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K Ajith *et al*
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Original Article

Liposome's: Microscopic Vesicles Formulated Using Diclofenac Sodium

K Ajith*, P Hyma, Anu Tumma

Department of Pharmaceutics, St Paul's College of Pharmacy, Telangana, India

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ABSTRACT

The present research deals with liposome are formulated with diclofenac sodium. Liposomes are sphere shaped vesicles containing one or more phospholipids bilayer, these are usually used to maintain drugs from any concentrations at target sites for alonger period of time (sustained effect) & even increase the bioavailability of the drug. The main reason to formulate diclofenac sodium into liposome is that diclofenac has a plasma t1/2 of 2-3 hours and there is a need for patients to administer the drug 2-3 times a day to persist the desired effect. We have produced liposomes using thin film hydration technique & different excipients like cholesterol, soya lecithin were used. When they were formulated as liposome it showed asustained effect when evaluated in vitro.The different evaluation studies like particle size determination, vesicular entrapment, drug release profile, SEM studies and FTIR spectrum studies were done in this research & these tests were used as parameters to determine the optimized formulation and even the sustain release effect of the system.

Keywords: Bilayers, Bioavailability, Cholesterol, Liposome's, Sustained effect, Soya lecithin,thin film hydration technique.

1. INTRODUCTION

Drug delivery systems are the means that carry drug to the desired parts of the body. Phospho-lipid vesicles (Liposome's) were first discovered decades ago by Bangham. The hydration of the dry lipid film was found to lead to the formation of the enclosed spherical vesicles, which resemble to the cellular organelles with lipid layer[1]

Oral administration of drugs is central in the development of pharmaceutical research due to its extensive application to most patients. With the aim to improve the oral bioavailability several strategies have been proposed to

Corresponding author *
K Ajith
E-mail: ajithk187@gmail.com

reduce dosing frequency and/or gastrointestinal side effects of many drugs. A tremendous amount of work has been done to formulate drugs in sustained and controlled release dosage forms for oral and parental administration.[2]

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task for which the carrier itself should be non-toxic, biodegradable, and suitable shape and size to accommodate awide variety of substances[3]. Liposomes have been widely evaluated for controlled and targeted drug delivery for treatment cancer, viral infections, and other microbial diseases. The importance of liposomes as drug delivery vehicleis now becoming well established[4]. This applies to particularly to the ability of liposomes to buffer the toxicity of entrapped drugs while maintaining efficacy, some areas in which liposomes display therapeutic promise are as carriers for anticancer agents, antiparasitic agents, antibacterial agents, antifungal agents, antiviral and ocular liposomes[5]

In this project, we have used diclofenac sodium.[6]The behavior of liposomes in vivo is strongly dependent on vesicle size, lipid composition, and lipid dose. In the absence of cholesterol, liposomes usually leak substantially when introduced intravenously.[7]

The circulation time is also sensitive to lipid dose; higher the dose leads to longer circulation time. These effects are presumably related to saturation of RES at ahigher dose.[10]

Diclofenac is used to relieve pain, swelling (inflammation), and joint stiffness caused by arthritis. Reducing these symptoms helps you do more of your normal daily activities. The medication is known as a nonsteroidal anti-inflammatory drug (NSAID). For diseases where diclofenac is used for achronic period of time, there is a lot of drug intake as because the plasma t1/2 of diclofenac is 1 to 2 hr, where approximately 65% is excreted in the urine and 35% in the bile as conjugates of unchanged diclofenac plus metabolites[9].

In the current research, we have formulated the liposomes with diclofenac sodium with the help of the thin film hydration technique and different excipients like cholesterol, soya lecithin & suitable solvent systems were used& they were evaluated with different *in- vitro evaluation* studies like particle size determination, vesicular entrapment efficiency, SEM studies, drug release profile of the different formulation & FTIR spectrum studies of the formulations were performed

The aim of the current study is to determine optimised formulation and even the sustained release effect of the delivery system were determined by using the evaluation parameter results.

2. MATERIALS AND METHODS

Materials: The materials like diclofenac sodium, cholesterol & soya lecithin were purchased from M.H. Enterprise and all other chemicals were of analytical grade

Methods :

Formulation of liposomes:

Large lamellar vesicles of diclofenac were prepared by thin film hydration technique using rotary flash evaporator. The liposomes were prepared using soya lecithin (neutral charge), cholesterol (neutral charge) and ethanol as avehicle by thin film hydration. The speed of rotation, temperature, vacuum, were kept constant and the ratio of soya lecithin and cholesterol were varied and evaluated for the optimal formulation, entrapment efficiency and diffusion time were studied.

