Liposomes : A Novel Drug Delivery System

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ABSTRACT

Liposomes are spherical vesicles composed of concentric phospholipid bilayers that can entrap hydrophilic, hydrophobic and amphiphilic drugs. Liposomes can be prepared from natural phospholipids, synthetic lipids or bacterial lipids. As per the economic point of view, bacterial lipids can be suitable for preparation of liposomes. The aim of this investigation was to prepare and evaluate the liposomes extracted from the bacteria E. coli and to evaluate antimicrobial activity of liposomes along with the two antibiotics-Gentamycin and Streptomycin. Extraction of lipids was done according to the Bligh-Dyer method. Liposomes were characterized and assessed by thin layer chromatography. The extracted liposomes were tested along with the two different antibiotics Gentamycin and Streptomycin against laboratory bacterial pathogens. When liposomes were used in combination with the antibiotics, it was very much effective against the tested bacteria Proteus vulgaris, Pseudomonas aeruginosa and Klebsiella spp.

KEYWORDS: *Liposomes, Escherichia coli, Antibiotics*

1. INTRODUCTION:

A liposome is an artificially-prepared spherical vesicle composed of a lamellar phase lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs (Torchillin, 2006). The word *liposome* derives from two Greek words: *lipo* ("fat") and *soma* ("body"); it is so named because its composition is primarily of phospholipid. Liposomes are widely used as universal carriers of chemical substances in the cosmetic and pharmaceutical industries. Intensive research is being carried out on liposomal formulation of antibiotics to increase their pharmacokinetics properties and antimicrobial activity. Liposomes have significant effect as antibiotic carriers on improving drug distribution and decreasing a drug's toxic properties (Sachetelli *et al*., 2000; Schiffelers *et al*., 2001).

Fluid liposomal drug formulation was developed to increase the bactericidal efficacy of antibiotics by promoting effective interaction between the bacteria and liposomes. Liposomes containing fluoroquinolones and aminoglycosides demonstrated reduction in minimum inhibitory concentration (MIC) compared with the free drug against Gram-positive and Gram -negative bacteria (Puglisi *et al*., 1995; Furneri *et al*., 2000). Proteoliposomes and cationic liposomes were investigated for their potential targeting ability to the bacterial biofilms produced by skin and oral bacteria. Furthermore, it has been proved that same cationic lipids used for liposomes preparation might act by themselves as anti-infective agents. There are many benefits of using liposomes as antibiotic carrier, and studies to find new liposomal forms of drugs are still in progress. Liposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life or reducing toxicity. Drug distribution is then controlled primarily by properties of the carrier and no longer by physico-chemical characteristics of the drug substance only.

The applicability of drugs is always a compromise between their therapeutic effect and side effects. Liposomal drug delivery systems not only enable the delivery of higher drug concentrations but also a possible targeting of specific cells or organs. Harmful side effects can therefore be reduced owing to minimised distribution of the drug to non-targeted tissues. Like all other carrier systems, the

use of liposomes in drug delivery has advantages and disadvantages. The amphiphilic character of the liposomes, with the hydrophobic bilayer and the hydrophilic inner core, enables solubilization or encapsulation of both hydrophobic and hydrophilic drugs. Along with their good solubilization power, a relatively easy preparation and a rich selection of physicochemical properties have made liposomes attractive drug carrier systems (White *et al*., 2000).

Biologically active materials encapsulated within liposomes are protected to varying extent from immediate dilution or degradation, suggesting drug carrier systems for the transport of drugs and other bioactive capsules to disease-affected organs. The unique ability of liposomes to entrap drugs both in an aqueous and a lipid phase make such delivery systems attractive for hydrophilic and hydrophobic drugs. Because of advancements in the methods of preparing and formulating liposomes, high-entrapment efficiencies are possible for incorporating drugs into liposomes, creating a tremendous pharmaceutical impact. Furthermore, such encapsulation has been shown to reduce drug toxicity while retaining or improving the therapeutic efficacy. Several laboratories have reported the use of liposomes as drug carriers in the treatment of cancer (Ahmad, 1993; Mayhew *et al*., 1987), leishmaniasis (Alving, 1998) and metabolic disorders and fungal diseases (Lopez-Berestein, 1985). Liposomes may have a use in gene delivery to correct gene-associated disorders or for vaccine therapy (Gregoriadis, 2006).

