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Development and Assessment of Herbosomes

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ABSTRACT

Some of the approaches for bioavailability enhancement are formulating at nano scale as nanoparticles, binding with lipids as liposomes or herbosomes/phytosomes, delivery in the form of micro emulsions, modification in chemical structures, delivery as prodrug and complexation with cyclodextrins etc. 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. In addition, 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 body. The aim of this study was to prepare the phytosome of Curcumin and evaluate it. The phytosomes containing 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

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 under developed 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. Considerable attention has been focused on the development of novel drug delivery system (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, over the period of 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]

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 is 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 the recent time. Some of the approaches for bioavailability enhancement are formulating at nano scale as nanoparticles, binding with lipids as liposomes or herbosomes/phytosomes, delivery in the form of micro emulsions,

modification in chemical structures, delivery as prodrug and complexation with cyclodextrins etc. have a number of 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] To overcome of these problems and to make herbal therapy more effective, these drugs have been incorporated into several novel delivery systems in the recent time.

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 a number of 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 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 colour, odour and appearance.

b. Boiling Point

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

c. Physical Compatibility Test

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

2. Formulation and Development

a. Selection of plant drugs

A natural colouring carotenoid phyto-constituent was selected for the preparation of phytosome.

b. Selection of phospholipids

Selection of a phospholipid for the preparation of phytosome was on the basis of compatibility and interaction with drug.

c. 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)

3. Anti-solvent precipitation method

The required quantities of Curcumin and phospholipid were separately taken in a 100 ml round base flask and refluxed with 30 ml of methanol at a temperature not surpassing 60ºC 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 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 was prepared by mentioned methods in Table No.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 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

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 cover slip. 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:

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

5.3 Determination of particle size

The mean diameter of each formulation was estimated by dynamic light scattering (DLS) using particle size analyser (Zetasizer 2000, Malvern Instruments Ltd., UK) at a settled scrambling point of 90^{o}at 25^{o}C.

5.4 Determination of entrapment efficiency and drug loading

The entrapment efficiency and drug loading of formulations were dictated by centrifugation method (Remi Elektro Technik Ltd, Vasai, India). The formulations were separately centrifuged with 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:

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

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

6. Preparation of final batches of Phytosomes

The different molar ratio of phytosome of drug was prepared by the selected optimized working method, mentioned in Table No.2.

Table No.2: Final Batches of Phytosome of Drugs

Sr. No.	Curcumin Phytosomes	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 phytosomes)

Evaluation of final batches of phytosomes

The phytosomes of Curcumin were 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.

Physical Appearance

All the prepared phytosomes of curcumin were visually inspected for colour, odour and physical state.

Determination of % yield

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

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

Solubility study

Solubility study for phytosome of drug was determined in different solvents.

***In vitro* drug release**

On the basis of 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. Dissolution study was completed utilizing dissolution apparatus (Electrolab TDT-08L, Mumbai) by 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 drug was dipped into 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 the similar measure of phosphate buffer. The samples were assayed at respective wavelength 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).

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 No.3.

Table No.3: Organoleptic Properties of Curcumin

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

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

2. UV Spectroscopy studies

Wavelength of maximum absorption (λmax) of curcumin in phosphate buffer pH 7.4 and methanol are shown in Table No.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 No.6 & 7 and Figure No.1 & 2, 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	

