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DEVELOPMENT OF LIPOSOMAL DRUG DELIVERY SYSTEM FOR TARGETED DELIVERY OF 5-FLUOROURACIL (5-FU)

E. Beaula Rani*[1], Yadiki Poojitha[2], Boppajada Praveen[2], Ramavath Priyanka[2], Prudhvi Gorati[2], Bathula Raju[2]

[1]Assistant Professor, Department of Pharmaceutics,Teegala Ram Reddy college of pharmacy, Meerpet, Hyderabad, Ranga Reddy Telangana - 500097, India.

[2]Students, Teegala Ram Reddy college of pharmacy, Meerpet ,Hyderabad, Ranga Reddy Telangana - 500097, India.

Abstract:

The present study was aimed at the formulation and evaluation of 5-Fluorouracil-loaded liposomes to enhance drug encapsulation, provide sustained drug release, and improve formulation stability. Liposomes were prepared using the thin-film hydration method by varying the concentration of cholesterol while maintaining a constant concentration of phosphatidylcholine. FTIR compatibility studies demonstrated the absence of significant interactions between the drug and excipients, confirming their compatibility.SEM analysis revealed that the prepared liposomes were spherical in shape with smooth surface morphology. The particle size of the formulations ranged from 175 to 258 nm, with formulation F3 exhibiting the smallest particle size (175 nm). Drug entrapment efficiency was found to vary among the formulations, with F3 showing the highest entrapment efficiency of 85.19%.In vitro drug release studies demonstrated sustained release behavior over an 8-hour period, with formulation F3 exhibiting the highest cumulative drug release of 98.51%. Stability studies conducted under various storage conditions (25°C/60% RH, 30°C/75% RH, and 40°C/75% RH) for three months indicated no significant changes in the physicochemical characteristics or drug release profile of the optimized formulation, confirming its stability.The findings of this study suggest that 5-Fluorouracil-loaded liposomes represent a promising drug delivery system capable of providing enhanced drug entrapment, sustained release, and improved stability. Among the developed formulations, F3 was identified as the optimized formulation based on its superior physicochemical characteristics and release profile. Further in vivo studies are warranted to establish the therapeutic efficacy and clinical applicability of the developed liposomal formulation.

Keywords: *5-Fluorouracil, Liposomes, Thin-Film Hydration Method, Entrapment Efficiency, Sustained Drug Release, Stability Studies.*

Corresponding author:

E. Beaula Rani ,

Assistant professor, Department of Pharmaceutics,
Teegala Ram Reddy College of Pharmacy,
Hyderabad - 500097, Telangana, India.
Mobile:
Email Id:

QR CODE

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INTRODUCTION:

Liposomes

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by membrane composed of lipid molecules. Various amphipathic molecules have been used to form liposomes. The drug molecules can either be encapsulated in aqueous space or intercalated in to the lipid bilayers. The exact location of drug will depend upon its physico-chemical characteristics and the composition of the lipids.

Phospholipids are the major structural components of the biological membranes in the human body, where two types of phospholipids exist i.e. phosphodiglycerides and sphingolipids, together with their corresponding hydrolysis products. The way they work and form membranes are elegant and miraculous. Each phospholipid molecule has three major parts, one head and two tails. The head is made from three molecular components: choline, phosphate, and glycerol. The head is hydrophilic in other words, it is attracted to water. Each tail is a long, essential fatty acid chain. These fatty acids are hydrophobic that is, they are repelled by water. When phospholipids are put in an aqueous (water-based) solution, the hydrophilic heads of the phospholipids form a line side by side with their tails behind much like swimmers at a starting gate. Then because the tails are hydrophobic, another phospholipid layer will line itself up tail-to-tail in response to the same aqueous environment. This natural alignment creates two-rows of tightly fitted phospholipid molecules, called a phospholipid bilayer. It is these phospholipid bilayers that form the membranes around and within every cell in our bodies.

