FORMULATION AND EVALUATION OF PHYTOSOMAL NANOCARRIERS FOR ENHANCED CHRYSIN DELIVERY

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ABSTRACT

The present work was designed to improve solubility and bioavailability of Chrysin by the development of Chrysin loaded nanophytosomes by rotary evaporation method. The formulation of phytosome evaluated for particle size ,zeta potential, SEM, FTIR, DSC, entrapment efficiency, drug content, solubilty , *in vitro* drug release and release kinetics. The particle size by DLS was found to be in nano size range. The SEM, FTIR, DSC and solubility analysis confirmed the formation of phytosome. Chrysin nanophytosome showed better *in vitro* drug release when compared to Chrysin. The kinetics of the drug release was found that it predominately follows zero order and Higuchi kinetics with non fickian diffusion mechanism.

KEYWORDS: Chrysin, flavanoid, nanocarrier, nanophytosome, rotary evaporation.

1. INTRODUCTION

Flavonoids are among the biggest group of polyphenols, widely distributed in plant-based foods. A plethora of evidence supports the health benefits and value of flavonoids can play in the physiological function treatment and in the prevention of disease particularly in the prevention of degenerative conditions including cancers, cardiovascular and neurodegenerative diseases. Water solubility and gastric stability are the major limiting factors for flavonoids to pass the biological membrane. Flavonoids when administered through nano-sized delivery systems show much better stability and absorption profile. Nanocarriers could be useful to enhance biovailability and bioefficacy of flavonoids because they can increase solubilization potential, alter absorption pathways, and prevent the metabolic degradation within the gastrointestinal tract. The technology of nanoscale

modification could overcome obstacles in the development of functional dietary supplements and medicines.[1,2]

The phytosome technique has emerged as one of the leading methods of improving bioavailability of phyto-pharmaceuticals having poor competency of solubilising and crossing the biological membranes.[3] Phytosome is a patented technology of Indena where plant polyphenolics are complexed with phospholipids to improve bioavailability.[4] Phospholipid mainly phosphatidylcholine, are lipophilic substances and readily form complex with polyphenolic compounds. Phosphatidylcholine is a major structural constituent of all biological membranes. Phosphatidylcholine is a major component of soybean lecithin which provides free choline in the blood for the manufacture of acetylcholine; regulates digestive, cardiovascular and liver functions.[5]

Chrysin belongs to the flavonoids and has been used as traditional medicine from ancient. Chrysin (5, 7-dihydroxyflavone) is a natural flavone present in many plant extracts, flowers such as the blue passion flower (Passiflora caerulea), honey and propolis. Chrysin has multiple biological activities, such as antitumor activity, anti-inflammatory, antioxidant, anti-allergic, anti-aging, anti-hypertensive, anti-angiogenesis, antiviral, anti-atherogenic, antibacterial, anti-diabetic, neuroprotective, hepatoprotective, nephroprotective and positively effect on reproductive system. Although, chrysin has multiple health benefits in humans, it has limited therapeutic use. The major constraint in the use of chrysin is its poor aqueous solubility which results in low bioavailability.[6,7]

Therefore the present work was designed to improve the solubility and the bioavailability of Chrysin by the development of Chrysin loaded nanophytosomes by rotary evaporation method.

2. MATERIALS AND METHODS
2.1 Materials
Chrysin and Soyabean lecithin (Soya lecithin / Phosphatidyl Choline) was obtained from Chemical House, Cochin (Make – TCI, Japan). All other reagents used were of analytical grade.

2.2 Method

2.2.1 Formulation of Nanophytosomes

Phytosomes were prepared by using rotary evaporation method with molar ratio of 1:1 of Chrysin and Soya lecithin. Chrysin was dissolved in ethanol, while Soya lecithin was dissolved in dichloromethane. The mixture was taken in a round bottom flask and evaporated in a rotary vacuum evaporator (VARIAC, JSGW) at 45 °C until evaporation of all solvents and producing thin dry film in the round bottom flask. The vacuum drying evaporates the organic solvents completely. The film was hydrated with distilled water in rotary evaporator at the same temperature and the phytosomal suspension was obtained. The phytosomal suspension was probe sonicated (SONICS, Ultracell) for 10 minutes to obtain the nanophytosomes.[8,9] The stages of nano-phytosome preparation depicted in figure 1.
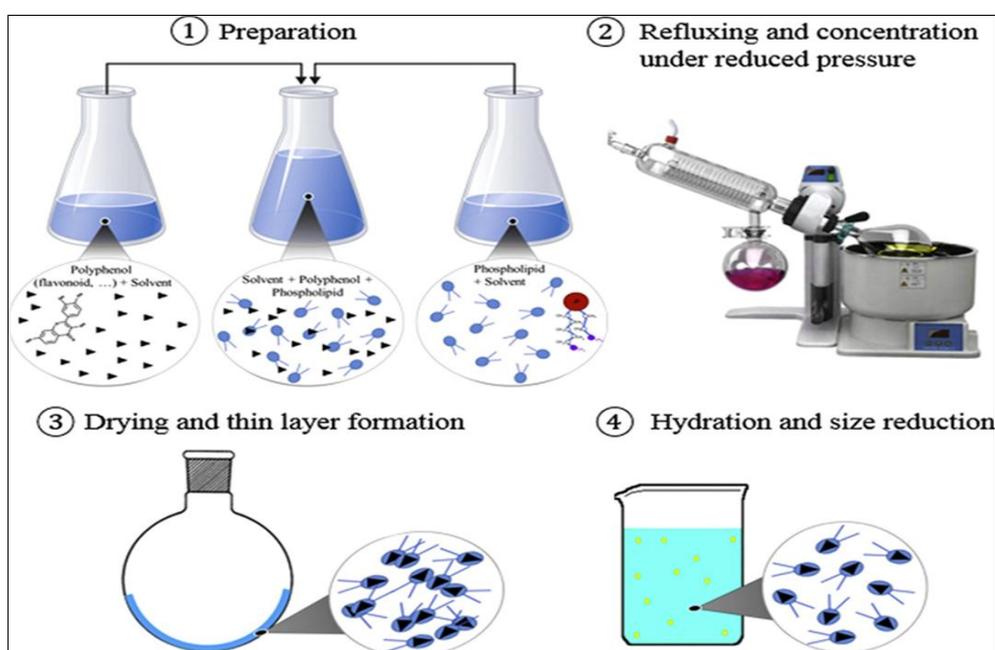

