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Preparation and Evaluation of Curcumin Phytosomes

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ABSTRACT

Curcumin, a hydrophobic polyphenol derived from the rhizome of Curcuma longa L. (Zingiberaceae or dietary Curcumin) has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, anti-amyloid, and antitumor activities. Also, the nontoxic nature of Curcumin has been demonstrated by its long history of dietary use and clinical trials. Curcumin has low bioavailability because it is less soluble in water and it is rapidly eliminated from the body. This study aimed to prepare the phytosome of Curcumin and evaluate it. The phytosomes containing a molar ratio of 1:1, 1:2, 2:1 and 2:2 of Curcumin and soya lecithin were prepared by the anti-solvent precipitation technique. The phytosome was characterized by SEM, DSC, and FTIR. The *in-vitro* drug release rate of the prepared phytosome was evaluated. DSC data showed that phytosome has irregular size vesicles consisting of soya lecithin and Curcumin was found to be intercalated in the lipid layer. FT-IR spectrum of the phytosome confirmed the formation of cell-like structure through interaction with soya lecithin. SEM data has shown the irregular particle size and crystalline shape of the prepared phytosome of Curcumin.

INTRODUCTION

Considerable attention has been focused on the development of novel drug delivery systems (NDDS) for herbal drugs in the past few decades. The novel carriers should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, throughout treatment. Secondly, it should channel the active entity of herbal drug to the site of action. Conventional dosage forms including prolonged-release dosage forms are unable to meet none of these.[1]

Plant-derived drugs have gained immense popularity and access to the medicine markets throughout the globe as safer and effective substitutes of modern synthetic medicines which are considered to be full of adverse and toxic interactions. In underdeveloped and developing nations all over the world plant drugs in traditional form or as alternative medicine have been supposed to satisfy the primary healthcare needs of about 80% of the population and even in developed nations, these medicines are being utilized by about 65% of the population.

Currently, as many as one-third to approximately one-half of all the drugs available are derived from plants or other natural sources. The plant drug formulations of traditional systems of medicine like the Chinese and Indian systems usually contain crude extracts of different plants which incorporate in them unwanted and many times harmful principles along with the active principles. With the developments in the field of phyto and analytical chemistry, specific ingredients or a group of similar ingredients from plants are being extracted, isolated and tested for their different medicinal applications. However, the bioavailability of active principles of plants has become an issue of concern for researchers because of poor oral bioavailability of many of them specifically those containing polyphenolic rings in their structures such as flavonoids and other water-soluble constituents like terpenoids and tannins. Some of the basic reasons for the poor bioavailability of these substances are low aqueous or lipid solubility, high molecular weight/size, and poor plasma membrane permeability. Moreover, the standardized extracts when administered orally lose some of their constituents in the presence of gastric fluids. This has restricted the use of pharmacologically effective polyphenolic plant actives for treating different disorders.

To counter these problems and to make herbal therapy more effective these drugs have been incorporated into several novel delivery systems in recent times. Some of the approaches for bioavailability enhancement are formulating at the nanoscale as nanoparticles, binding with

lipids as liposomes or herbosomes/phytosomes, delivery in the form of microemulsions, modification in chemical structures, delivery as prodrug and complexation with cyclodextrins, etc. have several advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation, etc. Thus the nano-sized novel drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines.[2]

The bioavailability of active principles of plants has become an issue of concern for researchers and scholars because of poor oral bioavailability of many plants specifically those containing polyphenolic rings in their structures such as flavonoids and other water-soluble constituents like terpenoids and tannins. Some of the basic reasons for the poor bioavailability of these substances are low water or lipid solubility, high molecular weight/size, and poor plasma membrane permeability. [2, 25-27] To overcome these problems and to make herbal therapy more effective, these drugs have been incorporated into several novel delivery systems in recent times.

The technique of complexion of plant drugs or extracts with phospholipids has emerged as challenging and one of the most successful methods for improving bioavailability and therapeutic efficacy of several poorly absorbed plant constituents.

This technique incorporates the phospholipid molecules containing phosphatidylcholine in their structure to form complexes with standardized herbal extracts and/or the specific bioactive ingredient of the plant to improve the membrane permeability, water-oil partition coefficient, enhance the systemic bioavailability, enhancement of solubility, ability to cross the cell membranes, protection from toxicity, enhancement of stability. These complexes of drug and phospholipids are called as phytosomes.[3-7]

The objective of the research work was to formulate the phytosomes of plant drug Curcumin by different methods.

MATERIALS AND METHODS

Curcumin was purchased from Sigma Aldrich Pvt Ltd., Bangalore, India. Soya Lecithin was obtained from Himedia Laboratories Pvt Ltd, Mumbai. Whereas all other reagents used were of the highest quality and commercial grade.

1. Pre-formulation Studies

a. Organoleptic Properties

The sample of plant drug Curcumin was studied for organoleptic characters such as color, odor, and appearance.

b. Boiling Point

The melting point of curcumin was determined by a capillary method using melting point apparatus.

c. Physical Compatibility Test

The selection of drugs and phospholipid to prepare the phytosome is mainly based on the physical compatibility studies and solubility study. The pre-formulation study was carried out with potential formulation phospholipids or polymer to determine drug-phospholipids interaction/compatibility.

i. FTIR Spectroscopy

A FT-IR range of the drugs was acquired using the Shimadzu-8400S FT-IR Spectrophotometer (Tokyo, Japan) by the KBr pellet method. The Curcumin was separately blended with IR grade KBr in the proportion of 1:100 and the active was also mixed with excipients to check compatibility. Each blend was compacted in the form of a pellet by applying 10 tons of pressure in a hydraulic press. The pellets were scanned over a wavenumber range of 4000 to 400 cm^{-1} in the Fourier Transform Infrared instrument and spectral analysis was done. Software used for the data analysis was Perkin-Elmer Spectrum 5.3.[8]

ii. Differential Scanning Calorimetry Studies

Thermogram Curcumin was obtained using Shimadzu DSC-60 Differential scanning calorimeter (Shimadzu DSC-60, Japan) using aluminum pans. The dry samples of drugs (2.00-10.00 ± 5 mg) were separately weighed, fixed in aluminum pans hermetically and warmed at an examining rate of 10°C/min between 30°C to 300°C. The ideal environment was provided by cleansing nitrogen stream at the rate of 40 mL/min.[9]

d. Determination of Solubility

Saturation solubility of Curcumin was determined in acidic pH 1.2 (0.1 N HCl), phosphate buffers pH 6.8 and 7.4, organic solvents such as acetone, acetonitrile, dimethyl sulfoxide (DMSO), dichloromethane (DCM), ethanol (EtOH), methanol (MeOH) and distilled water (DW). The abundance measure of each drug was separately added exclusively to 5 ml of every media in screw-capped tubes. A vortex mixture was utilized to encourage the solubilization. After 48 h, 1 ml of aliquots were taken out from each sample through filtration using Whatman filter paper No 41. Absorbance was estimated in the range of 200-400 nm on UV Visible Spectrophotometer (Shimadzu-1800, Japan) and calculations for solubility were done. [10-11]

e. UV Spectroscopy Study (Determination of λ max)

The standard stock solution of the drug was filtered between 200-400 nm using a UV spectrophotometer (Shimadzu-1800, Japan) in acidic pH 1.2 (0.1 N HCl), phosphate buffers pH 7.4, organic solvents such as acetonitrile and methanol (MeOH).

f. Formulation and Development

1. Selection of plant drugs

A natural coloring carotenoid Phyto-constituents was selected for the preparation of the phytosome.

