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Optimization and Development of Liposomal Formulation Enthusing Leuten and Its In-Vitro Evaluation

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ABSTRACT: Liposomes are small, cylindrical the ability to produce artificial vesicles from cholesterol and phospholipids that are both secure and healthy. Because compact structure, hydrophilic and hydrophilic makeup, and biocompatibility, liposomes are an appealing drug delivery technique. In this study, varied ratios of cholesterol and soy lecithin were used to physically disperse the marigold extract lipid membranes. All preparations were classified by chemical dissolution, UV and FTIR spectroscopy examination, as well as other physicochemical studies. With different formulations, the cumulative % of pharmaceutical publication be located related. As according estimations, the cumulative % drug release over 8 hours for formulations F8, F2, and F3 was 99.032.47, 91.922.72, and 82.122.51, respectively. The manufactured lipid membranes F 1 though F 8 displayed sustained drug release. The mechanical dispersing technique of planning and preparation also enhanced the drug's capacity to maintain its release when the soy phospholipid concentration was increased.

Keywords: Liposomes, Lutein, Sustained Release, Soy lecithin, Cholesterol, Marigold extract

I. INTRODUCTION:

Liposomes are spherical, synthetic vesicles composed of one or more lipid bilayers enclosing aqueous compartments.[1] They are typically made from natural phospholipids and cholesterol, offering biocompatibility and the ability to carry both hydrophilic and hydrophobic drugs, including peptides, proteins, enzymes, and anti-cancer agents.[2] Their properties, such as size, surface charge, lipid composition, and bilayer rigidity or fluidity, vary depending on preparation methods and lipid types. Hydration of phospholipids in aqueous environments leads to self-assembly into lamellar structures due to their amphipathic nature.[3] Liposome sizes range from 30 nm to several microns, with the polar lipid head groups interfacing with the surrounding aqueous phases, making them versatile systems for drug delivery.[4]

II. MATERIAL AND METHODS:

Material:

The marigold extract was purchased from local market of Lucknow. Ethanol, cholesterol and chloroform were obtained from the Chemicals for laboratories Reachem, Chennai. All the chemicals were analytical grade.

Analytical Procedure:

In a volumetric flask, the segments were made up to a total of 100 ml using ethanol after the precisely measured 12 mg of marigold extract was dissolved in it.[5] Then, the solution's absorbance was determined at 446 nm using an ultraviolet double beam spectrophotometer with ethanol used as a blank, and its max, or maximum absorbance wavelength, was recorded.[6]

- 100µl of stock solution was transfer of 10 ml volumetric flask and volume makeup 1 to10 ml.
- 200µl of the stock solution were transferred to a 10 ml volumetric flask with a 1 to 10 ml volume makeup.
- A 10 ml volumetric flask with a volume makeup of 1 to 10 ml received 400µl of stock solution.
- A 10 ml volumetric flask with a 1 to 10 ml volume makeup was filled with 600µl of stock solution.
- 800 µl of the stock solution were transferred to a 10 ml volumetric flask with a 1 to 10 ml volume makeup.
- A 10 ml volumetric flask was filled with 1000µl of stock solution, with a volume makeup of 1 to 10 ml.

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Preparation of pH 6.8 phosphate buffer
Dissolve potassium dihydrogen phosphate (11.45 gm.) and sodium hydrogen phosphate (28.20 gm.) in 1000 ml of water.[7]

Preparations of marigold extract using pH 6.8 phosphate buffer solution:

In a volumetric flask, 10 mg of precisely measured marigold extract was accurately diluted in 100 ml of methanol. To create a stock solution with a concentration of 100 µg/ml, 10 ml of the aforementioned solution was pipetted into a 100 ml volumetric flask and brought up to that volume using phosphate buffer pH 6.8. 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml, and 2.0 ml from this stock solution were pipetted into a series of 10 ml volumetric flasks and made up to mark with phosphate buffer pH 6.8 to produce a concentration in the range of 2 to 20 µg/ml. The solution's absorbance was then measured at 440 nm with an Ultra Violet Double Beam spectrophotometer using phosphate buffer pH 6.8 as the reference. Plotting concentration (µg/ml) values on the X-axis.[8]