Figure 1: Common stages of nano-phytosome preparation.

2.2.2 Evaluation of Nanophytosomes

➤ **Particle Size**

Particle size and Polydispersity index (PDI) of phytosome were measured using Dynamic light scattering (DLS) particle size analyzer with a computerized system (Malvern, Zetasizer). Sample was diluted with distilled water before measurement.

➤ **Zeta Potential**

The zeta potentials of the samples were determined at 25° C after suitable dilution with distilled water using a Malvern Zetasizer Version 7.2.

➢ **Scanning Electron Microscopy (SEM)**

Scanning electron microscopy (Model TESCAN VEGA 3 SBH) study was done to determine the surface morphology, size and shape of prepared phytosomes formulation. Sample was placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of phytosomes were taken by random scanning of the stub.

➢ **FTIR**

The FTIR spectra of Chrysin, physical mixture of Chrysin-Soya lecithin and Chrysin nanophytosomes were taken using FTIR spectrophotometer (SPECTRUM 400). Infrared spectra of the test samples were determined using the KBr disc technique. The FTIR measurements were performed in the scanning range from 4000 to 400 cm-[1].

➢ **Differential scanning calorimetry (DSC)**

The DSC of Chrysin, and physical mixture of Chrysin–Soya lecithin and Chrysin Nanophytosomes were analyzed in DSC analyzer (Q20 V24.10 Build 122). Each sample was placed in an aluminum pan separately with heating and cooling rates of 10°C/min and 250°C/min, respectively. Measurements were performed over 50-300°C under nitrogen purge at 50 ml/min.

➢ **Solubility**

Solubility of chrysin and phytosome formulation was carried out in solvents like chloroform, ethyl ether for evaluating whether flavonoids are incorporated into the phytosome structures. As flavonoids, in their pure forms, are insoluble in these solvents, if there was any un- incorporated flavonoid to the phytosome structure it can be seen by precipitation of flavonoids. They convert to be soluble after incorporating into the phytosomes. Hence, the phytosome generate a stable lipid compatible molecular complex.[10]

➢ **Entrapment efficiency**

The entrapment efficiency of phytosome was determined by centrifuging 2 mL of the phytosome formulation at 1500 rpm for 30 min at room temperature. The supernatant was taken carefully using pipette. Pure supernatant was then dissolved in ethanol to disrupt the vesicles and appropriate dilution was made and measured using UV spectrophotometer (SHIMADZU 1800) at 270 nm.[11] The percentage of drug entrapped was determined using the formula

Entrapment efficiency % =   Total drug added – un entrapped drug × 100

                                                    Total drug

Drug Content

Drug content of was determined by dissolving accurately weighed quantity of phytosome dispersion in 10 ml ethanol. After suitable dilution absorbance was determined by UV spectrophotometer (SHIMADZU 1800) at 270 nm and drug content was determined by using the formula.[12]

Drug Content (%) =   Actual drug content in Phytosomes   × 100

                                      Theoretical yield

In-vitro drug release study

• Preparation of egg membrane

From local department store egg was purchased. The egg yolk was separated carefully by means of hole on the surface of the egg. After that the egg shell was immersed in HCl for 2 hours with constant stirring followed by the complete separation of egg membrane. The membrane was washed with phosphate buffer pH 7.4 and further used for the experimental work.

• Drug release through egg membrane

The *invitro* drug release studies were carried out in an open diffusion tube which was opened at both the ends. The phytosome sample (2ml) was spread uniformly on the surface of egg membrane and was fixed to the one end of tube such that the preparation occupies inner circumference of the tube. The whole assembly was fixed in such a way that the lower end of tube containing phytosome was just touched (1-2 mm deep) the surface of diffusion medium i.e., 50ml pH 7.4 phosphate buffer contained in 100 ml beaker which was placed in water bath and maintained at $37\pm2°C$. The egg membrane acts as a barrier between the phytosome and pH 7.4 phosphate buffers (sink condition). A quantity of 2 ml samples were withdrawn from receptor fluid at the time interval of 15min, 30min, 45min, 1, 2, 3, 4, 5, 6 hrs and 2 ml phosphate buffer pH 7.4 was replaced at each time interval. The released drug was estimated spectrophotometrically at 270 nm.

Release kinetics of *in vitro* drug release study

To understand the drug release kinetics and mechanism of drug release, the *in vitro* drug

release study was fitted to mathematical equations of different kinetics model such zero order (cumulative percentage of drug release versus time), first-order (log cumulative percentage of drug remaining versus time), Higuchi (cumulative percentage of release versus square root of time) and Korsmeyer- Peppas (log cumulative percentage of drug released versus log time) equation models. The equation with the high regression coefficient (R^2) for formulation will be the best fit of release data.

