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NOVEL *Plumbago Indica L.* EXTRACT HERBOSOMES: DEVELOPMENT AND OPTIMIZATION USING EXPERIMENTAL DESIGN

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Abstract:
Aim: This study was to develop and evaluate a novel herbosome formulation of *Plumbago Indica L.* Root Extract with phospholipid.

Methods: The impact of many methods and formulation factors was examined in order to synthesize the Herbosome-loaded *Plumbago Indica L.* roots extract. The technique of thin-film hydration was utilized, with soya-lecithin serving as the phospholipid of choice. The *Plumbagin* herbosome was confirmed by taking into account a number of factors, including as particle size, zeta-potential, scanning electron microscopy and entrapment efficiency (%) compared with drug.

Results: The optimized technique provided that particle size within the range and good polydispersity index, an average Zeta potential of -45.3 mV, and an entrapment efficacy (%) of 69.42. Shape and morphology analysis using a scanning electron microscope revealed a smooth surface and spherical shape. The formulation adheres to the zero order kinetic models based on the greatest correlation (R2) value, as evidenced by further *in-vitro* drug release studies of the improved formulation.

Conclusion: Formulation research aims to develop a dosage form that is elegant, reliable, safe, and compatible with other components. The herbosomal complex was observed better solubility, melting point. *In-vitro* studies for hepatic malignancy were conducted to further confirm the efficacy of the optimized formulation.

Keywords: *Efficacy, Herbosome, In-vitro studies, Plumbago Indica L, soyalecithin.*

Introduction

Certain components of extracts may be degraded in the stomach environment when consumed orally. It has been shown that complexion these extract and their separate components with a few other therapeutically beneficial nutrients significantly increase their bioavailability (1). The efficacy

of a medication can be significantly impacted by the way it is administered. Certain medications have a range of ideal concentrations where the most benefit can be obtained, and vice versa (2). This resulted in the creation of new approaches for regulating pharmaceutical pharmacokinetics, pharmacodynamics, immunogenicity, biomarker identification, non-specific toxicity, and efficacy. These revolutionary processes, known as "drug delivery systems" (DDS), are based on multidisciplinary approaches that combine molecular biology, pharmaceutics, polymer science, and bio-conjugate chemistry. The term "novel drug delivery system" (NDDS) primarily refers to the creation of novel pharmacological forms with optimal properties such targeted sites of action, increased permeability, and reduced particle sizes (3). When compared to the effects of bio therapeutic drugs in traditional dose forms, NDDSs can be utilized to improve their performance (4, 5). Once considered a pipe dream or, at most, a possibility, regulated and unique medication delivery is now a reality. Phospholipid Phosphatidylcholine, which is mostly used to create Herbosomes, is obtained from soybeans (Glycine max). Herbosomes have a greater ability to pass through the lipoidal bio membrane and eventually enter the systemic circulation, making them more accessible than traditional herbal extracts. A developing method for delivering herbal medications and Neutraceuticals is the use of Herbosomes (6, 7). In this area of medication research, experts from the pharmaceutical sector as well as other fields have conducted in-depth studies during the past fifteen years. The capacity to guide the drug-loaded system to the desired location is known as targeting.

Material and Methods

Material

The *Plumbago indica L.* (Family: *Plumbaginaceae*) plant that was used for that study gathered from Maharashtra and prepared root extracts with chloroform, ethyl acetate, and methanol. All other chemicals and reagents were of the Lab grade.

Methods

Preformulation studies

Pre-formulation refers to a set of investigations centered on the physicochemical characteristics of a novel drug candidate that may impact the efficacy of the medication and the creation of a dosage form. This might support the necessity for molecular change or offer crucial information for formulation design (8). The pre-formulation study's goal was to create a dose form that was tasteful, reliable, safe, and compatible with other components by determining the kinetic rate profile and the physicochemical parameters of novel pharmacological compounds. Pre-formulation studies consider features such as medication solubility, melting point, polymorphic forms, and stability to be crucial (1). In that work, solubility was analysed in different solvents and the best solubility was observed in methanol solvent of *Plumbagin*. In accordance with the recommendations of the International Conference on Harmonization (ICH Q2 R1), the approach was designed and verified. (9). The study was carried out in methanol and a standard stock solution was prepared with 1000µg/ml, from this 100µg/ml solution prepared used to λ_{max} was found to be 266nm and 410nm calibration were also found. Characterization was done by UV, FT-IR, DSC, melting point and pure drug concentration was prepared in the range of 10-70µg/ml and the linear regression analysis data showed good linear relationship with an R^2 value of 0.942 which indicated that the developed method was precise, specific, rapid and suitable for the analysis of commercial samples.

