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### FORMULATION AND *IN VITRO* EVALUATION OF LIPOSOMES LOADED WITH MUPIROCIN

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#### ABSTRACT

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of a lipid moiety and are well known to alter the bio distribution of entrapped substances by protecting the enclosed materials. They are widely used as vehicles or carriers to target the specific molecule to a specific organ especially in condition of bacterial infections. A development of system which controls the release and enhances the bioavailability of Mupirocin, an anti-bacterial agent is in demand. This study aimed at developing and optimising liposomal formulation of Mupirocin, a broad spectrum antibiotic of maximum therapeutic efficacy with minimal side effects by lipid film hydration technique using various ratios of soya lecithin and cholesterol. Upon pre-formulation studies and optimization, the various formulations (of varying proportions) were prepared and subjected for various physico chemical evaluation studies i.e., morphology, particle size, drug entrapment efficiency, *in-vitro* drug release, release kinetics and stability studies. Among five formulations (F1- F5) F4 formulation emerged as the most satisfactory formulation in all the evaluation parameters. The liposomes were found to be stable during their stability studies when stored at different temperatures. Hence it can be concluded that Mupirocin can also be loaded in liposomal carriers which found to be effective, stable and can be proceeded for further future studies.

#### KEYWORDS

Cholesterol, Liposomes, Mupirocin and Soya lecithin.

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#### INTRODUCTION

Liposomes are colloidal, concentric layered vesicles in which an aqueous volume is entirely enclosed by membranous lipid bilayers mainly composed of natural or synthetic phospholipids. This vesicle encapsulates a liquid interior which contain substances such as peptides, proteins, hormones, enzymes, antibiotics, antifungal and anticancer

agents. A free drug injected in blood stream typically achieves therapeutic level for short duration due to metabolism and excretion. Drug encapsulated by liposomes achieves therapeutic level for long duration as drug must first be released from liposomes before metabolism and excretion<sup>1,2</sup>. In the past decade, liposomal formulations have been extensively employed to enhance the efficiency of drug delivery via several routes of administration. In a number of instances, liposomal drug formulations have been shown to be markedly superior to conventional dosage forms, especially for intravenous and topical modes of administration of drugs. The major advantages of topical liposomal drug formulations accrue from their demonstrated ability: (i) to reduce serious side effects and incompatibilities that may arise from undesirably high systemic absorption of drug; (ii) to enhance significantly the accumulation of drug at the site of administration as a result of the high substantivity of liposomes with biological membranes; and (iii) to readily incorporate a wide variety of hydrophilic and hydrophobic drugs. Liposomes are also non-toxic, biodegradable and are readily prepared on a large scale<sup>3</sup>. In addition they also provide selective passive targeting to tumour tissues (Liposomal doxorubicin), Increased efficacy and therapeutic index, Increased stability via encapsulation, Reduction in toxicity of the encapsulated agents, Improved pharmacokinetic effects (reduced elimination, increased circulation life times), Flexibility to couple with site specific ligands to achieve active targeting<sup>4</sup>. Structurally liposomes mainly consist of two components namely Phospholipids and cholesterol.

A particularly promising area concerns topical administration using liposomal drug formulations. The first report indicating that topical application of liposomal encapsulated drugs altered drug deposition was presented at the FIP 1979 congress<sup>5</sup>. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body, to achieve promptly and then maintain the desired drug concentration. Liposomes are Microscopic, Fluid-filled pouches, and whose walls

are made up of layers of Phospholipids identical to the Phospholipids that make up cell membrane<sup>6</sup>. Liposomes can be classified either on the basis of their structural properties or on the basis of the preparation method used. These two classification systems are in principle, independent of each other. Depending on the selection of lipids, preparation technique and preparation condition, liposomes can vary widely in size and number and position of lamellae and can be classified based on the structure and preparation methods.

Liposomes being one of the novel drug carriers and because of their lipophilic nature they find many applications in the field of science, some of the important applications of liposomes are as follows.