Preformulation Studies

Pre-formulation testing is the process of analyzing the physical and chemical properties of a pharmacological ingredient both on by itself and in conjunction with excipients. This is the first logical step in the development of a dosage form. Generating data is the primary goal of pre-formulation testing. This will facilitate the creation of stable, stable, and bioavailable dose forms during the formulation process. Pre-formulation variables raise the probability of producing a stable, safe, effective, and acceptable product.[9]

Formulation of liposomes loaded with marigold extract: Marigold extract-loaded liposome formulation was created using the physical dispersion approach.[10]

Physical dispersion method:

Using a varied mix of soya lecithin and cholesterol while maintaining a steady temperature, liposomes were created using the physical dispersion method. This procedure involved dissolving cholesterol and soy lecithin in chloroform.[11] It is then spread out completed a conical flask with a flat bottom, where it was left to evaporate over night at room temperature without disturbing the solution to prevent the creation of a lipid layer. In phosphate buffer with a pH of 6.8, the medication was dissolved. As an aqueous media, it serves.[12] The lipid layer was then hydrated by the addition of the aqueous medium. This was accomplished by tilting the flask to one side, adding aqueous medium along its side, and then gradually bringing the flask back to an upright position. To finish hydrating, the conical flask was then placed in a water bath that was maintained at 37°C for two hours. The liposome suspension was created by gently shaking the conical flask to remove the lipid layer from the flask's wall. The created liposome suspension was then kept at 4°C for a day to allow the liposomes to mature. The overall procedure outlined above was used to create various batches of liposomes, and the ingredients used to create liposomes.[13]

Table No. 1: Formulation of Marigold extracts liposomes

Formulation Code	Soya Lecithin	Cholesterol	Marigol d extract	Ethanol	Chloro form	Dis. water	Phosphate buffer pH 6.8	Sonication time (min.)
F1	200 mg	100 mg	100 mg	10ml	10ml	10ml	10ml	10 min.
F2	400 mg	100 mg	100 mg	10ml	10ml	10ml	10ml	10 min.
F3	200 mg	200 mg	100 mg	10ml	10ml	10ml	10ml	10 min.
F4	400 mg	200 mg	100 mg	10ml	10ml	10ml	10ml	10 min.
F5	200 mg	400 mg	100 mg	10ml	10ml	10ml	10ml	10 min.
F6	400 mg	400 mg	100 mg	10ml	10ml	10ml	10ml	10 min.

Characterization Of Liposomes:

Vesicle Size Analysis: The liposomes that were generated were examined to observe the production of vesicles and the distinctness of scattered vesicles. The liposome's particle size was determined using an Optical microscope. The determination of particle size as the mean diameter relies on direct observation using a microscope. The process consists of two distinct stages: Eye-piece micrometre calibration. Globule size measurement.[14]

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The shape and surface morphology of liposomes were examined using scanning electron microscopy (SEM) in a study on vesicle morphology. The liposomes that were created were attached to an aluminium stub using double-sided adhesive carbon tape. The vesicles were coated with a layer of gold/palladium using a vacuum evaporator. They were then analyzed using a scanning electron microscope that had a digital camera, with an accelerating voltage of 25 kV.[15]

Drug Encapsulation Efficiency Determination: The entrapment efficiency of liposomes was measured using the centrifugation method. Liposomal dispersion samples were centrifuged using a Remi R4C laboratory centrifuge at a speed of 3500 revolutions per minute (rpm) for a duration of 90 minutes. The transparent liquid portion was carefully extracted to separate medications that were not trapped, and the intensity of light absorption was measured. The sediment in the centrifuge tube was mixed with a 100 ml volume of phosphate buffer, and the absorbance of this solution was measured. The combined quantity of drug in the liquid portion and solid portion resulted in the total amount of drug in the mixture. The drug entrapment percentage was determined using the following formula:
The entrapment efficiency is calculated by dividing the difference between the total amount of the drug and the amount of free drug by the total drug, and then multiplying the result by 100.

Evaluation of Liposomes:
Determination of drug content of liposomes:

Centrifuging samples is a very helpful procedure that is used in many different laboratories. High-speed sample spinning in a centrifuge causes denser materials to fall quickly to the bottom of the tube due to centrifugal force. This makes it possible to separate samples of liquid and solid.[16]

The prepared liposome suspension required 20 minutes of 15,000 rpm centrifugation for further investigation, the obtained solid was collected and cleaned with distilled water[52]. Under UV Spectroscopy at wavelength 446 nm, the various Liposome concentrations were investigated while creating a curve.