2. Selection of phospholipids

The selection of a phospholipid for the preparation of the phytosome was based on compatibility and interaction with the drug.

3. Selection of method[12-13]

The following methods were tried in a trial or error base to prepare the phytosome:

1. Anti-solvent precipitation method (AP)

2. Solvent evaporation method (SE)

3. Rotary evaporation technique (Film hydration technique) (RE)

Anti-solvent precipitation method

The required quantities of Curcuminand phospholipids were separately taken in a 100 ml round base flask and refluxed with 30 ml of methanol at a temperature not surpassing 60^{o}C for 2 h. The blends were concentrated to 5 ml and n-hexane (20 ml) was added deliberately with constant mixing to get the precipitate which was sifted, gathered and put away in vacuum desiccators for overnight. Powdered phytosomes formulations were set in a golden shading glass bottle and put away at room temperature.

4. Preparation of preliminary trial batches for selection of method

The preliminary batches of phytosomes of drugs with phospholipid of 1:1 molar ratio were prepared by the mentioned methods in Table 1.

Table No. 1: Preliminary Batches of Phytosome of Drugs

Sr. No.	Methods	Drug	Phospholipids	Molar ratio	Solvents
1.	AP	Curcumin	Soya lecithin	1:1	Methanol + n – hexane
2.	SE	Curcumin	Soya lecithin	1:1	Tetrahydrofuran
3.	RE	Curcumin	Soya lecithin	1:1	Ethanol + n – hexane

5. Evaluation of preliminary batches for selection of working method

For the selection of the working method, the preliminary trial batches of phytosomes of drugs were characterized and evaluated for shape using microscope, % yield, % drug loading and % entrapment efficiency and particle size.

5.1 Shape of the phytosomes

An optical microscope was used for the characterization of the formulations. The formulations were separately suspended in phosphate buffer pH 7.4 and a drop was set on a slide and secured with a coverslip. Microscopic view of the complex was observed at a magnification of 10x10.[14-15]

5.2 Determination of % yield

Assurance of % yield of formulations was calculated by the accompanying equation:[16]

$$(\%)\ \text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

5.3 Determination of particle size

The mean diameter of each formulation was estimated by dynamic light scattering (DLS) using particle size analyzer (Zetasizer 2000, Malvern Instruments Ltd., UK) at a settled scrambling point of 90ºat 25ºC. [17-19]

5.4 Determination of entrapment efficiency and drug loading

The entrapment efficiency and drug loading of formulations were dictated by the centrifugation method (Remi Elektro Technik Ltd, Vasai, India). The formulations were separately centrifuged with a 10 ml volume of methanol at 5000 rpm for 10 min. The free amount of the drug in the filtrate was determined by UV/Vis spectroscopy (Shimadzu-1800, Japan) at 283 nm. Estimations were performed in triplicate. The entrapment efficiency and drug loading were figured by the accompanying formula: [20-24]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{amount of free drug}}{\text{Total amount of drug}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{Weight of the entrapped drug}}{\text{Weight of the Formulation}} \times 100$$

6. Preparation of final batches of Phytosomes

The different molar ratio of the phytosome of the drug was prepared by the selected optimized working method, mentioned in Table 2.

Table No. 2: Final Batches of Phytosome of Drugs

Sr. No.	Curcumin Phytosomes	Molar ratios	
		Curcumin	Soyalecithin
1.	TOP1	0	1
2.	TOP2	1	1
3.	TOP3	1	2
4.	TOP4	2	1
5.	TOP5	2	2

#TOP1 (Blank phytosomes)

7. Evaluation of final batches of phytosomes

The phytosomes of Curcuminwere characterized by physical appearance, FT-IR and DSC compatibility study and evaluated for % yield, % entrapment efficiency, % drug loading, particle size, zeta potential and *in vitro* drug release.

7.1 Physical Appearance

All the prepared phytosomes of curcumin were visually inspected for color, odor and physical state.[25]

7.2 FT-IR compatibility study

The prepared phytosome of curcumin were characterized using Shimadzu-8400S FT-IR Spectrophotometer (Tokyo, Japan) by KBr pellet method.[26]

7.3 Differential Scanning Calorimetry (DSC) study

The prepared phytosome of curcumin were characterized by Shimadzu DSC-60 Differential scanning calorimeter (Shimadzu DSC-60, Japan) using aluminum pans. [27]

7.4 Determination of % yield

Assurance of % yield of phytosomes of curcumin was calculated by the accompanying equation:

$$(\%)\ \text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

7.5 Solubility study

The solubility study for the phytosome of the drug was determined in different solvents.

7.6 Determination of entrapment efficiency and drug loading

The entrapment efficiency and drug loading of phytosomes of curcumin were detected by the centrifugation method (Remi Elektro Technik Ltd, Vasai, India). The entrapment efficiency and drug loading were figured by the accompanying formula:[28-31]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Amount of free drug}}{\text{Total amount of drug}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{Weight of the entrapped drug}}{\text{Weight of the Formulation}} \times 100$$

7.7 Determination of particle size and zeta potential

The average diameter and surface charge property of phytosomes of curcumin were estimated by dynamic light scattering (DLS) using particle size analyzer (Zetasizer 2000, Malvern Instruments Ltd., UK).

7.8 Scanning Electron microscope (SEM) study

Scanning electron microscopy (Hitachi Ltd., S-3400N type II model, Tokyo, Japan) was utilized to decide the surface morphology of the optimized phytosome of the drug. Dry samples of Curcumin was separately set on an electron magnifying instrument metal stub and covered with gold in a particle sputter. Digital pictures of the formulation were taken by irregular examining of the stub at various amplifications.[32]

7.9 *In-vitro* drug release

Based on the literature survey phosphate buffer pH 7.4 (900 ml) was utilized as a dissolution medium for 24 hours and maintained at 37 ± 0.5°C. The dissolution study was completed utilizing a dissolution apparatus (Electrolab TDT-08L, Mumbai) by the USP II paddle method at 50 rpm. The cotton paper pack was utilized to complete the *in-vitro* drug release.