Preparation of Herbosomes complex

Herbosomes are essentially herbal-liposomes that are specifically made to increase the bioavailability of herbals in order to provide a specific site of action. In that work, we created Herbosomes utilizing the traditional liposome manufacturing process, known as thin film hydrolysis (TFH), with a few adjustments (10). A rotary evaporator was used to evaporate the mixture after soy lecithin and cholesterol were briefly mixed in varying concentrations of chloroform-methanol (1:1) (11). The mixture also included 5mg/mL of *Plumbagin*. Phosphate buffer 7.4 was added to the

round-bottomed flask to hydrate the thin layer that had developed there. The suspension was sonicated for a few hours after being magnetically agitated for thirty minutes (12, 13). Table 1 list the several formulation variables that were taken into account for this study. Afterwards, herbosome was effectively gathered in containers and applied to additional medication research.

Table 1 Composition of Ingredients

S. No	Code	Soya-Lecithin (mg)	Cholesterol (mg)	Sonication Time (min)
1.	F1	50	20	2
2.	F2	112.5	10	3
3.	F3	175	10	2
4.	F4	50	15	3
5.	F5	175	15	3
6.	F6	112.5	15	2
7.	F7	112.5	15	2
8.	F8	50	15	1
9.	F9	112.5	20	1
10.	F10	50	10	2
11.	F11	175	20	2
12.	F12	112.5	10	1
13.	F13	112.5	20	3

Design of Experiment

Box-Behnken design with three factors and three layers was used to finish the optimization. Independent variables were the Soya-Lecithin (X_1), Cholesterol (X_2) and Sonication time (X_3) for two dependent variables i.e. Entrapment Efficacy (Y_2) and Particle size (Y_1) as shown in table 2. The experimental nature based on this mixture of the component has resulted in 13 separate herbosomes formulation batches. As indicated, numerous herbosomes lots were prepared. This data was statistically analysed and validated by Design Expert® (Version I.3.0, Stat-Ease) using ANOVA as a linear model to find an optimised set of process parameters. The best fit model was the quadratic model for the two dependent variables (14). The significance of the model with that of comparing with the other model for the analysis by analysis of variance (ANOVA) and the polynomial equations to find out the optimized formulation (15).

Table 2 Independent and Dependent Variables

Type of variables	Code	Variables
Independent	X_1	Soya-Lecithin
	X_2	Cholesterol
	X_3	Sonication time
Dependent	Y_1	Particle size
	Y_2	Entrapment Efficacy

Formulation of Herbosomes (Optimized formulation)

The results of formulations as per the design when fitted into various models, linear model was observed to be significant for entrapment efficacy with F value 3.80 and P value 0.0472. The results when analysed and optimized had generated numerical optimized solutions based on this experimental design. From the numerical optimization results, a solution was selected coded as optimized formulation and considered as optimized herbosome formulation shown in table 3.

Table 3 Composition of optimized formulation (Based on Design of expert software)

Drug (mg)	Soya-Lecithin (mg)	Cholesterol (%)	Sonication time (Min.)
5mg/ml	175.000	10.000	2.000

Characterization of optimized formulation

Particle size

The Malvern Zeta sizer (Malvern Instruments) was used to measure the size of herbosomes. The sample was put in a disposable sized cuvette after the dispersions were diluted with Millipore filtered water to the proper scattering intensity at 25°C (16).

Zeta potential

In order to ascertain the particle charge and movement velocity of the particles in an electric field, the zeta potential was measured. In the current study, Zetasizer Malvern equipment was used to evaluate herbosomes that had been diluted ten times with distilled water. A 5-10min sonication was performed on every sample prior to zeta potential measurements (8, 17).

