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PREPARATION AND EVALUATION CURCUMIN PHYTOSOMES BY REFLUX 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-phytosomes were prepared by using Reflux 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 Reflux method showed better practical yield, the drug content, SEM, measurement of particle size & zeta potential and other *in vitro* drug release studies were 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 ability to cross lipid-rich biological membranes and which results in increase oral bioavailability.

INTRODUCTON

Plant Preparations or their parts have been widely used in medicine since ancient times and till today use of phytomedicines is wide spread. Most of the biologically 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 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 growth of new blood vessels in tumors and reduce metastasis and it inhibit transformation of cells from normal to tumor and it also inhibit vascular epithelial growth factors every tumor need a blood supply the growth factors build one but curcumin 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]

MATERIALS AND METHODS

Materials: Curcumin powder was obtained as gift sample from LobaChemieherbspvt ltd, Mumbai; Soy lecithin from VitaeGen Life Sciences, and solvents are Dichloromethane, used were of analytical grade. Equipments: UV-Visible spectrophotometer (Shimadazu UV-1800), Particle size analyzer (Microtrac), FTIR (Agilent Technologies).

Characterization of powdered drug Curcumin: Organoleptic properties: Curcumin was observed for its organoleptic properties like color, solubility and wavelength maxima. Solubility profile of curcumin: Solubility of curcumin was determined in different solvents such as dichloromethane, Ethanol, Acetone,Phosphate buffer 6.8. Determination of wavelength maxima: Concentration 10 µg/ml of curcumin dissolved in mixture of 3% tween 80 and 6.8 phosphate buffer (2:8) scanned over a wavelength range of 400-800nm.[6]

Compatibility Studies: FTIR spectroscopy can be used to investigate and predict any physicochemical interactions between Curcumin and soy lecithin in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients. The aim of the present study was to test, whether there is any interaction between the carriers and drug.[7]

Differential scanning Calorimetry (DSC): Curcumin, soya lecithin phospholipids, physical mixture of Curcumin and soya lecithin and phytosome complex of Curcumin were placed in the aluminum crimp cell and heated at 10^0C/min from 0 to 400^{o}C in the atmosphere of nitrogen. Peak transition onset temperatures were recorded by means of an analyzer.[8]

Preparation of Curcumin Phytosomes Complex (Cpc): Curcumin phytosomes complex were prepared by using Reflux method.

Solvent evaporation method: Curcumin phytosomes complex in the ratios of (1:1, 1:2, 1:3, 1:4, and 1:5) were prepared by reflux method. Curcumin and soy lecithin were placed in a 100ml round bottom flask and refluxed in dichloromethane for 1hr not exceeding 40°C. The resultant clear solution was then evaporated and 15ml of n-hexane was added and than add phosphate buffer 6.8 until precipitate was formed. The precipitate was collected and placed in desiccator.[9]

Characterization of Curcumin Phytosomes complex (Cpc)

Scanning Electron Microscopy (SEM): 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: 7.

Measurement of particle size: 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]

Measurement of Zeta potential

Zeta potential is the most important parameter for physical stability of phytosomes. The higher the electrostatic repulsion between the particles the greater is the stability. Zeta potential value more than +20 mV or less than -20 mV predicts good physical stability of dispersion. Zeta potential measurement of the optimized phytosomesuspension 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]

Determination of % yield: Determination of % yield of phytosome complex was calculated by the following formula: [13]

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

Determination of drug content: Drug content of phytosome complex was determined by dissolving accurately weighed 10 mg of complex in 10 ml methanol. After suitable dilution

absorbance was determined by UV – Spectrophotometer at 426nm and drug content was determined.[14]

Entrapment efficiency (EE)

Curcumin phytosomes were centrifuged at 12000 rpm for 45 min using a Remi centrifuge to separate phytosomes from unentrapped drug. 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]

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

***In vitro* drug release studies:** The release of drug was determined by using the treated cellophane membrane mounted on the one end of 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]

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 phytosomerelease kinetics was shown in Fig: 13-16 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 ± 3^0C, 30^0C ± 2^0C/65%±5% RH, 40^0C± 2^0C/75%±5% RH for a period of 3 months and studied for drug content and drug release.[19]

RESULTS AND DISSCUSSION

Characterization of powdered drug Curcumin: Organoleptic properties: Curcumin drug was analyzed for their organoleptic properties like color, Solubility and wave length maxima of 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.

