MS.ID-IJPBS-7722
International Journal of Pharmacy and Biological Sciences-IJPBS® (2023) 13 (3): 70-75
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Design, Prepare, and Characterization of Erythrina Variegata Loaded Solid Lipid Nanoparticles

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Received: 12 Mar 2023 / Accepted: 9 Apr 2023 / Published online: 1 Jul 2023
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Abstract
The goal of this study was to assess the efficacy of a method based on the creation of solid lipid nanoparticles as an innovative formulation of Erythrina Variegata with enhanced therapeutic efficacy. The Erythrina Variegata solid lipid nanoparticles were created using the Sonicator to apply ultrasonic energy during the emulsification solvent evaporation process. The numerous formulations with varied drug-lipid and surfactant ratios were analysed and improved. Erythrina Variegata solid lipid nanoparticles containing soy lecithin were created using the solvent evaporation method, then the particle size was decreased by sonication. Particle size, surface morphology by SEM, drug excipient compatibility by FTIR, and in-vitro drug release experiments were used to characterize the produced nanoparticles. The formulation with the best encapsulation efficiency was (F-4) A drug encapsulation effectiveness of up to 95.36 % has been attained in this study. It was discovered that the efficiency of encapsulation improved along with the soy lecithin content. According to the results of the current investigation, the manufacture of Erythrina Variegata solid lipid nanoparticles can be done using a solvent evaporation process followed by sonication.

Keywords
Erythrina Variegata extract, solid lipid Nanoparticles, Solvent Evaporation, lipid, FTIR, in-vitro drug release.

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INTRODUCTION
Nanocarriers are drug transport systems that have gained a great deal of attention over recent decades for their ability to facilitate site-specific drug delivery, including brain delivery. Solid lipid nanoparticles, in particular, are regarded as interesting drug delivery systems.[1]

Their preparation techniques have gained a great deal of attention. In contrast to emulsion and liposome, the particulate matrix of SLNs is composed of solid lipids. They are colloidal particles of submicron size, with a diameter between 50 to 1000 nm for colloid drug delivery applications.[2] They provide advantages including stabilization of incorporated compounds, controlled release, and conclusiveness. In the current study, SLNs loaded with Erythrina Variegata extract were prepared using the Solvent Evaporation technique.[3] The prepared SLNs were characterized and on the basis of obtained results, the best formulation was selected amongst various formulations and further characterized for in-vitro performance evaluation. [4] Medicinal plants are majorly an important therapeutic aid for alleviating the ailments of humankind. The current widespread and strong belief about plant-derived drugs is that “Green medicine” is safe and more

dependable than costly synthetic drugs due to their adverse side effects.[5] Different parts of *E. Variegata* have been used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. The leaves are used in fever, inflammation, and joint pain. They have a potential use in the prevention of postmenopausal bone loss. It is also known to possess pharmacological activities including antimicrobial, anti-bacterial effects.[6]

MATERIALS

Erythrina Variegata extract was obtained from Synpharma research labs, HYD. Soya Lecithin, Poloxamer 407 were procured from Synpharma research labs, HYD and other chemicals and the reagents used were of analytical grade.

METHODOLOGY

Preparation of leaf extract [7]

For extraction, fresh leaf of Erythrina was collected from Synpharma labs, HYD, India. Collected leaves were cleaned well with normal water and again cleaned with double distilled water. The leaf is dried under sun with closed pack to free from dust. The dried leaf is ground it to fine powders and 5g of powder is mixed with 100 ml of distilled water then it is boiled to 60ºC for 15 min. After cooling down to normal room temperature, the extract was filtered through normal filter paper to get free from powder and again filtered using whatman filter paper to get clear leaf extract. The filtered extract is stored in the refrigerator at 4 ºC and used for further synthesis process.

Method of preparation of Erythrina variegata loaded nanoparticles[8].