Entrapment efficiency

The centrifugation technique was used to calculate the herbosomes entrapment efficiency. The herbosome was diluted with methanol and spun using a high speed cooling centrifuge equipment at 1000rpm for 30minutes at -4^0C. The quantity of free medicine in the supernatant was measured using a UV visible spectrophotometer set to 266nm for *Plumbagin* (8, 18).

Scanning Electron Microscopic (SEM)

The optimized herbosome morphological characteristics were obtained with scanning electron microscope electron beam. A sputter coater operating under vacuum was then used to coat the herbosomes with a thin coating of metal, such as platinum, palladium, or gold, ranging in thickness from 2 to 20nm (19). Following the preparation, the specimen was exposed to an electron beam, which caused secondary electrons known as auger electrons to develop. Only the electrons dispersed at a 90° angle were chosen from this interaction between the electron beam and the specimen's atoms, and these were then processed using Rutherford and Kramer's Law to provide the surface topography photographs (8).

In-vitro drug release study of optimized formulation

Formulations of *Plumbagin*-loaded herbosomes were evaluated for in-vitro drug release using the dialysis bag diffusion method. The dialysis bag was filled with *Plumbagin*-loaded herbosomes, and the bag was then kept in a beaker with 100mL of pH 7.4 phosphate buffer. Throughout the experiment, the temperature of the assembly was maintained at 37±1°C by placing the beaker over a magnetic stirrer. The experiment's speed was kept constant at 100rpm. At certain intervals, samples were taken out and swapped out with equal volumes of brand-new pH 7.4 phosphate buffers. The samples were examined using a UV-Visible spectrophotometer set at 266nm after being suitably diluted. Many kinetic models were used to characterize the release kinetics in order to assess the in-vitro drug release data (5).

Different kinetic models were used to characterize the release kinetics in order to interpret the *in-vitro* release data. The zero order kinetic models describe settings where the drug release rate is independent of its concentration. The first order kinetic theory describes the release from a system where the release rate is concentration-dependent. Higuchi characterized the release of drugs from an insoluble matrix as the square root of a time-dependent mechanism based on Fickian diffusion. Plotting of the *in-vitro* release profile results for each formulation was done using data treatment modalities (19).

Results and Discussion

Pre-formulation studies

Solubility was analysed in different solvents and the best solubility observed in methanol. Melting point range by open capillary method was found 79°C.

The λ_{max} of *Plumbagin* in methanol was observed 266nm and 409nm. The linear regression analysed data showed good relationship with correlation coefficient R^2 value was 0.942 and calibration curve

shown in figure 1. The FT-IR showed characteristic peaks *Plumbagin*, Soya-Lecithin, Cholesterol and also drug with soya-lecithin complex shown in figure 2. The DSC plot of *Plumbagin* showed from 30° to 300°C under nitrogen atmosphere (60mL/min). All the validations parameters were as per the guidelines. The linearity range was 10-70µg/mL. The DSC plot showed drug with soya-lecithin complex shown in figure 3.