Drug: soya lecithin: cholesterol, in particular, aratio based on formulation was dissolved in 10ml of 99.9% ethanol. This solution was taken in 250 ml round bottom flask. The flask was rotated at 80 rpm at 45^{o}C for 15 minutes. The organic solvent was slowly removed by this process such that a very thin film of dry lipids was formed on the inner surface of the flask. The dry lipid film was slowly hydrated using 10 ml of distilled water. The flask was once again rotated at the same speed as before and at room temperature for 20 minutes. The liposomes suspension was left mature overnight at 4^{o} C to ensure full lipid hydration.[11]

Evaluation of liposomes:

Liposomal formulations after their formulation and processing for a specified purpose and characterized to ensure their predictable *in-vitro* performances. There are several examples demonstrating the importance of proper selection of liposomes structure to optimize therapeutic effect[12]. The characterization parameters for the purpose of evaluation could be classified as physical, chemical & biological categories. Physical characterisation evaluates various parameters including size, shape, surface features, and drug release profile

Determination of entrapment efficiency

The amount of entrapped diclofenac sodium was determined by the lysis of vesicles with absolute ethanol. A 1ml of liposomal suspension is taken and added to 10ml of ethanol and sonicate it for 10 minutes to break the vesicle. Later on collect 1 ml of the sonicated solution and make up the volume to 10ml using ethanol. The concentration of diclofenac sodium was determined spectrophotometrically at 277nm using UV spectrophotometer.

Vesicle morphology analysis

The freshly formulated liposomes were observed under a calibrated eyepiece micrometer to analyze the particle size.

The average was taken for their size distribution range and mean diameter were calculated by roughly about 100 liposomes individually. Even the morphology was studied by using SEM technique.

In vitro drug release studies

In vitro diffusion studies were carried out using Franz diffusion cell. Apparatus with adiameter of 25mm and a diffusional area of 4.90cm2.An egg membrane was isolated and soaked in pH 6.8 phosphate buffersolutions for 24hrs and was sandwiched between the lower cell reservoir and the glass cell top containing the sample and secured in place with a pinch clamp. The receiving compartment was filled with pH phosphate buffer. The system was maintained at 37oC by magnetic heater resulting in a membrane surface temperature of 32oC stirred in the receiving medium to avoid diffusion layer effects. 2ml of receptor fluid were withdrawn from the receiving compartment at 15, 30, 45, 60, 120, 180, 240, 300, 360 minutes and replaced with 2ml of fresh solution. Samples were assayed spectrophotometrically for drug content at 277nm.

3. RESULTS AND DISCUSSIONS

The particle size of the liposome was determined by using microscopy studies and the particle size was found to 116-170 µm. The FTIR spectrum studies show no interactions between drug and polymers. The peaks of drug and polymer were found in the optimized formulation. In the SEM studies discrete particles were seen with no visible oil particles. Liposomes were found to be round and spherical. The diffusion studies were performed and the drug release percentage for the optimised formulation was found to be 63.8% for 8 hours and other formulations like F2, F3, F4& F5 were found to be (38.8%, 32.6%, 30.2%, 33.7%) for 8 hours.

Particle size distribution:

The mean particle sizes for the formulated liposomes were calculated and a graph is plotted & the SEM images of the particles are mentioned in figure 4

Vesicle morphology study

Observation of liposomes under optical microscope

The formulated liposomes were observed under anoptical microscope and the particle observed was spherical in shape and surrounded by the formation of lipid layer. The microscopic images are shown in fig 1& graph of particle size is mentioned in figure 3.

Drug release profile

The formulation code of f1 was found to be significant because it has shown a percentage drug release of 63.8% over a period of 8 hours. The rate of drug release data obtained for diclofenac sodium was plotted according to different modes of formulated was shown in figure 2.