Using a solvent emulsification/evaporation process, Erythrina variegata-loaded SLN was created. The ingredients in each formulation Drug and lipid solutions were combined after 200 mg of the substance had been dissolved in 10 ml of methanol and 20 ml of chloroform, respectively. Using a rotary evaporator, the organic solvent combination was totally evaporated at 70°C to extract the organic solvent. After adding the drug-embedded lipid layer to 100 ml of an aqueous solution containing poloxamer 407 surfactants, the mixture was Sonicated for 15 minutes using a Sonicator before being homogenized for 15 minutes using a high-speed homogenizer at various speeds. It was then allowed for the suspension to cool to room temperature. Through a membrane filter, the suspension was filtered. The filtrate was centrifuged (1000 rpm for 10 minutes) and nanoparticles were collected.

Table -1: composition of Erythrina variegata for preparation of solid lipid nanoparticles

Ingredients	F1	F2	F3	F4
Erythrina variegate extract	20	20	20	20
Phosphatidylcholine	50	75	100	50
Poloxamer 407	50	100	150	200
Solvent (Methanol)	10	10	10	10
Chloroform	20	20	20	20

Evaluation of Erythrina variegata loaded nanoparticles:

Particlesize[9]

All of the generated batches of nanoparticles were observed under a microscope to establish their sizes. The average size of the nanoparticles was determined by measuring the size of each batch's nanoparticles in a small drop of nanoparticle dispersion on a slide.

SEM analysis[10]

The morphology of nanoparticles was examined using the scanning electron microscope (SEM, Hitachi, Tokyo, Japan). After being properly diluted (1:100) in double-distilled water, Erythrina variegata-freeze-dried SLNs were added to a drop of the nanoparticle formulation and left to air dry. The sample was then observed under various magnifications and a 15,000-volt accelerating voltage. The imaging was performed in a high vacuum.

Drug encapsulation efficiency [11]

A set volume of the SLNs dispersion (10 ml) was poured into a centrifuge tube at room temperature, and it was spun at 18,000 rpm for 20 minutes (Remi Instruments Pvt. Ltd, India). The drug's absorbance in the supernatant was measured spectrophotometrically at a maximum wavelength of 270 nm after the lipid component was removed (Shimadzu 1800, Japan).

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped.}}{\text{Total drug loaded.}} \times 100$$

***In-vitro* drug release studies[12]**

Utilizing the dialysis bag approach, in vitro release tests were carried out. Prior to the release trials, the dialysis membrane (molecular weight cutoff between 12,000 and 14,000) was immersed in double distilled water for an overnight period. As releasing media, phosphate buffer pH 6.8 and hydrochloric acid (0.1 N) were also employed. A donor compartment and a receptor compartment make up the experimental unit. A boiling tube that was cut open at one end and tied with a dialysis membrane at the other end serves as the donor compartment, into which 3 ml of SLN dispersion was injected for the release research. The receptor compartment is made up of a 250 ml beaker that contains 100 ml of release media and was kept at a temperature of 37 0.5 °C. Every 3 ml sample was taken out of the receiver compartment and replaced with the same amount of release medium at the 1, 2, 3, 4, 5, 6, 7 and 8h time periods. The collected samples were appropriately diluted before being examined at 345 nm with a UV-visible spectrophotometer.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug

Da = The amount of drug released

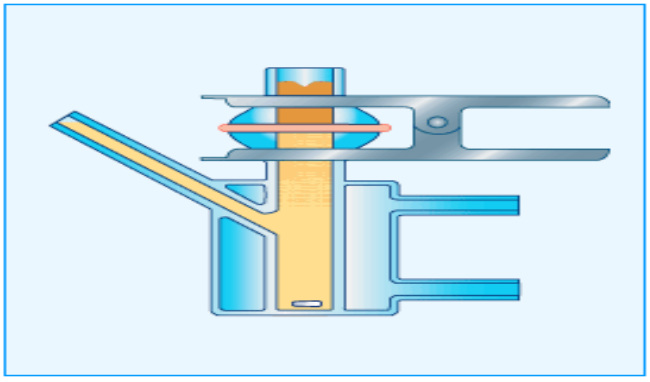

Fig-1: Franz diffusion cell

Stability studies[13]

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability testing is to obtain a stable product which assures its safety and efficacy up to the end of shelf life at defined storage conditions and peak profile. The prepared Erythrina liposomes were placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2°c and refrigerator 2-8°c for a period of 90 days.

RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

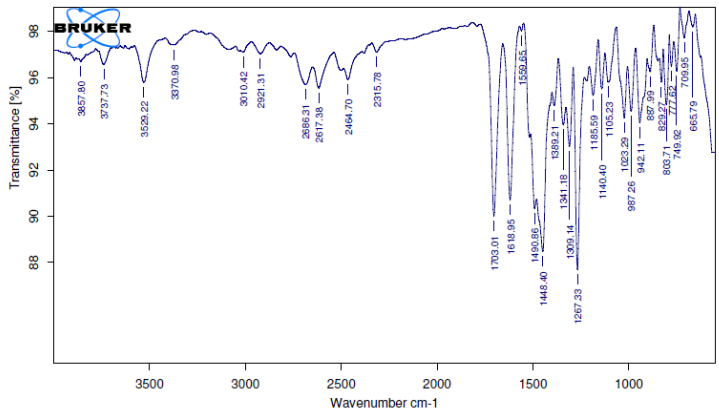

Fig-2: FT-IR Sample for Erythrina variegate.

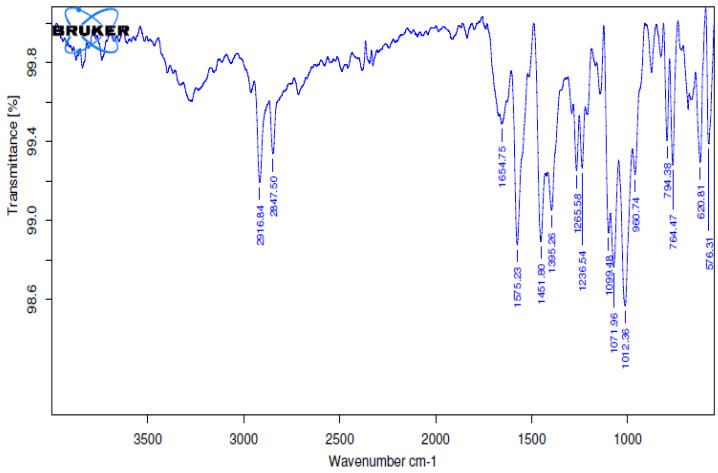

Fig-3: FT-IR Sample for Optimized Formulation

EVALUATION PARAMETERS

Particle size:

With an increase in lipid concentration, the particle size increased. based on entrapment effectiveness and particle size distribution.

Surface morphology:

According to scanning electron microscopy (SEM), the solid lipid nanoparticles were round, smooth, and free of any aggregation.

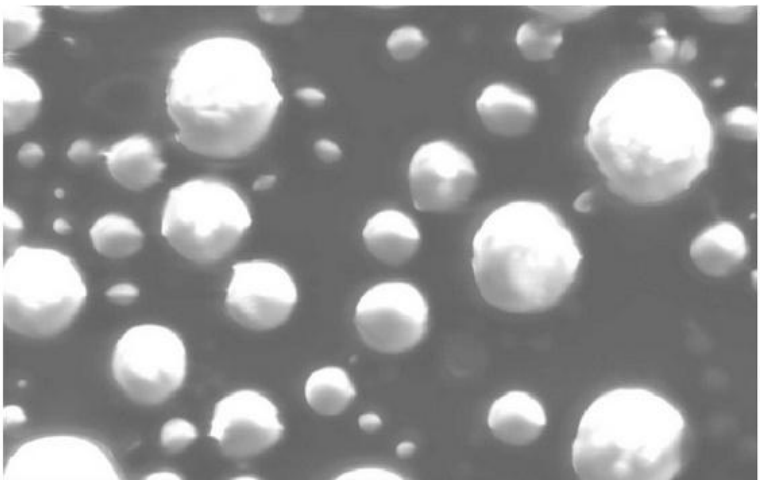

Fig-4: SEM analysis of Optimized Solid lipid nanoparticle

Drug entrapment efficiency:

Optimizing the lipid concentration to be used in the creation of solid lipid nanoparticles was the first step of the work plan. Based on the particle size and entrapment effectiveness of the discovered solid lipid nanoparticles, the lipid content was optimized.

