SSRG International Journal of Pharmacy and Biomedical Engineering
ISSN: 2394 – 2576 / doi:10.14445/23942576/IJPBE-V6I1P104
Volume 6 Issue 1, 29-34, Jan-Apr 2019

Preparation and Evaluation of Curcumin Phytosomes by Rotary Evaporation Method

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Abstract
The objective of the study was to develop a novel Phytosome formulation of curcumin by incorporation of phospholipids, for the improved permeability, solubility and better physical characteristics. The Curcumin-pyrosomes were prepared by using Rotary evaporator method and were optimized. The formations of phytosomes were analyzed for SEM, measurement of particle size & zeta potential, drug content, drug entrapment efficiency, Percentage yield,In-vitro drug release studies and also the release kinetics of curcumin phytosomes complex. The Curcumin phytosomes prepared by using Rotary evaporator method showed better practical yield, the drug content, drug entrapment efficiency, SEM, measurement of particle size & zeta potential and other In-vitro drug release studies resulted as anticipated. The Curcumin phytosomes were found to show better solubility and compatibility with the excipients. It is concluded that Curcumin phytosomes has better physical characteristics and improved permeability, solubility than that of curcumin drug to overcome the ability to cross lipid-rich biological membranes and which results in increase oral bioavailability.

Keywords - *Curcumin, Curcumin phytosome complex, Soy lecithin.*

I. INTRODUCTION

Plant preparations or their parts have been widely used in medicine since ancient times and till today use of phytomedicines is widespread. Most of thebiologically active constituents of plants are polar or water-soluble. However, water-soluble phytoconstituents are poorly absorbed due to macromolecular 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.[1]

Phytosomes are defined “Phyto” means plants and some means cell-like, which is a novel drug delivery system,Phytosome is a newly introduced patented technology developed to incorporate the water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called phytosomes.[2] Phytosomes provide better absorption and bioavailability than the Conventional herbal drugs. When a stoichiometric amount of the phospholipid was made to react with the purified herbal drug in an aprotic solvent, phytosomes were formed. This project aims in improving the drug release characteristics of curcumin by formulating Curcumin phytosome.[3]

Curcumin longa, commonly known as “Turmeric” (Family: Zingiberaceae) is well known for its therapeutic use in the Ayurvedic system of traditional medicine. Chemical constituents include Curcuminoids, Curcumin (curcuminin I), Demethoxycurcumin (Curcumin II), Bisdemethoxy curcumin (Curcumin III),Cyclocurcumin.[4] The Curcumin longa are reported to show anticancer properties, including the prevention of regrowth of cancer cells, reduce angiogenesis it means the growth of new blood vessels in tumours and reduce metastasis, and it inhibitsthe transformation of cells from normal to the tumour,and it also inhibits vascular epithelial

Growth factors every tumour need a blood supply the growth factors build one, butcurcumin was shown to stop them. In this study, a complex of curcumin and soy lecithin was prepared, and the physicochemical properties of the complex were investigated.[5]

II. MATERIALS AND METHODS

Materials: Curcumin powder was obtained as a gift sample from Loba Chemieherbs Pvt Ltd, Mumbai; Soy lecithin from VitaeGen Life Sciences and solvents are Dichloromethane, used were of analytical grade.
Equipment: UV-Visible spectrophotometer (Shimadzu UV-1800), Particle size analyzer (Microtrac), FTIR (Agilent Technologies).

A. Characterization of powdered drug Curcumin:

a) Organoleptic properties

Curcumin was observed for its organoleptic properties like colour, solubility and wavelength maxima.

b) Solubility profile of curcumin:

The solubility of curcumin was determined in different solvents such as Dichloromethane, Ethanol, Acetone,Phosphate buffer 6.8.

c) Determination of wavelength maxima

Concentration 10 µg/ml of curcumin dissolved in a mixture of 3% tween 80 and 6.8 phosphate buffer (2:8) scanned over a wavelength range of 400-800nm.[6]

d) Compatibility Studies

FTIR spectroscopy can be used to investigate and predict any physicochemical interactions between curcumin and soy lecithin in a formulation. Therefore it can be applied to the selection of suitable chemically compatible excipients. The present study aimed to test whether there is any interaction between the carriers and drug.[7-8]

B. Preparation of Curcumin Phytosomes Complex (Cpc)

Curcumin phytosomes complex was prepared by using Rotary evaporator method.

