ORIGINAL ARTICLE

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JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

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Formulation, characterization and antibacterial evaluation of liposomal delivery system for azithromycin

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Article History

Received on: 04/07/2022

Revised on: 11/08/2022

Accepted on: 17/08/2022

Published on: 06/12/2022

Keywords

Azithromycin

liposomes

reverse phase evaporation

antibacterial

release

ABSTRACT

Drug loaded liposomes were prepared by a reverse phase evaporation method using phospholipid (Soy lecithin), cholesterol and stearylamine for having stable particles. The characterization of the liposomes was carried out by estimation of Azithromycin, estimation of encapsulation efficiency, *in vitro* drug release, particles size, and stability of the liposomes. The antibacterial action of the liposome solution was assessed by disc diffusion method. The particle size of the formulations ranged from 3.12 ± 5.429 to 3.60 ± 6.519 µm and the entrapment efficiency ranged from 42.17 to 67.33 %. All the formulations released 62 to 75% drug at the end of 8 hours of the study. The antibacterial action of the liposomal formulation was compared to that of the pure drug solution and it was found that the liposomal formulations loaded with Azithromycin were able to exhibit comparable antibacterial activity against Staphylococcus aureus in the disc diffusion assay, as measured using the zone of inhibition.

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JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

ISSN No. 2456-8244

Publication Hosted by
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Introduction

Azithromycin is a potent broad spectrum antibiotic indicated in the treatment or prevention of infections that are proven or strongly suspected to be caused by susceptible bacteria (Indian Pharmacopoeia, 2007). It binds to the 23S rRNA of the bacterial 50S ribosomal subunit. It stops bacterial protein synthesis by inhibiting the transpeptidation/translocation step of protein synthesis and by inhibiting the assembly of the 50S ribosomal subunit. This results in the control of various bacterial infections (drugbank, 2021). It is BCS III drug with low permeability and has an oral bioavailability of 37%. It a well-known fact that liposomes have the capacity to improve the permeability and stability of incorporated drug molecules (Armaan et al., 1990; Tiwari et al., 2019; Hemmingsen et al., 2021; Fang et al, 2021; Souto et al., 2021). Hence it was hypothesized that use of liposomes loaded with azithormycin would be helpful in topical delivery of the drug. The objective of this project is to develop, optimize and characterize azithromycin loaded liposomes and to determine its antibacterial efficiency and stability.

Material and Methods

Azithromycin was obtained as gift sample from Medreich Pharmaceuticals, Bengaluru; Soy lecithin and cholesterol were purchased from Merck.

Calibration curve of Azithromycin

The calibration curve was obtained using different concentrations of the drug at the 285 nm using UV –Visible spectrophotometer. The stock solution was freshly prepared by dissolving 10 mg of Azithromycin in 1 ml of ethanol in a 10 ml volumetric flask and then made up the solution upto the mark using phosphate buffer pH6.8 for obtaining the solution of strength 1000 µg/mL (stock I). 1 ml of this solution is diluted to 10 ml with phosphate buffer pH6.8 to obtain a solution of strength 100 µg/mL (stock II). From this solution with draw 1, 2, 3, 4 & 5 ml of solution in to the 10 ml volumetric flask and volume made up to 10 ml by using phosphate buffer pH6.8 to get the solutions of 10, 20, 30, 40 & 50µg/ml.

Preparation of Liposomes

Drug loaded liposomes were prepared by a reverse phase evaporation method. Required amounts of phospholipids, cholesterol and stearylamine were dissolved in chloroform-methanol mixture (1:2 v/v) in a round bottom flask (Almeida dos Santos et al., 2020; Golhar et al., 2020). The flask was attached to the rotary vacuum evaporator and the solvent was evaporated at 40°C leaving behind a thin film of the lipin on the inner wall of the flask. The lipid film redisperesed in 10 mL ether and the drug (azitrhomycin) solution (100 mg) in 10 mL acetone along with 10 mL phosphate buffered saline (PBS, pH 7.4) added. The mixture was then placed on the rotary evaporator to evaporate organic solvent. The liposomes were allowed equilibrating at room temperature, and then the liposomal suspension was completed to 10 mL with PBS, which was kept in the refrigerator overnight. All liposome disper-

sions were characterized immediately after preparation.