The cotton paper packs containing phytosomes of the drug were dipped into the jar containing medium and it was shut with cover to avoid vanishing of the dissolution medium. At predetermined time intervals, aliquots were withdrawn from the discharge medium and supplanted with a similar measure of phosphate buffer. The samples were assayed at respective wavelengths by UV spectrophotometer (Shimadzu-1800, Japan). The experiment was repeated three times for both the drugs and the values recorded as mean ± standard deviation (SD). [33]

7.10 Stability Study

In-vitro stability study is a standout amongst the most basic variables of preparation and in ensuring the safety and adequacy of the product. Optimized phytosome and phytosome loaded complex were packed individually in glass vials with nitrogen purging and sealed by rubber stoppers and crimped using aluminum seals. The samples were divided into three sets for the stability study and stored at:

- In refrigerator (5 ± 3℃) for 3 months
- In humidity control chambers
  - 25 ± 2℃/60 ± 5 % RH for 3 months
  - 40 ± 2℃/75 ± 5% RH for 3 months

Samples were withdrawn on 0, 1, 2 and 3 months and evaluated for changes in physical appearance, % entrapment efficiency, % drug-loading and average particle size as per ICH Q1A (R2) guidelines.

RESULT AND DISCUSSION:

(I) Pre-formulation Studies

1. Organoleptic properties

The organoleptic properties of curcumin were characterized by color, odor and appearance and the results were shown in Table 3.

Table No. 3: Organoleptic properties of Curcumin

Drug	Color	Odor	Melting point
Curcumin	Yellowish to orange	Spicy and Pungent	183°C

FT-IR Spectroscopy

FT-IR spectrum of drug samples showed all the characteristic peaks as reported in the literature indicating the presence of functional groups of Curcumin.

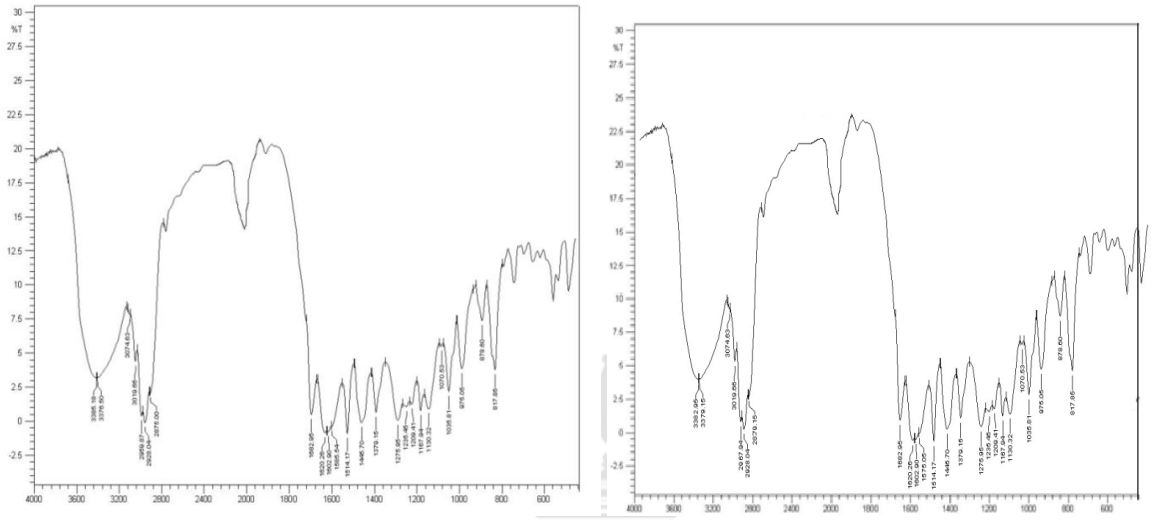

Figure No. 1: FTIR spectra of Curcumin and physical mixture

The FT-IR spectrum of Curcumin and its physical mixture with soya lecithin was determined. The functional group with corresponding peaks of curcumin and in its physical mixture of curcumin & soya lecithin was found to be correlative (Figure 21). The prominent peaks of curcumin at 3385.18cm^{-1} due to $V_{(o\text{-}H)}$ O-H stretching, while peaks at 3376.50 & 2959.87 cm^{-1} correspond to $V_{(c\text{-}h)}$ C-H asymmetric stretching and strong absorption peaks were 1585.94 cm^{-1} corresponds to v(c=o), v(c=o) C=C stretching, C=0 stretching was found in the FT – IR spectra of the physical mixture. As evident from the results, there was no interaction between curcumin and selected soya lecithin as prominent peaks were present in the physical mixture. Hence, curcumin and selected phospholipid were compatible with each other. Therefore, from the FT-IR study, it can be concluded that curcumin and selected phospholipid are compatible with each other.

2. Differential scanning calorimetry studies

Differential scanning calorimetry studies of Curcumin and its physical mixture with soya lecithin were determined using DSC 60. The DSC thermogram of curcumin showed a sharp melting endotherm at a temperature of 182.01°C and a Physical mixture of 185.16 as presented in Figure 2.

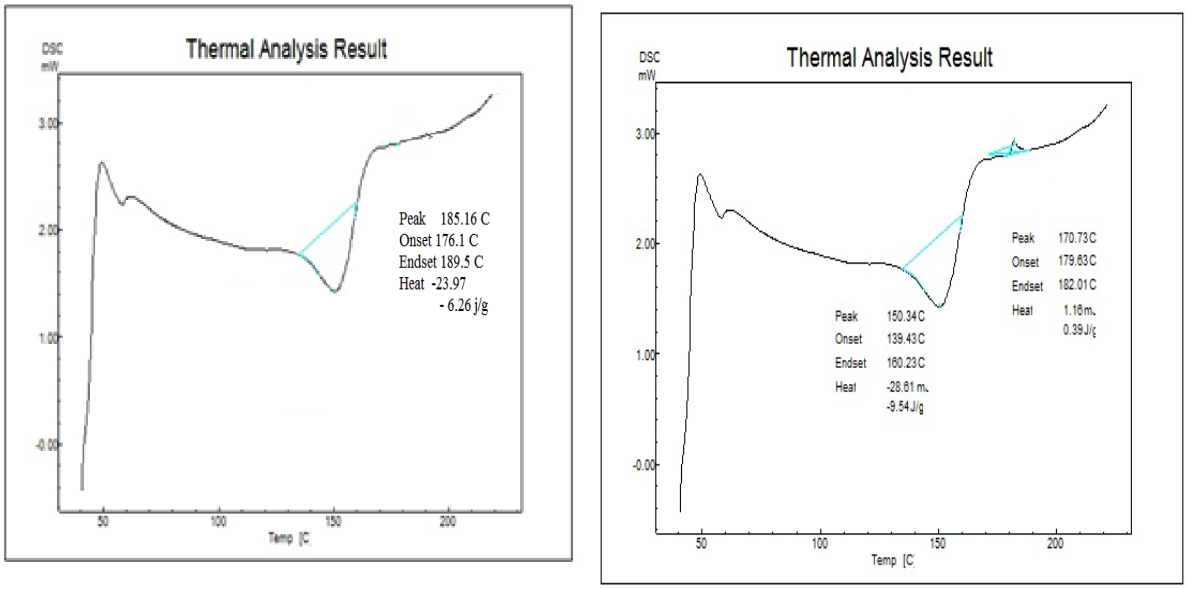

Figure No. 2: DSC Thermogram of Curcumin and physical mixture

DSC study shows that the characteristic peaks of Curcumin were present in the physical mixture and hence no interactions between Curcumin and selected phospholipid were seen.