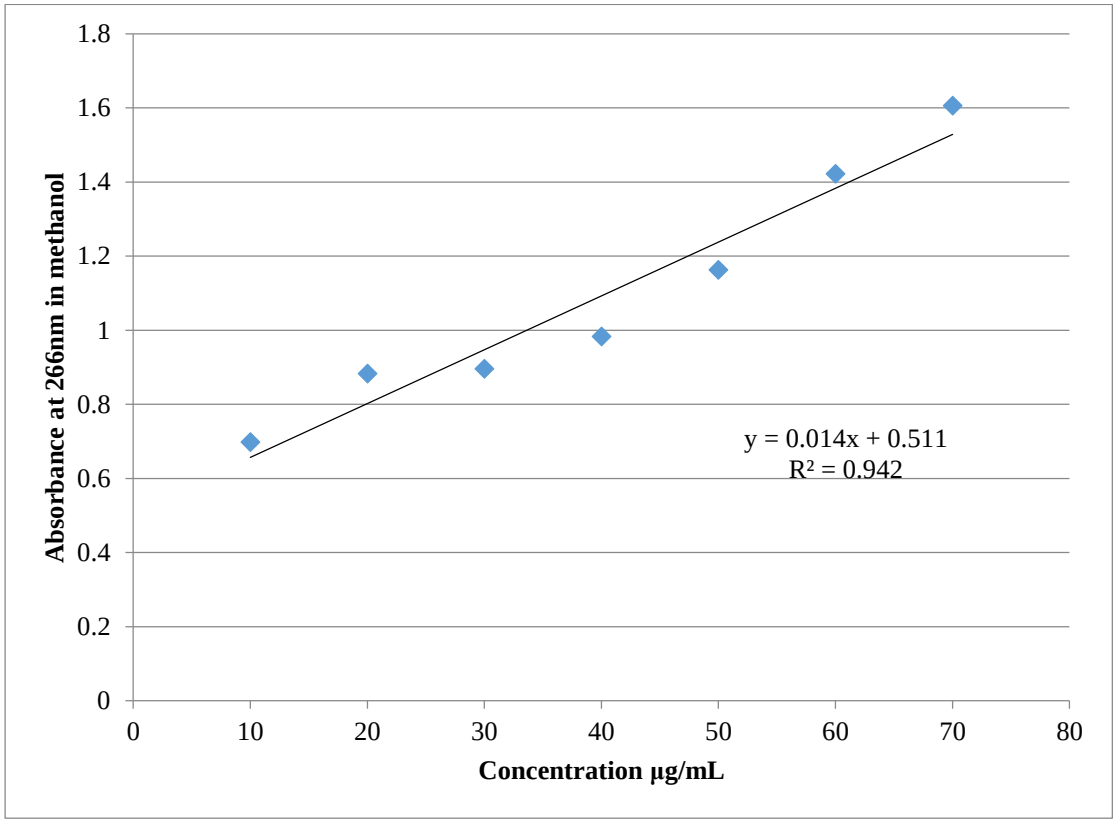

Figure1 Calibration curve of *Plumbagin*

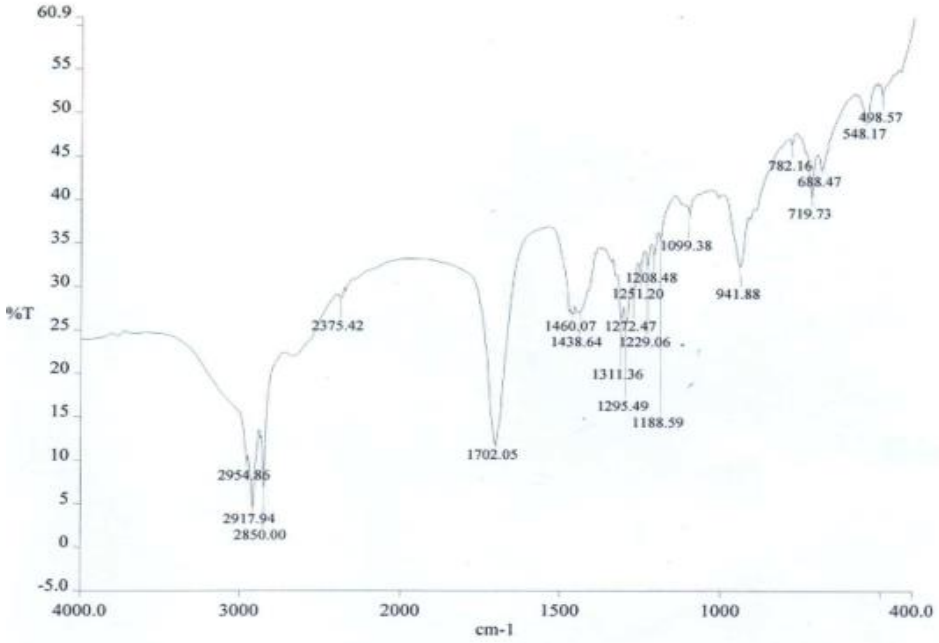

Figure 2 FT-IR of Herbosome Complex (*Plumbagin* with Polymer)

