of tablet and maximum percentage of deviation allowed.²⁵

5) In-vitro dissolution studies:

In-vitro dissolution studies for all the prepared *Terminalia*

arjuna phytosomal tablets were carried out using USP paddle dissolution apparatus (model no. Electrolab 152) at 100 rpm in 900 ml of phosphate buffer pH 6.8 as dissolution media maintained at 37±0.5 °C. 5 ml of the solution was withdrawn from the dissolution apparatus at a specific time intervals, and the samples were replaced with fresh dissolution medium. The samples were filtered through Whatman filter paper and from the filtrate 1ml was taken and diluted to 10 ml. Absorbance of these solutions was measured at 265nm using UV spectrophotometer (Shimadzu (UV-1800), Japan).²⁶

6) Kinetic analysis of dissolution data

The mathematical models were used to evaluate the mechanism

of drug release from the tablets and kinetics. Based on the correlation coefficient (r) value in various models, the model that best fits the release data was selected. The model giving the high ' r ' value was considered as the best fit of the release data. Goodness of fit test is the criterion for selecting the best fit model. The mathematical models used were.¹⁸

- i. Zero-order kinetic model
- ii. First- order kinetic model
- iii. Hixson- Crowell model
- iv. Korsmeyer-peppas model

The statistical analysis was carried out by using the software GraphPad Prism (San Diego, CA).

7) Stability study

Stability studies were carried out for 3 months on the optimized formulation (F4) of *Terminalia arjuna* phytosomal tablets. Sufficient number of tablet formulations were packed in a stability container and kept in a stability chamber at Temperature 45 °C and RH 75 %. Samples were taken for 90

days for drug content estimation; also the appearance, weight, pH, content uniformity and in-vitro disintegration studies were performed to determine the drug release profile.²⁷

3. STATISTICAL ANALYSIS

The statistical analysis was carried out by using the software GraphPad Prism (San Diego, CA).mathematical models were used to evaluate the mechanism of drug release from the tablets and kinetics. Based on the correlation coefficient (r) value in various models, best fits model was selected.

4. RESULTS

4.1 Determination of λ_{max}

The concentration 100 $\mu\text{g/ml}$ *Terminalia arjuna* extract in distilled water was found to be 265 nm,graph was shown in Fig. No. 1.

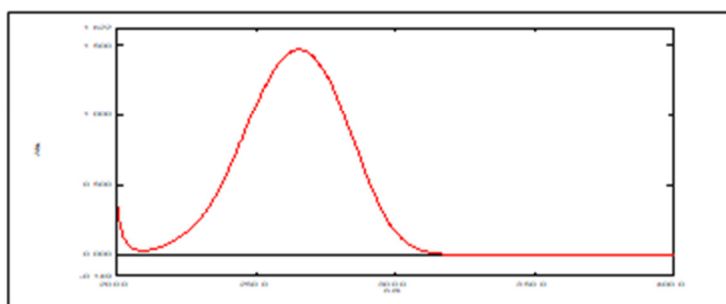


Fig No.1: UV spectra of *Terminalia arjuna*

4.2 Preparation of standard calibration curve of *Terminalia arjuna* extract

The UV absorbance of *Terminalia arjuna* extract standard solution in the range of 2-12 $\mu\text{g/ml}$ distilled water showed linearity at λ_{max} 265 nm. The linearity was plotted for absorbance against concentration with R^2 value 0.997 for distilled water was shown in Fig. No. 2.

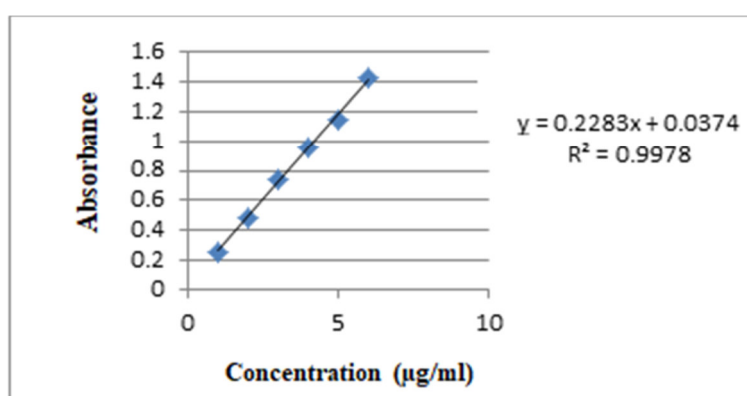


Fig No.2: Calibration curve of *Terminalia arjuna*

4.3 HPTLC analysis of *Terminalia arjuna*

The estimation of quantity of arjunolic acid in *Terminalia arjuna* bark extract was performed using high performance thin layer chromatography technique. The hydro-alcoholic

extract of *Terminalia arjuna* was developed in the mobile phase chloroform: methanol (9:1v/v). TLC plate was visualized under white light. The extract showed the presence of arjunolic acid, shown in Fig. No. 3.