a) Rotary evaporation technique:The specific amount of curcumin and soya lecithin were dissolved in Dichloromethane in a rotary round bottom flask followed by stirring for 1 hour at a temperature not exceeding 40ºC. Thin-film of the sample was obtained to which n-hexane was added and continuously stirred until a monolayer of phospholipid and then add phosphate buffer 6.8 and precipitate obtained was collected, placed in an amber-coloured glass bottle and stored at room temperature.[9]

C. Characterization of Curcumin Phytosomes complex (Cpc)

a) Scanning Electron Microscopy (SEM)[10]

Scanning electron microscopy study was done to determine the surface morphology, size and shape of prepared Curcumin phytosomes formulation. The optimized freeze-dried Phytosomes was subjected for Scanning electron microscopy and photographed in fig: 6.

b) Measurement of particle size (PS)

The particle size of Curcumin phytosomes was measured by particle size analyzer (Microtrac). For the measurement, 300µm of the formulation was diluted with an appropriate volume of PBS pH 6.8, and the vesicle diameter was determined.[11]

c) Measurement of Zeta potential (ZP)

Zeta potential is the most important parameter for the physical stability of phytosomes. The higher the electrostatic repulsion between the particles, the greater is the stability. Zeta potential value ±20 mV predicts good physical stability of the dispersion. Zeta potential measurement of the optimized phytosome suspension was done by using the Microtrac. For the measurement, 1ml of the sample was diluted to 10ml with water, 5ml of this diluted sample was transferred to a cuvette, and the zeta potential was measured.[12]

d) Determination of % yield

The following formula13 calculated determination of % yield of phytosome complex:

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

e) Determination of drug content

Drug content of phytosome complex was determined by dissolving accurately weighed 10 mg of a complex in 10 ml methanol. After suitable dilution absorbance was determined by UV – Spectrophotometer at 426nm and drug content was determined.[14]

f) Entrapment efficiency (EE)

Curcumin phytosomes were centrifuged at 12000 rpmfor 45 min using a Remi centrifuge to separate phytosomes from the unentrapped drug. The concentration of the free drug as the supernatant was determined by measuring absorbance at 426nm using UV-Visible spectrophotometer. The percentage drug entrapment was calculated by using the formula.[15]

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of Encapsulated Drug X 100}}{\text{Amount of Drug added}}$$

g) In vitro drug release studies

The release of drug was determined by using the treated cellophane membrane mounted on the one end of the open tube, containing phytosome(equivalent to 50mg curcumin). The dialysis tube was suspended in 500ml beaker, containing 250ml of phosphate buffer 6.8. The solution was stirred at 100 rpm with the help of magnetic stirrer at 37±0.5°C, and then 1 ml sample of was withdrawn at definite time intervals, and equivalent volumes of fresh PBS were added in. All samples were filtered, diluted and analyzed by UV spectrophotometer. The permeation of the complex was compared with the Curcumin drug.[16]

h) Determination of release kinetics of Curcuminphytosomes complex

To study the release kinetics of the Curcumin phytosome from the formulation, data obtained from diffusion studies were computed in different kinetics model of (a) zero-order (cumulative percent drug released vs time) (b) first order (Log cumulative percent drug retained vs time) (c) Higuchi (Log cumulative percent drug released vs square root of time) (d) Peppas release kinetics equation (Log of cumulative % release Vs log time). The regression coefficient values of different release kinetics equations were evaluated by computing the data of release profiles of optimized Curcumin phytosomeformulation. The computed Curcumin phytosome release kinetics was shown in table

number3, and the data are summarized; the value of K (release rate constant) was calculated from the slope of the diffusion profiles.[17-18]

Stability Studies:The stability of Phytosomes was carried out as per ICH guidelines. The optimized formulations were stored at different temperature ranges $5^0C \pm 3^0C$, $30^0C \pm 2^0C/65\%\pm5\%$ RH, $40^0C\pm 2^0C/75\%\pm5\%$ RH for 3 months and studied for drug content and drug release.[19]

III. RESULTS AND DISCUSSION

A. Characterization of powdered drug curcumin

Organoleptic properties: Curcumin drug was analyzed for their organoleptic properties like colour, Solubility and wavelength maxima of the drug. From the results, it was concluded that curcumin was found to be soluble in phosphate-buffered saline (PBS pH 6.8) and Dichloromethane. The concentration 10µg/ml of Curcumin drug in phosphate-buffered saline was found to be 426nm.