3. Determination of solubility

The phytosome consist of drug and phospholipid. The selection of the right solvent for the preparation of the phytosome is mainly based on the solubility and compatibility of the drug with phospholipid in the respective solvents. The solubility of Curcumin was determined in different solvents such as acidic pH 1.2 HCl, phosphate buffer pH 6.8 & pH 7.4, organic solvents acetone, acetonitrile (ACN), dimethyl sulfoxide (DMSO), dichloromethane (DCM), ethanol (EtOH), methanol (MeOH) and distilled water (DW). The solubility analysis of Curcumin in different solvents is presented in Table 4. Except for distilled water, all other solvents showed good solubility profile of Curcumin.

Table No. 4: Solubility analysis data of Curcumin

Sr. No.	Solvents	Concentration (mg/mL)
1.	pH 7.4	4.51 ± 0.03
2.	Acetonitrile	1.02 ± 0.16
3.	Methanol	5.03 ± 0.27
4.	Ethanol	3.64 ± 0.31
5.	DCM	4.76 ± 0.00
6.	Water	0.81 ± 0.47
7.	DMSO	3.97 ± 0.05
8.	Ethyl acetate	2.07 ± 0.01

Mean ± SD, n = 3

4. UV Spectroscopy studies

The wavelength of maximum absorption (λmax) of curcumin in phosphate buffer pH 7.4 and methanol are shown in Table 5.

Table No. 5: Maximum absorbance wavelength (λmax) of curcumin

Solvents	Wavelength of maximum absorption (λmax) (nm)	
	Observed	Reported
Phosphate buffer pH 7.4	338	340
Methanol	416	425

The calibration curve of Curcumin was prepared in phosphate buffer pH 7.4 and methanol is depicted in Table 6&7 and Figures 3 and 4, respectively. The regression coefficient (R2) of Curcumin was found to be 0.9998 in phosphate buffer pH 7.4 and 0.9998 in methanol, respectively. The results indicate a linear relationship between concentration and absorbance in the range of 30-210 µg/ml & 20-140 µg/ml of Curcumin in phosphate buffer pH 7.4 & in methanol, respectively).

Table No. 6: Calibration curve data of Curcumin in pH 7.4 Phosphate buffer

Concentration (µg/ml)	Absorbance at 416 nm
0	0
20	0.145± 0.06
40	0.276± 0.04
60	0.411± 0.01
80	0.543± 0.02
100	0.688± 0.05
120	0.819± 0.03
Mean ± SD, n=3	

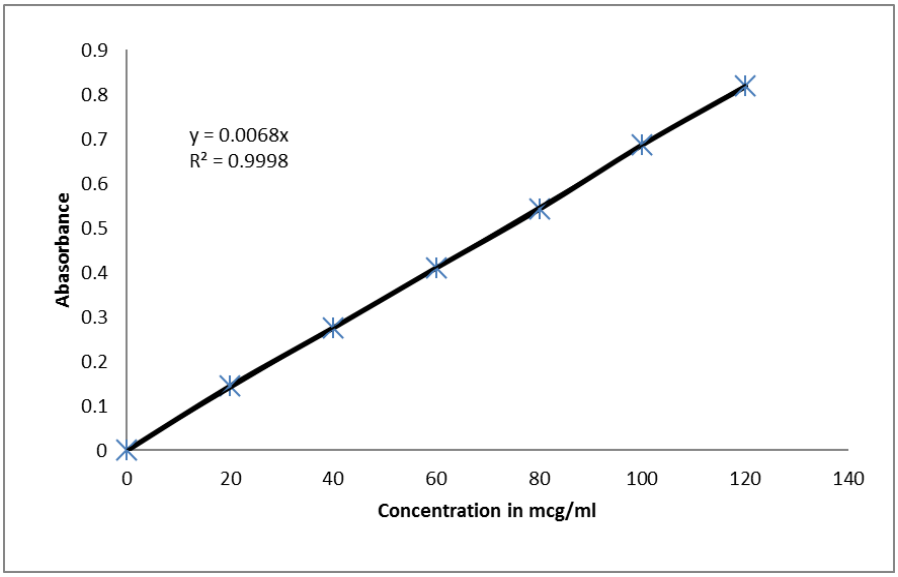

Figure No. 3: Calibration curve of Curcumin in pH 7.4 Phosphate buffer

Table No. 7: Calibration curve data of Curcumin in methanol

Concentration (µg/ml)	Absorbance at 416 nm
0	0
20	0.128± 0.06
40	0.245± 0.04
60	0.359± 0.01
80	0.483± 0.02
100	0.598± 0.05
120	0.72± 0.03
140	0.837± 0.07

Mean ± S.D, n = 3

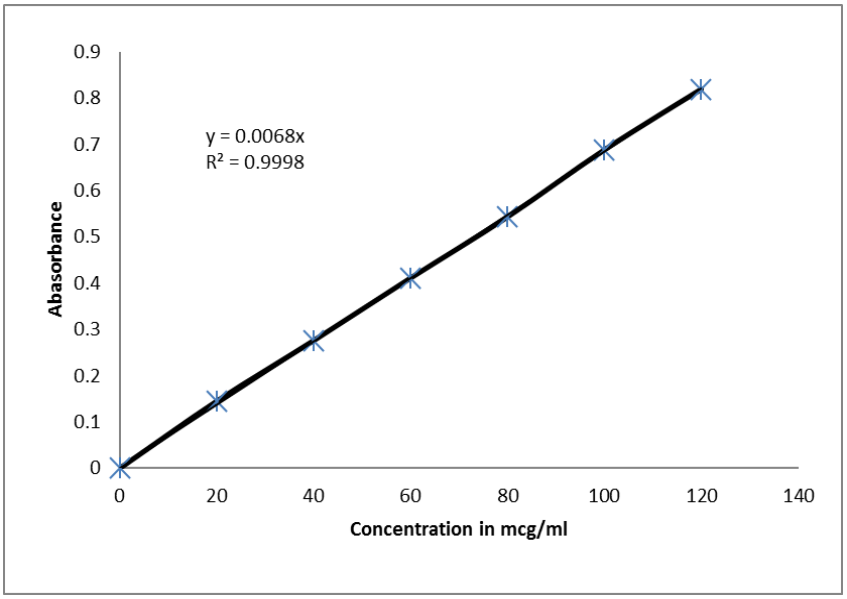

Figure No. 4. Calibration curve of Curcumin in methanol

(II) Formulation and Development

1. Selection of plant drug and phospholipids

The plant Phyto-constituents polyphenolic carotenoid drug Curcumin was selected as an antibacterial drug for the preparation of phytosome**.** Soya lecithin was selected as a phospholipid for the preparation of phytosome based on compatibility and interaction with the drug.