3. RESULT AND DISCUSSION

3.1 Particle Size

The vesicle stability depends on particle size and polydispersibilty index (PDI). The particle size by dynamic light scattering (DLS) was found to be in nano size range. The peak particle size was found to be 87.46nm. The Z average was found to be 150.5 nm.

The PDI refers to width of a particle size distribution. PDI values > 0.5 are unstable and indicate the sample has broad size.[13] The PDI value of nanophytosome was found to be 0.199 which indicates the formulation is stable and homogeneous distribution. (Figure 2)
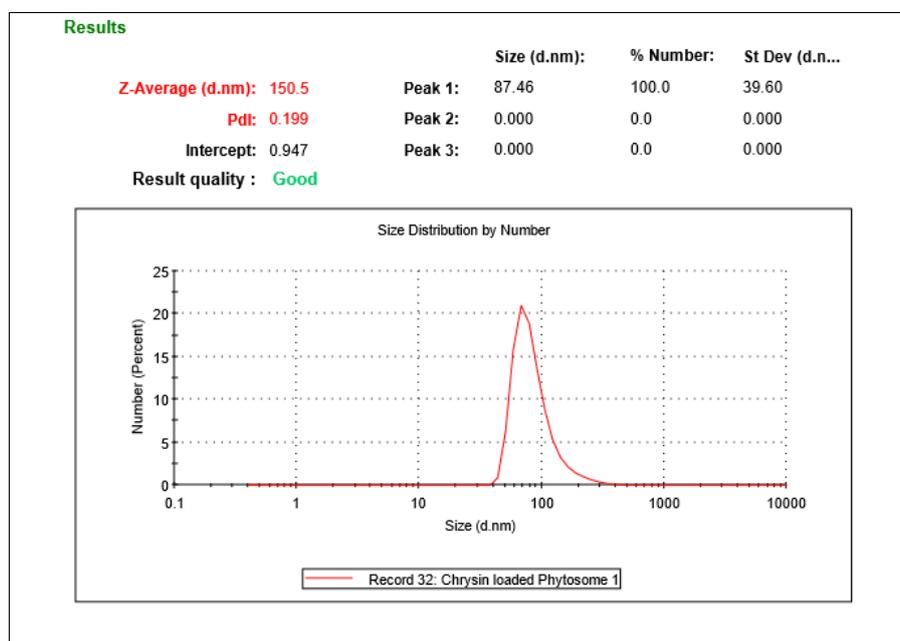

Figure 2: Particle size result by DLS.

3.2 Zeta Potential

Zeta potential is the most important parameter for physical stability of phytosomes. The zeta potential value $\geq \pm 30$mV are considered stable for phytosomes.[13] The zeta potential value

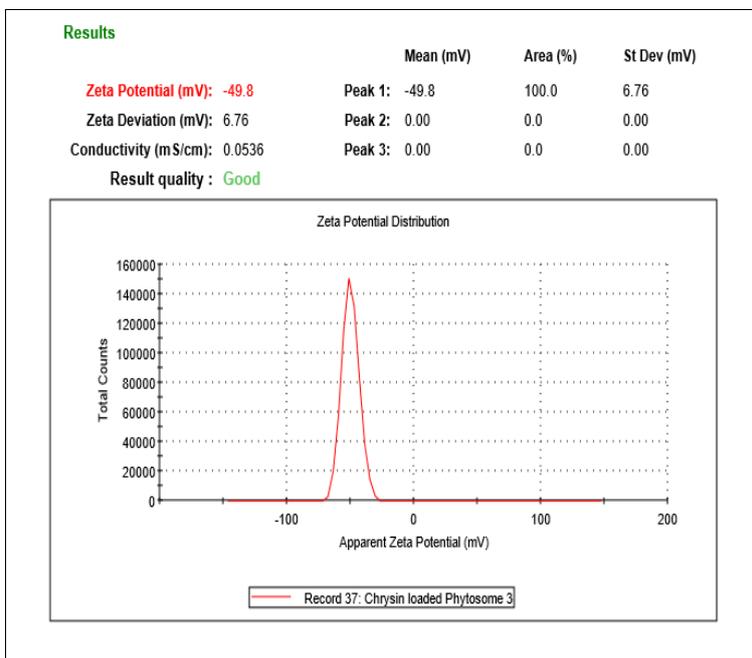of nanophytosomes was found to be -49.8mV which indicates the formulation is stable. (Figure 3)

Figure 3: Zeta potential result.

3.3 Scanning Electron Microscopy (SEM)

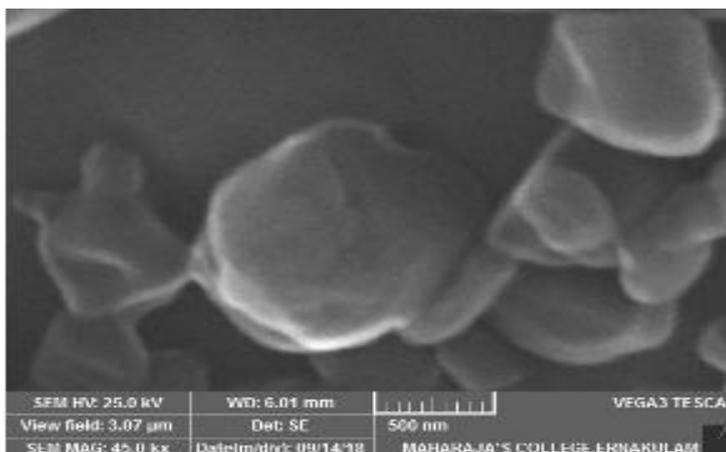The surface morphology of Chrysin nanophytosomes as shown in Figure 4 indicated the presence of spherical shape.