B. Standard calibration curve of Curcumin in UV spectrophotometer

The UV absorbance of Curcumin standard solution in the range of 10-60µg/ ml of drug in phosphate-buffered saline pH 6.8 showed linearity at λ max 426nm. The linearity was plotted for absorbance against concentration with R^2 value 0.999 and with the slope

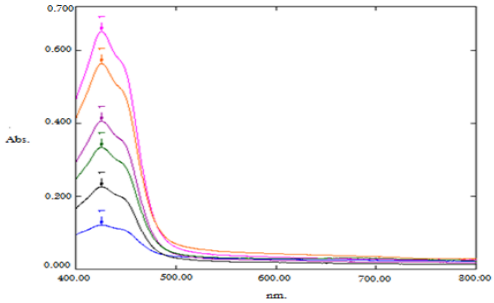

Figure1: UV Spectra of curcumin

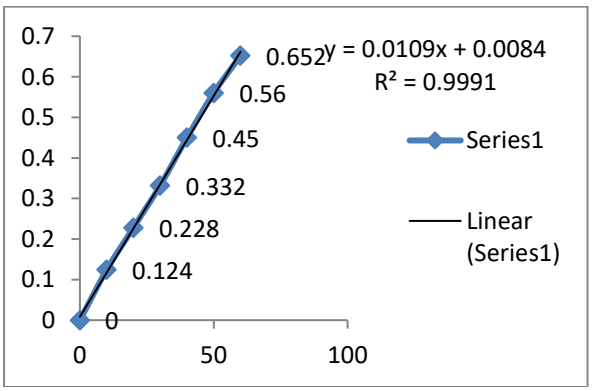

Figure 2: Standard calibration curve of curcumin

C. Compatibility studies

The compatibility between the Curcumin and Soy lecithin 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 as shown in Figure 3 and 4

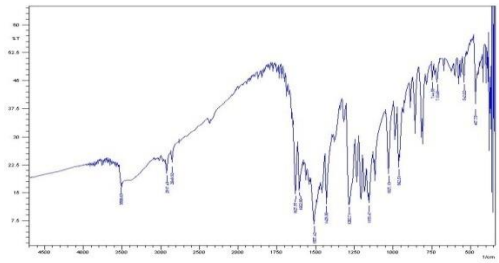

Figure 3: FTIR graph of Curcumin

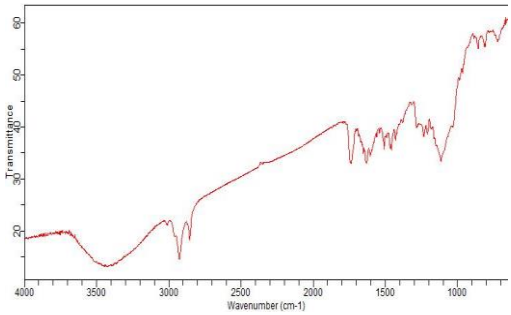

Figure 4: FTIR graph of curcumin soya lecithin

D. Preparation of Curcumin phytosomes complex

Curcumin phytosomes complex prepared by rotaryevaporator method in the ratios of (1:1, 1:2, 1:3, 1:4, and 1:5) by varying the polymer concentration (Table 1)

Table 1: Formulation of curcumin phytosomes

SL. No	Formulation Code:	Ratio of Drug: Soy lecithin	Dichloro methane (ml)	Hexane (ml)	PBS (ml)
01	FB1	1:1	20ml	15ml	5ml
02	FB2	1:2	20ml	15ml	5ml
03	FB3	1:3	20ml	15ml	5ml
04	FB4	1:4	20ml	15ml	5ml
05	FB5	1:5	20ml	15ml	5ml

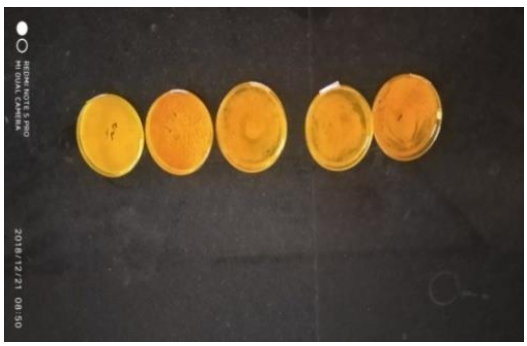

Figure5: FB1-FB5 Curcumin phytosomes

E. Characterization of Surface morphology of Curcumin Phytosomes Complex by scanning electron microscopy view

The SEM view of the Curcumin phytosomes complex indicated the presence of sphere-shaped vesicle