Entrapment Efficiency (Pal et al., 2022)

5 ml of liposome formulation was taken and transferred to a 100 ml volumetric flask containing 25 ml of phosphate buffer (skin pH 6.8), and sonicated using an probe sonicator for 6 minutes at 35% impulse and 1 min cycles and filtered through a 0.45µm membrane filter. The filtrate was finally diluted with phosphate buffer (pH 6.8) and absorbance was recorded by UV visible spectrophotometer at 285 nm.

Particle Size Determination

The particle size of the microspheres was determined by using microscope, employing the calibrated eye piece and stage micrometer method. Size of liposomal vesicles was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles was determined.

***In vitro* drug release** (Sharma and Tripathi, 2021)

In-vitro drug release study of liposomal formulations was performed using franz diffusion cell. An egg membrane was placed between donor and receptor compartments. The receptor compartment contained phosphate buffer pH 6.8 was continuously stirred by magnetic bead and maintained at temperature of 37 ± 1ºC. One ml liposomal suspension was loaded on the donor compartment. The drug concentrations in aliquot were withdrawn at different time intervals and analyzed at 285 nm against appropriate blank.

Stability of Liposomes

The stability of the liposomal preparations was evaluated as a function of storage time. The liposomal samples were stored in a refrigerator at 4°C for 3 months immediately after preparation. The particle size of the samples was determined at the end of the third month.

Evaluation of antibacterial activity

Lyophilized bacterial culture of *Staphylococcus aureus* was revived using previously sterilized nutrient broth by incubation at 37°C for 24 h. The liposome solution was diluted in sterile distilled water to obtain a concentration of 100 µg/mL azithromycin. 1mL of this solution was soaked in cellulose acetate circular paper disc for testing the antibacterial action. The antibacterial action of the liposome solution was assessed by disc diffusion method. The sterilized media (nutrient agar) was cooled to 45°C and inoculated with the revived bacterial culture in a laminar air flow bench. This was poured in to sterile Petri dish and allowed to solidify and the test sample disc was carefully placed on the solidified media by using sterilized forceps. These Petri dishes were kept in the laminar air flow unit undisturbed for one-hour diffusion at room temperature and then for incubation at 37°C for 24 h in an incubator. The antibacterial action of the liposome was assessed by measuring the zone of inhibition of bacterial growth exhibited by the test sample disc.

Results and Discussion

Calibration Curve of Azithromycin

Calibration curve of Azithromycin was plotted as absorbance versus concentration (µg/ml) at 285 nm (Figure 1).

Liposomal formulation

The process and formulation and parameters strongly affect the properties of drug-loaded liposomes. The parameters used to characterize the liposomes in the preliminary experiments

included particle size, the encapsulation efficiency and the in vitro drug release profile. Stability studies using particle size as an indicator of stability were also conducted for a 3-month period.

The particle size of the formulations ranged from 3.12 ± 5.429 to 3.60 ± 6.519 µm. It can be observed from the results that the particles size was very slightly affected by the concentration of soy lecithin. An increase in particle size was observed on increasing the Lectihin concentration. Cholesterol is commonly added in liposomes to provide rigidity to the bilayer and improve the physical stability of liposomes. The presence of stearylamine in the formulations provides the required zeta potential to maintain stability of the liposomes.

The entrapment efficiency ranged from 42.17 to 67.33 % (Table 2). The increase in entrapment of drug enhances by increased cholesterol content as an increased cholesterol concentration in lipid bilayer increases the rigidity of bilayer, resulting in higher stability and reduced permeability of the liposomal membrane and hence greater drug retention. The *in vitro* release of Azithromycin from the liposomes was studies using Franz diffusion cell. The release was found to be not significantly affected by the lecithin to cholesterol ratio. All the formulations released 62 to 75% drug at the end of 8 hours of the study (Figure 2).

Stability of liposomes

The change in particle size over a period of three months was considered to ascertain the stability of the liposomal formulation. No significant change in particle size was observed suggesting that the formulations were stable at the storage conditions.