The number of components of the liposomes is varied; however phospholipids and cholesterol are the main components. The most commonly used phospholipids include phosphatidyl choline (PC). PC is an amphipathic molecule in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains, with a hydrophilic polar head group, phospho choline.Phosphatidyl choline, also known as “lecithin”, can be derived from natural and synthetic sources. At various temperatures, lecithin membranes can exist in different phases. At elevated temperatures lipid membranes pass from tightly ordered gel to liquid crystal phase where freedom of movement of individual molecules is higher. In general, increasing the chain length, or increasing the saturation of the chains, increases the transition temperature. Incorporation of sterols (cholesterol) in liposome bilayer can bring about major changes in the preparation of these membranes. Cholesterol does not by itself form bilayer structure, but can be incorporated in to phospholipid membranes in very high concentrations up to 1:1 or even 2:1 molar ratios of cholesterol to PC. Cholesterol inserts in to the membrane with its hydroxyl group oriented towards aqueous surface and aliphatic chain aligned parallel to the acyl chains in the center of the bilayer. Cholesterol incorporation increases the separation between the choline head groups and eliminates the normal electrostatic and hydrogen bonding interactions. The high solubility of cholesterol in phospholipid liposomes has been attributed to both hydrophobic and specific head group interactions.

MATERIALS:

5-Fluorouracil was procured from Aurobindo ltd, Hyd, Cholesterol, Lecithin, Phosphatidylcholine, Chloroform and Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

Preparation of liposomes

Method

5-Fluorouracil -loaded liposomes can be prepared by a modified thin-film hydration technique. First, the required lipids such as phosphotidyl choline and cholesterol are accurately weighed and dissolved in a volatile organic solvent such as chloroform–methanol (2:1 v/v). The solvent mixture is evaporated under reduced pressure in a rotary evaporator at about 40 °C to form a uniform dry lipid film on the inner wall of the flask, and any traces of solvent are removed by flushing with nitrogen or argon. An aqueous phosphate buffer (pH ≈ 7.4) containing the desired concentration of 5-Fluorouracil is then added to the dried lipid film, and the flask is gently rotated or vortexed so that the film hydrates and detaches, entrapping the 5-Fluorouracil solution within multilamellar vesicles. The size of the vesicles is reduced and a more uniform population obtained by probe sonication, high-pressure homogenisation or extrusion through polycarbonate membranes of decreasing pore size. Unencapsulated drug is removed by centrifugation or by passing the suspension through a size-exclusion (gel-filtration) column. Finally, the liposomal suspension is sterilised—preferably by aseptic processing or, if particle size permits, by filtration through a 0.22 µm sterile filter—and stored under refrigeration at 2–8 °C to maintain stability and sustain the controlled-release characteristics of the 5-Fluorouracil liposomes.

Table-1: Composition of lipids for preparation of liposomes

Ingredients	F1	F2	F3	F4
Phosphatidylcholine (mg)	100	100	100	100
Cholesterol(mg)	100	200	300	400
Chloroform (ml)	10	10	10	10
Methanol	5	5	5	5
Drug(mg)	10	10	10	10
Phosphate buffer pH 7.4(ml)	10	10	10	10

Evaluations of liposomes

Encapsulation efficiency determination

The encapsulation efficiency of 5-Fluorouracil liposomes is determined by first separating the free (unencapsulated) drug from the liposome-entrapped drug and then measuring the amount of each fraction. A known volume of the liposomal suspension is subjected to ultracentrifugation at about 100,000 g for roughly one hour at 4 °C, or alternatively passed through a size-exclusion (gel filtration) column such as Sephadex G-50 or G-75. This step allows the liposomes, which elute in the void volume or form a pellet, to be separated from the free cytarabine that remains in the supernatant or later fractions. The entrapment efficiency of the liposomes was estimated using the following equation. (Eq 1).

$$\text{Entrapment efficiency (\%)} = [(TD - FD)/TD] \times 100 \quad (1)$$

Where

FD is the quantity of drug estimated in the filtrate and

TD is the theoretical amount of tacrolimus that is present in liposomal preparation.

Particle size analysis

First, an accurately measured aliquot of the liposomal suspension is diluted with filtered deionised water or isotonic buffer to an appropriate concentration so that the sample is optically clear enough for light-scattering measurements but still gives a strong scattering signal. The diluted dispersion is transferred to a clean, dust-free cuvette, taking care to avoid formation of air bubbles. The cuvette is then placed in the sample holder of the DLS instrument, which is typically maintained at a controlled temperature (for example 25 °C). A laser beam of fixed wavelength is directed through the suspension, and the instrument detects fluctuations in the intensity of scattered light arising from the Brownian motion of the liposomes.