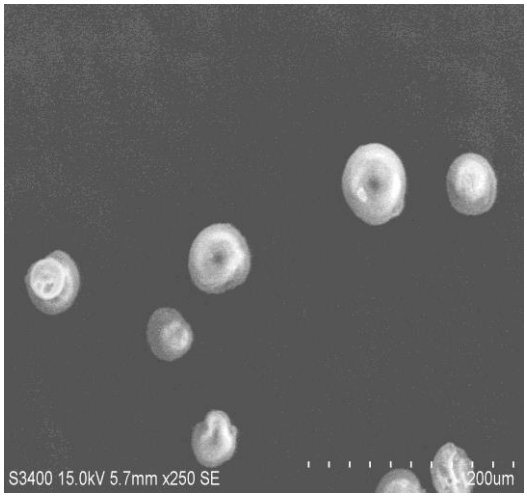

Figure6: SEM of Curcumin phytosomes FB5

F. Particle size and Zeta potential of Curcumin phytosomes

An idealbatch of prepared phytosomes was analyzed to determine their particle size distribution and zeta potential values. It was observed that the average particle size was found to be 181.6nm for formulation FB5 and zeta potential value was found to be 33.2 mV indicating better stability of the formulationFB5. The results were graphically represented in Figure 7.

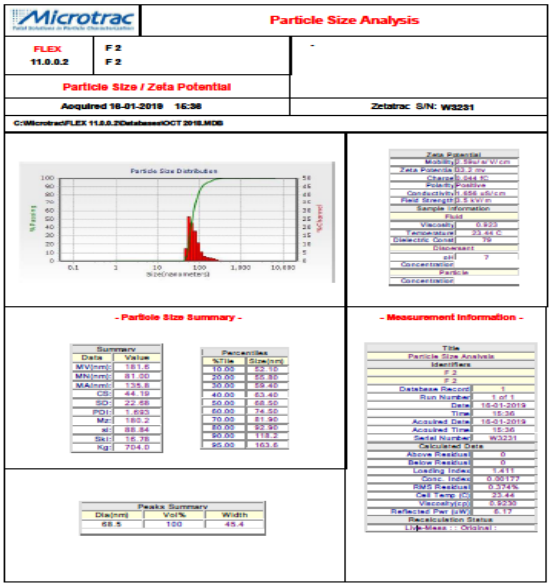

Figure7: PS & ZP of Curcumin phytosomeFB5

G. Percentage yield, % Drug content, % Entrapment Efficiency of Curcumin phytosomes

The percentage yieldof curcumin phytosomes FB5 that contains curcumin and soy lecithin in the ratio 1:5 showed maximum percentage yield than compared to FB1-FB4. % Drug content of the phytosome complexes was studied andpresented in table number2. The percentage of drug content was foundin a range of 78-88%. The % entrapment efficiency of the phytosome complexes was studied andpresented in Table number2. The % entrapment efficiency was foundin a range of 76-87%. The results showed better % Drug content & % Entrapment Efficiency.

Table2: Formulation and physicochemical characterization of Curcumin phytosomes prepared by Rotary evaporation method.

Sl. No	Formulation Code	Percentage yield	%Drug content	%Entrapment Efficiency
01	FB1	70.28	78.98	76.95
02	FB2	75.65	83.43	81.21
03	FB3	80.92	86.89	83.56
04	FB4	86.84	87.64	86.42
05	FB5	89.57	88.91	87.61

H. In vitro release data

The prepared Curcumin phytosomes complex was loaded in a diffusion cell, and the receptor compartment was filled with PBS. The diffusion cell was maintained at 37 ± 5^0C. For all the prepared formulations 50mg equivalent phytosomes were taken. Cumulative drug released for FB5 was found to be 77.12% respectively. It was apparent that the *In-vitro* release of curcumin showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggest that some drug was localized on the surface of the phytosomes.