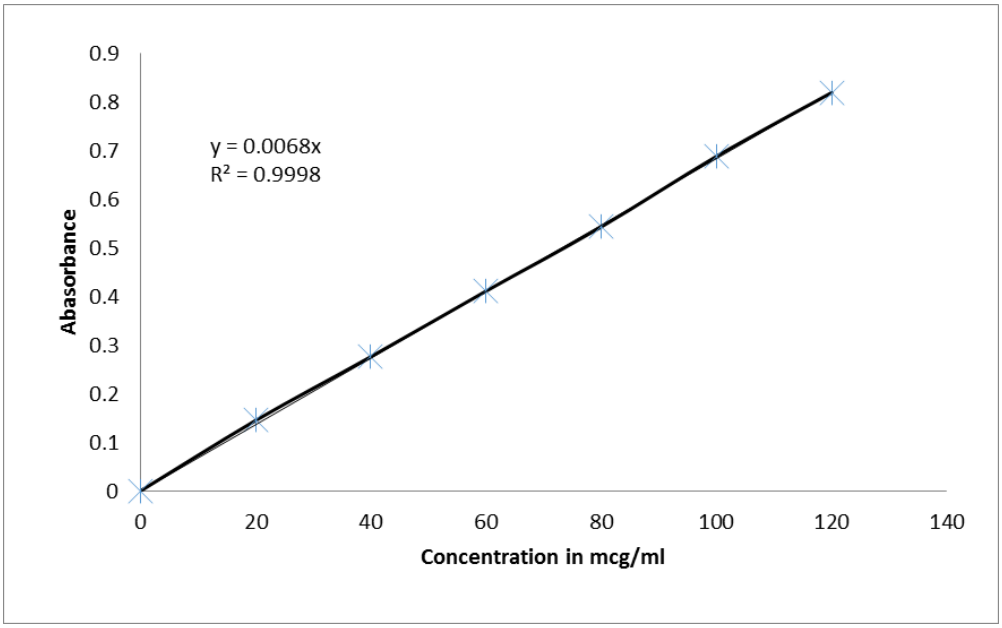

Figure No.1: Calibration Curve of Curcumin in pH 7.4 Phosphate Buffer

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

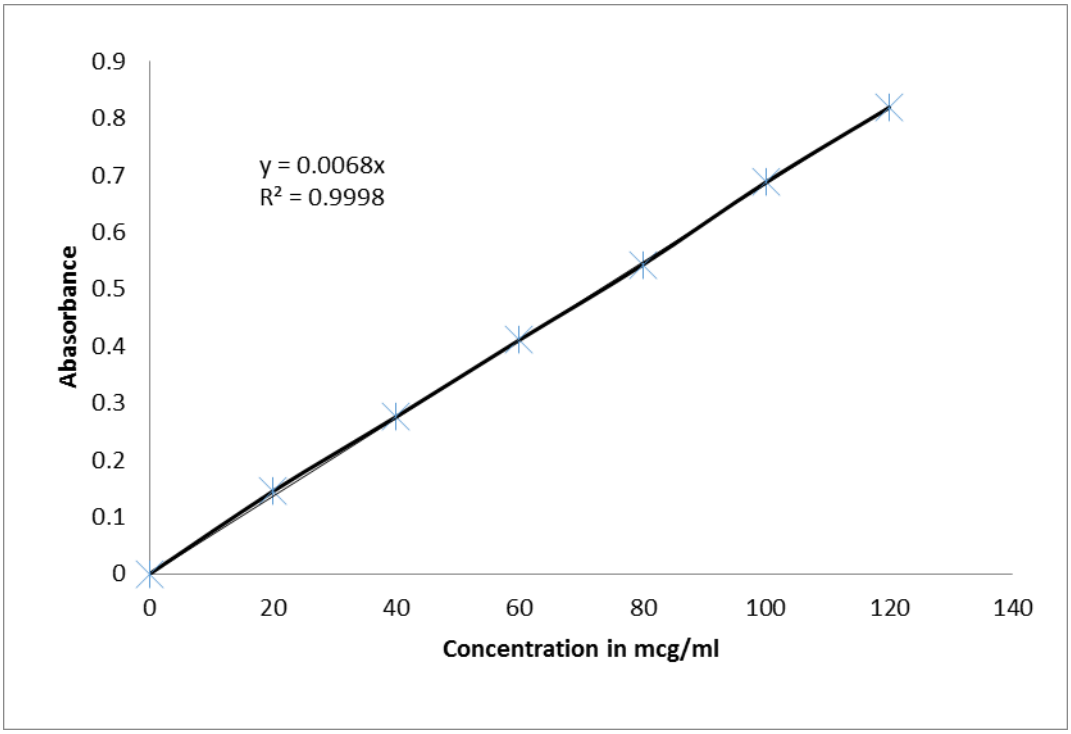

Figure No. 2: Calibration Curve of Curcumin in Methanol

(II) Formulation and Development

1. Selection of plant drug and phospholipids

The plant phyto-constituent polyphenolic carotenoid drug Curcumin was selected as antibacterial drug for the preparation of phytosome**.** Soya lecithin was selected as a phospholipid for the preparation of phytosome on the basis of compatibility and interaction with drug.