Figure 4: SEM image of Chrysin nanophytosomes.

3.4 Ftir

The stability of a formulation primarily depends on the compatibility of the drug and excipients. Hence, it is important to detect any possible chemical or physical interactions,

since they can affect the bioavailability and stability of the drug. The FTIR Spectra of Chrysin, physical mixtures of Chrysin and Soya lecithin, and Chrysin nanophytosomes were obtained and these are shown in Figure: 5-8.
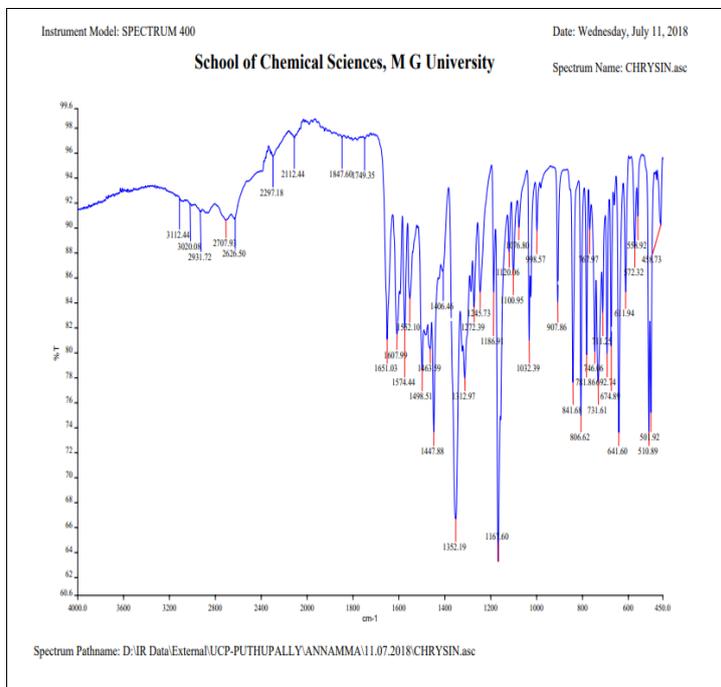

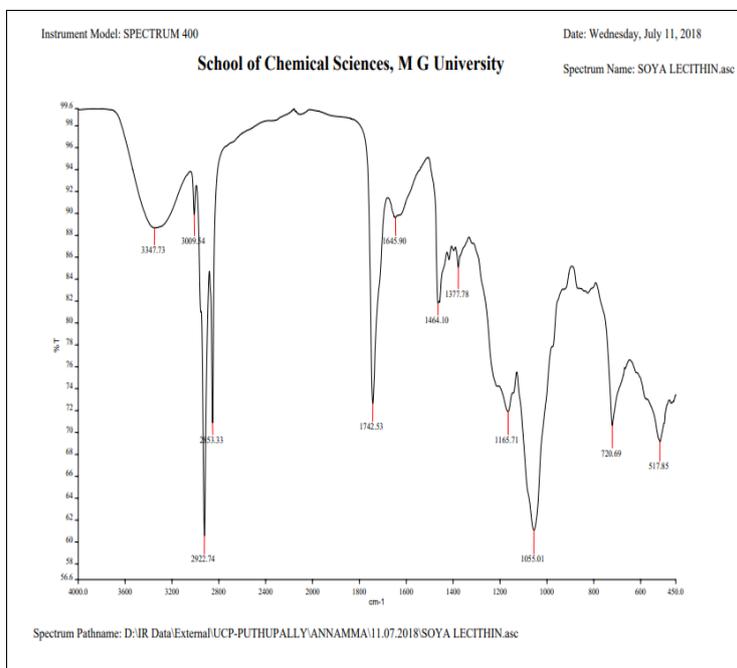**Figure 5: FTIR spectrum of Chrysin.**
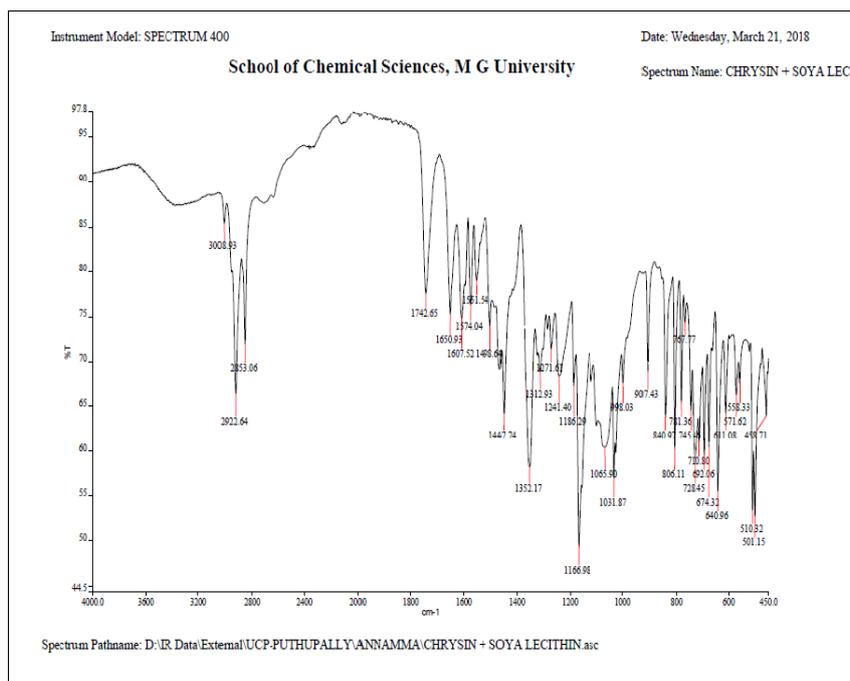

Figure 6: FTIR spectrum of Soya lecithin.

