



DEVELOPMENT AND CHARACTERIZATION OF LIPOSOMAL DRUG DELIVERY SYSTEM FOR NIMESULIDE

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ABSTRACT

The focus of this study was to develop and characterize liposomal drug delivery system for Nimesulide. The encapsulation of Nimesulide into liposomes significantly improves their properties. In spite of the numerous advantages of using liposomes as carriers to deliver Nimesulide over the free form of the drug, *in vitro* studies of liposome-encapsulated Nimesulide have been mainly focused on evaluation of better method of Nimesulide liposomes which have high drug entrapment, vesicle size and drug release. In this study the Nimesulide loaded liposome was prepared by two methods ethanol injection method and rotary evaporator technique. The average particle size, percent drug entrapment, drug release at the end was found to be 270-703, 49-58 %, and 65.71 % in case of ethanol injection method while in case of rotary evaporator it was found to be 1-12 μ m, 69-86 and 76.97 % respectively. The Zeta potential for Nimesulide loaded liposomes of ethanol injection method (batch- 1) and rotary evaporator method (batch – 3) were -21.23 and -26.78 mV respectively.

Thus rotary evaporator technique was better for Nimesulide liposomes preparation on the basis of stability, drug entrapment efficiency and ethanol injection method was better on the basis of small size of liposomes and sustains release of drug when compared to rotary evaporator method and pure drug.

Keywords: Nimesulide, Dipamitoyl Phosphatidyl Choline (DPPC), Cholesterol (CH), Stearic acid, Small Unilamellar Vesicles (SUV), Multilamellar vesicles (MLV).

INTRODUCTION

Liposome was discovered about 40 years ago by Bangham and co-workers and was defined as microscopic spherical vesicles that form when phospholipids are hydrated or exposed to an aqueous environment¹. Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids. They can encapsulate and effectively deliver both hydrophilic and lipophilic substances²⁻³ and may be used as a non-toxic vehicle for insoluble drugs⁴. Liposome as a microstructure consist of one or more concentric spheres of lipid bilayer separated by water or aqueous buffer compartments⁵. Liposomes have many of the requirements for good drug delivery systems as they are relatively non-toxic and bio-degradable⁶. They have been found to be useful carriers for both hydrophilic and hydrophobic drugs⁷. Liposomal encapsulation of a drug can dramatically alter the pharmacokinetic properties of a drug, targeting the drug to particular organs and/or enhance the efficacy of the encapsulated drug⁸.

The formulation of an appropriate liposomal system as a carrier for a given drug is dependent on the type of the lipid used and the method of preparation⁹. According to their size they are known as small unilamellar vesicles (SUV) or large unilamellar vesicles (LUV). If more bilayers are present they are referred to as multilamellar vesicles (MLV). Depending on the composition liposomes can have a positive, negative, or neutral surface charge. Lecithin can provide liposomes with a neutral surface; stearylamine and phosphatidic acid components provide positive¹⁰ and negative surface charge, respectively. Depending on the lipid composition, methods of preparations and the nature of the encapsulated agents, many types of liposomal products can be formulated. The ideal drug candidates for liposomal encapsulation are those that have potent pharmacological activity and are either highly lipid or water soluble. If a drug is water soluble, it will be encapsulated within the aqueous compartment and its concentration in the liposomal product will depend on the volume of the entrapped water and the solubility of that drug in the encapsulated water.

The lipophilic drug is usually bound to the lipid bi-layer or 'dissolved' in the lipid phase. A lipophilic drug is more likely to remain encapsulated during storage due to its partition coefficient.

Since the lipophilic drug is associated with the lipid bi-layers it will not leach out as readily to the 'external' water phase. Generally the encapsulation efficiency is higher for lipophilic drugs than hydrophilic drugs¹¹. The applications of liposomes as nanoscale containers for drugs¹², vitamins¹³, enzymes or genetic material¹⁴⁻¹⁵ require control and prediction of the liposome dispersion stability¹⁶.

Nimesulide is a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoea. The encapsulation of Nimesulide into liposomes significantly improves their therapeutic index by altering their pharmacokinetics and pharmacodynamics¹². In spite of the numerous advantages of using liposomes as carriers to deliver Nimesulide over the free form of the drug, *in vitro* studies of liposome-encapsulated Nimesulide have been mainly focused on evaluation of better method of Nimesulide liposomes preparation which has high drug entrapment, drug release and vesicle size. In this study the Nimesulide encapsulated liposomes were prepared by two methods (I) ethanol injection method and (II) rotary evaporator techniques and further characterized to determined vesicle size, percent drug entrapment and drug release.

MATERIALS AND METHODS

Materials

Nimesulide was obtained from Redson Pharmaceuticals (Ahmedabad, India). Dipamitoyl phosphatidyl choline was kind gift sample from Lipoid Germany. Cholesterol was purchased from Qualigens Fine Chemicals, Mumbai. Stearic acid purchased from Research Lab, Mumbai India. Ethanol (AR), Methanol and Chloroform purchased from Merck, India.

