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Formulation of Azithromycin loaded liposomes for improved bioavailability on topical application

Nagendra Kumar Pal*, Reena Shende, Satyawan Dangi

RKDF School of Pharmaceutical Sciences, Bhopal, Madhya Pradesh, India

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ABSTRACT

This investigation was performed with an objective to improve the bioavailability and stability of azithromycin by formulating as liposomes and using it for topical delivery of azithromycin. Drug loaded liposomes were prepared by a modified ethanol injection method using soy lecithin (20, 40, 60 mg/ml) and cholesterol (2 & 4 mg/ml) as the lipids require to prepared the liposomes and maintain its stability. The particle size of the formulations ranged from 2.29 ± 6.48 to 2.65 ± 6.48 µm and the entrapment efficiency ranged from 27.35 to 62.18 %. Formulations AL4, AL5 and AL6 were found to exhibit slightly lower drug release throughout the study period. The maximum release was obtained in AL3 (84%) while the lowest was found in AL4 (57%). No significant change in particle size was observed over the three month storage duration suggesting that the formulations were stable at the storage conditions. 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.

*Corresponding Author

Nagendra Kumar Pal

Email: nagendrapal167@gmail.com

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Introduction

A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell. They have been receiving a lot of interest as a carrier for advanced drug delivery (Langer 2001). Liposomal membranes often include cholesterol (CH) and its derivatives as component which helps in providing the necessary lipophilicity for travelling across the membrane. They are suitable for delivery of hydrophobic, amphipathic as well as hydrophilic drugs. A few liposomal preparations have been approved for clinical administration of the incorporated drugs and are reviewed elsewhere (Pierre and Costa, 2011).

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. It is known to have a very long half life (68 h) and 37% bioavailability (Indian Pharmacopoeia, 2007; drugbank, 2022). Liposomes have been known to improve the bioavailability as well as stability of the incorporated drugs molecules (Fang et al., 2021; Vanic et al., 2021; Hemmingsen et al., 2021). In persuasion of this fact, some reports have been found for topical application of azithromycin in liposomal formulations and improvement in the bioavailability of the drug therein (Rukavina et al., 2018 ; Liu et al., 2016; Solleti et al., 2015).

Hence it was envisioned to use liposomes loaded with azithromycin for topical delivery of the azithromycin. The most common components cholesterol and soy lecithin would be used for formulating the liposomes and the evaluation of the formulations would be carried out.

Material and Methods

Azithromycin was obtained as a gift sample from Ind Swift Pharmaceuticals, Baddi; cholesterol and soy lecithin were purchased from Merck and all reagent and chemical used in the study were of analytical grade.

Preformulation Studies

The organoleptic properties like color, odor, taste and appearance were observed in well lit and ventilated area. The solubility of the drug was qualitatively observed in various solvents of varying polarity by shaking in test tube. The melting point was determined by open capillary method, loss on drying was monitored by drying in hot air oven at 105°C. The calibration curve was plotted in phosphate buffer by dissolving the drug in small amount of ethanol.

Preparation of Liposomes

Drug loaded liposomes were prepared by a modified ethanol injection method. Required amounts of phospholipids (20, 40, 60 mg/ml) and cholesterol (2 & 4 mg/ml) were dissolved in ethanol and Azithromycin (200 mg) was added to the organic phase (Table 1). Resulting organic phase was injected by means of a syringe pump to aqueous phase under magnetic stirring at 45 ± 2 °C. A spontaneous formation of liposome occurred as soon as the ethanolic solution was in contact with the aqueous phase. Liposome suspension was then kept under stirring for 1h at room temperature to remove the traces of solvent. The unloaded drug was removed by ultracentrifugation of liposome suspension at 10,000 rpm for 1 hour and stored at 4°C.

Evaluation of liposomes (Ambika et al, 2021)

The characterization of the liposomes was carried out by estimation of Azithromycin, estimation of encapsulation efficiency, *in vitro* drug release, particle size and stability of the liposomes.

Entrapment Efficiency

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* dissolution**

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 4ºC 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 procured from Institute of Microbial Technology, Chandigarh. The lyophilized culture 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

The preformulation studies confirmed the identity and purity of the azithromycin sample. The results obtained are presented in Table 2. The calibration curve was having a R^2 value of 0.993.

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.

Particle size

The particle size of the formulations ranged from 2.29 ± 6.48 to 2.65 ± 6.48 µm (Table 3). It can be observed from the results that the particles size was very slightly affected by the concentration of soy lecithin. The particles size was found to slightly increase by increasing the concentration of cholesterol in the formulations. Cholesterol is commonly added in liposomes to provide rigidity to the bilayer and improve the physical stability of liposomes. As the concentration of cholesterol increases more cholesterol molecules get distributed in the phospholipid bilayer, leading to an increase in the liposome mean size.

Entrapment Efficiency

The result of drug entrapment efficiency of liposomes indicates that as the concentration of lecithin increases, drug entrapment efficiency of liposomes decreases which may be due to the saturation of lipid bilayer. The encapsulation efficiency of liposomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane in the presence of biological fluids such as blood/ plasma. The entrapment efficiency ranged from 27.35 to 62.18 (Table 3).