Figure 7: FTIR spectrum of Chrysin –Soya lecithin mixture.
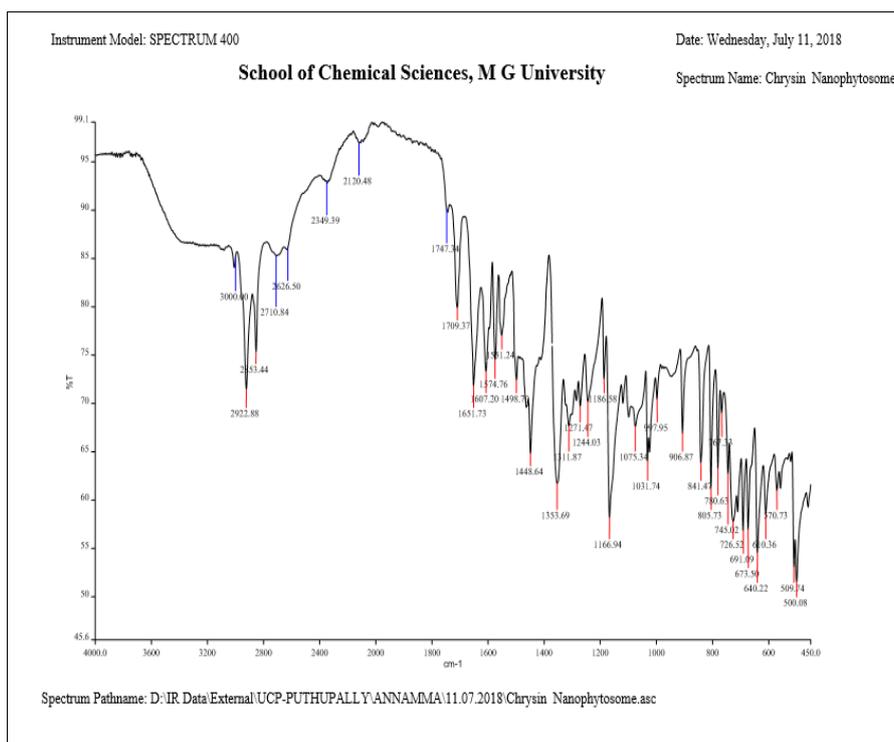

Figure 8: FTIR spectrum of Chrysin nanophytosome.

The characteristic peaks of Chrysin at 3020.08 cm^{-1} (O-H), 2707.93 cm^{-1} (C-H), 1651.03 cm^{-1} (C=O) and 1607.99 cm^{-1} (C=C). The band of N(CH3) group in soya lecithin spectra (1464.10 cm^{-1}) is shifted to a higher frequency in Chrysin nanophytosome spectra (1561.24 cm^{-1}) indicated the interaction between soya lecithin and Chrysin is at the level of choline moiety. The band of the long chain fatty acids in the phospholipid molecule (2922.75 &

2853.34 cm^{-1}) is maintained unchanged in phytosome spectra indicating that the long chain fatty acids are not involved in phytosomes formation. The spectrum of the nanophytosome showed significant changes, and the absorption peak of hydroxyl stretching of chrysin showed broadening from 3000.90 -2710.84cm^{-1}. It can be concluded that the phytosome complex is generated.