Liposomes can be prepared from natural phospholipids egg or soya or synthetic lipids such as dioleoylphosphatidyl ethanolamine. In fact, these liposome preparation methods require the use of expensive lipids that are commercially available only from a few sources in the world. The cytoplasmic membrane of the *E. coli* includes a high proportion (70-75%) of phosphatidylethanolamine (White *et al*., 2000). The aim of this investigation was to prepare and evaluate the liposomes extracted from the bacteria *E. coli,* which is extremely economical compared to the synthetic lipids and to evaluate antimicrobial activity of liposomes along with the two antibiotics- Gentamycin and Streptomycin.

2. MATERIALS AND METHODS:

Collection of Microorganisms: The bacterial cultures of these bacteria *Escherichia coli* NCIM-2207, *Proteus vulgaris* NCIM 2027, *Klebsiella* spp. NCIM 2079 and *Pseudomonas aeruginosa* NCIM 2036 were obtained from National Chemical Laboratory (NCL), Pune.

Extraction of Lipids from *E. coli*: *E. coli* cells were grown in nutrient broth at 37^0c for 24h. Final cell concentration was 108 cfu/ml according to the McFarland turbidometry. Extraction of lipids was done according to the Bligh-Dyer method (Gupta *et al*., 2008). Chloroform-methanol mixture (1:2) was used as the extraction solvent. Briefly, 3.75 ml of the extraction solvent was added to 1.0 ml of the bacterial culture and vortexed for 10 min. Then 1.25 ml of methanol was added. After vortexing again for 1 min, 1.25 ml of distilled water was added. The final solution was centrifuged at 1000 rpm for 5 min at room temperature. The inferior organic phase containing the lipid was separated using pasture pipette.

Characterization of Liposomes: The liposomes must be diluted 1:10 in water and 10µl of this dilution was assayed by placing it in a glass tube (150 x15mm). Thus 10µl of the liposome used would be 0.15µ mole. A 0.7ml of 1:1(v/v) mixture of 60% Perchloric acid: Sulphuric acid (conc.) was added to the tube. The tube was heated over an open flame until the solution turns yellow. For the liposome sample, the solution was 1st turns brown then clear and then turns yellow (Zhong *et al*., 2012).

Thin Layer Chromatography: TLC was performed on glass silica gel slide which was first activated by heating at 150ºC for 30 min. The integrity of the liposome (phospholipids) was assessed by thin layer chromatography. The major degradation product is fatty acid and lysophospholipid. The latter could be easily detected by TLC. The liposomes were diluted in chloroform to 3µ mole/ml. This was 1:50 dilution of the liposome used in the experiment. A 25µl of diluted liposome was spotted on the previously prepared silica gel plate in a 1cm long area with a Hamilton syringe. 1-myristoyl-sn-glycero-3-phospho-rac-(1-glycerol) (lyso PG) (Avanti polar lipid) was dissolved in chloroform at 2mg/ml and 25µl was spotted on the plate as a standard. The plate was developed in TLC tanks in

188ml of Chloroform: Methanol: Water (65: 25:4), (v/v). After drying, liposomes were visualized with molybdenum blue spray. Liposomes appeared blue on a white background. The Rf value was found to 0.23 which was similar with that of the standard (Kargar *et al*., 2014).

Drug Delivery Using Liposomes: The extracted liposomes were tested along with the two different antibiotics Gentamycin and Streptomycin. The antimicrobial activity of these drugs was carried out against the standard bacteria such as *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella* spp. For each organism, a lawn was prepared by using sterile cotton ear bud on the nutrient agar plate. Three wells were prepared on the plate by using a sterile cork borer. A 20µl of the liposome suspension was added to the first well, in second well the antibiotic solution was added and in third well a mixture of liposomes and antibiotic was added. The plates were incubated for 24 hours at 37^0C. After incubation zone of inhibition (mm) was observed (Gubernator *et al*., 2007).

3. RESULTS AND DISCUSSION:

Liposomes have been widely investigated since 1970 as drug carriers for improving the delivery of therapeutic agents to specific sites in the body. Liposomes, which are biodegradable and essentially non-toxic vehicles, can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug delivery systems. As a result, numerous improvements have been made, thus making this technology potentially useful for the treatment of certain diseases. In the present project extraction of liposomes was carried out from *Escherichia coli*. It was reported that fluid liposomal drug formulation was developed to increase the bactericidal efficacy of antibiotics by promoting effective interaction between the bacteria and liposomes. Therefore this investigation was carried out to prepare and evaluate the liposomes using bacteria *E. coli*.