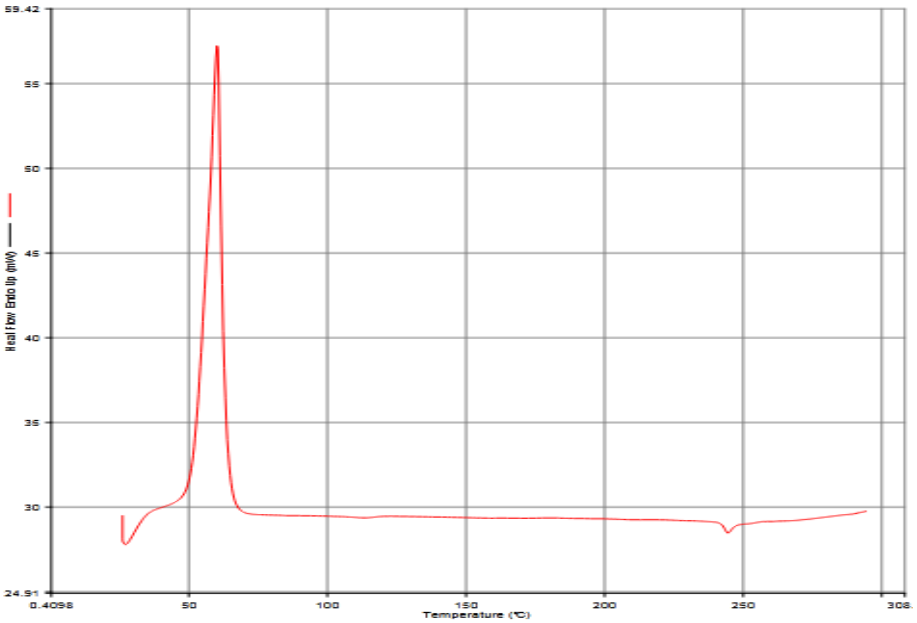

Figure 3 DSC of Herbosome Complex (*Plumbagin* with Polymer)

Preparation of Herbosomes complex

The optimized formulation was observed by variables *i.e.* Particle Size (Y_1) and Entrapment Efficacy (Y_2). The optimized formulation (F3) was maximum 0.988. Regression equation of the fitted quadratic model for both the responses was as follows –