3.5 DSC

DSC is a fast and reliable method to screen drug-excipient compatibility and provides maximum information about the possible interactions. These interactions are observed as the elimination of endothermic peak, the appearance of new peak, the change in peak shape, onset temperature/ melting point, relative peak area or enthalpy.[10] DSC thermograms of Chrysin, and physical mixture of Chrysin–Soya lecithin and Chrysin Nanophytosomes were obtained as in Figure:9-11
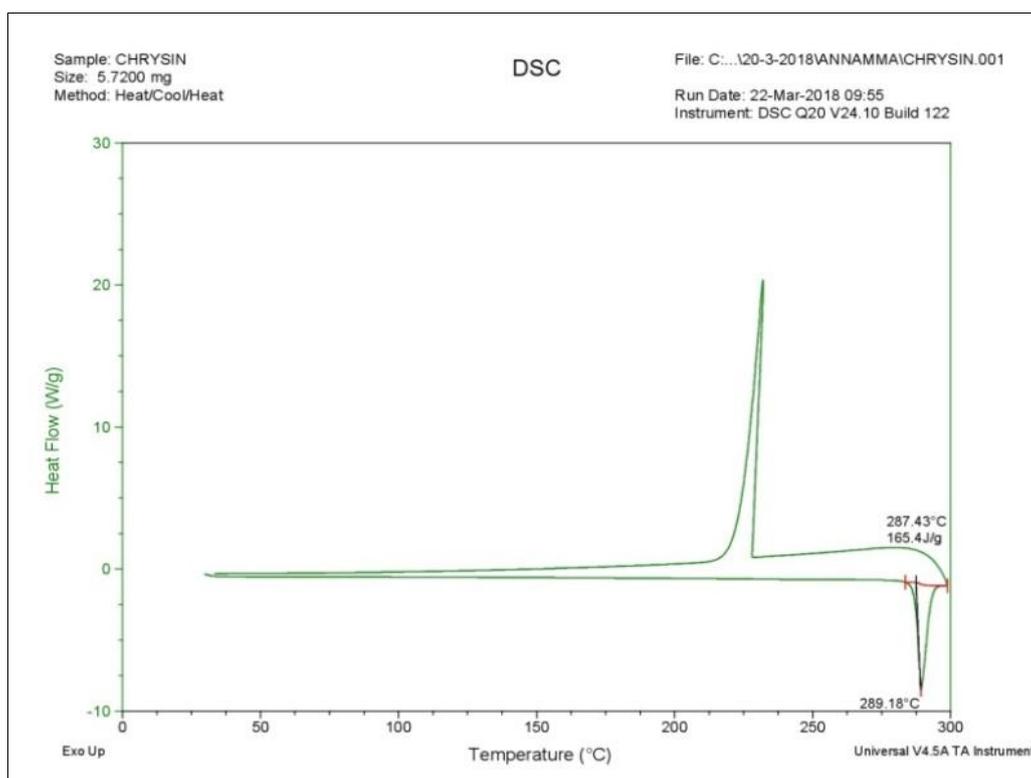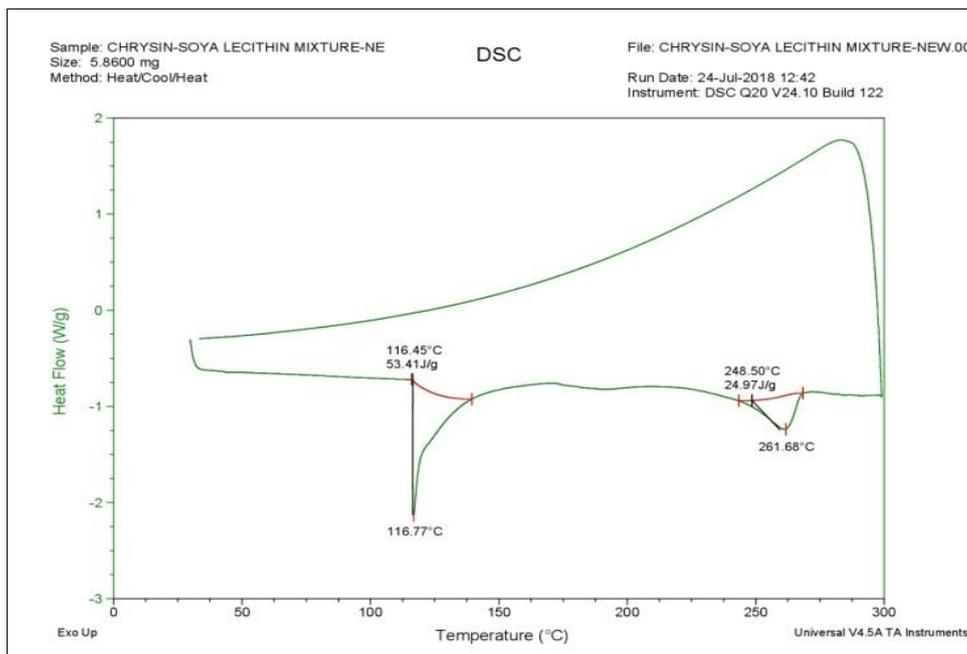

Figure 9: DSC thermogram of Chrysin.

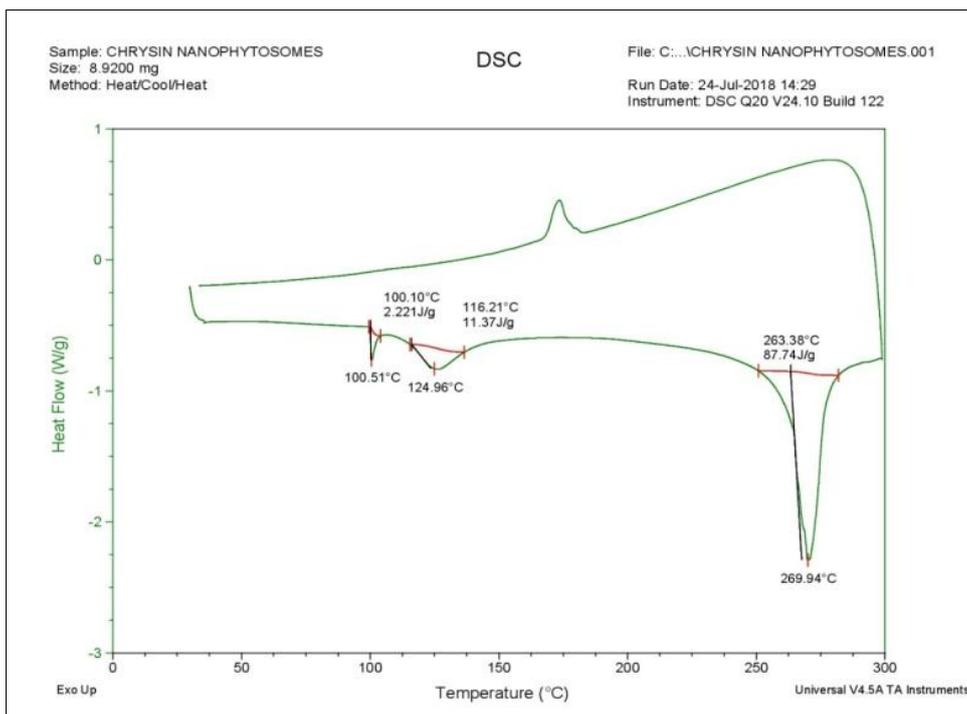**Figure 10: DSC thermogram of Chrysin-Soya lecithin mixture.**

Figure 11: DSC thermogram of Chrysin nanophytosomes.