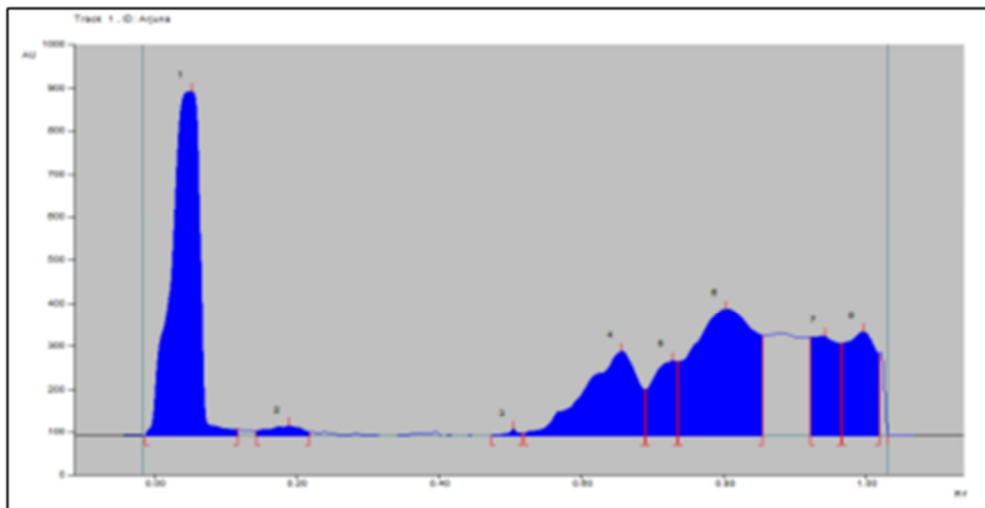


Fig No. 3: HPTLC Chromatogram of Terminalia arjuna 10 µL bark extract (265 nm)

4.4 Preparation of Terminalia arjuna Phytosomes Complex

kept at lower level i.e. phospholipids-drug ratio 1gm and the counter plot showing combined effect of reaction temperature and reaction time kept at lower level of phospholipids-drug ratio 1 gm.

Design of Experiment: The response surface diagram showing combined effect of reaction temperature and reaction time

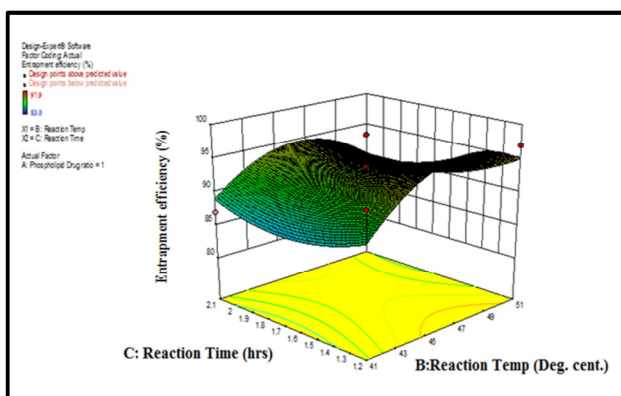


Fig No.4: Response surface diagram showing combined effect of reaction temperature & reaction time kept at lower level i.e. phospholipids- drug ratio 1 gm.

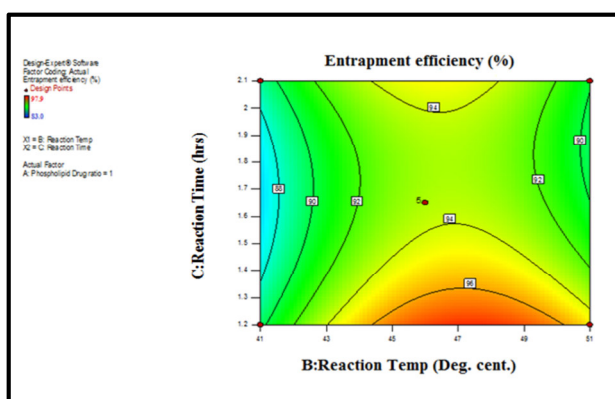


Fig No.5: Counter plot showing combined effect of reaction temperature and reaction time kept at lower level of phospholipids-drug ratio 1 gm.

4.5 Characterization Of Terminalia Arjuna Phytosomes Complex

4.5.1 Microscopic view

Optical microscopy was used for the surface morphology of Terminalia arjuna phospholipids complex. The microscopic view, as shown in Fig. No. 6 indicated the presence of spherical structures of the complex.