2. Selection of phospholipids

Soya lecithin was selected as a phospholipid for the preparation of phytosome based on compatibility and interaction with the drug.

3. Selection of method

Preparation and Evaluation of preliminary batches for selection of working method

The preliminary batch of the phytosome of Curcumin was prepared by different methods as mentioned earlier. The quantitative results indicate that the phytosome of curcumin obtained from various methods shown in Table 29. It was found that the anti-solvent precipitation method showed 73.98±0.86% yield, 576.39±5.89 nm particle size, 86.21±1.20% entrapment efficiency and 15.67±2.70% drug loading as compared to 89.38±0.23% yield, 645.45±7.43

nm particle size, 82.69±1.33% entrapment efficiency, 15.31±5.60% drug loading by solvent evaporation, 77.25±0.78% yield, 806.82±5.72 nm particle size, 81.82±1.35% entrapment efficiency, 11.89±5.71% drug loading by rotary evaporation technique.

Table No. 8: Evaluation of Preliminary Batches of Curcumin Phytosomes

Sr. no	Methods	Evaluation Parameters			
		Yield (%)	Particle size (nm)	Entrapment efficiency (%)	Drug loading (%)
1.	AP	73.98±0.86	576.39±5.89	86.21±1.20	15.67±2.70
2.	SE	89.38±0.23	645.45±7.43	82.69±1.33	15.31±5.60
3.	RE	77.25±0.78	806.82±5.72	81.82±1.35	11.89±5.71

Mean ± SD, n = 3

The phytosome of Curcumin by anti-solvent precipitation method showed better % yield, particle size, % entrapment efficiency and % drug loading as compared to other methods. The microscopic view of the phytosome of Curcumin indicated the presence of sphere-shaped vesicles. Thus, the anti-solvent precipitation method was selected to prepare the final batches of the phytosome of Curcumin.

4. Preparation of final batches of Phytosome

The different molar ratio of the phytosome of Curcumin was prepared by the anti-solvent precipitation method, mentioned in Table 9.

Table No. 9: Final Batches of Curcumin Phytosome

Sr. No.	Phytosome	Molar ratios	
		Curcumin	Soya lecithin
1.	TOP1	0	1
2.	TOP2	1	1
3.	TOP3	1	2
4.	TOP4	2	1
5.	TOP5	2	2

 # TOP1 (Blank phytosome)

5. Evaluation of final batches of Phytosome

All the prepared phytosome of Curcumin were characterized by FT-IR, DSC and evaluated for % yield, entrapment efficiency, drug loading, particle size, zeta potential and *in vitro* drug release, etc.

i. Physical Appearance

The physical appearance of the phytosomes of Curcumin was found to be yellowish to orange color powder and spicy and pungent odor.

ii. Percentage Yield, Entrapment Efficiency, and Drug Loading

The evaluation results of phytosome (TOP1 – TOP5) of curcumin shown in Table 31. TOP1 and TOP3 showed the lowest 41.51±1.24 % and highest 89.45±5.13 % yield, respectively.

iii. Entrapment Efficiency and Drug Loading

The entrapment efficiency and drug loading of phytosome of curcumin vary from 61.23±0.12 to 84.69±0.51 % and 01.82±0.23 to 13.93±0.40 %, respectively as shown in Table 31. It was found that the phytosome of curcumin (TOP4) showed the highest 84.69±0.51% entrapment efficiency and 09.45±0.61 % drug loading due to the proper bounding of curcumin with the polar head of soya lecithin as compare to the others.

iv. Particle Size and Zeta Potential

The particle size varies from 195.76±4.64 nm to 496.23±6.43 nm as shown in Table 10 and Figure 5 (blank) and Figure 6. It was found that the phytosome of curcumin (TOP5) showed the lowest 438.77±5.55 nm particle size due to the availability of numbers of curcumin molecules and combined interaction with soya lecithin as compared to the others, indicating uniformity in the particle size distribution. The zeta potential of the phytosome of curcumin (TOP4) was found to be -15.34 mV. These results suggest that the selected ratio of soya lecithin favored the formation of a phytosome, resulting in the formation of uniformly distributed nanosized phytosome.

Table No. 10: Evaluation of Final Batches of Phytosome of Curcumin

Sr. No.	Formulations Phytosome	Evaluation Parameters			
		Yield (%)	Entrapment efficiency (%)	Drug loading (%)	Particle size (nm)
1	TOP1	41.51±1.24	00.00±0.00	00.00±0.00	195.76±4.64
2	TOP2	47.78±2.39	61.23±0.12	01.82±0.23	496.23±6.43
3	TOP3	89.45±5.13	83.78±0.23	13.93±0.40	453.21±7.67
4	TOP4	77.52±3.57	84.69±0.51	09.45±0.61	476.72±3.67
5	TOP5	69.38±5.22	78.87±0.81	07.51±0.32	438.77±5.55

Mean ± SD, n = 3

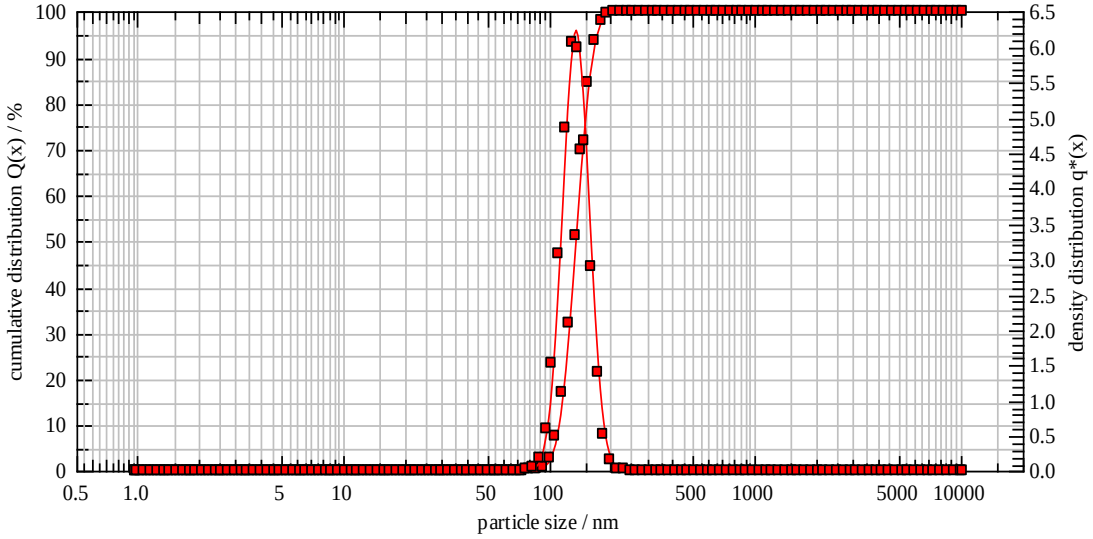

Figure No. 5: Particle size of blank phytosome of Curcumin (NOP1)