Liposome is used as a carrier for drug delivery. In the present project liposomes were prepared from *E. coli*. Liposome is a lipid which was obtained from the bacterial culture which is extremely economical compared to the synthetic lipids. This was correlated with the previous work of (Gupta *et al.,* 2008; Kargar *et al*., 2014) in the context of liposomal preparation by using the same organism *E. coli*. Their study demonstrated the procedures for convenient and reproducible preparations of 200–300 nm diameter liposomes from bacterial cells. They also showed a potential application of these bacterial liposomes in delivery of aqueous molecules to cancer cells.

Table1: Demonstration of Liposomal Activity in Combination with Antibiotics

Sr. No.	Organisms	Gentamycin			Streptomycin		
		Liposome (L)	Gentamycin (G)	L+G	Liposome (L)	Streptomycin (S)	L+S
1	*Proteus vulgaris*	NZ	12mm	23mm	NZ	29mm	31mm
2	*Pseudomonas aeruginosa*	12mm	18mm	21mm	NZ	13mm	14mm
3	*Klebsiella* spp.	12mm	27mm	31mm	NZ	31mm	33mm

Where, NZ= No Zone

In the present study when the prepared liposomes were tested against three bacteria, it was found that there was less or no activity of liposomes. The tested antibiotics have shown good antimicrobial activity against all the bacteria tested. When liposomes were used in combination with the antibiotics, it was very much effective against the tested bacteria. Gentamycin alone showed 12mm zone of inhibition against *Proteus vulgaris* but the antimicrobial activity was increased along with liposomes (23mm). Likewise, for *Pseudomonas aeruginosa*, Gentamycin (18mm) while Gentamycin with liposomes (21mm), for *Klebsiella* spp. Gentamycin (27mm) and in combination with liposomes (31mm) zone of inhibition was reported. Streptomycin alone showed (29mm) zone of inhibition against *Proteus vulgaris* but the antimicrobial activity was increased along with liposomes (31mm). Likewise, for *Pseudomonas aeruginosa*, Streptomycin (13mm) while Streptomycin with

liposomes (14mm), for *Klebsiella* spp. (31mm) and in combination with liposomes (33mm) zone of inhibition was reported (Table 1).

Thus the zone of inhibition of liposomes with antibiotic was found to be more as compared to that of the antibiotic alone. It was also reported by (Gubernator *et al.,* 2007) that liposomes can encapsulate both hydrophilic and hydrophobic materials, and is utilized as drug carriers in drug delivery systems. According to the literature a susceptibility-drug accumulation relationship has also been observed in the case of *P. aeruginosa* because it represents a poor outer membrane permeability (Mayhew et al., 1987), probably due to the presence of water filled channels in this bacteria, such as porin F, that are substantially smaller than those of other gram-negative bacteria. This fact determines a lower permeability of hydrophilic antibiotics through the bacterial outer membrane (Ropert *et al*., 1993). Thus, increased outer membrane permeability due to the liposome formulation is likely to contribute to the improved susceptibility of these organisms.

Liposomes, besides having a direct interaction with the bacterial cell outer membrane, can also ensure "contact" and/or "juxtaproximal" release, that is, a massive drug release close to the bacterial cell surface (both outer membrane and peptidoglycan), allowing the formation of a drug concentration gradient and hence a higher rate of entrance than that of the free drug. The latter aspect is particularly important in the case of gram-positive bacteria, which present a peptidoglycan barrier that can hamper a direct contact with the cytoplasmic membrane (Gustafsson *et al*., 1995).

4. CONCLUSION

The present work has successfully demonstrated reliable preparation of liposomes from lipids derived from bacterial cultures. The present project concluded that it is possible to formulate liposomes from bacterial lipids by using *E. coli*. The bacterial liposomes represent low cost in drug delivery systems. Thus, it is important to appreciate that this economical method for preparing liposomes from bacterial sources (in contrast to using expensive synthetic lipids, commercially available only from a very few sources in the world) is bound to open exciting avenues for biological research utilizing liposomes as model system. According to the results, loading of some antibiotics like Gentamycin and streptomycin in liposome may exhibit improved release properties. The flexibility of liposomes behavior can be exploited for the drug delivery through any route of administration and for any drug material irrespective of their solubility properties. More importantly, the study also demonstrated the utility of the economical liposomes in developing model system to study uptake of molecules by targeted cells. Thus liposomes have been realized as extremely useful carrier systems for targeted drug delivery.

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