Particle Size; Y1= +563.83-146.98X1+52.69X2+235.70X3

Entrapment Efficacy: Y2=+58.33+1.25X1-8.11X2+5.25X3

The contour Plots and 3D response curve were obtained as shown in figure 4

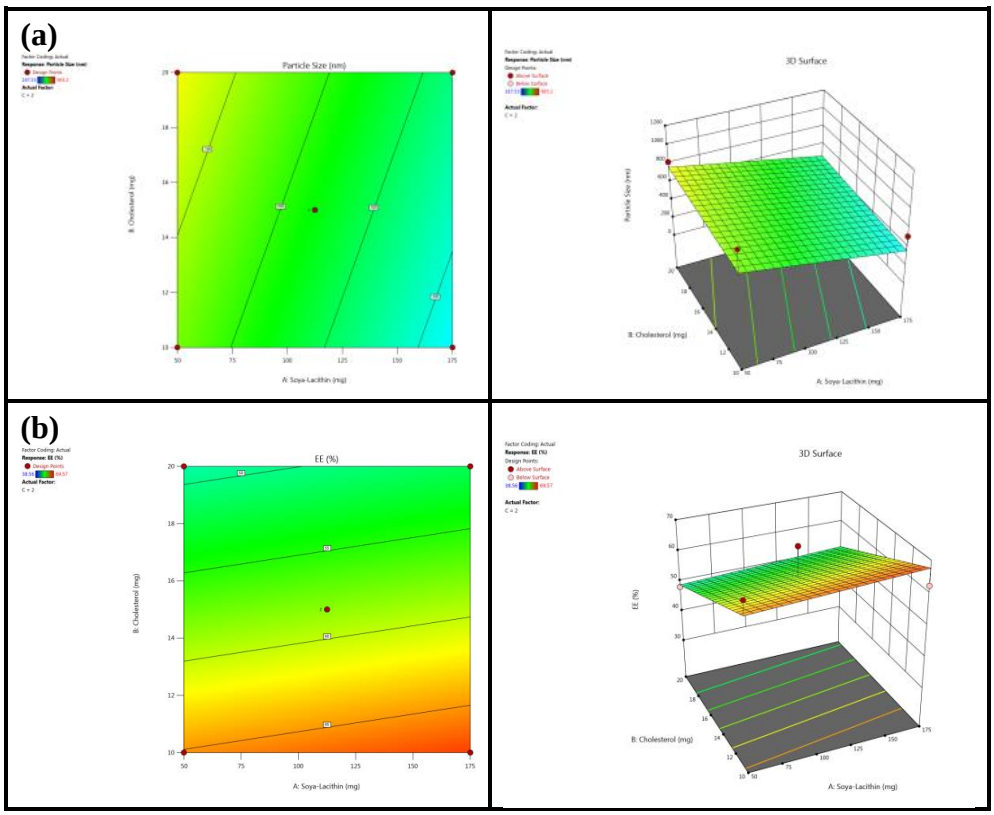

Figure 4 Contour plots and 3D surface curves for Particle Size (a) and Entrapment Efficacy (b)

Particle size distribution

The particle size is one of the most important parameters for the characterization of herbosome. The average particle size of the prepared herbosome was measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of herbosome was found to be 326.9nm with PDI value 0.405 shown in Figure 5.

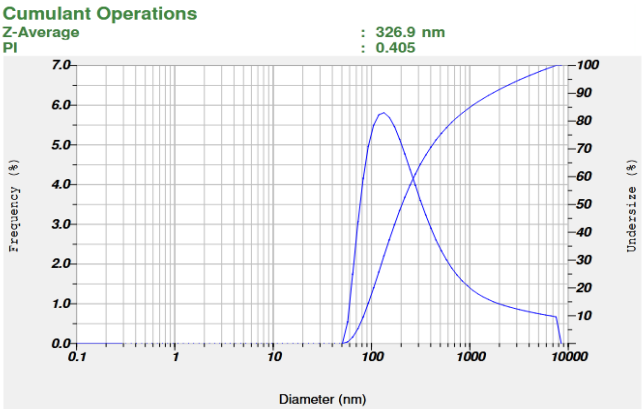

Figure 5 Particle Size and PDI of Herbosome Complex

Zeta potential

Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability. Herbosome with zeta potential value greater than +25mV or less than -25mV typically have high degrees of stability. If the particles in herbosome have a large positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating for herbosome. Zeta potential was found to be -45.3mV with peak area of 100% intensity. These values indicate that the formulated herbosomes are stable as shown in figure 6.

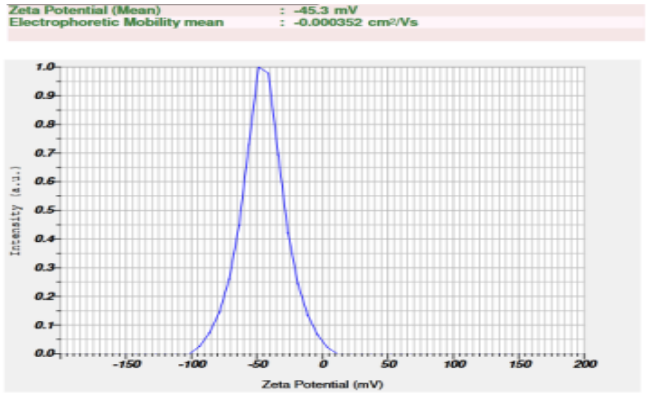

Figure 6 Zeta Potential of Herbosome Complex

Entrapment efficacy

This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer concentration and difference in the degree of lipid. The prepared Optimized herbosomes possess high drug entrapment efficiency and were found to be in the range of 69.42%.

Scanning electron microscope (SEM)

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared herbosome. Herbosomes were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared herbosome at 63.93kx magnification showed that the herbosome were porous with a

smooth surface morphology and spherical shape. The smooth surface of herbosome was clearly observed in the SEM images as shown in figure 7.