The endothermic peak of Chrysin was observed at 289.18ºC corresponding to its crystalline nature (Figure: 9). In DSC thermogram of physical mixture of Chrysin- Soya lecithin, the endothermal peaks of both are still detectable but shifted towards lower temperatures (Figure 10). On the other hand, the DSC thermogram of Chrysin nanophytosomes, a broad peak appeared at 100.51ºC. This peak may be attributed to the formation of a new complex peak

near the phosphatidyl choline peak. The broad peak of phytosome implies the crystallinity loss (Figure11). It was also concluded that Chrysin was molecularly distributed in nanophytosome matrix, indicating its reduction in crystallinity as the peak intensity of the Chrysin was found to be reduced.

3.6 Solubility

Chrysin (flavanoid) was found to be insoluble in chloroform and ethyl ether. Chrysin nanophytosome formulation was soluble in chloroform and ethyl ether which confirmed that the Chrysin was incorporated into the phytosome structure and stable complex was formed.

3.7 Entrapment Efficiency

The entrapment efficiency of chrysin nanophytosomes was obtained by centrifugal method and found to be 90.25%.

3.8 Drug Content

The drug content of chrysin in nanophytosome formulation was found to be 89.25%.

3.9 *In vitro* drug release study

The *in vitro* drug release of Chrysin and Chrysin Nanophytosome was done and compared. Chrysin nanophytosome showed better *in vitro* drug release when compared to Chrysin. The percentage cumulative drug release at 6th hour for chrysin and nanophytosome was found to be 33.21% and 65.51% respectively. The *in vitro* release graph for Chrysin and Chrysin Nanophytosome is graphically represented in figure: 12.
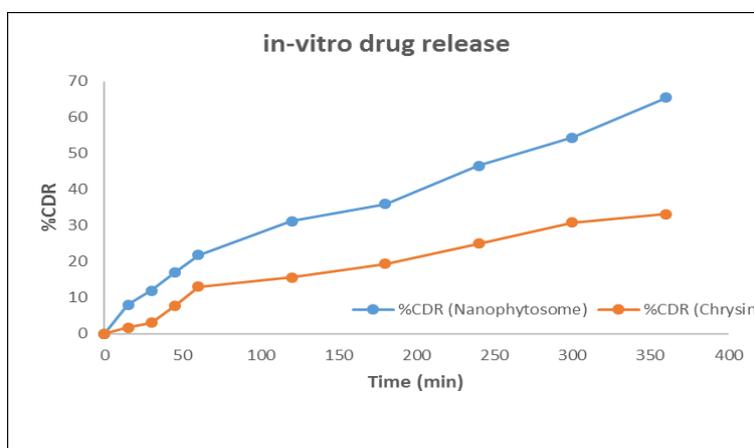

Figure 12: *In vitro* drug release graph for Chrysin and Chrysin Nanophytosome.

3.10 Kinetics of *in-vitro* drug release

The release data were computed in different kinetics model of a) zero order b) first order c) Higuchi d) Korsmeyer equation. The regression coefficient values of different release kinetics equations were evaluated by computing the data of release profiles of Chrysin nanophytosome formulation (Figure 13- 16). The results of *in vitro* release kinetics was summarised in table 2.

Table 2: Results of *in vitro* release kinetics of nanophytosome.

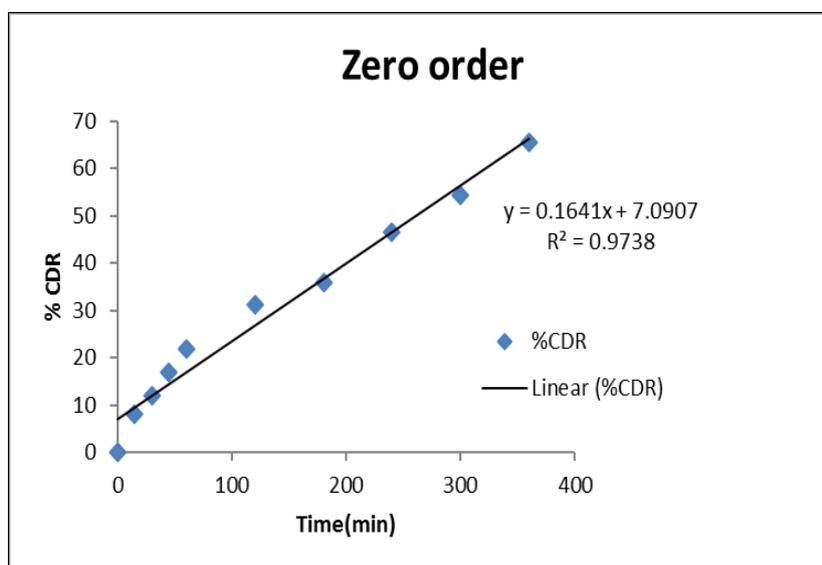| Formulation	Zero order	First order	Higuchi	Korsmeyer peppas	
	R^2	R^2	R^2	R^2	n
Nanophytosome	0.9738	0.6105	0.9785	0.9948	0.691