Zeta potential analysis

Take an accurately measured aliquot of the freshly prepared liposomal suspension and dilute it with filtered, deionized water or an appropriate low-conductivity buffer to obtain a clear, dust-free dispersion that still gives a stable scattering signal. Transfer the diluted sample into a clean, disposable or rinsed capillary zeta cell, ensuring no air bubbles remain. Place the cell in the instrument chamber and allow the temperature to equilibrate, commonly at 25 °C. When a voltage is applied across the cell, the charged liposomes migrate toward the oppositely charged electrode; the instrument detects the Doppler shift of laser light scattered from the moving particles and calculates their electrophoretic mobility. The software then converts the mobility to zeta potential (mV) using the Henry or Smoluchowski equation, depending on the ionic strength of the medium. Record the mean zeta potential and the standard deviation from several consecutive measurements to characterise the surface charge and colloidal stability of the 5-Fluorouracil liposomes.

SEM analysis

First, a small volume of the liposomal suspension is diluted with deionised water and a drop is placed on a clean, conductive specimen holder or on an aluminium SEM stub pre-covered with a thin layer of conductive adhesive such as carbon tape. The sample is allowed to air-dry at room temperature so that the vesicles form a dry film; sometimes a mild vacuum desiccator is used to ensure complete removal of surface moisture without collapsing the structure. To prevent charging of the non-conductive lipid material under the electron beam, the dried sample is then coated with a very thin (≈10 nm) conductive layer—commonly gold using a sputter coater. The prepared stub is mounted in the SEM chamber, the instrument is evacuated to the required

vacuum and operated at an accelerating voltage typically between 5 and 20 kV. Images are recorded at different magnifications to examine the overall morphology, surface texture and approximate size of the liposomes. The micrographs obtained provide qualitative confirmation of the spherical shape and surface characteristics and can be complemented with particle size measurements from other techniques such as dynamic light scattering.

In Vitro Drug release study

In-vitro drug release of 5-Fluorouracil from liposomes is usually evaluated using a dialysis method that maintains sink conditions. Typically, a known volume of the liposomal suspension containing a quantified amount of 5-Fluorouracil is placed inside a pre-soaked dialysis bag or dialysis tubing with an appropriate molecular weight cut-off (for example 10–12 kDa) that retains the vesicles but allows free 5-Fluorouracil to diffuse. The sealed bag is then immersed in a measured volume of release medium—commonly phosphate-buffered saline (PBS) at pH 7.4—maintained at 37 ± 0.5 °C and gently agitated on a shaker or in a water bath to simulate physiological conditions. At predetermined time intervals, fixed-volume samples are withdrawn from the external medium and replaced with an equal volume of fresh buffer to maintain constant volume and sink conditions. Each collected sample is analyzed for 5-Fluorouracil content by a validated method such as UV–visible spectrophotometry at approximately 266 nm. The cumulative amount of drug released at each time point is then expressed as a percentage of the total drug initially entrapped in the liposomes and plotted against time to generate the release profile.

Stability studies:

The stability of optimized liposomes formulation was assessed for a period of 3 Months by storing the liposomes at 3 specified temperature conditions, i.e. 25±20 /60% RH (Room temperature; RT), 40±2°C/75% RH (Accelerated conditions) and 2-80 C (Refrigerator; control). The liposomal formulation was kept in sealed laminated aluminum tubes (10 ml capacity) which were previously flushed with nitrogen. Samples were periodically taken at specified time period of 0, 1, 2and 3months. These were tested for drug release, in the manner described.

RESULTS& DISSUSSION:

Particle size

Vesicle shape: Vesicle shape of the prepared formulation was found to be spherical from the SEM (scanning electron microscope) analysis at 15.00kV.

