DEVELOPMENT AND IN VITRO EVALUATION OF LIPOSOMAL DRUG DELIVERY SYSTEM BY USING BRAHMI EXTRACT

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ABSTRACT: The drug release from Liposomes depends on many factors including the composition of Liposomes, the type of drug encapsulated and nature of the cell. Once it is released a drug that normally crosses the membrane of a cell will enter the cell, other drugs will not enter. Brahmi extract is a drug with narrow therapeutic index and short biological half-life. This study aimed at developing and optimizing liposomal formulation of Brahmi in order to improve its bioavailability. In evaluation study the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of Brahmi with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 h in all formulations. F1 emerged as the most satisfactory formulation in so far as its properties were concerned.

Keywords: Liposomes, Brahmi, FTIR studies, thin film hydration technique, In vitro drug release studies

I. INTRODUCTION

Liposome have a great potential to be carriers of herbal extracts owing to their biodegradable nature and ability to enhance paracellular and transcellular drug transport. Considering the variety of nanocarriers, liposomes (Lips), spherical vesicles consisting of at least one phospholipid bilayer, have been investigated as potential carriers for drug delivery applications due to their high biocompatibility, complete biodegradability, low toxicity, and ability to entrap both water- and lipid-soluble functional compounds and simplify specific drug delivery to tumor site.[1] Furthermore, the stability of the functional components encapsulated in Lips can be increased, therefore maintaining their activities in environments that typically result in rapid degradation. [2] Here in the formation of liposomes was prepared according to the thin film method. Liposomes are nano-sized spherical vesicles composed of an aqueous core surrounded by one (or more) phospholipid bilayer shells. Owing to their high biocompatibility, chemical composition variability, and ease of preparation, as well as their large variety of structural properties, liposomes have been employed in a large variety of nanomedicine and biomedical applications, including nanocarriers for drug delivery, in nutraceutical fields, for immunoassays, clinical diagnostics, tissue engineering, and theranostics formulations. [3] Multilamellar vesicles (MLVs) were prepared using thin lipid film hydration method. Various factors such as phosphatidylcholine and cholesterol ratio, lipid and drug ratio, incorporation of charged species and pH etc. were studied which may affect the size, shape and incorporation efficiency of liposomes. [4,5] Many herbal plants and extracts have already documented beneficial results when tested for antiamnesic effects. Brahmi (*Bacopa monniera*) is one such common herbal drug, which is employed for a long time in the Indian and Chinese medical system in order to treat several disorders.[6] Brahmi exerts many pharmacological effects including memory boosting capacity in the treatment of Alzheimer's disease and Schizophrenia, exhibiting antiparkinsonian, antistroke, and anticonvulsant potentials.[7]

II.MATERIALS

Brahmi procured from Synpharma Research labs, Hyderabad, phosphotidylcholine, Cholesterol and other chemicals, and the reagents used were of analytical grade.

Methodology

Preparation of leaf extract [8]

For extraction, fresh leaf of Brahmi 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 refrigerator at 4 ºC and used for further synthesis process.

Preparation of liposomes

Table-1: composition of preparation of liposome

Ingredients	F1	F2	F3	F4
Phosphatidylcholine	250	500	750	1000
Cholesterol	500	500	500	500
Methanol	10	10	10	10
Drug(Brahmi)	100	100	100	100
Phosphate buffer pH 7.4	10	10	10	10

Liposomes preparation: Brahmi liposomes were prepared using thin film-hydration method. Accurately weighed quantities of the phosphotidylcholine and cholesterol in different ratios, were dissolved in 10 ml of methanol in a round-bottom flask. Afterwards, Brahmi dissolved in 10 ml of 7.4 phosphate buffer was added to the lipid solution. The organic solvents were removed under vacuum in a rotary evaporator at 40 ◦C for 20 min to form a thin film on the wall of the flask, and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. After removal of the last trace of organic solvents, hydration of the film was carried out using 10mL of distilled water at 55◦C. The resulting Liposomal suspension was mechanically shaken for 1 h using a horizontal mechanical shaking water bath at 55 ◦C. Then, the vesicle suspension was sonicated in 3 cycles of 1min "on" and 1min "off" leading to the formation of multi lamellar liposomes. The Liposomal suspension was left to mature overnight at 4 ◦C and stored at refrigerator temperature for further studies.[9,10]

Evaluations of liposomes

Drug entrapment efficiency of liposomes[11]

Entrapment efficiency of liposomes were determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3500 rpm for a period of 90 min. The clear supernatants were removed carefully to separate non entrapped Brahmi and absorbance recorded at 245nm. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded at 245 nm.

Amount of Brahmi in supernatant and sediment gave a total amount of Brahmi in 1 ml dispersion.