***In vitro* release**

The *in vitro* release of Azithromycin from the liposomes was studies using Franz diffusion cell. The release was found to be affected by the amount of Lecithin as well as cholesterol in the formulation. While increasing the concentration of lecithin increased drug release, cholesterol was found to decrease the release. Formulations AL4, AL5 and AL6 were found to exhibit slightly lower drug release throughout the study period. The maximum release was obtained in AL3 (84%) while the lowest was found in AL4 (57%) (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 Activity of Azithromycin loaded Liposome

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

Ethanol injection 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 more than 8 hours. Liposomes composed of 4 mg/mL cholesterol and 60 mg/mL soy lecithin exhibited the highest drug entrapment. The drug release from the liposomes suggested that the higher level of cholesterol decreases the drug release while increasing the concentration of lecithin increases the release of Azithromycin form the formulation.

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Table 1. Composition of liposome formulations

Formulation	Soy Lecithin (mg)	Cholesterol (mg)	Azithromycin (mg)	Ethanol (mL)
AL1	200	20	200	100
AL2	400	20	200	100
AL3	600	20	200	100
AL4	200	40	200	100
AL5	400	40	200	100
AL6	600	40	200	100

Table 2. Micromeritic properties of formulation blends

Organoleptic features	Melting Point	LOD	Solubility	Partition coefficient (Log P)
White colored odorless powder with bitter taste	117-120°C	0.15%	Insoluble in water, slightly soluble in methanol and phosphate buffer and soluble in	4.05

Table 3. Entrapment Efficiency and Particle Size

Formulation	Average Particle Size	Entrapment Effi-
AL1	2.29 ± 6.48	27.35
AL2	2.40 ± 6.64	39.48
AL3	2.43 ± 6.75	49.26
AL4	2.65 ± 6.48	34.53
AL5	2.59 ± 6.75	44.17
AL6	2.58 ± 6.83	62.18

Figure 1. Calibration curve for azithromycin in phosphate buffer

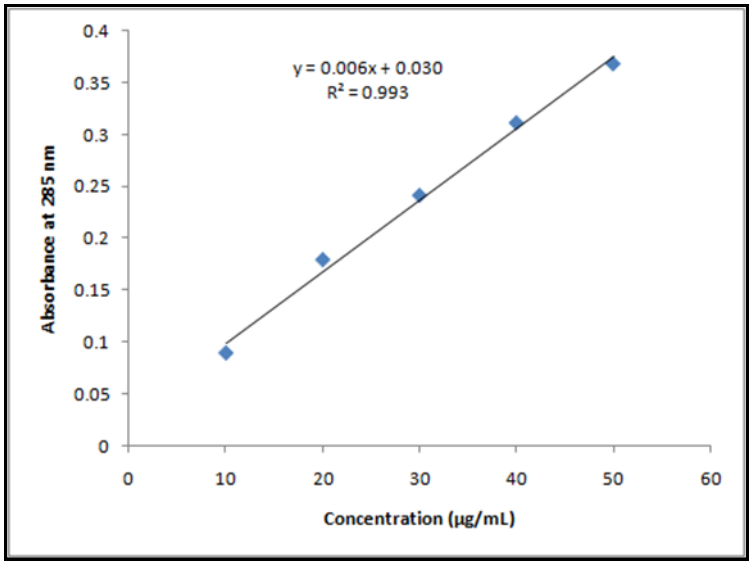
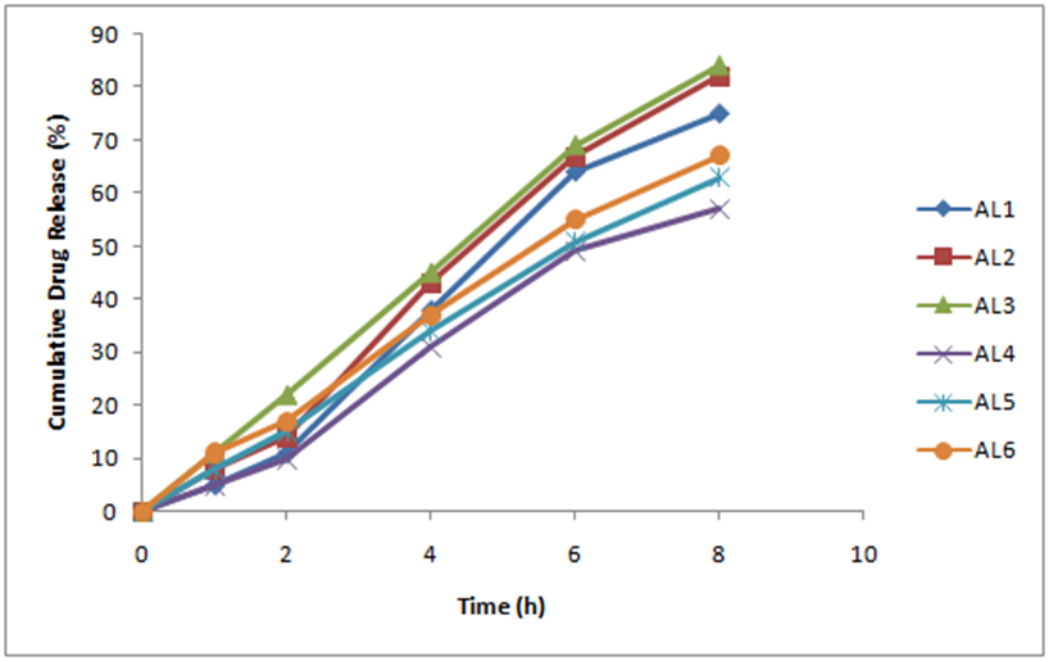


Figure 2. *In vitro* drug release profile of azithromycin from liposomes



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