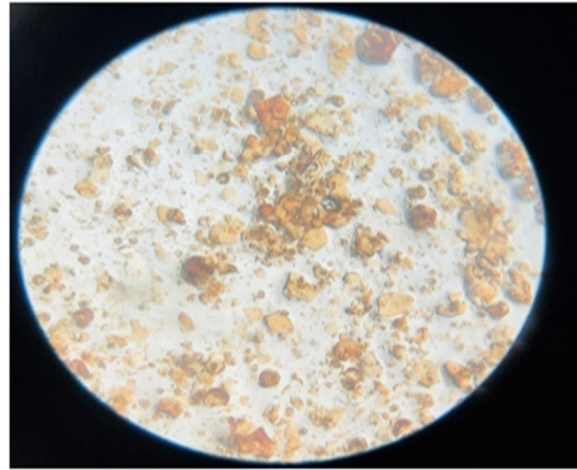
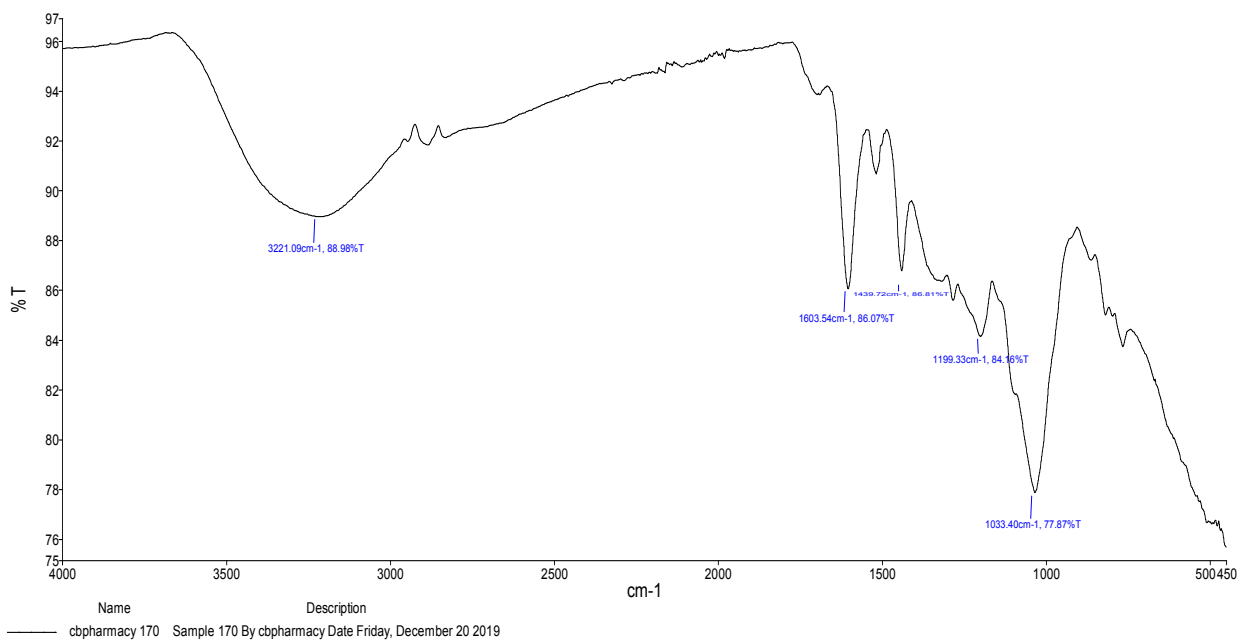


Fig No.6: Microscopic view of *Terminalia arjuna* phospholipids complex.

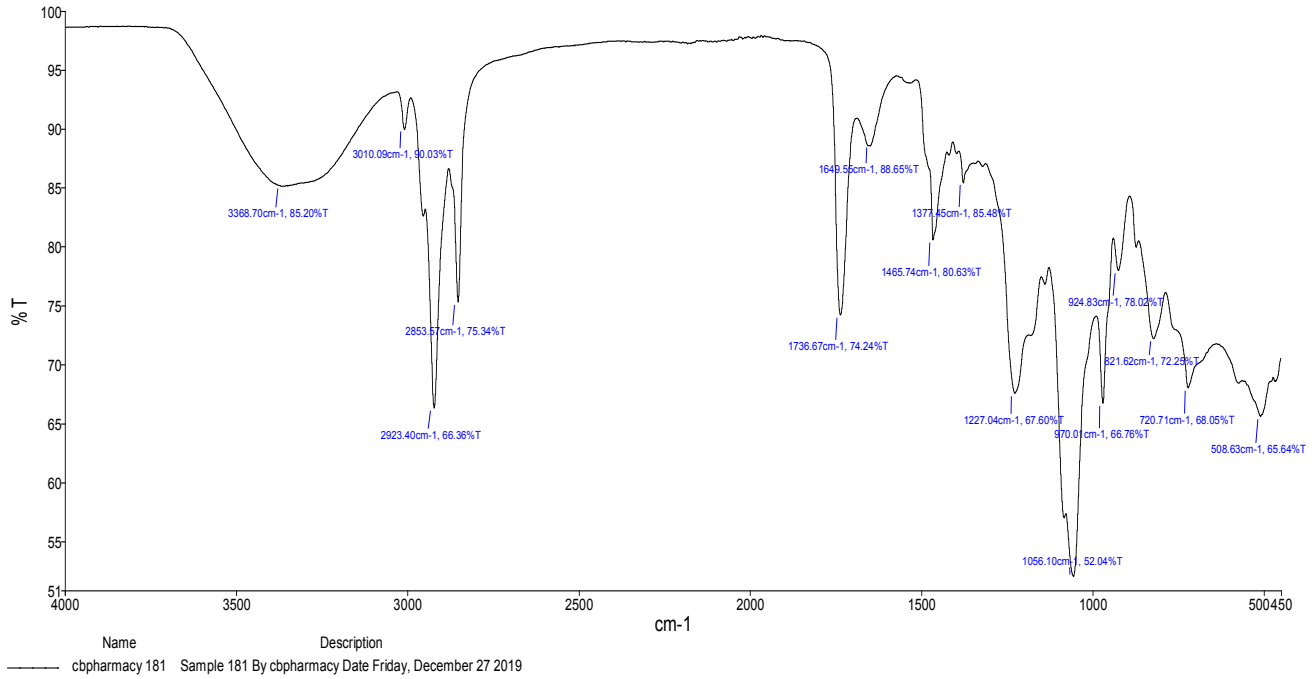
4.5.2 Compatibility studies

FTIR spectrum of *Terminalia arjuna* extract, soya phosphatidylcholine, *Terminalia arjuna* Phytosomes and the formulated *Terminalia arjuna* phytosomal tablet are shown in Fig. No. 7. The compatibility between *Terminalia arjuna* and