#### **Applications of liposomes in Basic sciences**

A number of new theoretical concepts were developed to understand their conformational behaviour. On the other hand they can be used as a model in order to understand the topology, shape fluctuations, phase behaviour, permeability, fission and fusion of biological membranes<sup>8</sup>.

#### **Applications of liposomes in medicine**

Sustained release system of systemically or locally administered drugs can be formulated in liposomes. Examples are doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin<sup>8,9</sup>.

#### **Macrophage activation and vaccination**

Since conventional liposomes are digested by phagocytic cells in the body after intravenous administration, they are ideal vehicles for the targeting of drug molecules into these macrophages, the automatic targeting of liposomes to macrophages can be exploited in several ways, including the macrophage activation and in vaccination<sup>10</sup>.

#### **Sterically Stabilized liposomes**

Liposomes can be sterically stabilized by the PEG head groups of synthetic phospholipids. Unlike conventional liposomes, which show dose-dependent blood clearance kinetics, the PEG-liposome formulations show prolonged circulation time in blood, with clearance kinetics that are completely independent of dosage over a wide

range. Most importantly, they produce a marked enhancement of the antitumor activity of encapsulated doxorubicin and epirubicin in mice<sup>11</sup>.

#### **Applications of Stealth liposomes**

The encouraging results of Doxorubicin encapsulated in Stealth liposomes in preclinical studies were observed also in clinical trials in humans. Blood circulation times around 45 hours were found and at reduced toxicity very good response in AIDS patients with Kaposi sarcoma was observed<sup>12</sup>.

#### **Application of liposomes in cosmetics**

In addition to the drug application, liposome pastes are used as replacement for creams, gels, and ointments for cosmetic products. And also they are used for formulating various extracts, moisturizers, and complex products containing recombinant proteins for wound or sunburn healing<sup>13</sup>.

#### **Application of liposomes in treatment of AIDS**

The liposomes also find its application in formulating the drugs for the treatment of AIDS<sup>14</sup>.

#### **Application of liposomes in agro-food industry**

Lipid molecules, from fats to polar lipids, are one of the fundamental ingredients in almost any food. For instance, lecithin and some other polar lipids are routinely extracted from nutrients, such as egg yolks or soya beans. Liposomes are also used in agro food industries<sup>15, 16</sup>.

#### **Liposomes in parasitic diseases and infections**

From the time when conventional liposomes are digested by phagocytic cells in the body after intravenous management, they are ideal vehicles for the targeting drug molecules into these macrophages. The best known instances of this 'Trojan horse-like' mechanism in several parasitic diseases which normally exist in the cell of MPS. They comprise leishmaniasis and several fungal infections<sup>17</sup>.

#### **Marketed products of liposomes**

Many of the drugs like anticancer, antibacterial, antifungal drugs, enzymes, hormones, NSAIDs, have been marketed in liposomal formulations<sup>18</sup>. The present study is aimed at developing and optimising liposomal formulation of Mupirocin, a broad spectrum antibiotic by lipid film hydration

technique using various ratios of soya lecithin and cholesterol, before which preformulation studies was also performed. Upon pre-formulation studies and optimization, the various formulations (of varying proportions) were subjected to various physico chemical characterization i.e., morphology studies, drug entrapment efficiency, *in-vitro* drug release, release kinetics and stability studies. Among the prepared five formulations (F1- F5) F4 formulation emerged as the most satisfactory formulation in all the evaluation parameters. The liposomes were found to be stable during their stability studies when stored at different temperatures.

## **MATERIALS AND METHODS**

### **Preformulation Studies**

#### **Thermal Degradation Test by Melting Point Apparatus**

The melting point of the Mupirocin was determined by taking a small amount of drug in a capillary tube and placed in the melting point apparatus, and the apparatus is turned on, the reading was taken in triplicate.