Preparation of nimesulide liposomes

In this study liposomes were prepared by ethanol evaporation and rotary evaporator method. For the preparation of liposome by both methods various batches were prepared using different quantities of lipid and cholesterol (Table 1).

Table 1: Various batches of nimesulide loaded liposomes.

| S.No. | Lipid (DPPC) (mg) | Cholesterol (mg) | Drug (mg) | Stearic acid (mg) |
|----------|-------------------|------------------|-----------|-------------------|
| Batch 1. | 40 | 40 | 10 | 10 |
| Batch 2. | 40 | 50 | 10 | 10 |
| Batch 3. | 50 | 40 | 10 | 10 |
| Batch 4. | 50 | 50 | 10 | 10 |
| Batch 5. | 60 | 40 | 10 | 10 |
| Batch 6. | 60 | 50 | 10 | 10 |

Ethanol injection method

Solvent injection method is suitable for preparing SUV or LUV. The lipid, cholesterol, stearic acid and lipid soluble component (drug) were dissolved in organic solvents (ethanol) and injected into 10 ml preheated distilled water at 55-65°C with continuous stirring at 500 rpm using magnetic stirrer. The solvent was evaporated by heating so as to obtain drug loaded liposomes.

Rotary evaporator method

Rotary evaporation method is the most frequently used method¹⁷⁻¹⁸. The lipids, cholesterol and lipophilic components are dissolved in methanol and chloroform mixture (2:3 ratio), which was removed under vacuum by rotary evaporation. The lipid residue forms a film on the wall of the container. An aqueous solution generally containing electrolytes and water soluble components of the product is added to the film. Agitation produces large MLV. SUV can be prepared by sonication or sequential filtration.

Physicochemical characterization

Mean particle size analysis

Mean vesicle size of Nimesulide loaded liposomes was determined using particle size analyzer (Malvern 2000SM) based on photon correlation spectroscopy at room temperature by keeping angle of detection at 90°.

Percent drug entrapment

PDE was determined by mini-column centrifugation method. In brief, Sephadex® G50 solution (10 %, w/v) was prepared in water and was kept aside for 48 hours for complete swelling. To prepare mini-column, Whatman filter pad was inserted in 1mL syringe and swollen Sephadex was added carefully to it to avoid air entrapment in the column. Excessive amount of water was removed by spinning the column at 2000 rpm for 3 min using centrifuge. Nimesulide liposomes suspension (100 µL) were slowly added on prepared column and centrifuged at 5000 rpm for 3 min, and then the same procedure was repeated by adding 100 µL of water. The remaining free drug bound to the gel, while liposomes passed through the gel and were collected from the first and second stage of centrifugation. The eluted liposomes obtained were ruptured using ethanol and percent encapsulation was calculated from total amount of liposomes present in 100 µL of liposomes by UV-Visible spectrophotometer (slope) using Eq. (1) the method is validated using free drug instead of liposomal dispersion. The free drug was analyzed by UV-Visible spectrophotometer.

$$\text{Encapsulation efficiency (EE)} = (Q_e / Q_i) \times 100 \text{ ----- (1)}$$

Where,

Q_e is the amount of encapsulated Nimesulide and

Q_i is the amount of Nimesulide in 100 µL of liposomes suspension.

Microscopic studies

Vesicles morphology of liposomes obtained from ethanol evaporation and rotary evaporator method was determined using a photomicroscope (Nikon Model UFX-II, Japan) at 1000 x magnification.

Drug release study

In vitro release studies of developed nimesulide liposomes by rotary evaporator, ethanol injection and pure drug suspension in phosphate buffer (pH 7.4) were evaluated in a customized and validated diffusion cell across cellophane membrane (12,000 MCO) for 10 hours using 200 ml of Phosphate Buffer (pH 7.4) as diffusion medium. Nimesulide liposomes prepared by above both methods and pure drug suspension were compared by taking 3 ml from each (equivalent to 3 mg) were separately transferred to the donor compartment and stirred at 50 rpm while the receptor compartment was stirred at 100 rpm. 1 ml of the sample was withdrawn from the receptor compartment at definite time intervals and equivalent amount of fresh medium was replaced to the receptor compartment. Samples were assayed for Nimesulide by UV spectrophotometer. To examine the % drug diffused, the data were subjected to the following equation (2):

The percent drug diffused was determined by the formula

$$\text{Percent drug diffused} = \frac{C_r V_r}{C_d V_d} \text{ ----- (2)}$$

Where,

C_r = Conc. of drug in receptor compartment

V_r = Volume of the receptor compartment

C_d = Conc. of drug in donor compartment

V_d = Volume of donor compartment

Zeta potential measurement

Zeta Potential of optimized liposomes obtained from ethanol evaporation and rotary evaporator method was determined using Zeta sizer 300HSA (Malvern instrument, Malvern, UK).

RESULT AND DISCUSSION

Mean particle size analysis

The particle size of drug loaded liposomes as determined by Malvern was found to be 270 - 703 nm by ethanol injection method and 1 - 12 µm by rotary evaporator method. (Table 2) Thus the ethanol injection method results in smaller particle size as compared to the rotary evaporator method.

