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Original Article

DESIGN, FORMULATION AND EVALUATION OF LIPOSOME CONTAINING ISONIAZID

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ABSTRACT

Objective: The objective of the present study was to formulate and evaluate liposomes loaded with isoniazid.

Methods: Liposome of isoniazid was made by thin layer film hydration method. L-α-phosphatidylcholine and cholesterol were used to make multiamellar vesicles. Six batches of liposomes were prepared based on the different weight ratio of L-α-phosphatidylcholine and cholesterol. Differential scanning calorimetry (DSC) study conducted to study in any incompatibility.

Results: The prepared liposomes were evaluated by particle size analysis, entrapment efficiency, release study and stability study. Particle sizes were determined from the scanning electron microscopy (SEM) photographs. When particle frequencies were plotted against particle diameter in the histogram, it showed that F1 batch had a skewed distribution towards smaller liposomes while F6 shows a proper bell-shaped curve with a mean at 225 µm. The percentage entrapment efficiency was found to be 8.99 ± 0.15 to 4.19 ± 0.12 % respectively. From the release profile, it was seen that F1 batch was fastest and F6 was slowest to release the drug. The satisfactory batch F1 was packed in Eppendorf tube and stored at 4 °C temperature for one month. At the end of one month, the samples were analyzed for their physical properties, drug entrapment and *in vitro* release profile. The percentage release was found to be 96.5 ± 3.2 after 4 h.

Conclusion: The F1 batch showed most promising results compared to other. No significant change was found during one month's stability study of final batch (F1).

Keyword: Liposome, Isoniazid, L-α-phosphatidylcholine, Cholesterol, Stability

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INTRODUCTION

Liposome has become an essential therapeutic tool most notably in drug delivery and targeting. Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids [1]. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of lipid crystalline bilayers become fluid and swell. The hydrated lipid sheets get detached during agitation and self-close to form large, multiamellar vesicles (MLVs), which prevent interaction of water with the hydrocarbon core of the bilayer at the edges. Once these MLVs are formed, a change in vesicle shape and morphology may require energy input in the form of sonic energy and/or mechanical energy. The former produce small unilamellar vesicles (SUVs) while the latter usually produces large unilamellar vesicles. Liposomal drug delivery system is very useful for delivery of anticancer, antifungal, antibacterial, antiparasites, ocular and antiviral drugs [2-4].

Phospholipids such as phosphatidylcholine (PC) and cholesterol were selected for the formation of liposomes into which the drug was incorporated. Phospholipids are amphipathic molecules as they have a hydrophobic tail and a hydrophilic or polar head. Cholesterol acts as a 'fluidity buffer' since below the phase transition it tends to make membrane less ordered while above transition it tends to make membrane more ordered thus suppressing the tilts and shifts in membrane structure specifically at the phase transition. Though cholesterol itself does not form bilayers, but it can be incorporated into phospholipid membrane in high concentration in the different molar ratio of cholesterol to PC [5].

Tuberculosis is an infectious bacterial disease, which most commonly affects the lungs. Isoniazid, also known as isonicotinic acid hydrazide (INH), is the first-line medication for tuberculosis and it is most widely used for treatment and prevention of tuberculosis [6].

The purpose of the present study was to design, formulate and evaluate the liposomes containing Isoniazid by *in vitro* methods. The liposomal formulations are designed for intramuscular administration for a sustained release action and at the same time automatic absorption of the delivery system, since all the components are biodegradable.

MATERIALS AND METHODS

Materials

Isoniazid, L-α-phosphatidylcholine (soya lecithin), cholesterol were purchased from yarrow Chem., Mumbai. Chloroform was purchased from Merck India. All the chemicals and reagents were used were of analytical grade.