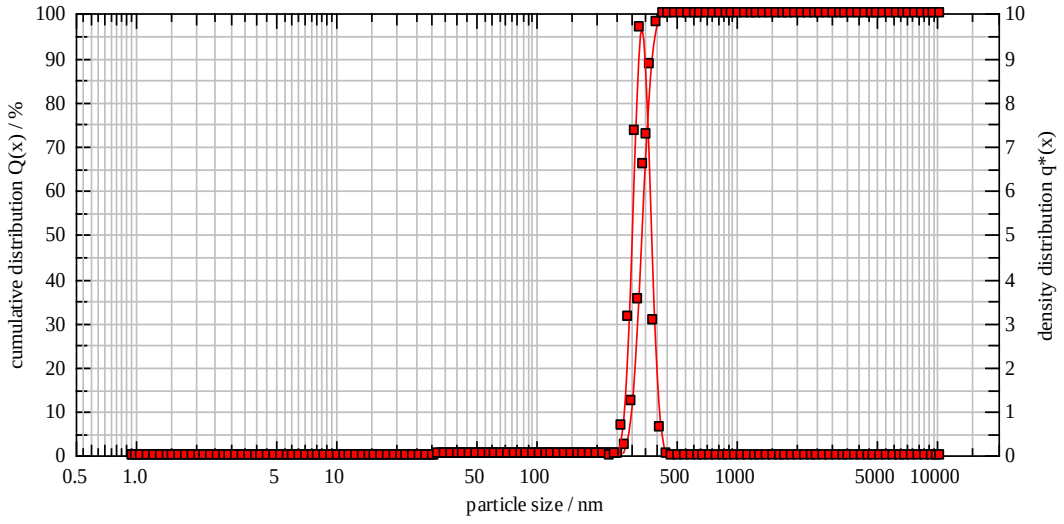

Figure No. 6: Particle size of Optimized Curcumin phytosome (TOP4)

v. FT-IR study

The optimized phytosome of curcumin TOP4 was characterized by the FT-IR spectrum as shown in Figure 7. The FT-IR spectrum of optimized phytosome TOP4 revealed the presence of a peak at 3875.00 cm^{-1} due to $V_{(o-H)}$ O-H stretching, while peaks at 3379.15 & 2970.53 cm^{-1} correspond to $V_{(c-h)}$ C-H asymmetric stretching and strong absorption peaks were 1585.94 cm^{-1} corresponds to v(c=o), v(c=o) C=C stretching, C=0 stretching was found in the FT – IR spectra of the physical mixture. In the FT-IR spectrum of the optimized phytosome TOP4, the major peak position of functional groups of curcumin and soya lecithin was not affected or changed.

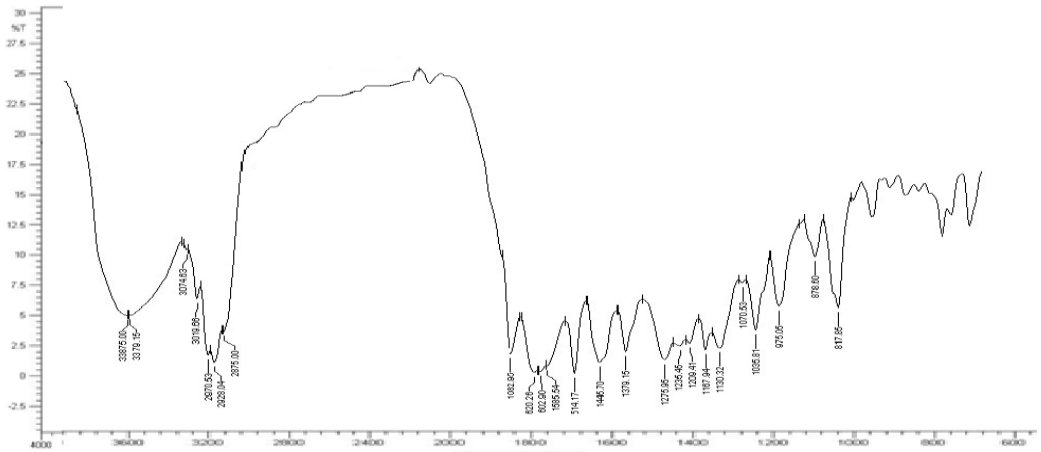

Figure No. 7: IR spectrum of Optimized Curcumin phytosome (TOP4)

vi. Differential Scanning Calorimetry (DSC) study

DSC thermogram compatibility study of optimized phytosome TOP4 is shown in Figure 8. The DSC thermogram of curcumin and optimized phytosome TOP4 showed a sharp melting endotherm at temperature 199.14°C indicating the absence of interaction between drug and excipients. Thus, it was concluded that the curcumin and soya lecithin did not interact with each other and optimized phytosome TOP4 was compatible.

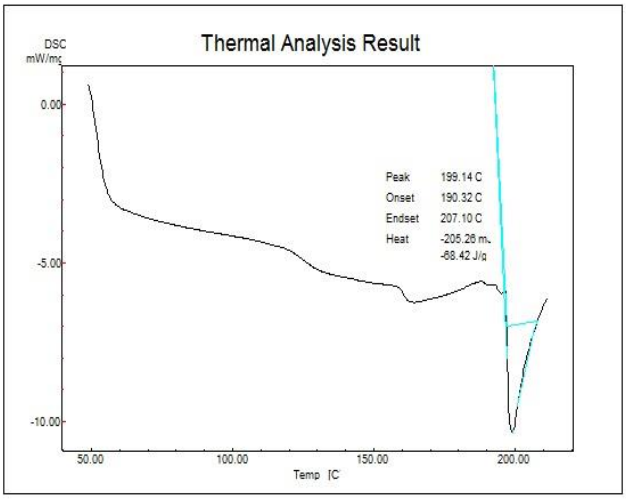

Figure No. 8: DSC thermogram of Optimized Curcumin phytosome (TOP4)

vii. Solubility Study

The solubility comparison of Curcumin and optimized phytosome TOP4 was observed in 0.1 N HCl (pH 1.2), pH 6.8 & pH 7.4 phosphate buffer, acetone, acetonitrile, methanol, ethanol, dichloromethane, distilled water, chloroform, and dimethyl sulfoxide are presented in Table 11. From the solubility comparison results, it was found that the phytosome of Curcumin TOP4 was showing better solubility profile (Figure 9) in all solvents as compared to the Curcumin due to the wettability and dispersion properties of soya lecithin.