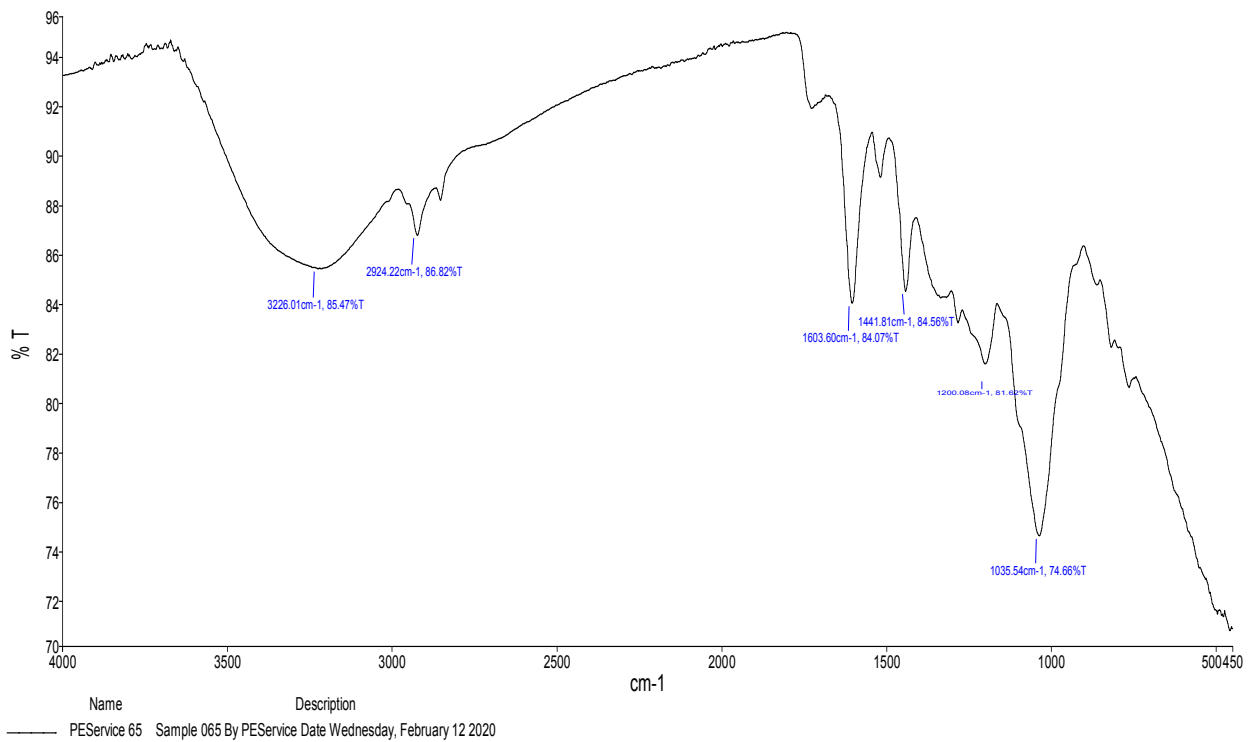
Soya phosphatidyl choline was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug and lipid.



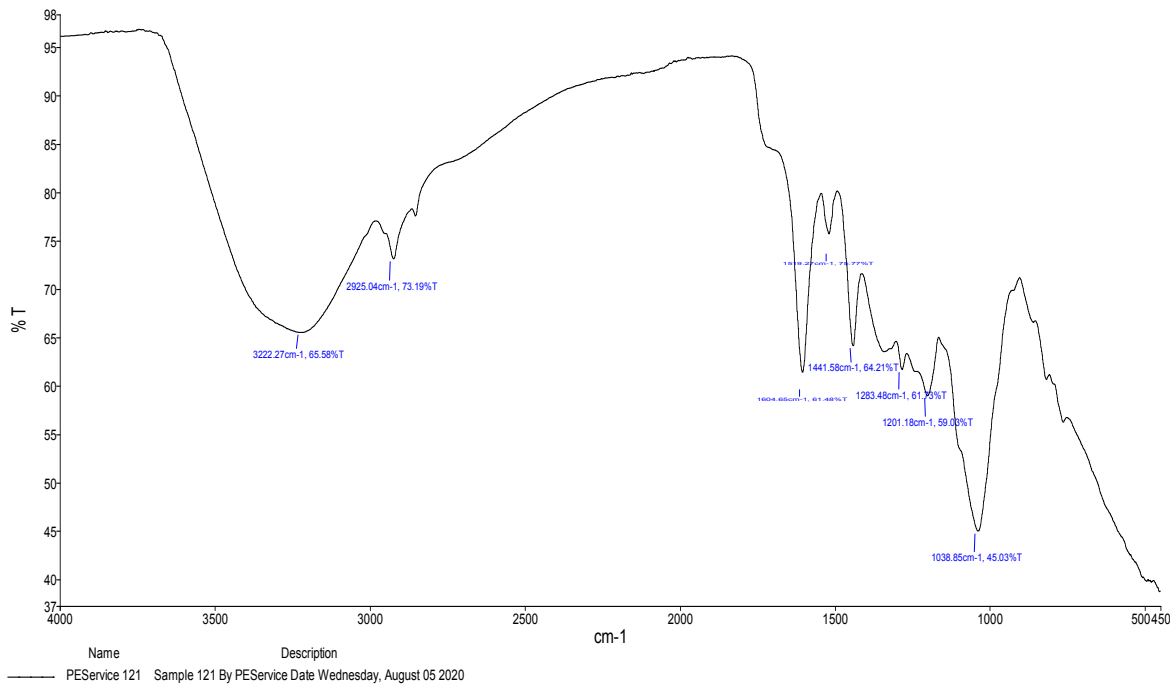
FTIR of *Terminalia arjuna* bark extract



FTIR of Soya phosphatidylcholine



FTIR of Terminalia arjuna extract phytosome sample



FTIR of *Terminalia arjuna* phytosomal tablet formulation

Fig No.7: FTIR Spectra of A) *Terminalia arjuna* bark extract B) Soya phosphatidylcholine C) *Terminalia arjuna* extract phytosomes D) *Terminalia arjunaphytosomal* tablet formulation.