Preparation of liposome by thin layer film hydration method

A solution of L-α-phosphatidylcholine and cholesterol in a specified weight ratio (F1=1:1, F2=2:1, F3=3:1, F4=4:1, F5=5:1 and F6=6:1) was dissolved in 5 ml chloroform. The chloroform solution was placed inside a 250 ml round bottom flask and rotated in the same direction. The flask was kept in a thermostatic water bath, and rotated while maintaining a temperature of 30 ° to 35 ° C. Rotation was continued till all the chloroform evaporates from the solution and a thin lipid film was deposited on the inner wall of the flask. An aqueous solution of isoniazid (5 ml containing 100 mg drug) was added to the flask and was rotated with the same speed as before for 30 min or until all the lipid was removed from the wall of the flask. The suspension was allowed to stand for a period of 15 min on water bath at 30 °C temperature for complete hydration.

Then, the suspension was taken into a bath type sonicator and sonicated for 30 min. Separation of non-entrapped drug was carried out by centrifugation at 3700 rpm for 40 min. The liposomal suspension was cooled by placing the test tubes in ice-cold water prior to centrifugation. Then liposome pellet was collected and re-suspended in distilled water [7, 13, 15].

Study of physical interaction between drug and excipients

Differential Scanning Calorimetry (DSC) thermograms were taken by scanning the samples of (i) pure isoniazid, (ii) excipients containing (lecithin, cholesterol) and (iii) the formulation (F1 and F6) using

DSC (Pyris Diamond TG/DTA, PerkinElmer, SINGAPORE) in nitrogen atmosphere (150 ml/min). Platinum crucibles were used with alpha alumina powder as reference [8, 16].

Particle size analysis

One drop of the Liposomal formulation was homogeneously spread onto a glass slide and left to dry overnight. After platinum coating the sample with a Polaron E5100 sputter coater (Polaron, England), the samples were observed under a Philips 505 scanning electron microscope at an accelerating voltage of 20 kV. Photographs were taken at 70, 100, 200, and 300 magnifications wherever necessary. Particle diameters were taken from the photographs by taking the scale shown in each photograph [9].

Drug entrapment studies

To aliquots of liposome sample (0.5 ml), 5 ml of 10% sodium lauryl sulphate (SLS) was added and the volume was made up to 50 ml. The sample was warmed on a water bath at 70 °C for 30 min. Similarly, a blank liposome (without drug) suspension (0.5 ml), 5 ml of 10% SLS were taken in 50 ml volumetric flask and the volume was made up with distilled water. The blank was warmed on a water bath at 70 °C for 30 min. The absorbance of the test solution was taken in UV-spectrophotometer at 263 nm against the blank solution [10].

Percentage of entrapment efficiency

It was determined by using the ratio of entrapped drug (mg) to the total drug (mg), which may be expressed by the following formula [10, 14].

$$\%\ Drug\ entrapped\ (PDE) = \frac{Amount\ of\ drug\ entrapped\ in\ liposome}{Total\ amount\ of\ drug\ taken\ initially} x\ 100$$

In vitro drug release study from liposomes

Concentrated liposomal suspension, 0.5 ml was taken in a test tube of opening diameter of 20 mm. The open end was covered with a semi-permeable dialysis membrane (Himedia Laboratories Pvt. Ltd.) and tied with a thread. The test tube was inverted and placed over the surface of 100 ml water present in a 250 ml beaker in such a way that the membrane just touched the water surface. The test tube was secured by a clamp fixed with a stand. The water in the beaker was stirred with a magnetic stirrer so that no vortex could form in the beaker. The temperature was maintained at 37 °C. The drug released from the liposomes permeates across the membrane and enters into the receptor chamber medium. Samples of 2 ml were taken out from the receptor chamber medium, suitably diluted, and the absorbances were taken by UV-spectrophotometer at 263 nm against a blank of fresh medium. At the same time, 2 ml of fresh medium was added to the beaker to keep the volume of the medium constant in the beaker [11, 17].

Stability test

The behaviour of the liposome to retain the drug was studied by storing the liposome at 4 to 8 ° C (refrigerator RF) for a period of 1 mo. The liposomal preparations were kept in sealed vials [11, 12].