#### **Solubility Studies**

The solubility of the Mupirocin was tested in various solvents including water, ethanol, acetone ether, chloroform etc<sup>19</sup>.

#### **Determination of Purity of the Drug sample**

##### **Preparation of stock solution**

A standard stock solution of mupirocin calcium was prepared by dissolving 100mg of drug in 100ml of 50:50v/v acetonitrile sodium phosphate buffer. Selection of solvent system was based on the solubility and stability of drug in solvent system as well as extraction of drug from its formulations. 10 ml of this solution was further diluted with same solvent to get the final concentration of 100 µg/ml and this was used as the standard stock solution<sup>20</sup>.

##### **Preparation of calibration curve**

From the stock solution various dilutions were made to obtain of 2, 4, 8, 10, 12, 14, 16 µg/ml. absorbance values of these solutions were measure at lamda max 220nm. The calibration curve was

plotted between concentration of mupirocin calcium and respective measured absorbance's.

### Compatibility studies for drug and Polymer (IR Study)

Infra-red spectra of mupirocin and Physical mixture of other ingredients were measured by KBr disc method from 400 to 2000cm<sup>-1</sup> using the Analytical Model Instrument.

### Preparation of KBr disc

Initially a pinch of Potassium Bromide was taken into granite made motor and pestle and a pinch of pure drug was added and mixed well and this is placed in the disc holder and made into a fine film by the aid of Hydraulic press and this disc was placed in the sample holder and is finally analysed by the IR instrument. Similarly separate discs were prepared for pure polymer and mixture of drug and polymer and analysed by using IR instrument<sup>21</sup>.

### Method

The method used for the preparation of liposomes is Lipid film hydration by hand shaking method. Initially cholesterol and soya lecithin were weighed accurately dissolved in a proposed ratio in ethanol at temperature of 60°C and stirred for two minutes. 100mg of Mupirocin was added to above solution and continued the stirring for 2min. Now the above solution is subjected for hydration with 10ml phosphate buffer pH 7.4 and added 2ml of 0.5% stearic acid and continued the stirring for about one hour for the formation of liposomal vesicles. Finally the product obtained is collected and cooled to room temperature and stored in a hermetically sealed container for further evaluation studies<sup>19, 22, 23</sup>.

### Physico-Chemical Characterization of Liposomes

#### Morphology Analysis

The particle size and morphology of the prepared liposomes was done by using the Scanning Electron Microscopy (SEM) at an accelerating voltage of 10kV. 9.3mm x 100SE. And the particle size determination of the liposomes was done by using Phase Contrast Microscopy where the size of the liposomal vesicles along with its shape and their distribution can be measured. The morphology of

vesicles of the liposomes was also analysed by Light Microscope<sup>24</sup>.

### Percentage Yield of liposomes

The prepared liposomes were collected and weighed. The measured weight was divided by the total amount of drug and ingredients which were used for the preparation of the liposomes.

$$\text{Percentage(\%)} \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug \& exceptients}} \times 100$$

### Drug Entrapment Efficiency (DEE)

The drug content in the Mupirocin loaded Liposomes was determined by calculating the difference between the total and the free drug concentrations in the liposomal suspension and the supernatant respectively. Supernatant was separated by centrifugation and the amount of free drug present in the supernatant (w) was assayed by UV spectrophotometer at 229nm. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added (W). In effect, (W-w) will give the amount of drug entrapped in the liposomes. The percentage drug entrapment was calculated by using the following formula<sup>25</sup>.

$$\text{Percentage drug entrapment} = \frac{(W - w) \times 10}{W}$$

### In-vitro Drug Release Study

The drug release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffers. Phosphate buffer pH 7.4, pH 6, pH 8(100 ml) was placed in a 250 ml beakers. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5°C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Mupirocin, liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (5ml) at specific intervals, filtered and the medium was immediately replaced with same quantity of fresh buffer solution. The Aliquots was measured for the amount of the drug by using UV spectrophotometer

at 229nm. The cumulative drug release was calculated with the help of standard calibration curve of Mupirocin pure drug. The drug release studies were carried out at three different pH's (i.e., at 6, 7.4 and 8) because the liposome carriers are best suited and formulated for the topical application and even the pH of the skin ranges from 4 - 8 and blood pH is 7.4<sup>19</sup>.