In vitro drug release study:
Franz diffusion method:

We employed Franz diffusion cells, 3.4618 cm2 in area, with a cellulose membrane. 30 minutes of membrane boiling in a 2% sodium bicarbonate solution. Then add 0.5 cc of EDTA with membrane and boil for 30 minutes. At 25°C for 24 hours, add 100 ml of distilled water to the membrane. The membrane was then permanently attached to the cell's contributor then receptor partitions. PH 6.8 for phosphate buffer.

An 8-hour in-vitro drug release study was performed on all of the designed Marigold extract liposomes in phosphate buffer, pH 6.8. The experiments were carried out at 37°C and 50 rpm in a Franz diffusion apparatus. The dissolution medium was phosphate buffer in a volume of 900 ml with a pH of 6.8. The magnetic beat was rotated at 30 rpm while liposomes containing 10 mg of marigold extract. Every 30 minutes up to 480 minutes, 3 ml of samples were taken using a double beam UV spectrophotometer73, made up to 3 ml with pH 6.8, and tested for the presence of marigold extract at 446 nm with pH 6.8 as a blank.[17]

Stability studies: These studies were performed on the optimal formulation, F1. The drug retention behaviour of vesicles was evaluated by subjecting the liposome dispersion to various temperatures, including 4-8 °C (refrigerator) and 27 °C. The temperature range is ±2 °C at room temperature and 38 ± 2 °C for a particular duration of 30 days.
The liposomal dispersion was stored in airtight jars made of aluminium foil and glass. It was then examined for particle size, in-vitro drug release, and the percentage of drug entrapment at a wavelength of 446 nm.[18]

Result and Discussion:
Preparation of Calibration curve:

Marigold extract was observed under UV Spectroscopy at wavelength 446nm and prepared calibration curve. (The absorbance of different concentrations is included in the table No.4)

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Table No. 2: Calibration curve data of marigold extract (Leutein)

Sr. N.	Marigold extract concentration (µg/ml)	Abs. (446nm)
1.	1.2	0.03
2.	2.4	0.061
3.	4.8	0.108
4.	7.2	0.161
5.	9.6	0.208
6.	12	0.266

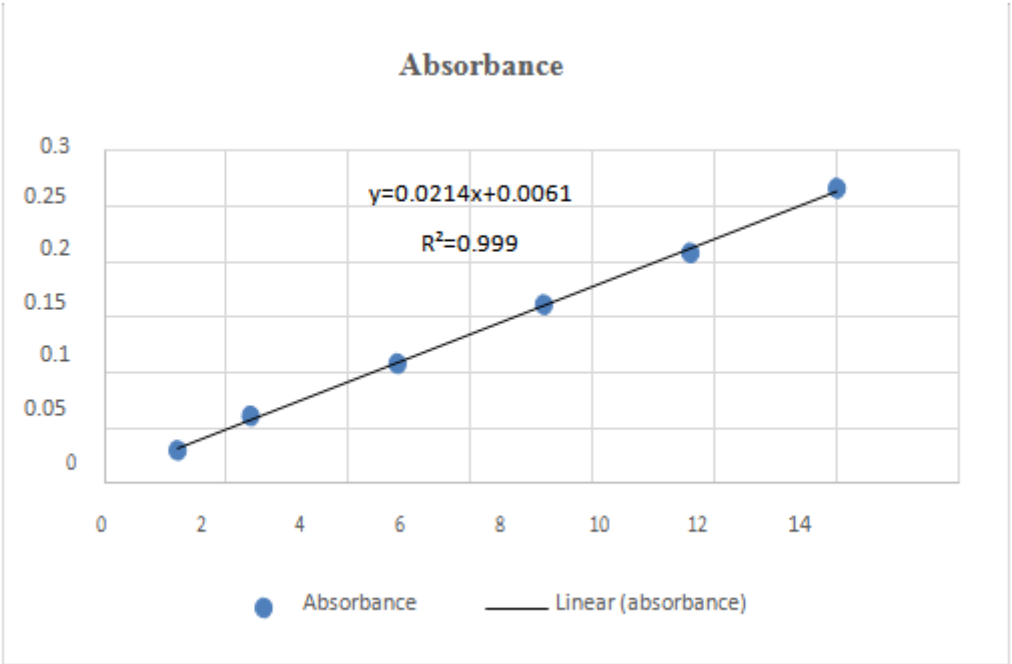

Figure 1: Calibration curve of marigold extract

Identification Results

FTIR spectra was recorded (Fourier Transform Infra-Red Spectrometer), at Babasaheb Bhimrao Ambedkar University, Lucknow, U.P. The genuine n drug's IR absorption spectra are investigated between 4000 and 450 cm^{-1}. Marigold extract was used; however no significant shifting or deprivation of functional peaks was seen in the spectra.FTIR Spectra of marigold extract:

FTIR Spectra of marigold extract:

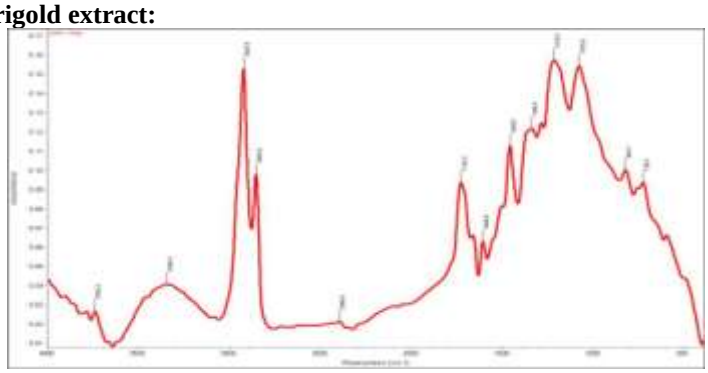

Figure 2: FT-IR Spectrum of Pure Marigold extract

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The FTIR spectra of marigold extract showed the characteristic peaks due to stretching at 3740.4, 3346.0, 2923.9, 2855.0, 2398.5, 1729.3 cm-1 (-OH) (symmetrical –CH3 group).

Drug – Excipients Interaction Studies:

Drug excipient interaction The IR absorption spectrums of the actual drug along the excipient were investigated in the range of 4000-450 cm. Observed peak are alike to notified peaks and there is no utmost shifting as well as deprivation of functional peaks between the spectra. FT-IR experiments with pure Marigold extract, cholesterol, soy lecithin, and Marigold extract + cholesterol + soya lecithin were used to examine the interaction between the medication and excipients. According to IR spectral analysis, the fundamental peaks and patterns of the spectra in both pure drugs and combinations combining drugs with the greatest number of excipients were similar. This demonstrated that the other excipients included in the formulations and the Marigold extract did not interact chemically. Figure shows spectral peaks graphically.

FT-IR Spectrum of Cholesterol:

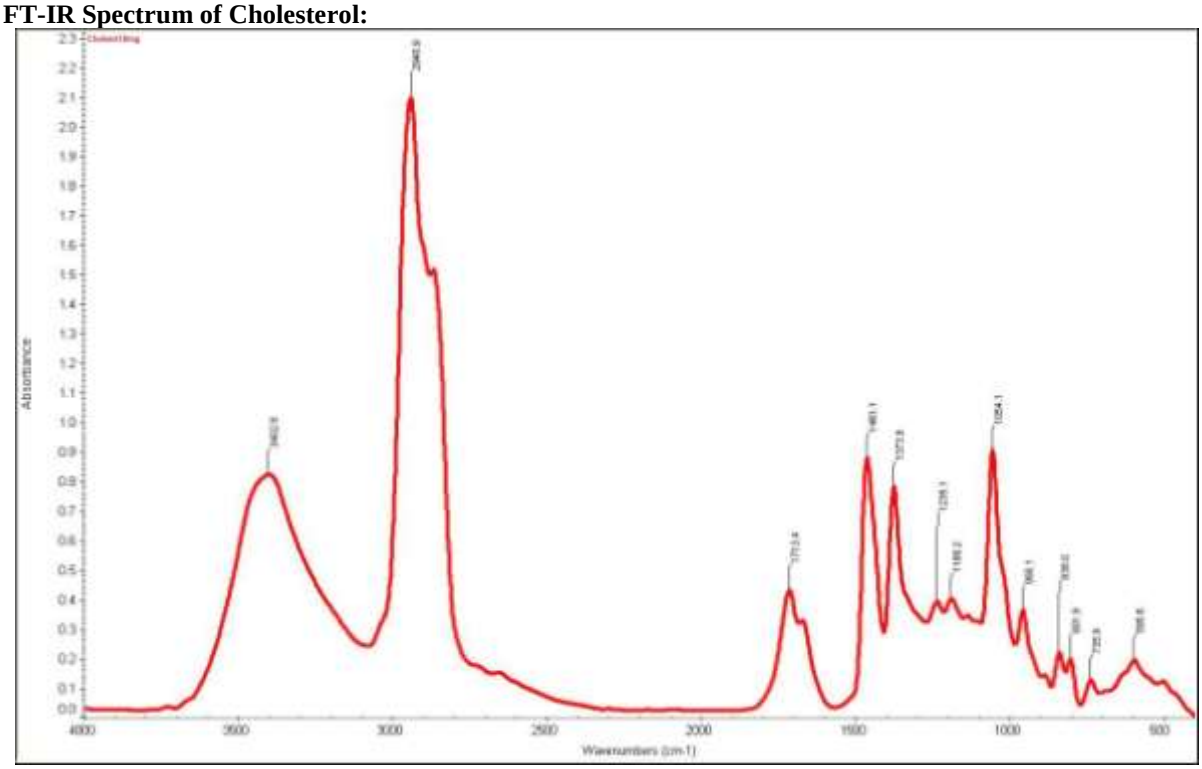

Figure 3: IR Spectrum of cholesterol

The FTIR spectra of cholesterol showed characteristic peaks due to stretching at 3402.8 (-OH) and 2940.9 cm-1 (symmetrical –CH3 group).

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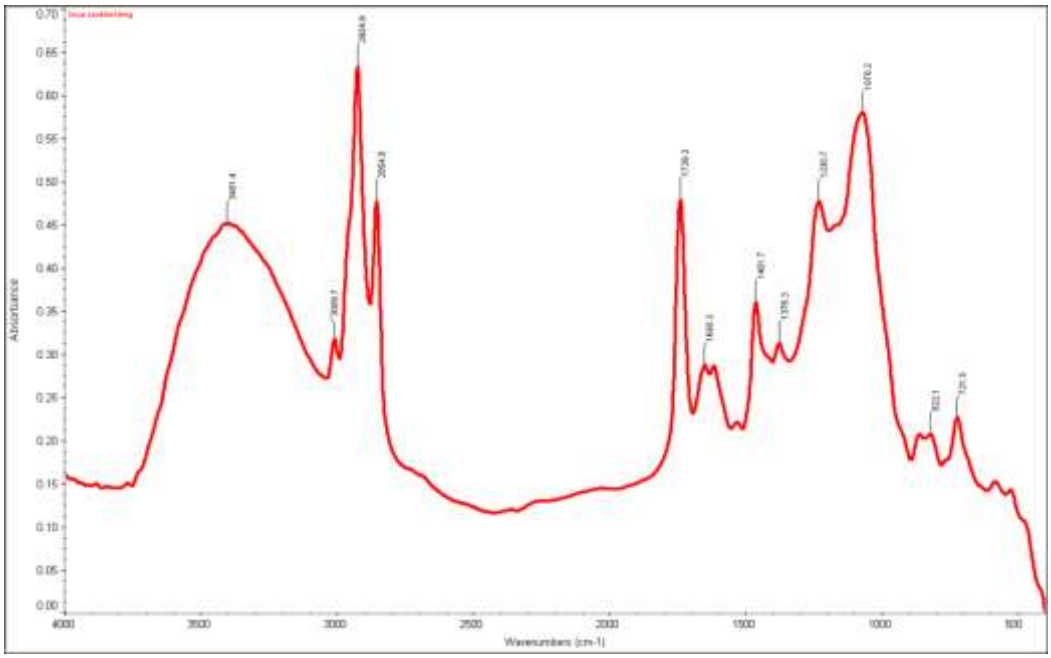

Figure 4: IR Spectrum of soya lecithin

The FTIR spectra of soya lecithin showed characteristic peaks due to stretching at 3464.1, 2923.8, 2854.5, 1733.8 cm-1 (C=O) and (C=C group).