Antibacterial action

The antibacterial action of the liposomal formulation was compared to that of the pure drug solution and it was found that the liposomal formulations loaded with Azithromycin were able to exhibit comparable antibacterial activity against *Staphylococcus aureus* in the disc diffusion assay, as measured using the zone of inhibition.

Conclusion

Reverse phase evaporation method was successfully applied for formulation of Azithromycin loaded liposomes. The liposomes were sufficiently stable and able to control the release of the drug for mor:e than 8 hours. Liposomes composed of soy lecithin and cholesterol in proportion 8:1 exhibited the highest drug entrapment and the lowest particles size. The drug release from the liposomes suggested that the drug release was not significantly affected by the lecithin to cholesterol ratio.

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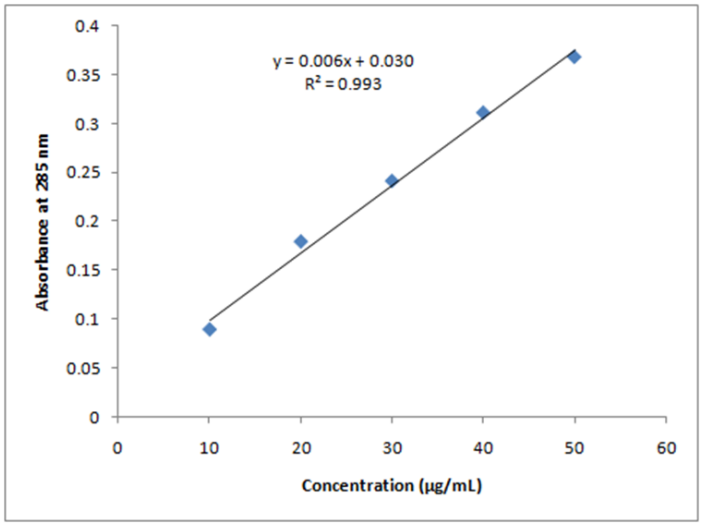

Figure 1 Calibration curve of Azithromycin in phosphate buffer

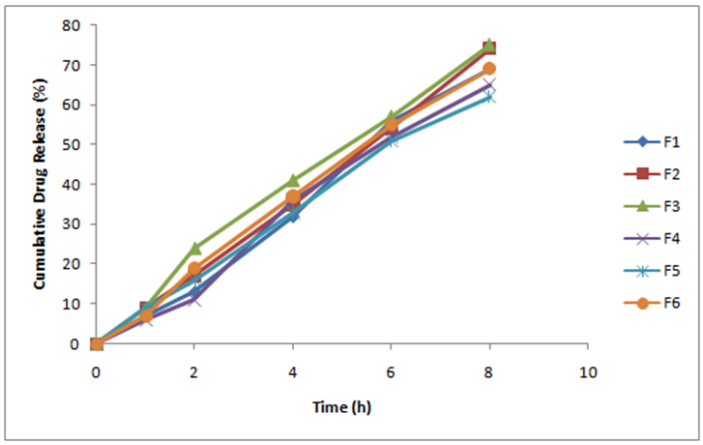

Figure 2 Drug release profile of Azithromycin from liposomes

Table 1 Composition of liposome formulations

Formulation	Soy Lecithin (mg)	Cholesterol (mg)	Stearyl amine (mg)	Azithromycin (mg)
F1	100	20	12	100
F2	120	20	14	100
F3	140	20	16	100
F4	160	20	18	100
F5	180	20	20	100
F6	200	20	22	100

Table 2 Particle size and entrapment efficiency of Azithromycin liposomes

Formulation	Average Particle Size (µm)	Entrapment Efficiency (%)
F1	3.12 ± 5.429	42.17
F2	3.28 ± 6.247	49.36
F3	3.56 ± 6.519	54.98
F4	3.34 ± 6.519	67.33
F5	3.46 ± 6.843	62.18
F6	3.60 ± 6.519	56.57

Cite this article as

Jain S, Jain S. Formulation, characterization and antibacterial evaluation of liposomal delivery system for azithromycin . J Pharmacol Biomed. 2022; 6(4): E559-E565