RESULTS AND DISCUSSION

Physical interaction between drug and excipients

The pre-formulation study of drug-excipient interaction was carried out by DSC, which showed no interactions of the drugs and excipients. The melting point of the pure Isoniazid was found from the peak of DSC thermogram at 172.81 °C (fig. 1a). The results are shown in fig. 1a to 1d. Phosphatidylcholine is a semisolid material that showed a wider peak at 136.61 °C (fig. 1b). Cholesterol exhibited a much sharper peak at 147.29 °C (fig. 1c). Finally, the liposome formulation F1 showed a melting point at 112.41 °C (fig. 1d) that is much lower than all the individual pure components. The peak is bifurcated also. Thus DSC thermogram showed some interaction with the three components.

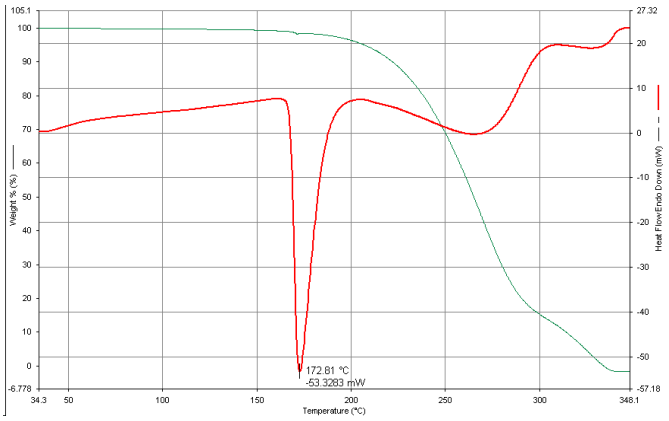

Fig. 1a: DSC curve of pure Isoniazid

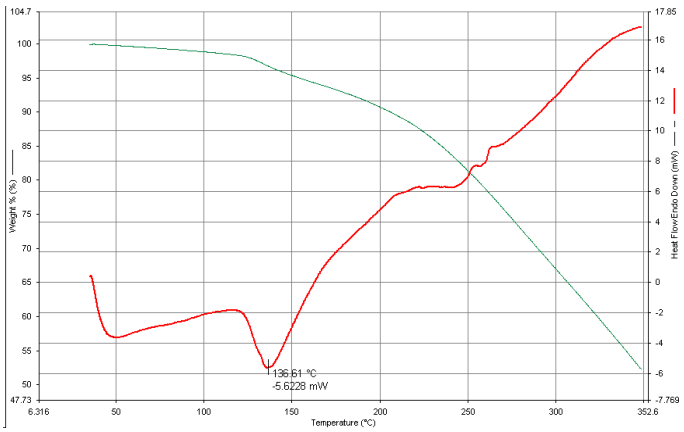