#### Stability Analysis

The behaviour of the liposome to retain the drug was studied by storing the liposome at 4 different temperature conditions, i.e., 4 - 8<sup>0</sup> (refrigerator RF), 25±2<sup>0</sup> (room temperature RT), 37±2<sup>0</sup> and 45±2<sup>0</sup> for a period of 1 month. The liposomal preparations were kept in sealed vials. Periodically samples were withdrawn and analyzed for the drug content following the same method described in % drug encapsulation efficiency. And also the liposomes were studied for their morphology at regular intervals<sup>26, 27</sup>.

#### RESULTS AND DISCUSSION

The melting point of the drug measured by using melting point apparatus was found to be 77.6°C and it complies with the standard value. Mupirocin is slightly soluble in water and is freely soluble in alcohol. The assay of Mupirocin was done by UV Spectrophotometer and the percentage purity of the sample was found to be 97.36%. FTIR graphs of the pure drug, soyalecithin, cholesterol and their physical mixtures were performed and the FTIR graphs are shown in (Figures No. 2, 3, 4, 5). From the figures and (Table No.2), it is noted that there is no possible interactions between mupirocin and the other ingredients within the formulation and the presence of functional groups are within the range, so this may not affect the formulation stability during its shelf life. The average vesicle size of the

liposomes was found to be between 18.3 to 35.8 μm (Table No.3) when analyzed under Phase Contrast Microscopy and was uniformly distributed. The SEM, Phase contrast and Light microscopic images of the liposomes are shown in (Figure No.6 a, b, c). The Percentage yields of the various Liposomal formulations are listed in (Table No.3) where, out of the five formulations the formulation F4 had the high percentage yield. The average percent drug entrapment efficiency of the five formulations ranges from 53% to 71.72 % where the Formulation F4 showed a maximum drug entrapment of 71.72 %. The Percent drug entrapment of all formulations was tabulated in (Table No.3). The standard Calibration curve of Mupirocin was constructed for calculation of drug release and it showed regression value of 0.999, so it passes linearity. The standard graph of mupirocin is shown in (Figure No.7). The drug release studies of the five formulations were studied and compared at three different pH's and is shown in (Figure No.8, 9, 10). By performing the comparative drug release studies for all the five formulations at three different pH and from the data obtained it is found that the formulation F4 showed good release pattern at all the three pHs. The release of the drug from the formulation F4 was immediate and it followed First order kinetics at all the three pH's as shown in (Figure No.11). After evaluating all the parameters the liposomal Formulations were tested for their stability studies by subjecting them to different temperatures for a period of one month and were found to be stable with very negligible variations. The entrapment efficiencies of all the formulations before and after stability studies are tabulated in (Table No.4). The light microscopic image and Drug release of the F4 formulations (at different pHs) are shown in (Figure No.13).

**Table No.1: Formulation of Mupirocin Liposomes**

S.No	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1	Mupirocin	100mg	100mg	100mg	100mg	100mg
2	Soya lecithin	500mg	600mg	700mg	800mg	900mg
3	Cholesterol	500mg	400mg	300mg	200mg	100mg
4	Phosphate buffer pH(7.4)	10ml	10ml	10ml	10ml	10ml
5	Stearic acid	2ml	2ml	2ml	2ml	2ml
6	Acetone	q.s	q.s	q.s	q.s	q.s
7	Ethanol	q.s	q.s	q.s	q.s	q.s