Percent drug entrapment

The percent drug entrapment in liposomes was determined by mini-column method and found to be 49 - 58 % by ethanol injection method, and 69 - 86 % by rotary evaporator method (Table 2). The drug entrapment in case of rotary evaporator method was higher. In this study the batch no. 1 and batch no.3 prepared from ethanol injection method and rotary evaporator method respectively were optimized for further study.

Table 2: Particle size and % drug entrapment of various batches

| Batch | Particle size (µm) | | % Drug entrapment | |
|----------|--------------------|--------------------------|-------------------|--------------------------|
| | Injection method | Rotary evaporator method | Injection method | Rotary evaporator method |
| Batch 1. | 0.270 | 1.501 | 58.38 | 71.22 |
| Batch 2. | 0.348 | 5.371 | 53.11 | 74.68 |
| Batch 3. | 0.553 | 7.075 | 54.44 | 86.41 |
| Batch 4. | 0.612 | 11.903 | 49.29 | 79.53 |
| Batch 5. | 0.639 | 12.137 | 51.67 | 69.13 |
| Batch 6. | 0.703 | 12.488 | 52.54 | 83.92 |

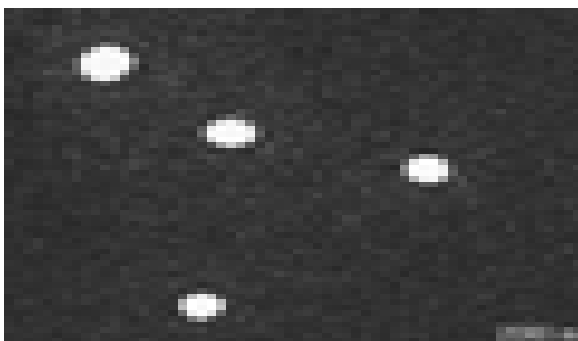


Fig. 1: Microscopic image of batch 1 of ethanol injection method

Microscopic studies

The microscopic study of drug loaded liposomes shows that small unilamellar vesicles were formed by ethanol injection method (Fig. 1) and multi-lamellar vesicles were obtained from rotary evaporator method (Fig. 2).

Drug release profile

The drug release studies in 7.4 phosphate buffer for pure drug, liposomes obtain by rotary evaporator method (batch - 3) and by ethanol injection (batch - 3) method was found to be 94.63 % 76.97 % and 65.71 % respectively after 540 min. The data shown (Fig-3) that liposomes obtain by ethanol injection method give more sustain release compared with liposomes by rotary evaporation method and pure drug.

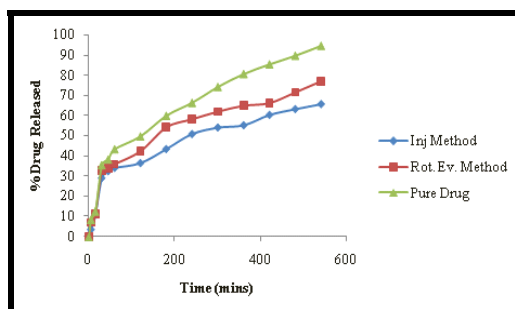


Fig. 3: In vitro drug released from Nimesulide Loaded Liposomes

Determination of zeta (ζ) potential

A ζ value $\geq \pm 30$ mV is essential for effective stability and to inhibits aggregation. In the present study the ζ potential for Nimesulide loaded liposomes of ethanol injection method (batch- 1) and rotary evaporator method (batch - 3) were -21.23 and -26.78 mV respectively. It was observed that ζ potential of prepared liposomes have sufficient charge to inhibit aggregation of vesicles. The highest ζ value of liposomes obtained by rotary evaporator method (-26.78mV) indicating most stable liposomes than liposomes obtained by ethanol injection method.

CONCLUSION

Thus for the preparation of nimesulide liposome an attempt has been made to study comparative evaluation of rotary evaporator and ethanol injection method. The particle size, percent drug entrapment, drug release was found to be 270-703, 49-58 %, and 65.71 % in case of ethanol injection method while in case of rotary evaporator it was found to be 1-12 μ m, 69-86 % and 76.97 % respectively. Thus rotary evaporator technique was better for Nimesulide liposomes preparation on the basis of stability, drug entrapment efficiency and ethanol injection method is better on the basis of small size of liposomes and sustain release of drug compare to rotary evaporator method and pure drug.

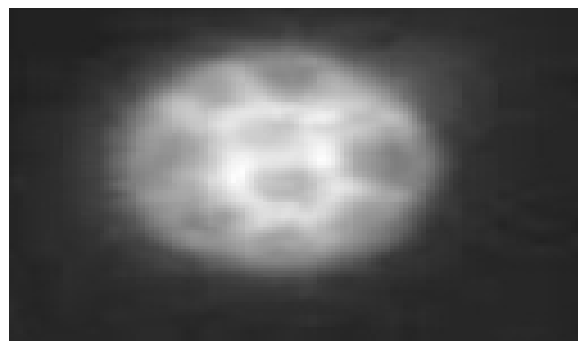


Fig. 2: Microscopic image of batch 3 of rotary evaporator method

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