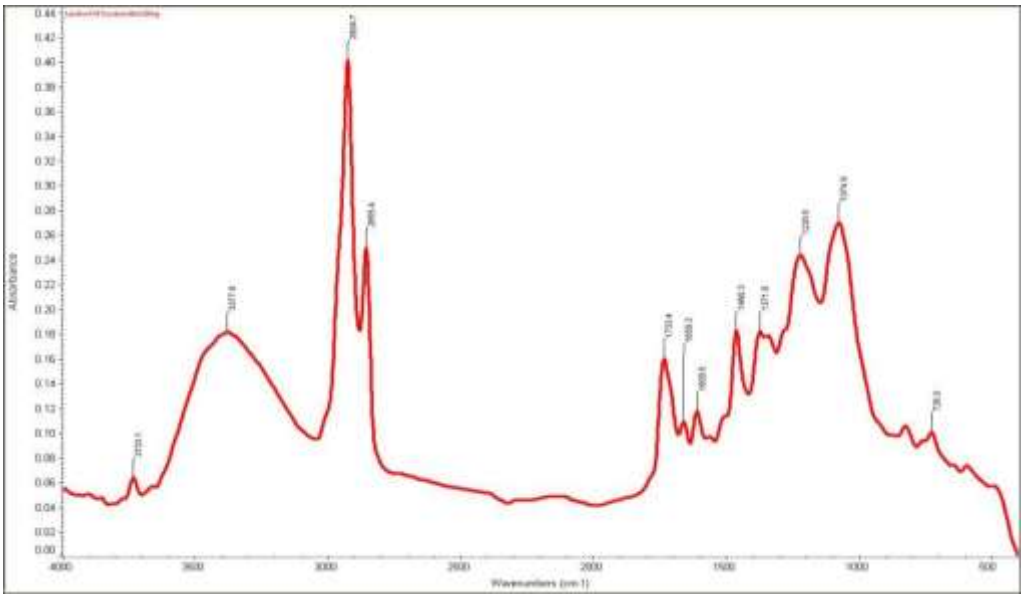

Figure 5: IR Spectrum of marigold extract, cholesterol and soya lecithin

The FTIR spectra mixture of marigold extract, cholesterol and soya lecithin showed peaks due to stretching at 3733.1, 3377.8, 2924.7, 2855.4, 1733.4

Characterization of Liposomes:

Vesicle Morphology: The structure of liposomes was examined using scanning electron microscopy. The scanning electron microscopy (SEM) pictures of formulation showed that the liposomes had a spherical form and were clearly defined with

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distinct boundaries, containing a significant amount of internal aqueous space.

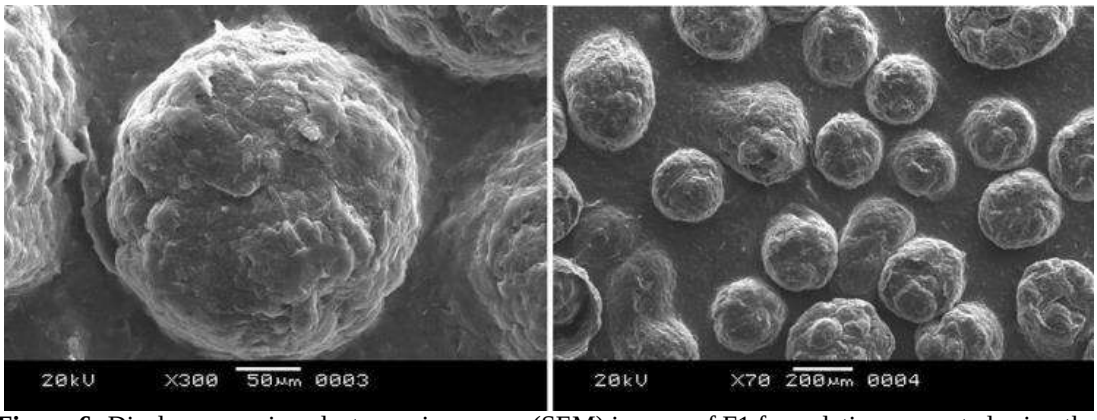

Figure 6: Displays scanning electron microscopy (SEM) images of F1 formulation generated using the optimised formulation.

Analysis of vesicle size: The average size of liposomal formulations is shown in Table 1. The results suggest that vesicles created with a concentration ratio of 2:1 of lecithin and cholesterol are smaller in size. The optimised liposomal formulation F1 had a vesicle size of 186.2 nm, as measured by the Zetasizer and depicted in Figure 4.