Fig. 1b: DSC curve of phosphatidylcholine

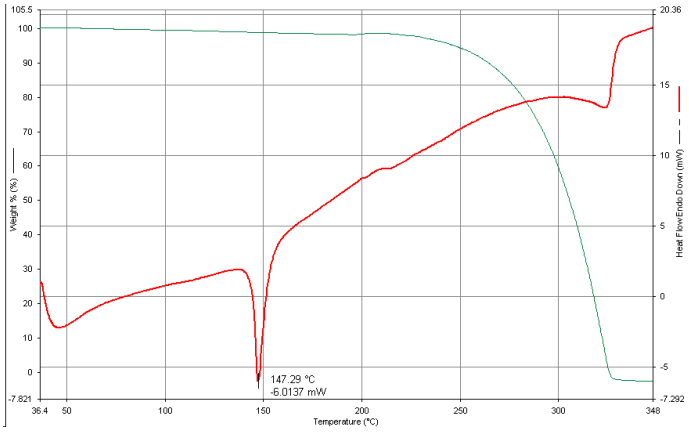

Fig. 1c: DSC curve of cholesterol

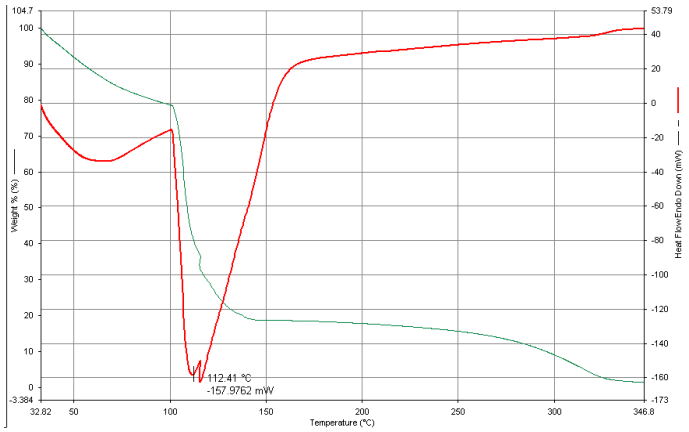

Fig. 1d: DSC curve of F1

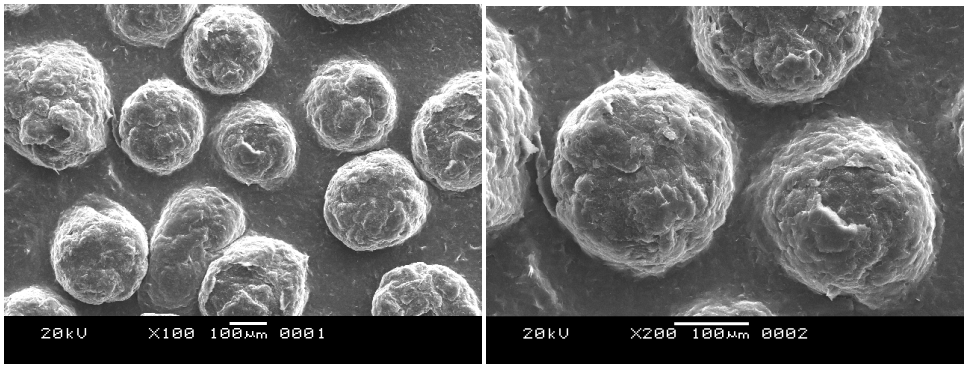

Fig. 2: SEM of F1

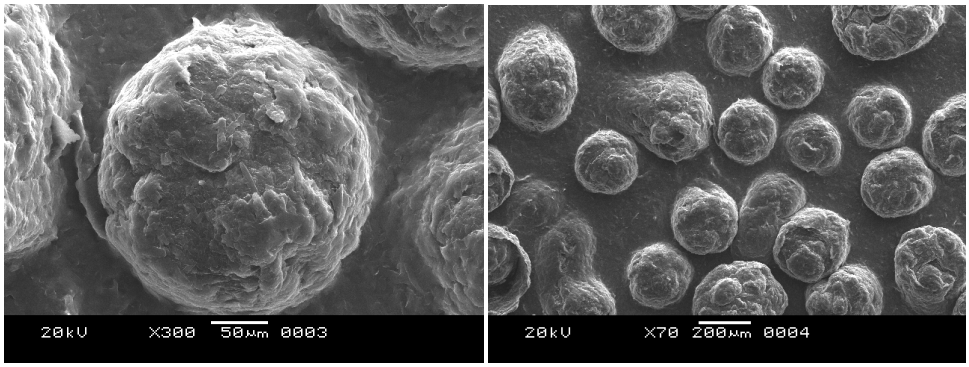

Fig. 3: SEM of F 6

Particle size analysis

Particle sizes were determined from the SEM photographs. Both F1 and F6, the two extreme formulations, were observed under SEM and the size of the liposomal vesicles was measured at different locations on photographs.

When particle frequencies were plotted against diameter the histogram in fig. 4 showed that F1 had a skewed distribution towards smaller liposome's while F6 shows a proper bell-shaped curve with a mean at 270 µm. The % entrapment efficiency of different batches is shown on the table 1.