**Table No.2: Drug - Excipient Interaction Studies by FTIR**

S.No	Observed Range $\text{Cm}^{-1}$			Characteristic peak Functional Groups
	Mupirocin	Cholesterol	Physical mixture	
1	1410.14 $\text{cm}^{-1}$	1466.08 $\text{cm}^{-1}$	1464.39 $\text{cm}^{-1}$	C-H Scissoring and bending 1470-1350 $\text{cm}^{-1}$
2	1379.27 $\text{cm}^{-1}$	1375.41 $\text{cm}^{-1}$	1378.19 $\text{cm}^{-1}$	CH <sub>3</sub> Bend 1400-1375 $\text{cm}^{-1}$
3	1251.95 $\text{cm}^{-1}$	1260.12 $\text{cm}^{-1}$	1231.94 $\text{cm}^{-1}$	C-N medium to sharp 1350-1000 $\text{cm}^{-1}$
4	1228.81 $\text{cm}^{-1}$	1219.23 $\text{cm}^{-1}$	1231.94 $\text{cm}^{-1}$	C-N medium to sharp 1350-1000 $\text{cm}^{-1}$

**Table No.3: Particle size, Percentage yield, DEE of the formulations**

S.No	Formulations	Average Particle Size ( $\mu\text{m}$ )	Percentage Yield (%)	Drug Entrapment Efficiency (%)
1	F <sub>1</sub>	35.8 $\mu\text{m}$	52.24	53
2	F <sub>2</sub>	29.78 $\mu\text{m}$	63.31	62.5
3	F <sub>3</sub>	26 $\mu\text{m}$	65.87	67
4	F <sub>4</sub>	18.3 $\mu\text{m}$	89.06	71.72
5	F <sub>5</sub>	21.35 $\mu\text{m}$	82.46	68

**Table No.4: DEE of formulations before and after stability studies**

S.No	Formulations	% Drug Entrapment Efficiency	
		Before Stability studies	After stability studies
1	F1	53±2.5	51±2.5
2	F2	62.5±2.5	61.5±2.5
3	F3	67±2.5	65±2.5
4	F4	71.72±2.5	70.72±2.5
5	F5	68±2.5	66±2.5

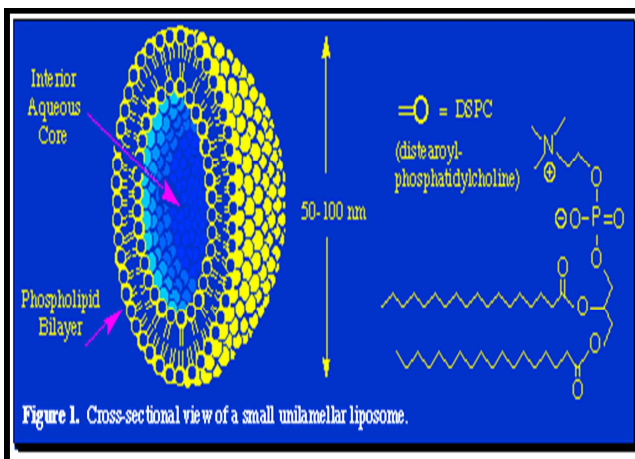
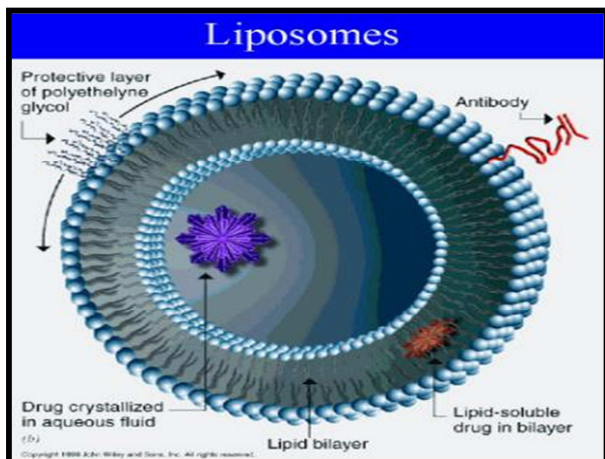