% entrapment of drug was calculated by the following formula

$$\% \text{ Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \text{ X } 100$$

Particle size analysis[12]

All the prepared batches of liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.

***In Vitro* Drug release study[13]**

The release studies were carried out in 10 ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10ml) was placed in a 10 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped Brahmi liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1sml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Stability studies[14]

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 Brahmi liposomes were placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40 ± 2^oc and refrigerator 2-8^oc for a period

of 90 days.

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR):

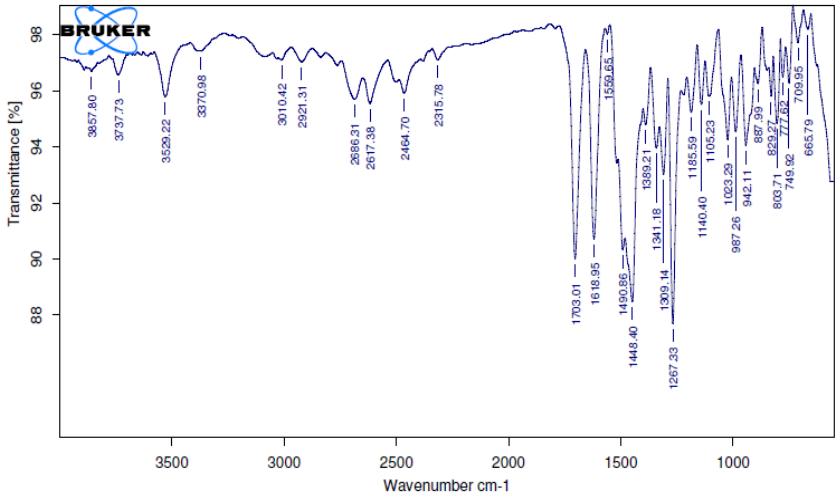

Fig.1. FT-IR Sample for Brahm

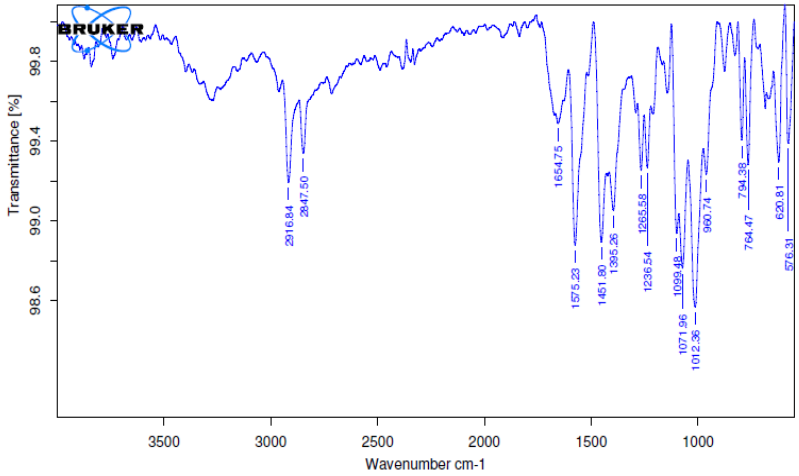

Fig.2. FT-IR Sample for physical mixture of extract and excipients

The IR spectrum of Brahmi and lipid mixture was shown in respectively. In the lipids used. From the figures it was observed that there were no changes in these main peaks in IR spectra of mixture of drug and lipids, which show there were no physical interactions because of some bond formation between drug and lipids. This further confirms the integrity of pure drug and compatibility of them with excipients.

EVALUATION PARAMETERS:

Entrapment Efficiency:

Separation of unentrapped drug from Liposomal suspension was done by exhaustive dialysis method. A measured quantity of Liposomal suspension was placed in a dialysis tube to which osmotic cellulose membrane was attached securely on one side and the dialysis tube was suspended in 100ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer. Through the osmotic cellulose membrane, the unentrapped drug was separated into the medium. For every one hour the whole medium was replaced with same quantity of fresh medium and the absorbance of collected medium reaches a constant reading indicating complete separation of unentrapped drug. The Liposomal suspension in the dialysis tube was further with propane–1–ol and the entrapped drug was estimated with the help of double beam UV spectrophotometer at 320 nm. The entrapment efficiency was measured in % with the help of following equation,

$$\%\ \text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

Table-3: Drug entrapment efficiency of all formulation

F.no	Drug entrapment efficiency
F1	87.96
F2	86.94
F3	79.81
F4	80.55

Determination of Vesicle morphology and Size

The morphological characteristics of formulated Liposomes were carried by using Scanning electron microscopy (SEM). A small drop of Liposomal suspension was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of -120^{0}c and voltage of 5kV.