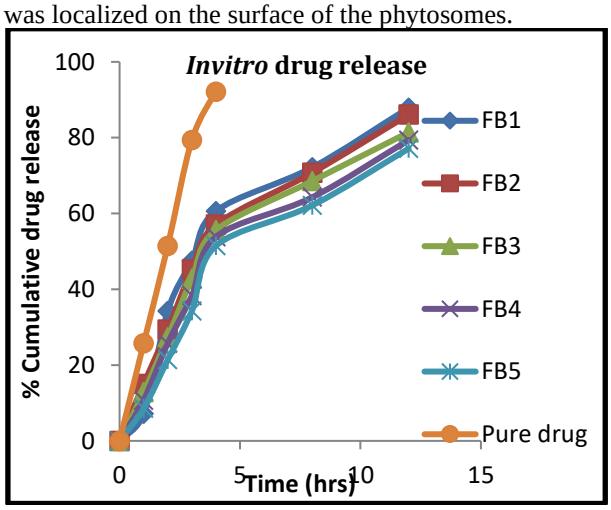

Figure8:*In-vitro* drug release of curcumin phytosomesFB1-FB5

I. Release Kinetics

In-vitro release profiles of all the formulation were fitted to various kinetic model and release profile represented in table number 3. It was found that all the formulation follows Zero order, First order, Higuchi, Peppas model. The 'n' values for all the formulation were found to be more than

0.5. This indicates that the release approximates non-Fickian diffusion mechanism.

Table3: correlation coefficients according to different kinetic equations of FB1-FB5

Formulation code	Zero-order r^2	First-order r^2	Higuchi plot r^2	Pappas plot	
				r^2	n
FB1	0.837	0.975	0.936	0.934	0.633
FB2	0.876	0.981	0.970	0.940	0.690
FB3	0.867	0.971	0.961	0.926	0.728
FB4	0.878	0.965	0.957	0.919	0.922
FB5	0.891	0.964	0.947	0.922	0.919

J. Stability Studies

Curcumin phytosomes were stored at refrigerated temperature and room temperature and for 3 months and Drug release & % Drug content was determined. Stability studies were conducted for optimized formulation FB5, which showed better drug release. The results showed no significant changes in % drug content and drug release with stored in refrigerator temperature and room temperature. Thus we conclude that the drug does not undergo degradation on storage.

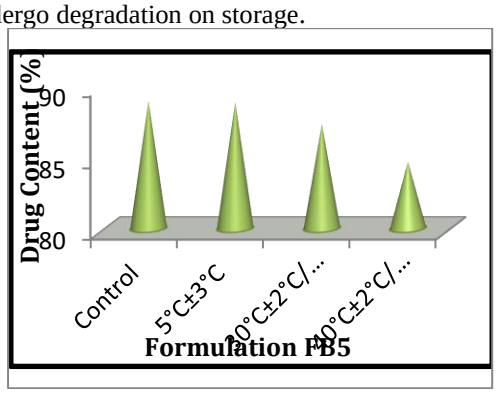

Figure9: Stability study: comparison of % drug content of formulation FB5
at 5±3℃, room temperature and 40℃ ± 2℃/75%RH

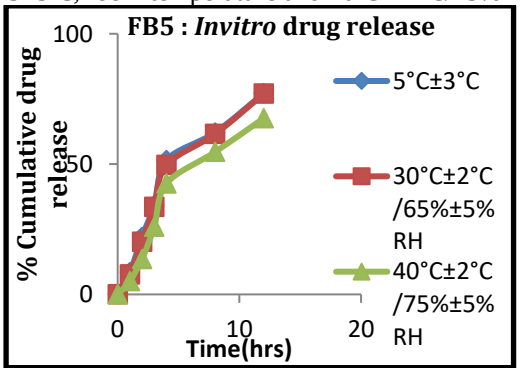

Figure10: Stability study: comparison of *In-vitro* the drug release profile for formulation FB5
at 5± 3℃, roomtemperature and 40℃ ± 2℃/75% RH after three months storage

V. CONCLUSION

The Phytosomes containing curcumin were prepared by Rotary evaporation method. The Phytosomes prepared byRotary evaporation methodwas found to be the discrete and spherical shape. The Phytosomes which were prepared by the Rotary evaporation method were having better particle size, % yield, which increases the absorption of the drug. Zeta potential was found to be more than 20mV, which shows better stability. The entrapment efficiency of formulated Phytosomes increases with increasing polymer concentrations. Based on drug content, entrapment efficiency, particle size, surface morphology, zeta potential and *In-vitro* release data's, the Rotary evaporation method was found to be the best method. The stability studies were carried out for the selected formulations, and the results showed that maximum drug content and closest *in-vitro* release to previous data was found for them, stored at 5˚C, room temperature and 40℃ ± 2℃/75% RH. Thus the Phytosomes of Curcumin by Rotary evaporation method with the core: coat ratio 1:5 was found to be spherical, discrete and able to control the drug release effectively.

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