Figure No.1: Liposome structure

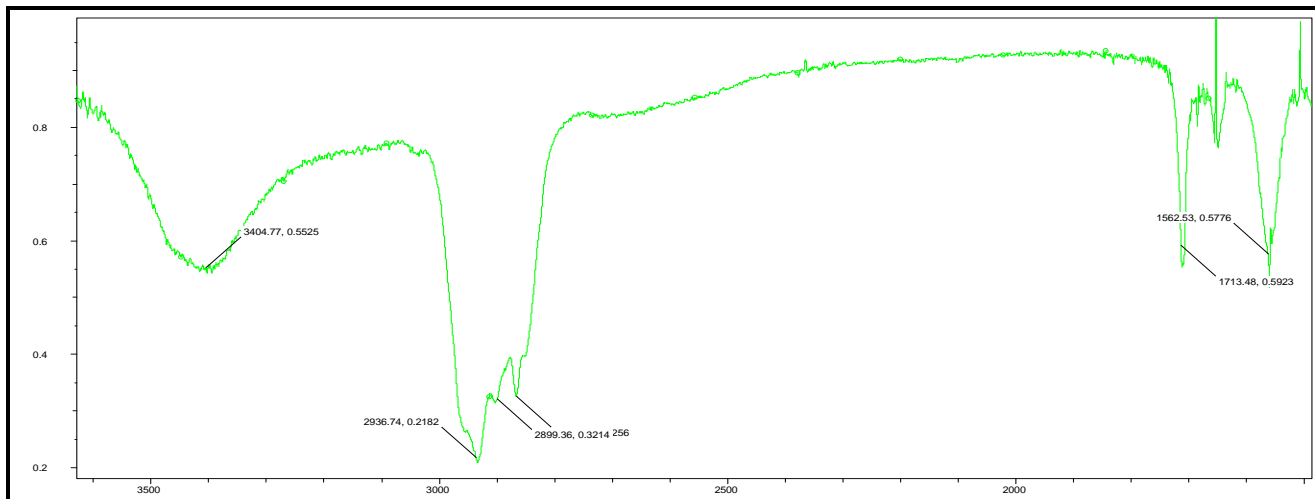


Figure No.2: FTIR spectrum of Mupirocin (Functional group region)