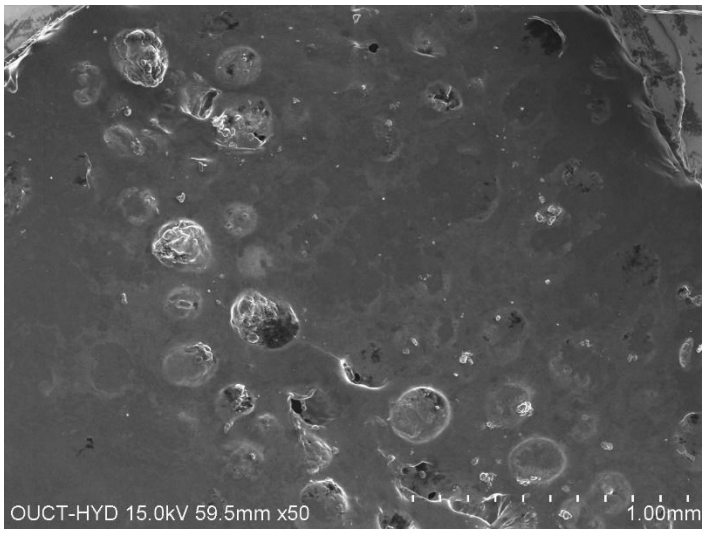

Fig.3. SEM analysis of Optimized liposomes

Table-4: Evaluation Studies of Vesicle size liposomes

Batch No	Particle size (nm)
F1	298.60
F2	302.26
F3	299.36
F4	358.25

***In vitro* drug release studies:**

The release of drug from Liposomal suspension was investigated using dialysis tubing method. All the formulations were separately placed in a dialysis membrane of 5cm length with closed ends which was washed and soaked in phosphate buffer pH 7.4 for about 15min. The membrane was suspended in a beaker containing 10ml of phosphate buffer pH 7.4 as diffusion medium maintained at a temperature of 37 ± 0.5^0 C and stirred continuously by means of magnetic stirrer at a constant speed. At a regular time, interval of one-hour 5ml of diffusion medium was withdrawn periodically for about 8hrs and immediately replaced with same amount of fresh diffusion medium to maintain sink condition. The collected samples were measured spectrophotometrically at 320 nm

Table-5: *In- vitro* drug release studies of (F1-F4) formulation

Time	F1	F2	F3	F4
0	0	0	0	0
1	11.15	15.45	12.18	14.27
2	23.06	25.42	23.42	22.84
3	35.56	33.87	34.67	33.65
4	54.4	45.56	41.98	48.17
5	64.2	55.70	55.18	58.37
6	77.89	68.83	70.46	65.42
7	86.82	73.10	76.81	82.87
8	94.80	89.98	88.69	90.55

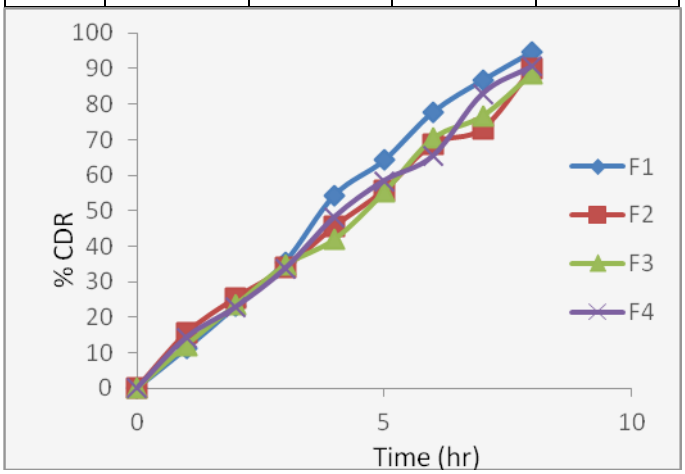

Fig.4. In vitro drug release studies of (F1-F4) formulation

Stability studies:

There was no significant change in physical and chemical properties of the liposomal formulation F-1 after 3 months. Parameters quantified at various time intervals were shown.

Table-6: Results of stability studies of optimized formulation F-1

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-1	**25^0C/60%RH % Release**	94.80	93.99	92.58	92.16	**Not less than 85 %**
F-1	**30^0C/75% RH % Release**	94.80	93.68	92.40	92.20	**Not less than 85 %**
F-1	**40^0C/75% RH % Release**	94.80	93.65	92.36	91.35	**Not less than 85**

IV. CONCLUSION

Brahmi possesses all requisite qualities required for liposomal drug delivery. Among the various formulation, the combination F1 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F1 comprising phosphatidylcholine, cholesterol 1:2, fulfills the requirement of good liposomal formulation. *In vitro* drug release upto 8 h and more than 94.80 % drug released. It shows encapsulation efficiency of 87.96 % and particle size of 298.60µm.

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