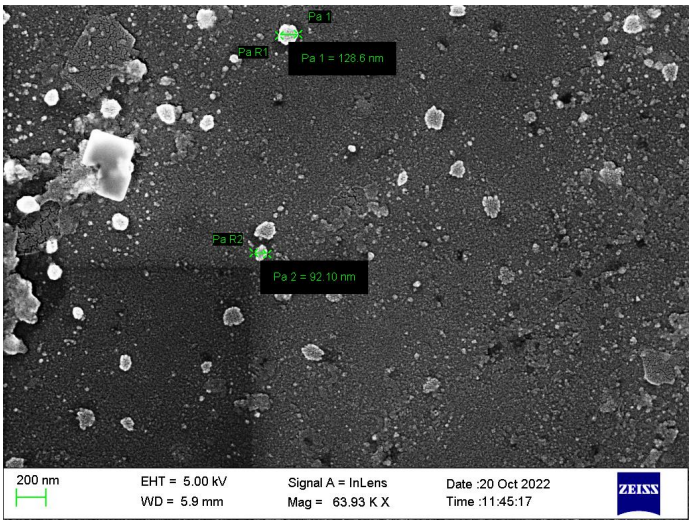

Figure 7 Scanning Electron Microscopy image of Herbosome Complex

***In-vitro* drug release kinetics study of optimized formulation**

The data of percentage drug release formulation were described as per kinetic study following plots were made cumulative (%) drug release vs. time (zero order kinetic models); log cumulative (%) drug remaining vs time (first order kinetic model); cumulative (%) drug release vs square root of time (Higuchi model); log cumulative (%) drug release vs log time (Korsmeyer–Peppas model). All Data and results are summarized in Table 3. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of optimized formulation showed the constant drug release from the herbosomes, the results of the zero order model was found to be y=5.972x+4.4111; R^2=0.988. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be y=-0.164x+2.341; R^2 = 0.807. The Higuchi model is used to describe the limits for transport and drug release. The Higuchi model of formulation was found to be, y= 25.18x-12.62; R^2=0.945 and the results of Korsmeyer Peppas kinetic model was found to be y=1.431x+0.399; R^2=0.881. *In-vitro* drug diffusion studies were carried out using dialysis bag method. On the basis of best fit with the highest correlation (R^2) (Table 5) value it is concluded that in the optimized formulation of herbosomes follow the Zero order kinetic model as shown in figure 8. *In-vitro* drug release of optimized formulation was 97.59% even after 16hr as shown in table 4.

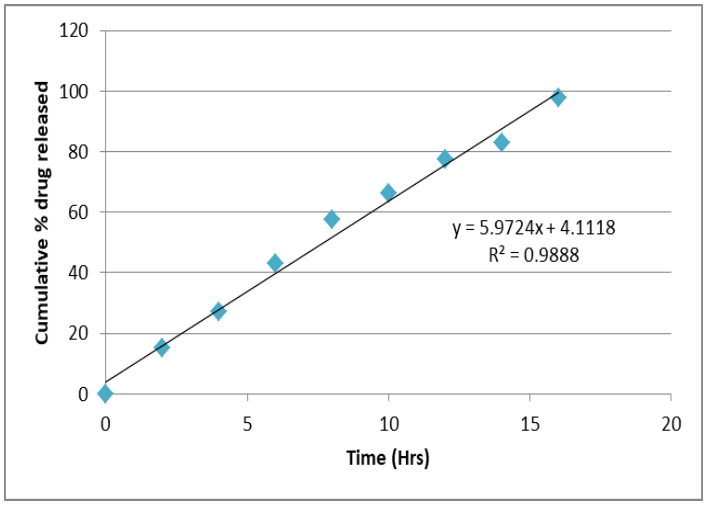

Figure 8 Zero Order Kinetic model (*In-vitro*)

Table 4 Release kinetics study of optimized formulation

S. No	Time (hr)	Cumulative Drug Release (%)	Drug Remaining (%)	Square Root Time	Log C_{umu} Drug Remaining (%)	Log Time
1.	0	0	100	0.000	2.000	0.000
2.	2	15.29	84.71	1.414	1.928	0.301
3.	4	27.11	72.89	2.000	1.863	0.602
4.	6	43.04	56.96	2.449	1.756	0.778
5.	8	57.61	42.39	2.828	1.627	0.903
6.	10	66.13	33.87	3.162	1.530	1.000
7.	12	77.42	22.58	3.464	1.354	1.079
8.	14	82.83	17.17	3.742	1.235	1.146
9.	16	97.59	2.41	4.000	0.382	1.204

Table 5 Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values
Herbosomes	Zero Order	$R^2 = 0.988$
	First Order	$R^2 = 0.807$
	Higuchi	$R^2 = 0.945$
	Korsmeyer Peppas	$R^2 = 0.881$

Conflict of interest: The authors declare that there is no conflict of interest.