Table 3: Average Vesicle Size of Liposomal Formulations

Formulation Code	Average Vesicle size in µm
F1	186.2±0.03
F2	413.1±0.05
F3	511.5±0.86
F4	326.1±0.12
F5	403.5±0.98
F6	352.7±0.04

Drug Encapsulation Efficiency: The process of enclosing drugs within a protective barrier.
The Encapsulation Efficiency of liposomal formulations varies between 81.052% and 94.135%. The liposome formulation comprising of lecithin, cholesterol and drug in the ratio of 2:1:1 exhibited superior encapsulation efficiency compared to other formulations.

Table 4: Percentage Encapsulation Efficiency

Formulation Code	Percentage Encapsulation Efficiency
F1	94.135±0.21
F2	81.052±0.42
F3	82.112±0.64
F4	84.231±0.21
F5	83.342±0.43
F6	87.532±0.52

All values represent mean ± standard deviation (SD), n=3

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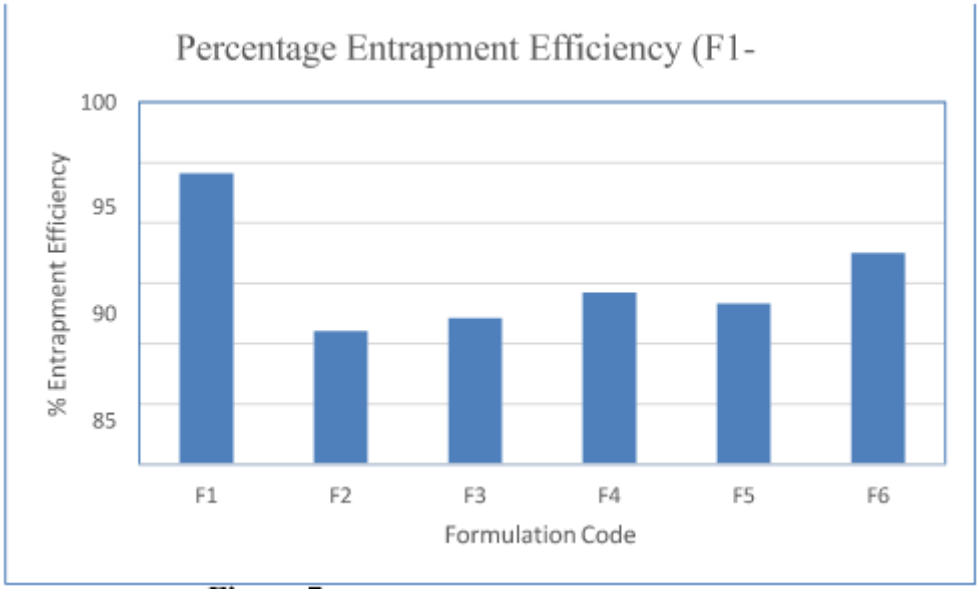

Figure 7:

Evaluation Of Liposomal Formulations

In-vitro drug release study results: All of the designed Marigold extract liposomes underwent an 8-hour in-vitro drug release study in phosphate buffer, pH 6.8.

Table 5: Cumulative % Drug released from formulations F1-F6 (marigold extract liposomal formulations)

Sr. N.	Time in minutes	F1	F2	F3	F4	F5	F6
1.	30	9.31±0.94	8.92±0.52	8.16±0.63	7.21±0.94	9.05±0.52	7.46±0.63
2.	60	15.76±0.59	12.19±0.61	11.24±0.80	14.22±0.59	13.64±0.61	12.65±0.80
3.	90	24.47±1.13	18.60±1.72	15.84±1.26	21.35±1.13	17.50±1.72	14.64±1.26
4.	120	32.70±2.54	25.22±1.47	20.78±2.4	36.12±2.54	22.22±1.47	18.48±2.4
5.	150	40.48±2.20	30.44±3.18	26.11±2.36	38.13±2.20	28.44±3.18	24.21±2.36
6.	180	45.49±1.85	36.58±3.54	30.60±2.44	41.84±1.85	33.58±3.54	34.60±2.44
7.	210	50.25±1.90	41.20±3.80	35.19±2.47	47.03±1.90	42.20±3.80	37.19±2.47
8.	240	57.43±1.72	47.65±3.87	38.49±2.61	51.11±1.72	48.65±3.87	40.49±2.61
9.	270	65.91±1.45	53.68±3.55	43.26±2.61	56.21±1.45	52.68±3.55	44.26±2.61
10.	300	74.00±3.11	58.45±3.00	46.98±2.38	62.04±3.11	57.45±3.00	48.98±2.38
11.	330	81.77±2.78	63.71±3.21	50.45±2.37	66.17±2.78	61.71±3.21	52.45±2.37
12.	360	88.04±2.81	69.84±3.56	55.85±2.37	71.89±2.81	66.84±3.56	56.85±2.37
13.	390	92.43±2.07	75.66±3.03	62.30±2.37	73.22±2.07	71.66±3.03	61.30±2.37
14.	420	95.83±2.11	80.70±2.63	69.23±2.51	76.06±2.11	78.70±2.63	67.23±2.51
15.	450	97.76±2.02	86.52±3.09	75.36±2.51	79.52±2.11	81.51±2.63	70.33±2.51
16.	480	98.03±2.47	91.92±2.72	82.12±2.51	81.11±2.11	83.42±2.63	75.86±2.51