2. Selection of phospholipids

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

3. Selection of method

Preparation and Evaluation of preliminary batches for selection of working method

The preliminary batch of phytosome of Curcumin was prepared by different methods as mentioned earlier. The quantitative results indicate that phytosome of curcumin obtained from various methods shown in the Table No.8. 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 phytosome of Curcumin.

4. Preparation of final batches of Phytosome

The different molar ratio of phytosome of Curcumin was prepared by the anti-solvent precipitation method, mentioned in Table No. 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 was 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 colour powder and spicy and pungent odour.

ii. Percentage Yield, Entrapment Efficiency and Drug Loading

The evaluation results of phytosome (TOP1 – TOP5) of curcumin shown in Table 10. TOP1 and TOP3 showed 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 varies 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 No.10. It was found that phytosome of curcumin (TOP4) showed 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 No. 10 and Figure. It was found that phytosome of curcumin (TOP5) showed lowest 438.77±5.55 nm particle size due to the availability of numbers of curcumin molecule and combined interaction with soya lecithin as compared to the others, indicating uniformity in the particle size distribution. The zeta potential of phytosome of curcumin (TOP4) was found to be -15.34 mV. These results suggest that the selected ratio of soya lecithin favoured the formation of 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

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

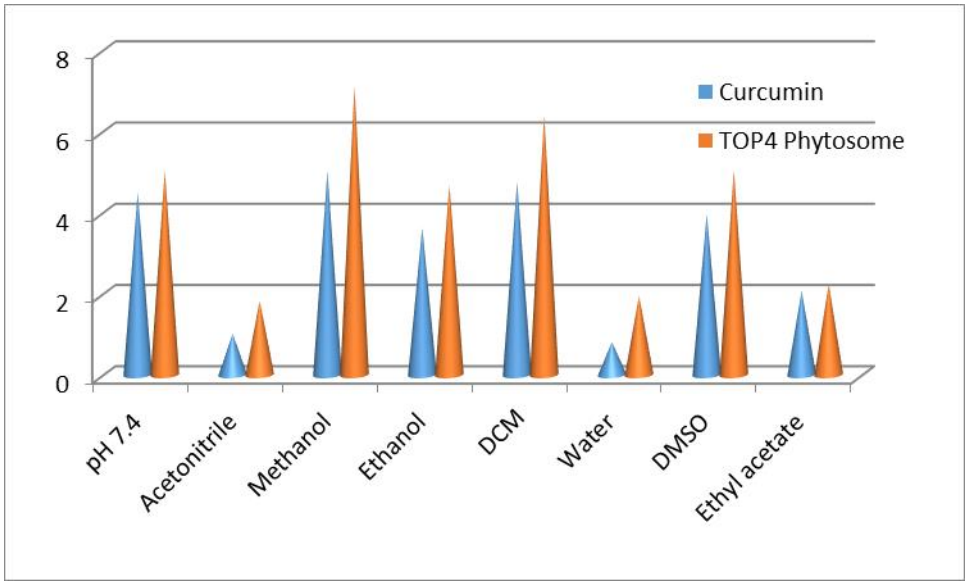

Figure No. 3: Solubility Comparison of Curcumin with Curcumin phytosome (TOP4)

v. Scanning Electron Microscope (SEM)

The surface morphology, shape and structure of the optimized phytosome of Curcumin TOP4 at various magnifications are shown in Figure, 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.