Table-2: Evaluation Studies of Prepared solid lipid nanoparticles: Entrapment Efficiency and Particle size

F. No	Particle size (nm)	Entrapment Efficiency (%)
F1	243	84.52
F2	224	86.30
F3	251	88.90
F4	235	90.32

***In vitro* drug release studies**

Using a dialysis membrane and a pH 7.4 buffer, the in vitro diffusion investigations were carried out for eight hours. The initial release of the medication from all three batches was discovered to be between 25 and 30 percent in 8 hours. This resulted from the drug's release from the surface of the solid lipid nanoparticles. Later, for 8 hours, a consistent and gradual medication release was seen. The lipid and surfactant ratio in the F4 formulation was shown to be the most effective one.

Table-3: *In vitro* drug release profiles of SLN (F1-F8)

Time	F1	F2	F3	F4
0	0	0	0	0
1	12.78	11.8	13.50	14.58
2	26.98	24.67	26.52	27.82
3	37.12	36.627	35.86	34.75
4	45.36	40.18	48.12	50.14
5	53.82	56.71	56.38	60.12
6	68.34	69.2	64.89	71.35
7	75.82	81.76	78.85	80.24
8	89.35	91.92	93.18	95.36

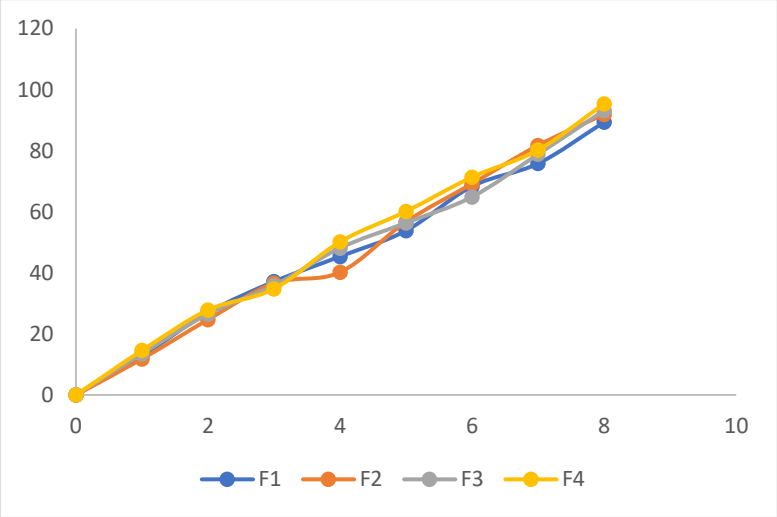

Fig-5: Drug release for all formulations

Stability studies:

After three months, the physical and chemical characteristics of the nanoparticles of formulation F-4 had not significantly changed. The parameters quantified at various times were displayed.

Table-4 : Results of stability studies of optimized formulation F-4

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	**25^{0}C/60%RH % Release**	95.36	95.12	94.53	93.53	**Not less than 85 %**
F-4	**30^{0}C/75% RH % Release**	95.36	95.08	94.26	93.42	**Not less than 85 %**
F-4	**40^{0}C/75% RH % Release**	95.36	95.02	94.18	93.20	**Not less than 85 %**

CONCLUSION

The current study suggested a unique Erythrina variegata a solid lipid nanoparticle formulation for regulated release. Investigation into the solid lipid nanoparticles' production, characterisation, and in-vitro release was done. *E.* variegata has been ethno medicinally used as a therapeutic agent for a variety of diseases. Alkaloids and flavonoids which were isolated from this plant may be responsible for its pharmacological activities. The numerous formulations with varied drug-lipid and surfactant ratios were analysed and improved. A drug encapsulation effectiveness of up to 98.85% has been attained in this study. Erythrina variegata solid lipid nanoparticles containing soy lecithin were created using the solvent evaporation method, then the particle size was decreased by sonication. This shows that the formulation procedure was suitable and reproducible in nature, and it provided a good yield. The formulation with the best encapsulation efficiency was (F-4) It was discovered that the percentage of encapsulation efficiency rose along

with the soy lecithin concentration. According to the method described permeation studies with dialysis membrane were conducted. The in vitro drug release profiles of all the formulations indicated an initial burst effect, followed by a gradual drug release. The formulations demonstrated good drug release from the lipid. These solid lipid nanoparticles contained more Erythrina variegata and released it more quickly.

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