SEM Analysis

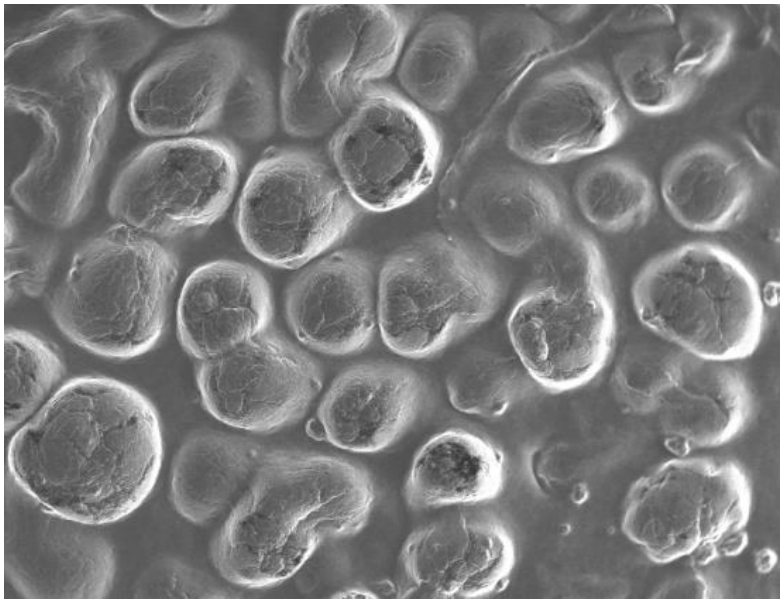


Fig-1: SEM Analysis of 5-Fluorouracil liposomes

Vesicle size:

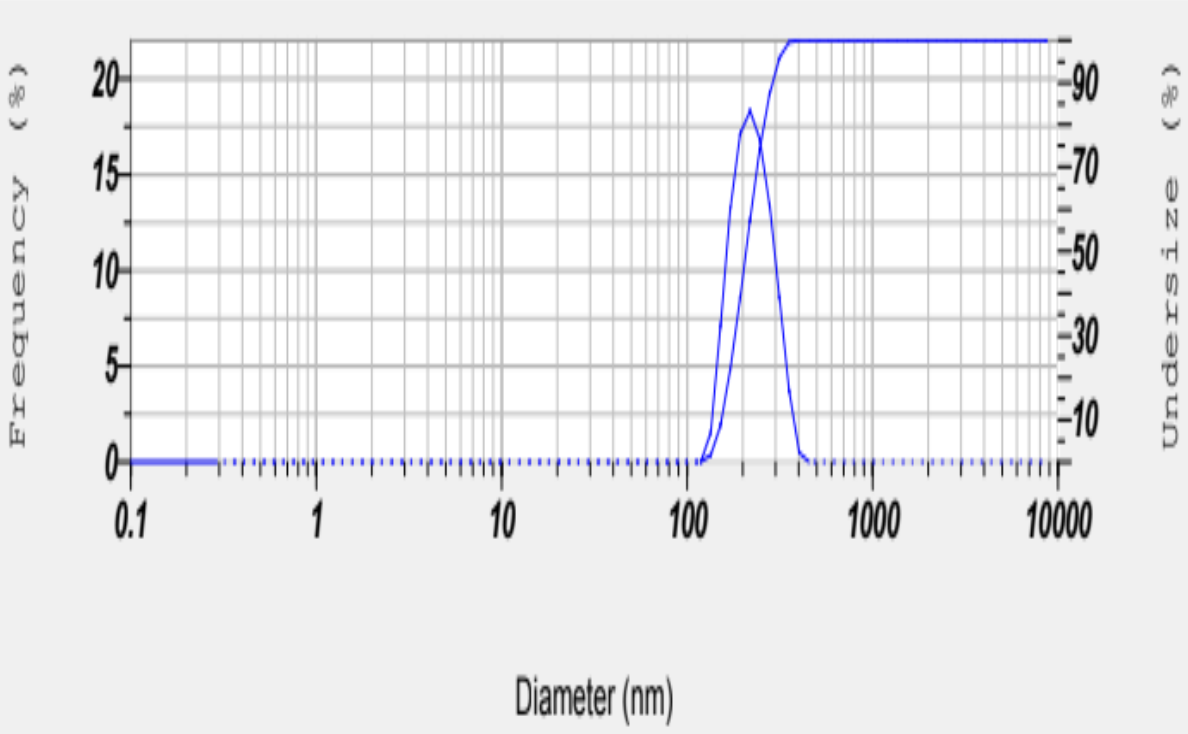


Fig-2: particle size of 5-Fluorouracil liposomes

Table-2: Mean particle size of different formulation of liposomes

Formulation No.	Particle size
F1	258
F2	179
F3	175
F4	243

Drug entrapment efficiency

Table-3: Different batches of liposome made by using different ratio of lipids

Formulation no.	% DEE
F1	82.39
F2	82.10
F3	85.19
F4	78.93

Drug release studies

Table-4: Cumulative percentage drug release from various formulation of liposomes

Time	F1	F2	F3	F4
0	0	0	0	0
1	14.89	16.13	18.10	17.58
2	26.65	28.19	28.93	27.82
3	34.52	36.82	38.59	37.17
4	46.98	48.19	48.53	47.35
5	58.49	57.58	59.28	58.10
6	65.55	68.55	71.25	69.85
7	79.88	81.29	83.65	82.35
8	92.20	96.30	98.51	95.58