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 equation y = 0.0109x +0.008 as shown in Figure 1 and 2

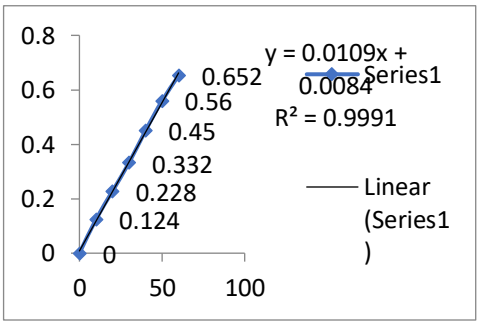

Figure 1: Standard calibration curve of Curcumin

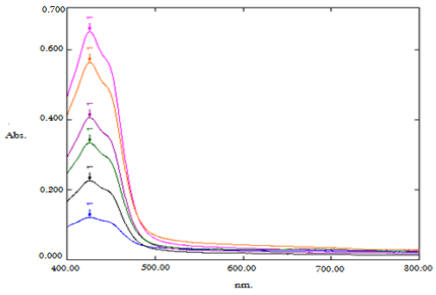

Figure2: UV Spectra of curcumin

Compatibility studies: The compatibility between the Curcumin and Soy lecithin was evaluated using FTIR peak matching method. There was no appearanceor 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

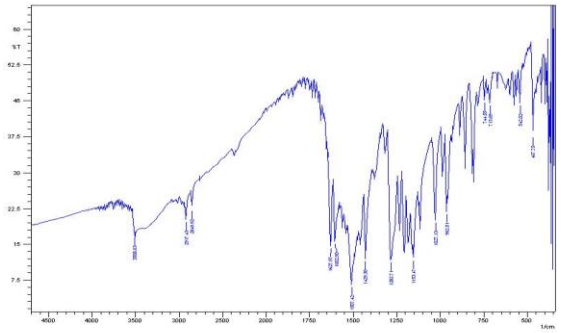

Figure 3: FTIR graph of Curcumin

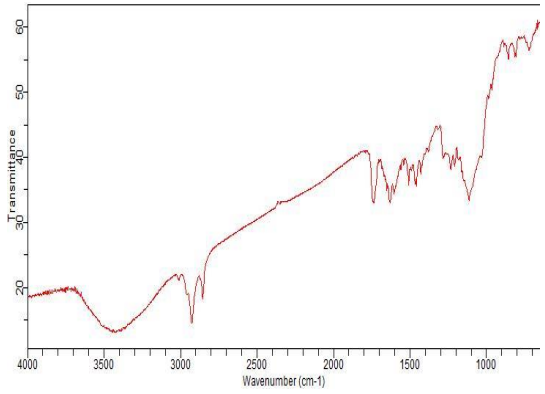

Figure 4: FTIR graph of curcumin soya lecithin

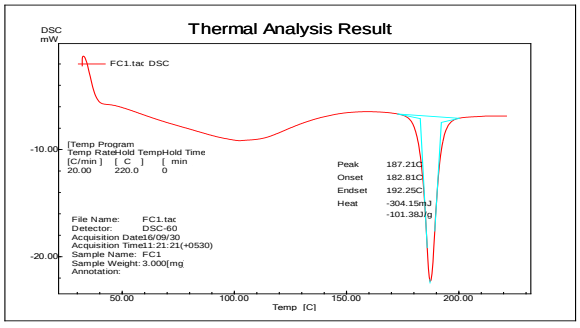

Figure 5: DSC Thermograph of Curcumin

Figure 6: FA1-FA5 Prepared Curcumin phytosomes

Differential scanning colorimetry of Curcumin: Melting point determination of curcumin was reported at 170-188°C by using differential scanning colorimetry (DSC) was observed in this range.

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

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 vesicles.

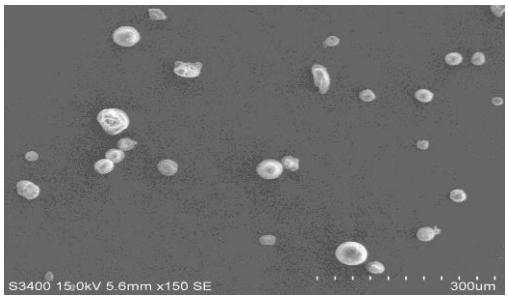

Figure7:FA5 Surface morphology of Curcumin phytosomes

Particle size and Zeta potential of optimizedCurcumin phytosomes: Optimized phytosomes were analyzed to determine their particle size distribution and zeta potential values. It was observed that the average particle size was found to be 152nm for optimized formulation FA5 and zeta potential value was found to be 30mv indicating better stability of the formulation FA5. The results were graphically represented in Figure 8.