4.6 Determination of Entrapment efficiency

According to the drug entrapment study conducted the maximum drug entrapment was shown by F1. The entrapment efficiency of all formulations was represented in Table No. 4. The *Terminalia arjuna* Phytosomes prepared by

solvent evaporation method have shown high entrapment efficiency compared to other methods. The formulation F1 showed the highest release entrapment efficiency of $97.9 \pm 0.4\%$ w/w indicating the optimum amount of lipid required for the formation of *Terminalia arjuna* Phytosomes.

Table No.4 – Different formulation of entrapment efficiency				
Formulation	X1 (w/w)	X2 (°C)	X3 (Hrs)	Entrapment efficiency (% w/w)
1	1	40	1.5	97.9 ± 0.4
2	2.5	40	1.5	96.3 ± 0.5
3	1	50	1.5	90.9 ± 0.3
4	2.5	50	1.5	85.0 ± 0.6
5	1	40	2.5	95.8 ± 0.8
6	2.5	40	2.5	96.7 ± 1.5
7	1	50	2.5	97.6 ± 1.2
8	2.5	50	2.5	84.0 ± 1.0
9	0.5	45	2	90.2 ± 1.7
10	3	45	2	96.8 ± 1.4
11	1.75	35	2	96.0 ± 1.1
12	1.75	55	2	93.5 ± 0.9
13	1.75	45	1	83.0 ± 0.6
14	1.75	45	3	96.2 ± 0.3
15	1.75	45	2	92.4 ± 0.8

4.7 Differential scanning calorimetry

In DSC studies of *Terminalia arjunaphytosome* formulation (F1), two peaks are observed at 140.72°C and 212.46 °C and

are shown in Fig. No. 8. This may be due to the melting of lipid components and their interaction with aqueous extract. This suggests that the drug extract has been entrapped into the lipid vesicles.

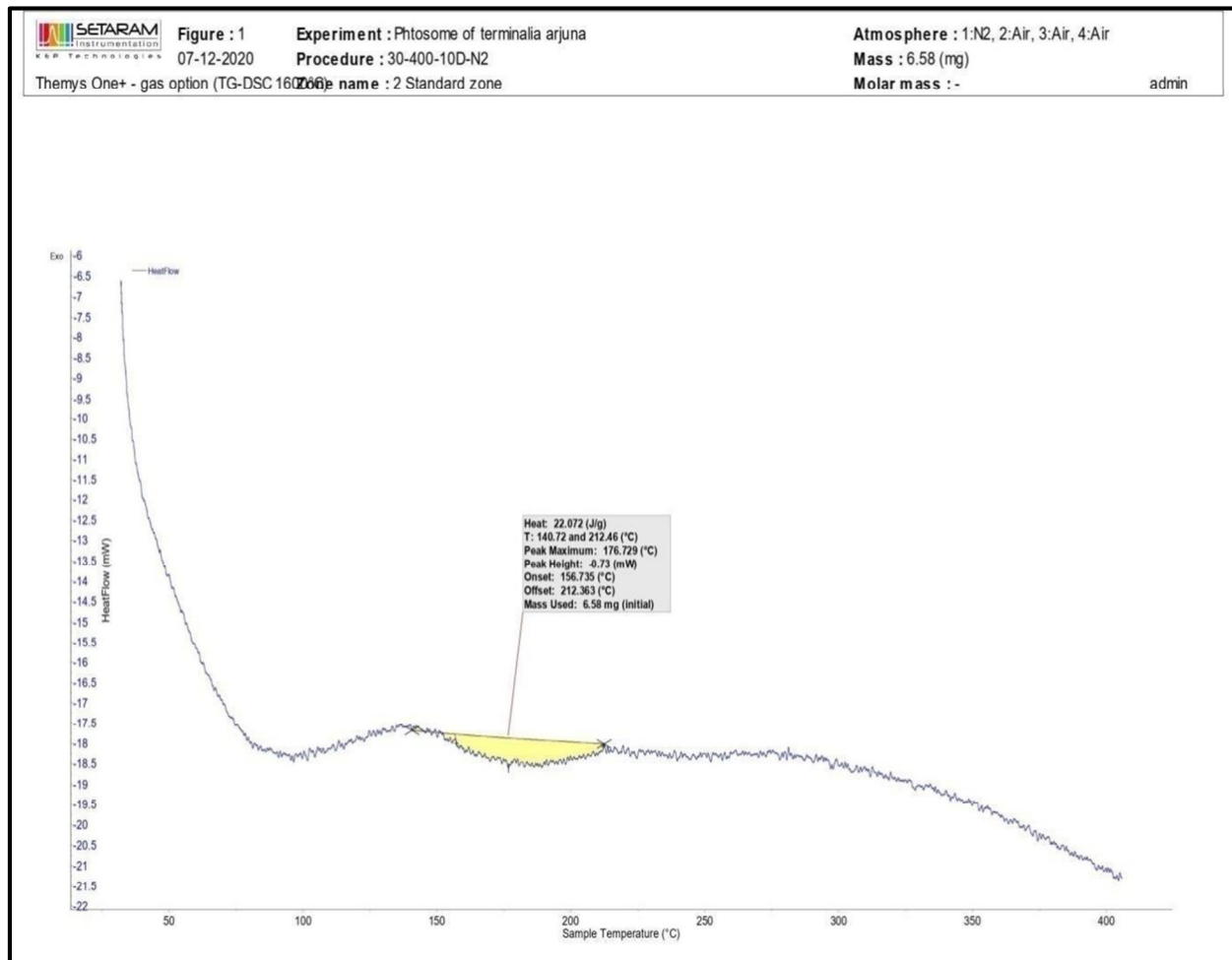


Fig No. 8: DSC Thermogram of Terminalia arjuna extract phytosomes.