The expressed values with mean ± standard deviation, n=3

By physically dispersing the marigold extract liposomes, varied ratios of soy lecithin and cholesterol were used. With various formulations, the cumulative percentage of medication release was compared.

For the formulations F1 to F6 the cumulative percent drug release was calculated over the course of 8 hours and found to be 98.03±2.47, 91.92±2.72, 82.12±2.51, 81.11±2.11, 83.42±2.63, and 75.86±2.51 respectively.

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Formulation F1 showed better release profile than other formulations.

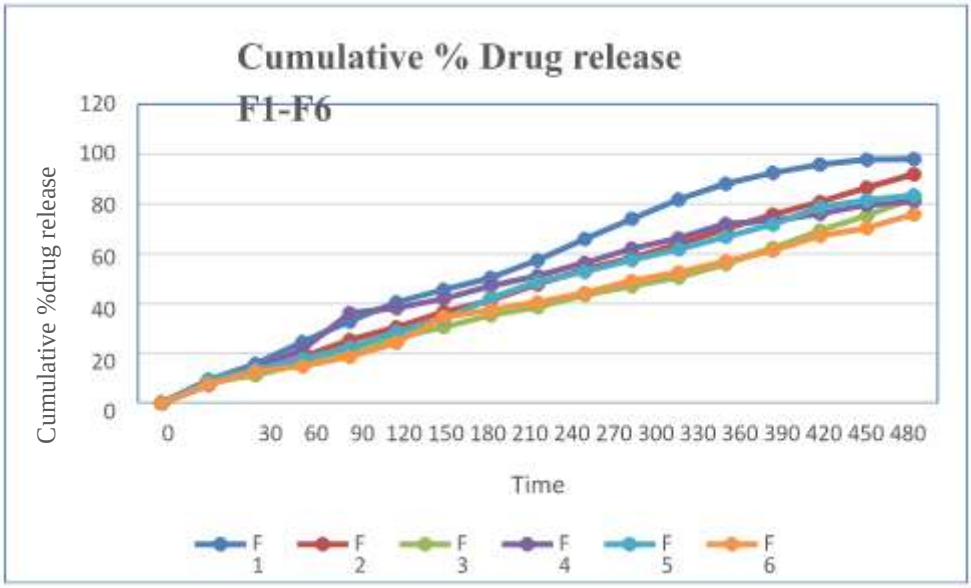

Figure 8: Shows cumulative % drug release from the formulations F1-F6 respectively in 8 hours (Comparative drug released from Marigold extract liposomes)

Stability Studies: The liposome formulation that had been optimised exhibited instability when exposed to temperatures of 37 ± 2 ºC and 40 ± ºC. Stability investigations indicated findings related to the physical characteristics, particle dimensions, in-vitro release of the drug. The entrapment of the F1 formulation has not changed when stored at 4-8 ºC.

III. CONCLUSION:

The physical dispersion method was proven to be a successful method for producing marigold extract as a liposomal drug delivery system in this investigation. The ratio of soya lecithin to cholesterol in these liposome formulations was (like 2:1, 4:1, 4:2….). Analysis of their physical features utilising in vitro drug release revealed that the rate of drug release slowed down as soy lecithin content increased. The manufactured liposomal formulation F1 had a higher percentage of drug entrapment. All of the formulations' stability investigations were carried out by maintaining the formulations at two distinct temperatures. The formulations were examined for in vitro drug release following the stability period. Based on all the results formulation F1 containing Soy lecithin, cholesterol and marigold extract in the ratio 2:1:1 showed the best results.

Conflict of Interest: The authors declare that they have no conflict of interest.

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