Percentage yield, % Drug content, % Entrapment Efficiency of Curcumin phytosomes: The percentage yield of curcumin phytosomes FA5 that contains Curcumin and soy lecithin in the ratio 1:5 showed maximum percentage yield than compare to the FA1-FA4. % Drug content of the phytosome complexes were studied and presented in figure10. The percentage of drug content was foundin a range of 78-88%. % entrapment efficiency of the phytosome complexes were studied andpresented in figure11. The % entrapment efficiency was found in a range of 76-87%.

SL. No	Formulation Code: (Reflux method)	Ratio of Drug : soy lecithin	Dichloromethane (ml)	Hexane (ml)	PBS (ml)
01	FA1	1:1	20ml	15ml	5ml
02	FA2	1:2	20ml	15ml	5ml
03	FA3	1:3	20ml	15ml	5ml
04	FA4	1:4	20ml	15ml	5ml
05	FA5	1:5	20ml	15ml	5ml

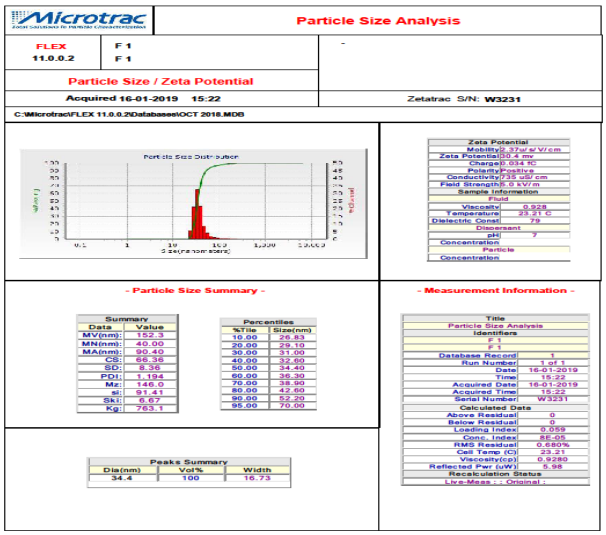

Figure8: FA5 PS & ZP of Curcumin phytosome

The results showed Better % Drug content & % Entrapment Efficiency.

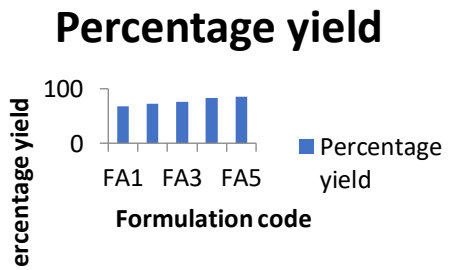

Figure 9: FA1-FA5 Percentage yield of phytosomes

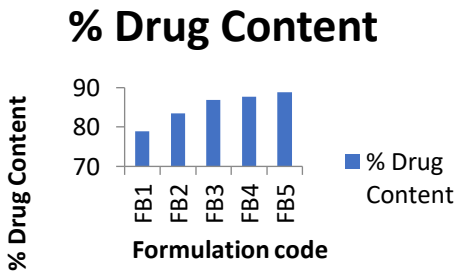

Figure10: FA1-FA5 % Drug content of C. Phytosomes

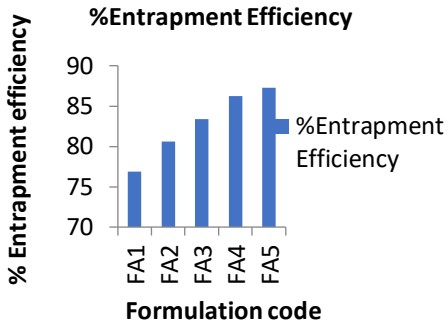

Figure11: FA1-FA5 % E.Efficiency of C.Phytosomes

***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^{0}C. For all the prepared formulations 50mg equivalent phytosomes were taken. It was observed that the formulation FA5 (79.36 %) showed controlled release compared to other formulations.

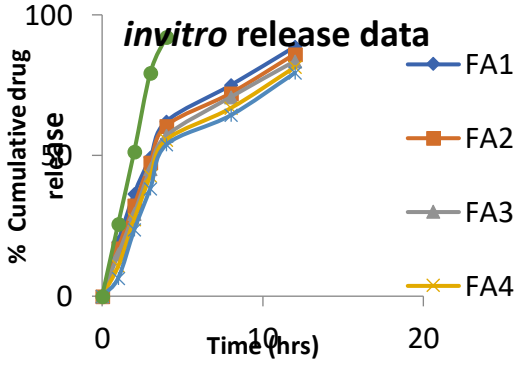

Figure12:FA1-FA5 *in-vitro* diffusion of curcumin phytosomes

Release Kinetics

*Invitro*release profiles of all the formulation were fitted to various kinetic model and release profile represented graphically in fig. 13-16. 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 then 0.5. This indicates that the release approximates non-Fickian diffusion mechanism.