4.8 Scanning Electron Microscopy

The surface morphology, shape and structure of the phytosome complex of Terminalia arjuna extract (FI) at

various magnifications are shown in Fig. No.9. It was observed that the drug particles are associated with the phospholipids forming complexes with irregular particles shape and crystalline structures.

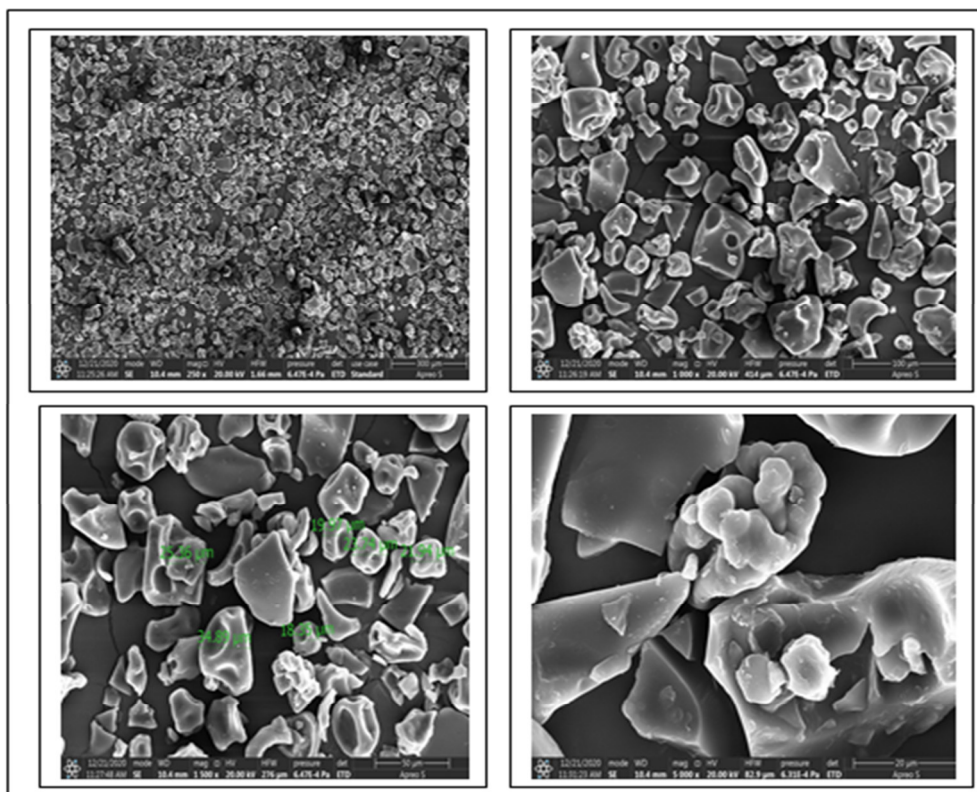


Fig No. 9: SEM Image of Terminalia arjuna extract phytosomes.

4.9 Preparation of Terminalia arjunaphytosomal tablet

Six different batches of Terminalia arjunaphytosomal tablets with different composition were prepared by direct compression process as shown in Table 3.

4.10 Pre-compression Parameters

Pre-compression parameters of all formulations were conducted for bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index. The two most important parameters for the direct compression technique are good flow and good compressibility. Pre-compression parameters are evaluated, these are mentioned in following Table No. 5.

Formulation	Bulk density (gm/ml)	Tapped density (gm/ml)	Angle of repose (°)	Hausner's ratio	Carr's index (%)
F1	0.476±0.02	0.543±0.03	26.37±0.75	1.14±0.04	12.38±0.46
F2	0.462±0.01	0.584±0.04	29.90±0.51	1.26±0.07	20.83±0.23
F3	0.454±0.03	0.561±0.02	28.67±0.41	1.23±0.08	19.08±0.58
F4	0.469±0.01	0.581±0.03	27.83±0.89	1.23±0.04	19.24±0.84
F5	0.473±0.03	0.552±0.02	28.76±0.49	1.16±0.06	14.21±0.57
F6	0.450±0.01	0.571±0.02	26.56±0.51	1.26±0.02	21.14±0.64

Values are mean±SD;(n=3)

4.11 Evaluation Of Terminalia arjunaPhytosomal Tablets

4.11.1 Post-compression Parameters

Evaluation of tablets was done by studying various parameters like hardness, thickness, friability, and weight variation results were presented in Table No. 6 and all the results were found to be within the Pharmacopeial standards.