Reference:

1. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 31st ed. Nirali Prakashan; 2005. p. 596.
2. Nalla A and Chinnala KM, 2017. Novel herbal drug delivery system-an overview. WJPPS, 6(8), pp.369-395.
3. Gandhi A, Dutta A, Pal A, et al. 2012. Recent trends of phytosomes for delivering herbal extract with improved bioavailability. J pharma and phyto, 1(4), pp.06-14.
4. Pawar HA, and Bhangale BD, 2015. Phytosome as a novel biomedicine: a microencapsulated drug delivery system. J Bio & Bio, 7.
5. Bhattacharya S, 2009. Phytosomes: the new technology for enhancement of bioavailability of botanicals and nutraceuticals. Int J Healt Res, 2(3), pp.225-232.
6. Barnes PM, Powell-Griner E, McFann K, et al. 2004, June. Complementary and alternative medicine use among adults: United States, 2002. In Seminars in integrative med (Vol. 2, No. 2, pp. 54-71). WB Saunders.
7. Faheem AM and Abdelkader DH, 2020. Novel drug delivery systems. In Engg drug del sys (pp. 1-16). Woodhead Publishing.
8. Nautiyal V, Roy A, Sahu RK, et al. 2021. Herbosomes: Impressive tool for better Bioavailability and Bioactivity of plant extracts and Botanicals. Res J of Pharma and Tech, 14(1), pp.471-476.
9. Validation of Analytical Procedures: Text and Methodology Q2(R1) https://www.ich.org/
10. Moghimipour E, and Handali S, 2012. Utilization of thin film method for preparation of celecoxib loaded liposomes. Adv pharma bull, 2(1), p.93.
11. More MS, Shende MA, Kolhe DB, et al. 2012. Herbosomes: herbo-phospholipid complex an approach for absorption enhancement. Int. J. Bio. Pharm Res, 3(8), pp.946-955.
12. Moghimipour E, Aghel N, Mahmoudabadi AZ, et al. 2012. Preparation and characterization of liposomes containing essential oil of Eucalyptus camaldulensis leaf. Jund. J. Nat Pharma Pro, 7(3), p.117.
13. Choubey A. 2011. Phytosome-A novel approach for herbal drug delivery. Int. J. Pharm. Sci. Res., 2(4), p.807.
14. Bhuyan C, Kalita B. and Das T. 2019. Design and development of herbosomes cream for the prevention and treatment of black fly bites.

15. Sopyan IYAN, Gozali DOLIH, and Guntina RK. 2022. Design-expert software (DOE): An application tool for optimization in pharmaceutical preparations formulation. Int. J. Appl. Pharm., pp.55-63.
16. Kalita B, Das MK, and Sharma AK. 2013. Novel phytosome formulations in making herbal extracts more effective. Res. J. Pharm. Tech., 6(11), pp.1295-1301.
17. Deleanu M, Toma L, Sanda GM, et al. 2023. Formulation of Phytosomes with Extracts of Ginger Rhizomes and Rosehips with Improved Bioavailability, Antioxidant and Anti-Inflammatory Effects in-vivo. Pharmaceutics, 15(4), p.1066.
18. Abhinav M, Neha J, Anne G. et al. 2016. Role of novel drug delivery systems in bioavailability enhancement: At a glance. Int. J. Drug Del. Tech., 6(1), pp.7-26.
19. Ortega-Pérez LG, Ayala-Ruiz LA, Magaña-Rodríguez OR, et al. 2023. Development and Evaluation of Phytosomes Containing Callistemon citrinus Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model. Pharmaceutics, 15(9), p.2178.