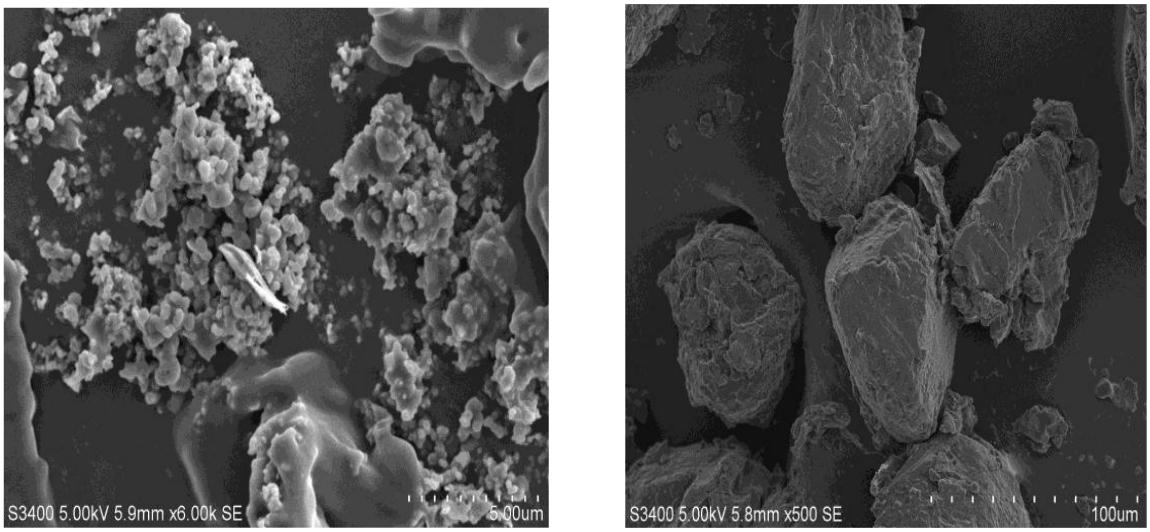

Figure No.4: Surface Morphology of Optimized Phytosome of Curcumin (TOP4)

vi. *In vitro* drug release

The *in vitro* cumulative drug release of phytosome of curcumin (TOP1- TOP5) given in Table No.12. It showed that 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

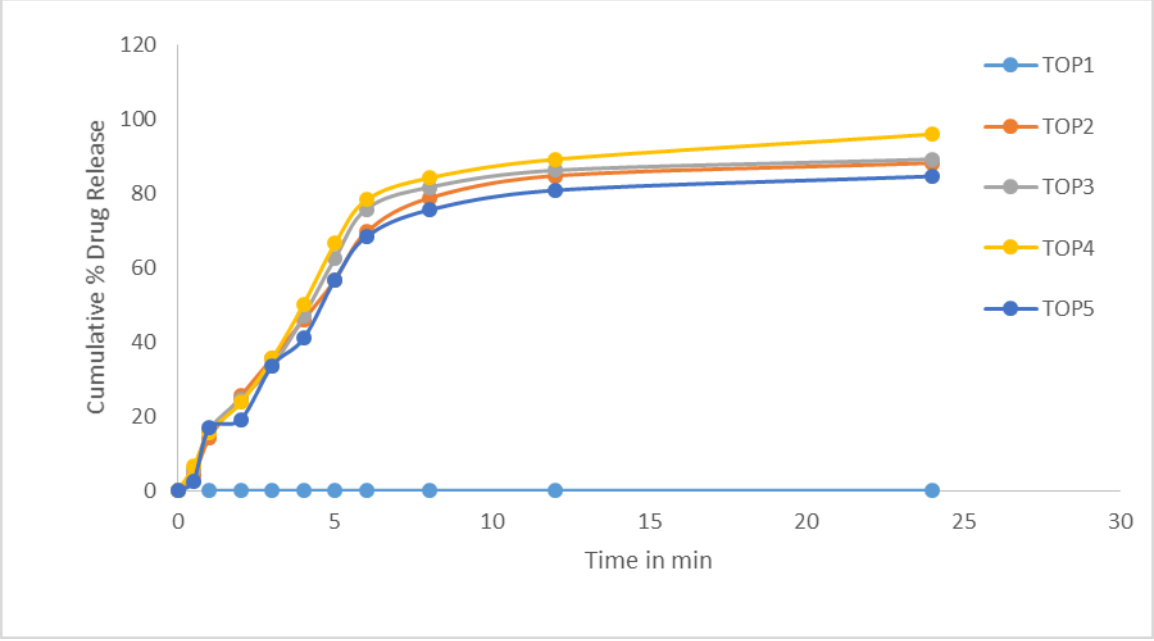

Figure No.5: *In vitro* Cumulative Drug Release of Phytosome of Curcumin

CONCLUSION

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

Plant phyto-constituent, phospholipids soya lecithin, and their molar ratio and selection of method play a vital role in the formulation of phytosome. Based on trial batches, anti-solvent precipitation technique was selected for the formulation of phytosome. Through anti-solvent precipitation technique, phytosomes in 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 phyto-constituent 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 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 phyto-constituent through the phytosome formulation.

Based on evaluation parameters 2:1 molar ratio of phytosome formulation of drug was selected for further evaluation like Scanning electronic microscopes that confirms that actual shape and size of the prepared phytosome of 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 phyto-constituents.

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