Table No. 11: Solubility Comparison of Curcumin with Curcumin phytosome(TOP4)

Sr. no.	Solvents	Solubility Concentration (mg/mL)	
		Curcumin	TOP4
1.	pH 7.4	4.51 ± 0.03	5.04 ± 0.21
2.	Acetonitrile	1.02 ± 0.16	1.83 ± 0.29
3.	Methanol	5.03 ± 0.27	7.12 ± 0.04
4.	Ethanol	3.64 ± 0.31	4.69 ± 0.03
5.	DCM	4.76 ± 0.00	6.4 ± 0.16
6.	Water	0.81 ± 0.47	1.95 ± 0.07
7.	DMSO	3.97 ± 0.05	5.05 ± 0.04
8.	Ethyl acetate	2.07 ± 0.01	2.24 ± 0.09

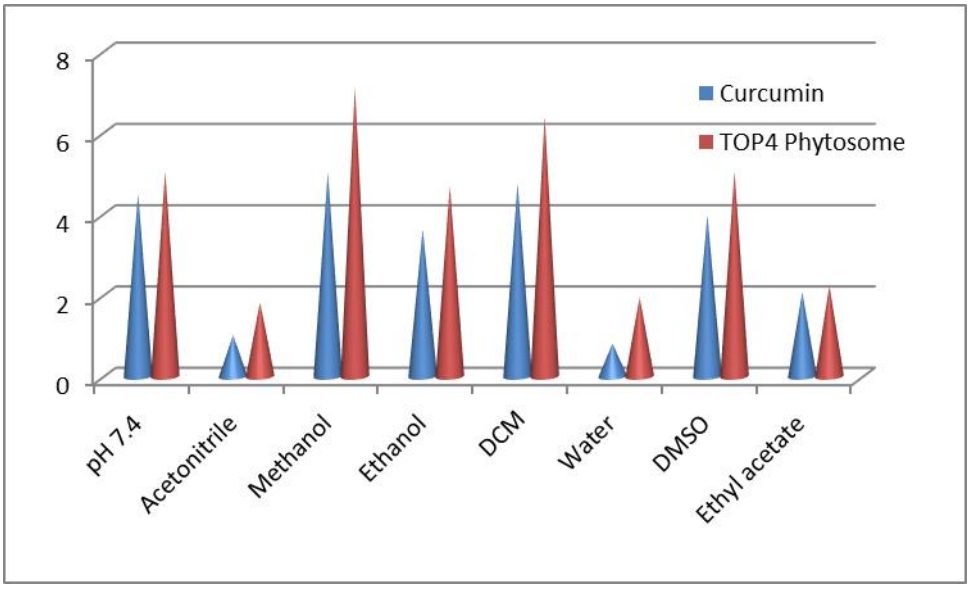

Figure No. 9: Solubility Comparison of Curcumin with Curcumin phytosome(TOP4)

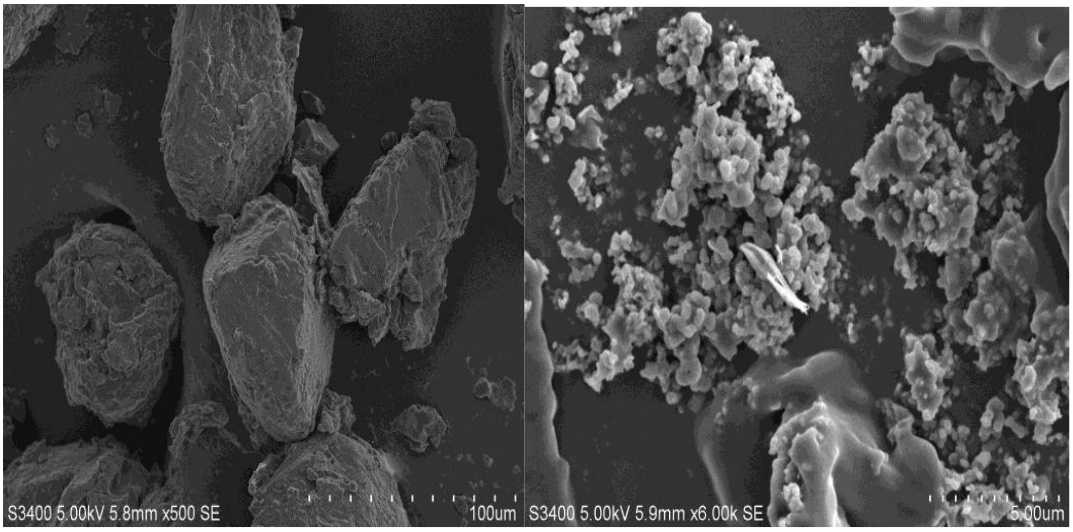

Figure No. 10: Surface morphology of optimized phytosome of Curcumin (TOP4)

viii. Scanning Electron Microscope (SEM)

The surface morphology, shape and structure of the optimized phytosome of Curcumin TOP4 at various magnifications are shown in Figure 10, by scanning electron microscope (SEM). It was observed that the Curcumin particles are associated with the polar head of soya lecithin that is forming phytosome TOP4 with irregular particle shapes, spherical and crystalline structures.

ix. *In-vitro* drug release

The in vitro cumulative drug release of phytosome of curcumin (TOP1- TOP5) given in Table 12. It showed that the highest 96.03±7.26% cumulative drug release of TOP4 at the end of 24 h.

Table No. 12: Cumulative percentage drug release of phytosome of Curcumin

Time (h)	TOP1	TOP2	TOP3	TOP4	TOP5
0.5	0.00±0.00	04.22±1.13	05.54±2.34	06.61±3.54	02.62±2.25
1	0.00±0.00	14.21±3.16	16.46±3.41	15.73±4.33	16.84±1.54
2	0.00±0.00	25.47±4.73	24.75±1.56	23.75±2.43	19.21±1.32
3	0.00±0.00	35.65±3.52	33.49±2.45	35.59±1.64	33.67±4.30
4	0.00±0.00	46.07±0.35	47.01±0.01	50.22±0.31	41.02±0.04
5	0.00±0.00	56.76±2.63	62.67±1.73	66.58±1.37	56.87±1.62
6	0.00±0.00	69.72±2.69	75.87±1.79	78.32±2.87	68.42±2.76
8	0.00±0.00	78.84±1.58	81.75±4.68	84.25±1.61	75.63±1.66
12	0.00±0.00	84.83±6.32	86.30±6.59	89.26±7.68	80.92±2.58
24	0.00±0.00	88.29±0.05	89.27±7.21	96.03±7.26	84.71±3.82