FTIR Studies

Even the FTIR studies were conducted to determine any interactions between the drug and other materials used in the formulation of liposomes. The images of the graph of FTIR studies were mentioned in figure 5

Table 2: Percentage entrapment efficiency of drug

Formulation code	Entrapment efficacy (%)
F1	88.6
F2	68.9
F3	54.7
F4	47,4
F5	44.6

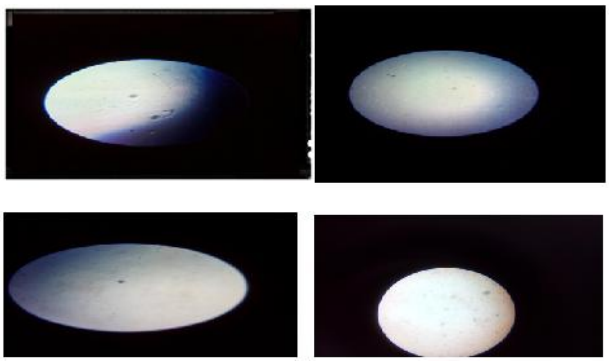

Fig 1: Microscopic images of Liposomes

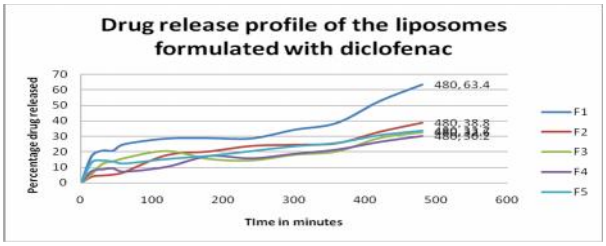

Fig 2: Graph of drug release profile

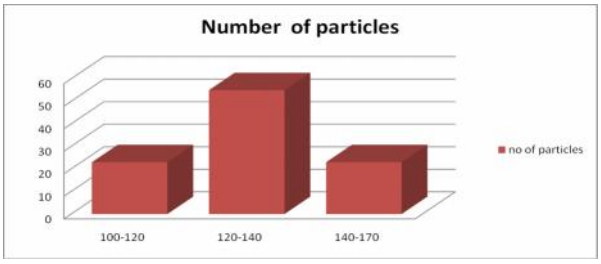

Fig 3: Particle size values (1-100 particles

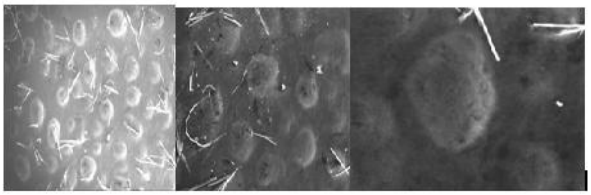

Fig 4: SEM Imaging of The Optimised formulation

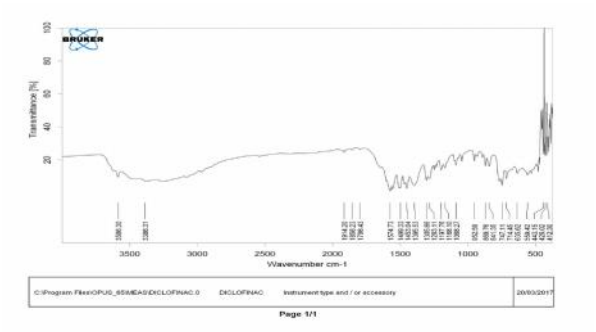

Fig 5.1: FTIR Spectrum of Diclofenac Sodium

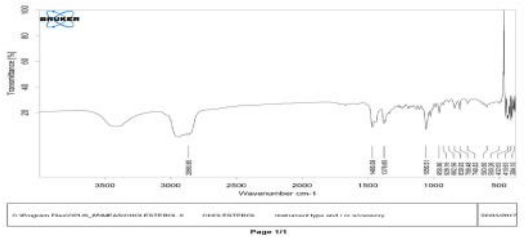

Fig 5.2: FTIR Studies of Cholesterol

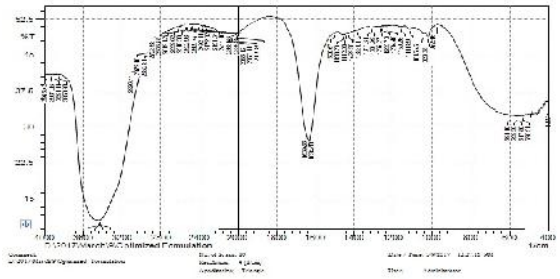

Fig 5.3: FTIR Spectrum of Optimised formulation

4. CONCLUSION

In the present research we have formulated diclofenac loaded liposome's using different lipids like cholesterol & soya lecithin. The SEM & microscopic studies have shown the decreased particle size in micron range for the liposomes. The absence of interactions between drug and polymer was confirmed by FTIR studies. The diffusion studies have shown sufficient release of drug from the delivery system. Hence, we can conclude by saying that liposomal formulation of the diclofenac sodium could be a better alternative to the conventional preparations available in the market.

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