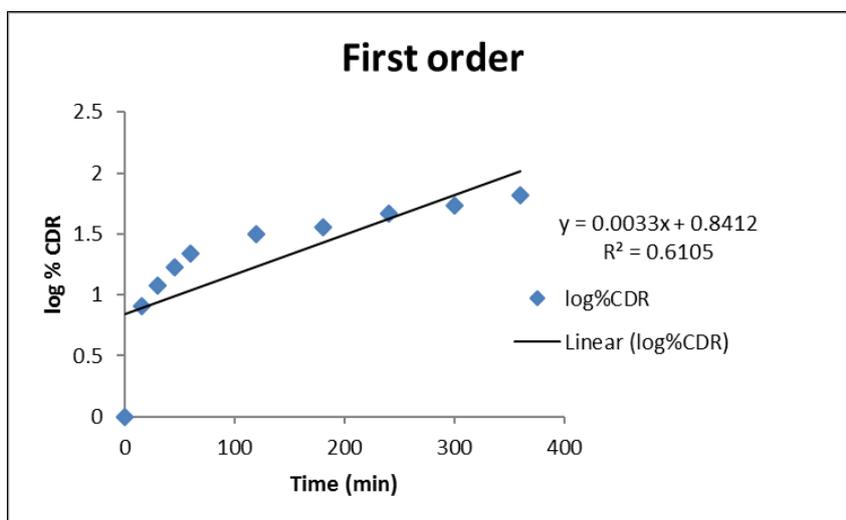**Figure 13: Zero order Plot.**
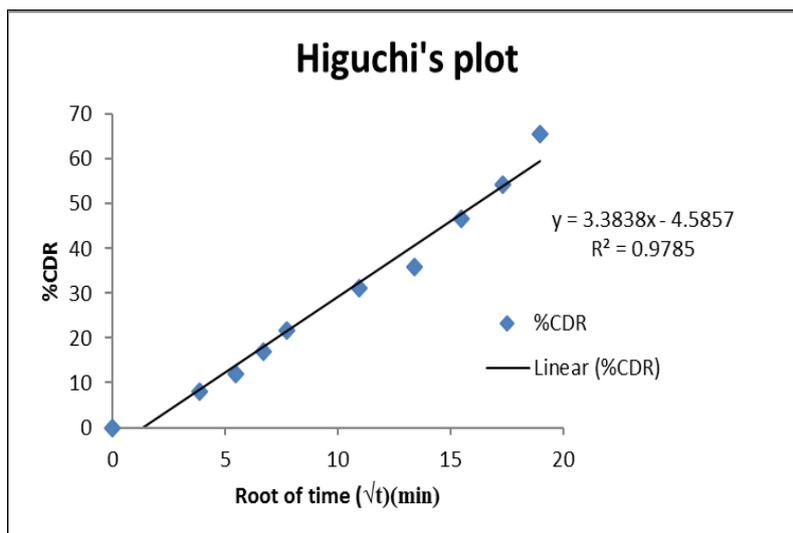

Figure 14: First order Plot.
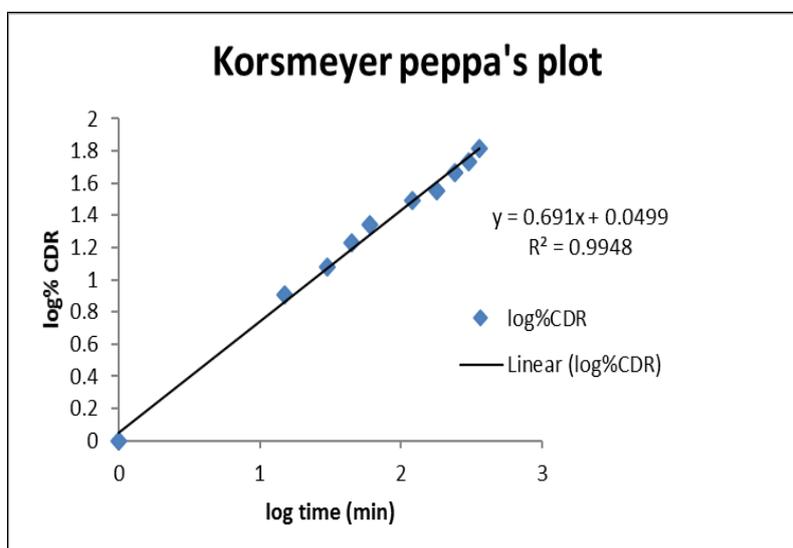
Figure 15: Higuchi Plot.

Figure 16: Korsmeyer Peppa's Plot.

Zero order plot for nanophytosome was found to be linear (R^2 - 0.9738) and indicated that it may follow zero order kinetics. Higuchi plot was found to be linear (R^2 - 0.9785), which indicates diffusion may be the mechanism of drug release for nanophytosome. Korsmeyer Peppas plot was found good linear, the n value was found to be 0.691 (n > 0.5), indicated that drug release may follow non fickian (anomalous) diffusion.

4. SUMMARY AND CONCLUSION

The Chrysin nanophytosomes was prepared by rotary evaporation method with 1:1 molar ratio of Chrysin: Soya lecithin.The prepared nanophytosomes was evaluated for particle size, zeta potential , SEM, FTIR, DSC, drug content, entrapment efficiency, solubility, *in-vitro*

drug release and release kinetics. The particle size by DLS was found to be in nano size range. The peak particle size was found to be 87.46nm. The Z average was found to be 150.5nm.The PDI of nanophytosome was found to be 0.199 which indicates the formulation is stable and homogeneous distribution. The zeta potential was found to be -49.8 m V which indicates the formulation is stable. The surface morphology of Chrysin nanophytosomes was done by scanning electron microscopy which indicated the presence of spherical shape. The percentage entrapment efficiency of Chrysin Nanophytosome formulation was found to be 90.25%. The drug content of Chrysin in nanophytosome formulation was found to be 89.25%. The *in vitro* drug release of Chrysin and Chrysin Nanophytosome was done and compared. Chrysin nanophytosome showed better in vitro drug release when compared to Chrysin. The percentage cumulative drug release at 6th hour for Chrysin and nanophytosome was found to be 33.21% and 65.51% respectively. The kinetics of the drug release was found that it predominately follows zero order and Higuchi kinetics with non fickian diffusion mechanism.

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