Batch no.	Hardness (kg/cm ²)	Thickness (mm)	Friability (%)	Weight variation (mg)
F1	4.5±0.51	3.85±0.48	0.24±0.11	300±0.58
F2	4.8±0.20	3.88±0.35	0.21±0.15	298±0.67
F3	4.6±0.46	3.50±0.13	0.27±0.10	300±0.61
F4	4±0.42	4.00±0.21	0.15±0.04	300±0.57
F5	4.6±0.46	3.85±0.15	0.25±0.08	301±0.63
F6	4.5±0.61	4.15±0.18	0.24±0.12	299±0.67

Values are mean±SD;(n=3)

4.11.2 In-vitro dissolution studies

The *in-vitro* dissolution studies were carried out by using USP type-II apparatus in 6.8 pH phosphate buffer to access the ability of the formulation for providing immediate drug

delivery. Among all the formulations (F1 to F6) prepared, batch F4 is the best formulation released 96.29 % at the end of 30 minutes. All the values were given in Table No. 6 and Shown in Fig. No. 10 and 11.

Time (min)	% Drug release					
	F1	F2	F3	F4	F5	F6
5	24.15±0.51	26.12±0.32	28.42±0.32	28.50±0.26	20.37±0.35	22.42±0.53
10	32.25±0.42	35.47±0.50	41.34±0.25	40.57±0.23	30.22±0.50	49.21±0.55
15	39.62±0.75	55.46±0.34	57.22±0.63	65.14±0.22	67.28±0.34	62.61±0.65
20	69.95±0.32	67.39±0.51	73.20±0.31	77.27±0.44	85.20±0.23	75.28±0.32
25	72.61±0.65	70.51±0.32	81.31±0.11	82.53±0.42	88.34±0.52	82.72±0.23
30	79.69±0.62	82.92±0.52	93.20±0.21	96.29±0.31	91.25±0.21	85.17±0.34

Values are mean±SD; (n=3)

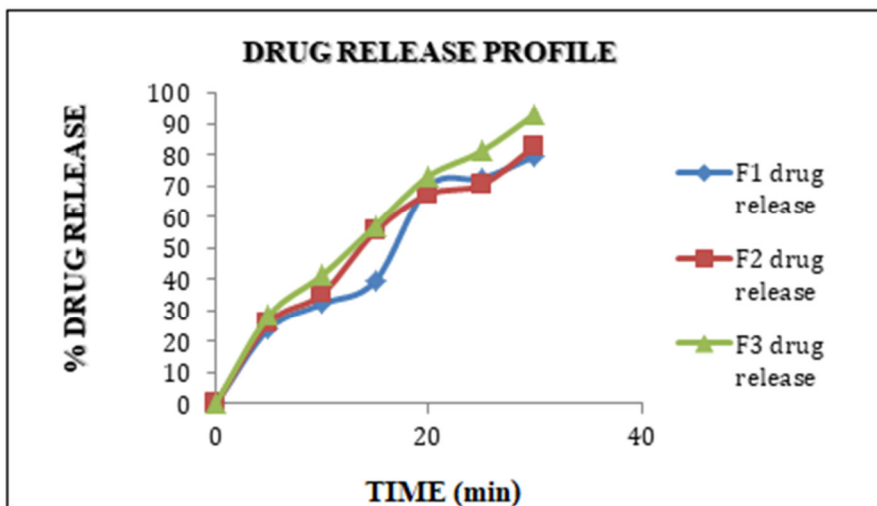


Fig. No.10: In-vitro dissolution profile of Terminalia arjunaphytosomal tablets of batches F1-F3.

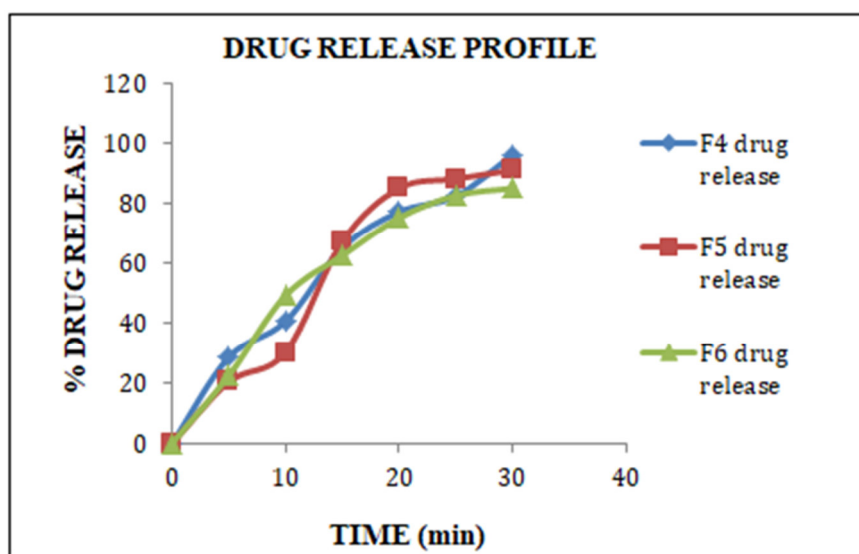


Fig. No.11: In-vitro dissolution profile of Terminalia arjunaphytosomal tablets of batches F4-F6.

4.11.3 Kinetic analysis of dissolution data

The in vitro data is fitted into different kinetic models and the best-fit was achieved with the Peppas model [Table No.8].