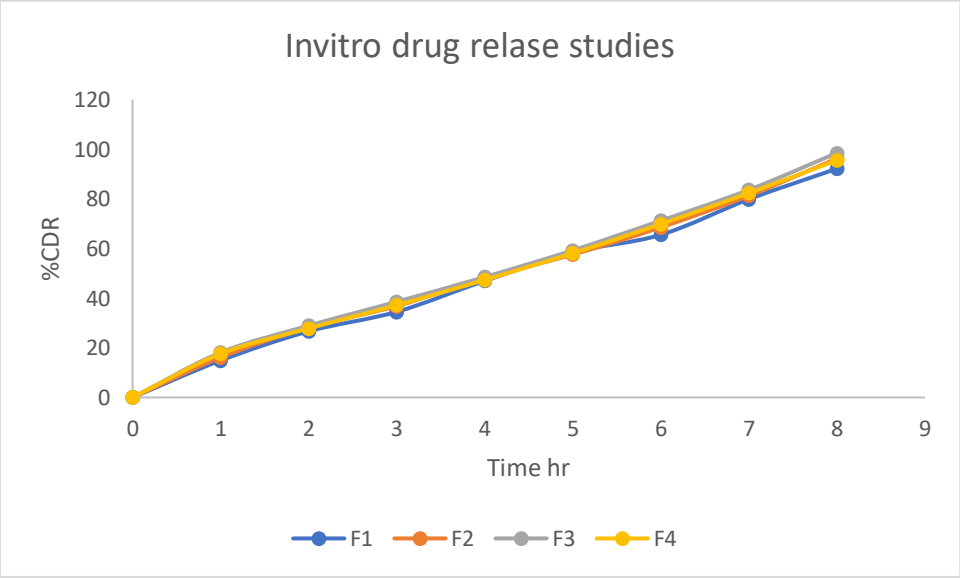


Fig-3: In vitro drug release of (F1-F4) formulations

Formulation F3 were found to release the drug in 8 h. The cumulative percentage release was found to be 98.51 %.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-3 after 3 months. Parameters quantified at various time intervals were shown;

Table- 5: Results of stability studies of optimized formulation F-3

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-3	**25^0C/60%RH % Release**	98.51	97.82	96.37	95.80	**Not less than 85 %**
F-3	**30^0C/75% RH % Release**	98.51	97.43	96.22	95.32	**Not less than 85 %**
F-3	**40^0C/75% RH % Release**	98.51	97.25	96.15	95.20	**Not less than 85 %**

SUMMARY AND CONCLUSION:

Summary

The present study was undertaken to formulate and evaluate 5-Fluorouracil-loaded liposomes using varying concentrations of cholesterol while maintaining a constant phosphatidylcholine concentration. Four formulations (F1–F4) were prepared and evaluated for physicochemical characteristics, drug-excipient compatibility, particle size, entrapment efficiency, in vitro drug release, and stability.Preformulation studies confirmed the suitability of 5-Fluorouracil for liposomal formulation development. Organoleptic evaluation showed that the drug was a white to almost white crystalline powder with a slightly bitter taste and was odorless in nature. The melting point of 5-Fluorouracil was found to be in the range of 282–286°C, indicating the purity of the drug sample. Solubility studies demonstrated that the drug was slightly soluble in water, freely soluble in DMSO, sparingly soluble in methanol, and practically insoluble in ethanol and chloroform.The calibration curve of 5-Fluorouracil in phosphate buffer pH 7.4 exhibited good linearity at 266 nm over the concentration range studied. FTIR compatibility studies confirmed the absence of significant interactions between the drug and formulation excipients, indicating their compatibility.

SEM analysis revealed that the prepared liposomes were spherical with smooth surface morphology. The particle size of the formulations ranged from 175 to 258 nm, with formulation F3 exhibiting the smallest particle size (175 nm). Entrapment efficiency studies demonstrated that formulation F3 showed the highest drug entrapment efficiency of 85.19%.

CONCLUSION:

In vitro drug release studies showed sustained release characteristics for all formulations over an 8-hour period. Among all formulations, F3 exhibited the highest cumulative drug release of 98.51%, indicating its superior release profile.

Stability studies conducted for three months under different storage conditions demonstrated no significant changes in drug release characteristics. The optimized formulation retained more than 95% drug release, confirming its stability during the study period.

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