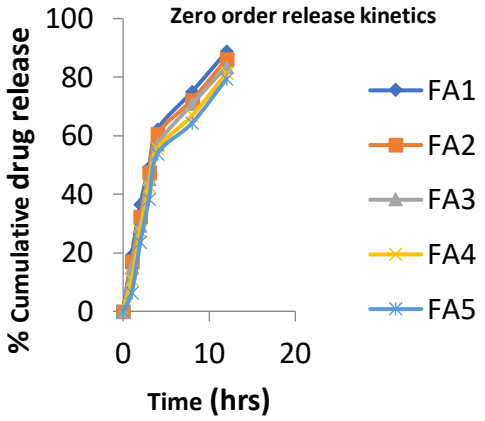

Figure13: FA1-FA5 Zero order release of C.Phytosomes

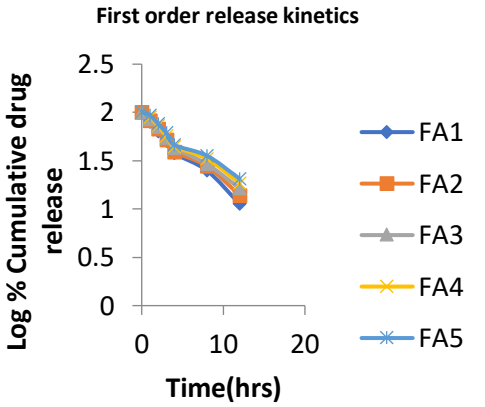

Figure14: FA1-FA5 first order release of C.Phytosomes

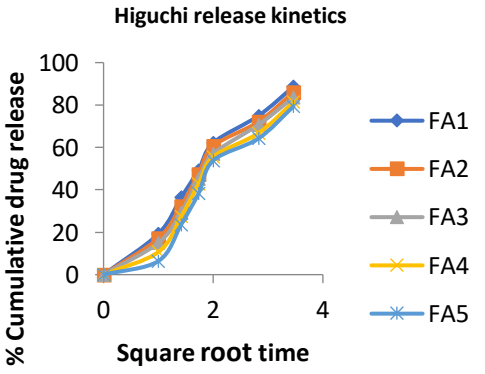

Figure 15: FA1-FA5Higuchi release of C.Phytosomes

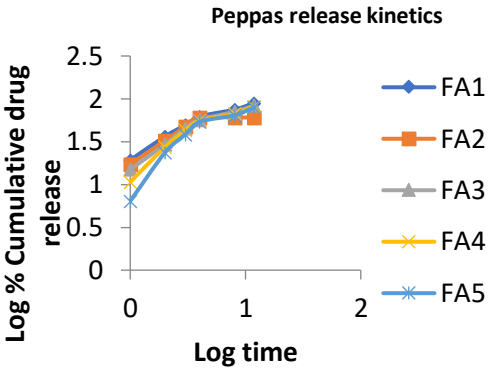

Figure 16: FA1-FA5 Peppas release of C.phytosomes

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 FA5 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.

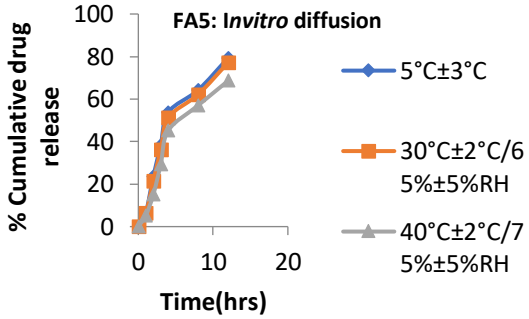

Figure 17: FA5*in-vitro* diffusion of Curcumin phytosomes

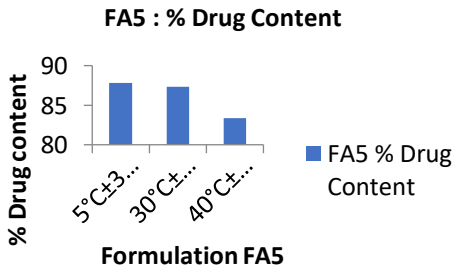

Figure18:FA5 % Drug content of Curcumin phytosomes

CONCLUSION

Curcumin phytosomes were successfully formulated using soy lecithin by Reflux method and evaluated by using various physiochemical parameters. The physiochemical parameters investigation showed that Curcumin formed complex with Soya lecithin with better bioavailability. The IR, DSC, SEM studies confirmed the formation of complex. The *in-vitro* release profile of the complex was found to be improved. Thus, it can be concluded that phospholipid complex of Curcumin may be of potential use for improving its bioavailability than compare to Curcumin Drug.

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