Table No.8: Kinetic analysis for different formulations							
Formulation	Zero Order (R)	First Order (R)	Matrix (R)	Hix-Crow (R)	Peppas		Best Model Fit
					(R)	(N)	
F1	0.9991	0.9884	0.9559	0.9955	0.9990	0.6472	ZERO
F2	0.9927	0.9553	0.9657	0.9798	0.9996	0.7686	ZERO
F3	0.9982	0.9503	0.9579	0.9784	0.9888	0.8858	PEPPAS
F4	0.9923	0.9846	0.9661	0.9956	1.0000	0.9373	PEPPAS
F5	0.9922	0.9904	0.9692	0.9945	0.9892	0.7570	HIXCROW
F6	0.9985	0.9921	0.9613	0.9978	0.9999	0.9181	PEPPAS

4.12 Stability studies

The stability study conducted by ICH guideline. It showed no significant change in properties of the optimized formulation and the drug release. Stability studies are performed in a stability chamber over a period of 3 month on the promising Terminalia arjuna bark extract phytosomal tablets formulation

F4. Sufficient number of tablet formulations were packed in a stability container and kept in a stability chamber at Temperature 45°C and RH 75%. Samples were taken for 90 days for drug content estimation; also the appearance, weight, pH, content uniformity and in-vitro disintegration studies were performed to determine the drug release profile[Table No.9].

Table No.9 – Stability parameters (Evaluation) of *Terminalia arjunaphytosomal* tablets.

Parameters	Initial	After 3 month
Shape	Round	Round (No change)
Colour	Reddish brown	Reddish brown (No change)
Hardness (kg/cm ²)	4	4
Friability (%)	0.15	0.15
Weight Variation (mg)	300	300
Thickness (mm)	4.00	4.00

5. DISCUSSION

In the present study, the work was an attempt to carry out standardization and extraction of active constituents of *Terminalia arjuna* bark and also to formulate and evaluate phytosome of *Terminalia arjuna* bark extracts and its tablet formulation. *Terminalia arjuna* bark extract was evaluated for physicochemical and phytochemical analysis by using different organic solvents. Standardization of *Terminalia arjuna* bark extract was done with loss on drying, determination of pH, total ash value, acid insoluble ash value, water soluble ash value and swelling.³¹ The water soluble constituents are poorly absorbed due to their poor lipid solubility thus unable to cross the highly lipid-rich biological membrane, which results in poor bioavailability.²⁹ *Terminalia arjuna* phytosome has more bioavailability because it is quickly soluble in water as compared to Lawsonia Phytosome because Lawsonia has low bioavailability it is less soluble in water and it is rapidly eliminated from body.²² The phytosomes formulation of *Terminalia arjuna* bark extract was done by varying drug concentration by using QbD approach.³⁰ The effect of phospholipids ratio on the physical characteristic of the formulated phytosomes was examined for various phospholipids ratios phytosomes at reaction temperature 40°C, reaction time 1.5 hrs and the phospholipids ratio was obtained 1:1.³¹ In DSC studies interactions can be observed by the appearance of new peak, disappearance of original peak, also by comparing the transition temperature, melting point and changes in the relative peak area. *Terminalia arjuna* phytosome formulation (F1), two peaks are observed at 140.72°C and 212.46 °C. This may be due to the melting of lipid components and their interaction with aqueous extract.³⁰ This suggests that the drug extract has been entrapped into the lipid vesicles. In the formulation (F1-F6) HPMC and microcrystalline cellulose used as a binding agent, crosscarmellose sodium used as a Superdisintegrating agent, starch used as a disintegrating agent and increases their dissolution profile.²⁹ Among all the formulations (F1 to F6) prepared, batch F4 is the best formulation released 96.29 % at the end of 30 minutes as compared to the *Carica papaya* phytosome complex tables which showed 21 % release in 2 hrs in 0.1 N HCL. The in vitro data is fitted into different kinetic model and the best-fit was achieved with the Peppas model.³² From the above results it is concluded that prepared *Terminalia arjuna* phytosomal tablets shows significant cardio protective activity.^{6,9}

6. CONCLUSION

The phytosome formulation of *Terminalia arjuna* bark extract

was done by varying drug concentration by using the QbD approach. In QbD approach entrapment efficiency is used to formulate. Soya phosphatidylcholine was used as a complexing agent. All 15 formulations was subjected to entrapment efficiency by using software of Central Design Expert. The presence of active constituents was also confirmed by the HPTLC analysis, calibration curve and FTIR of *Terminalia arjuna* bark extracts. Different formulations of *Terminalia arjuna* bark extract phytosomal tablets evaluation parameters were observed were F4 formulation was found to be the best formulation, it provided immediate release of *Terminalia arjuna* phytosomal tablets over 30 minutes and was considered as the good release i.e. (96.29 %). In the formulation (F1-F6) HPMC and microcrystalline cellulose used as a binding agent, crosscarmellose sodium used as a Superdisintegrating agent, starch used as a disintegrating agent and increases their dissolution profile up to 96,29% at the end of 30 minutes. A good *in-vitro* drug release study was observed for formulation F4 and kinetic release shows the best model fit is Peppas model.²⁷ Based on the above study it was concluded that the phytosome formulation of hydro-alcoholic extract of *Terminalia arjuna* and its Tablet formulation improved the bioavailability and may be used as herbal formulation in the treatment of cardiovascular disease for antioxidant activity.^{5,8}

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8. AUTHORS CONTRIBUTION STATEMENT

Waghmare Sagar gathered data, perceived the idea, carried out the research study with regard to this work. Dr Omprakash guided in conducting this research study and also reviewed the manuscript. Dr. Gaurav Agarwal analysed the data and gave necessary inputs towards the designing of the manuscript. All authors provided critical feedback, discussed the methodology, results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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