Mean ± SD, n = 3

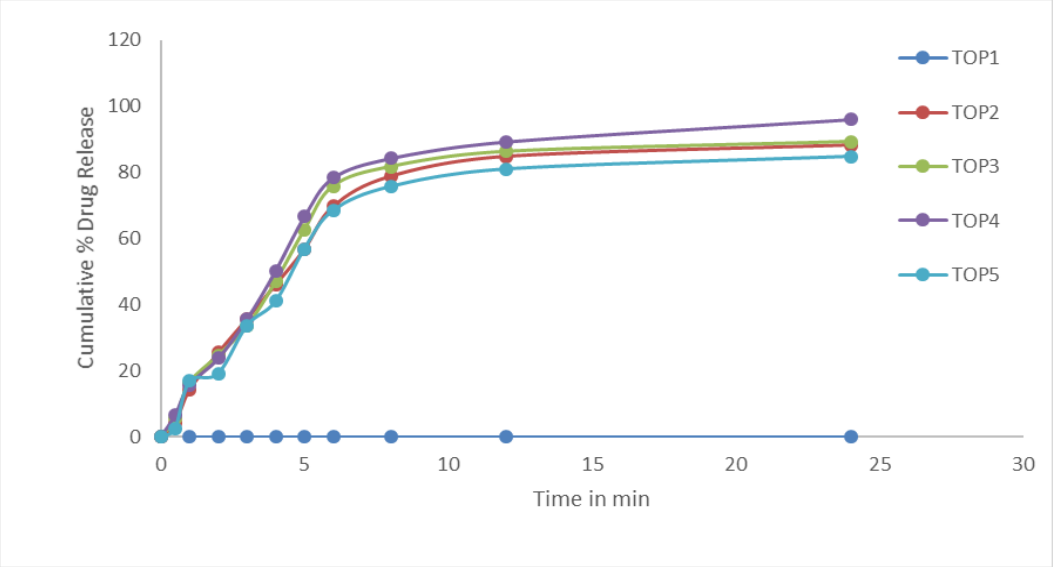

Figure No. 11: *In-vitro* cumulative drug release of phytosome of Curcumin

x. Stability studies

The results of the stability study of the optimized phytosome of Curcumin after 0 month, 1 month, 2 months and 3 months at different storage conditions are shown in Table 13 and are

illustrated in Figure 12. At predetermined intervals (0 month, 1 month, 2 months and 3 months), there was an insignificant increase in mean PS (particle size) of optimized phytosome of Curcumin that did not much affect % EE (Entrapment Efficiency) & % DL (Drug Loading) during the study period, indicating good stability of the optimized phytosomes. These results confirmed the compatibility of Curcumin with the polar groups of soya lecithin.

Table No. 13: Stability study data of phytosome of Curcumin

Time storage (month)	At 5±3˚C			At 25 ± 2˚C/60 ± 5 % RH			At 40 ± 2˚C/75 ± 5 % RH		
	% EE	%DL	PS (nm)	% EE	%DL	PS (nm)	% EE	%DL	PS (nm)
0	84.69± 0.51	9.45± 0.61	476.72± 3.72	84.69± 0.51	9.45± 0.61	476.72± 3.72	84.69± 0.51	9.45± 0.61	476.72± 3.72
1	84.53± 0.22	9.34± 0.21	474.42± 3.71	84.44± 0.61	9.34± 0.55	477.66± 3.34	83.43± 1.25	9.22± 0.23	471.73± 2.65
2	83.76± 1.67	9.67± 0.82	479.98± 2.83	84.98± 1.75	9.21± 1.93	479.43± 3.21	81.74± 7.22	9.21± 0.43	488.32± 3.51
3	83.32± 1.46	9.45± 0.61	483.65± 3.87	83.79± 16	9.11± 2.64	486.32± 2.87	79.22± 14	8.95± 3.31	489.21± 3.34

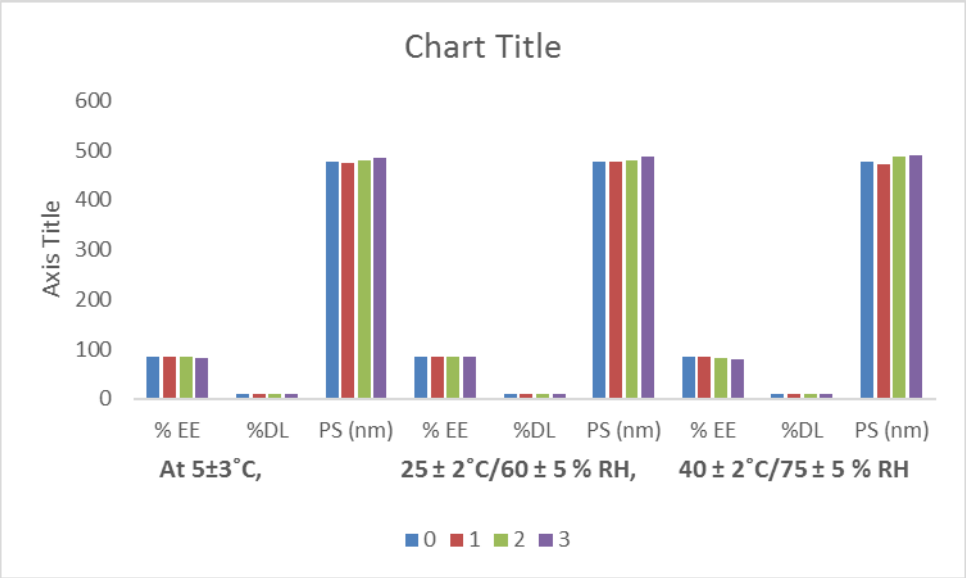

Figure No. 12: Stability study of phytosome of Curcumin

CONCLUSION

The present research work was directed towards the formulation of phytosome of plant drug, which would increase bioavailability and solubility of phytoconstituents through various mechanisms, improve the stability and also showed sustained drug release for 24 h.

Plant phytoconstituents, phospholipids soya lecithin, and their molar ratio and selection of method play a vital role in the formulation of phytosome. Based on trial batches, an anti-solvent precipitation technique was selected for the formulation of phytosome. Through anti-solvent precipitation technique, phytosomes in a different molar ratio such as 0:1, 1:1, 1:2, 2:1 and 2:2 were prepared for further studies.

Infra-red (IR) studies and Differential Scanning Calorimetry (DSC) revealed that there was no interaction between the phytoconstituents and phospholipids. The entrapment efficiency and drug loading rate of prepared phytosome of drug confirmed the effective loading of drug and also sustained delivery of drug at the specific target site. The particle size, zeta potential assured the nanoparticle size of prepared phytosome and confirmed the formation of phytosome within range. In vitro drug release showed the release rate of phytoconstituents through the phytosome formulation.

Based on evaluation parameters 2:1 molar ratio of phytosome formulation of the drug was selected for further evaluation like Scanning electronic microscopes that confirm that the actual shape and size of the prepared phytosome of a drug. Thus from the above observations, it can be concluded that the phytosome could be helpful for the treatment of various disorders and also to deliver the other plant phytoconstituents.

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