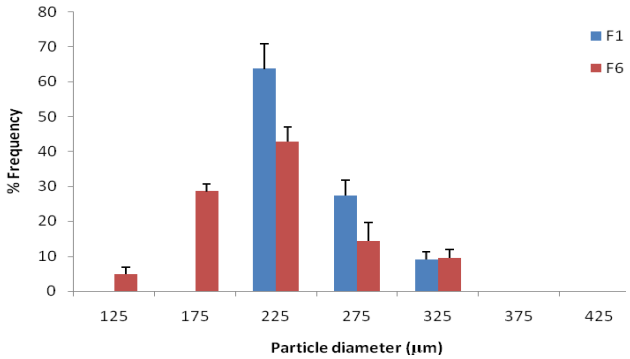

Fig. 4: Particle size distribution of F1 and F6 batches, *each value is represented as a mean±SD, n=3

Table 1: Drug entrapment and % entrapment efficiency of liposome formulations

Formulation batches	Drug entrapment (mg of drug/ml of liposomal suspension)	% entrapment efficiency
F1	17.98 ± 0.99	8.99 ± 0.15
F2	16.49 ± 0.51	8.25 ± 0.19
F3	13.71 ± 0.59	6.85 ± 0.15
F4	13.57 ± 0.32	6.79 ± 0.22
F5	9.52 ± 0.40	4.76 ± 0.10
F6	8.39 ± 0.37	4.19 ± 0.12

*Each value is represented as a mean±SD, n=3

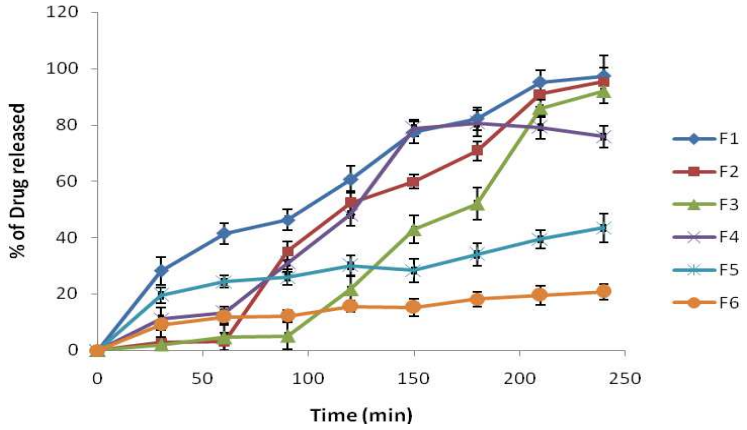

Fig. 5: Drug release (%) from all the liposomal formulations, *each value is represented as a mean±SD, n=3

The drug release pattern of all liposome formulations containing isoniazid was shown fig. 5. From the graph, it was seen that the drug release of F1 batch was fastest (97.3 ± 6.2 at 240 min) and F6 was slowest (20.40 ± 2.9 % at 240 min).

Many physicochemical parameters are investigated as major reasons for liposome instability. Oxidation and hydrolysis are the main reasons for chemical instability, as they occur in phospholipid unsaturated chains. Stored at low temperatures and light preservation can avoid such reactions. Proper entrapment, pH adjustment, temperature, ionic interaction and application of cholesterol in bilayer structure can successfully cause liposome stability. In this study, liposomes were stored at low temperature and far from light by application of cholesterol in the formulation. The efficacy of the cholesterol in various formulations as an indicator of stability was proved [18].

The satisfactory formulation (F1) was packed in the tube and stored at 4-8 °C temperature for one month. At the end of one month, the samples were analyzed for their physical property, drug entrapment and *in vitro* release study. The % entrapment efficiency was found 8.97 ± 0.14 and % release was found 96.5 ± 3.2. So there was no significant change was found in one-month stability.

CONCLUSION

This project was conducted to exploit the activity of isoniazid as a model drug by attempting targeting drug delivery in the form of a liposome. After the formulation of liposomes, they were evaluated to

estimate their particle size analysis, entrapment efficiency, release study and stability testing. The F1 batch showed most promising results compared to other. Though, long-term stability study and a clinical trial is required for future development of this formulation.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declared no conflict of interest

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