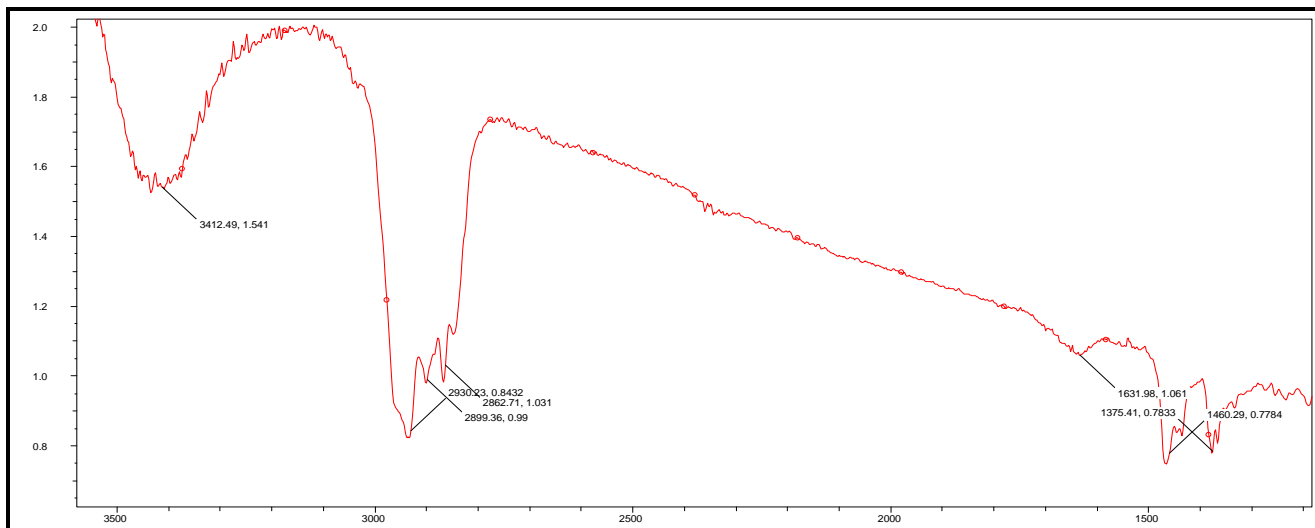
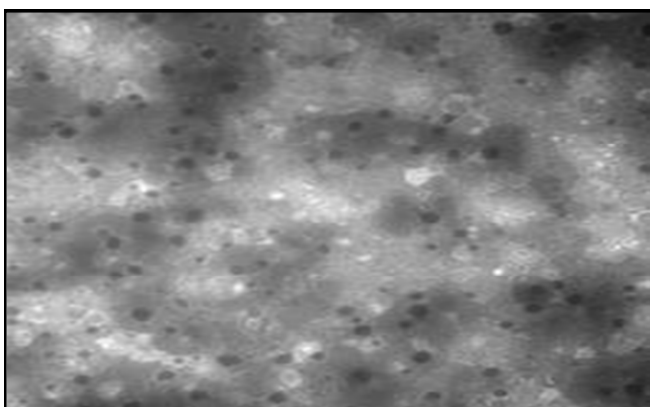
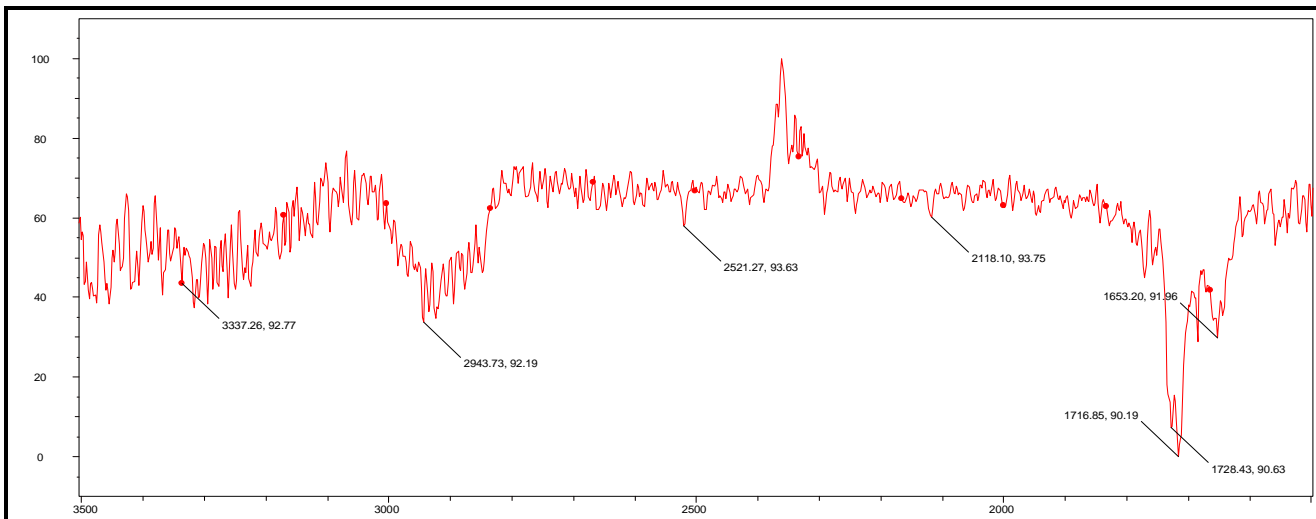
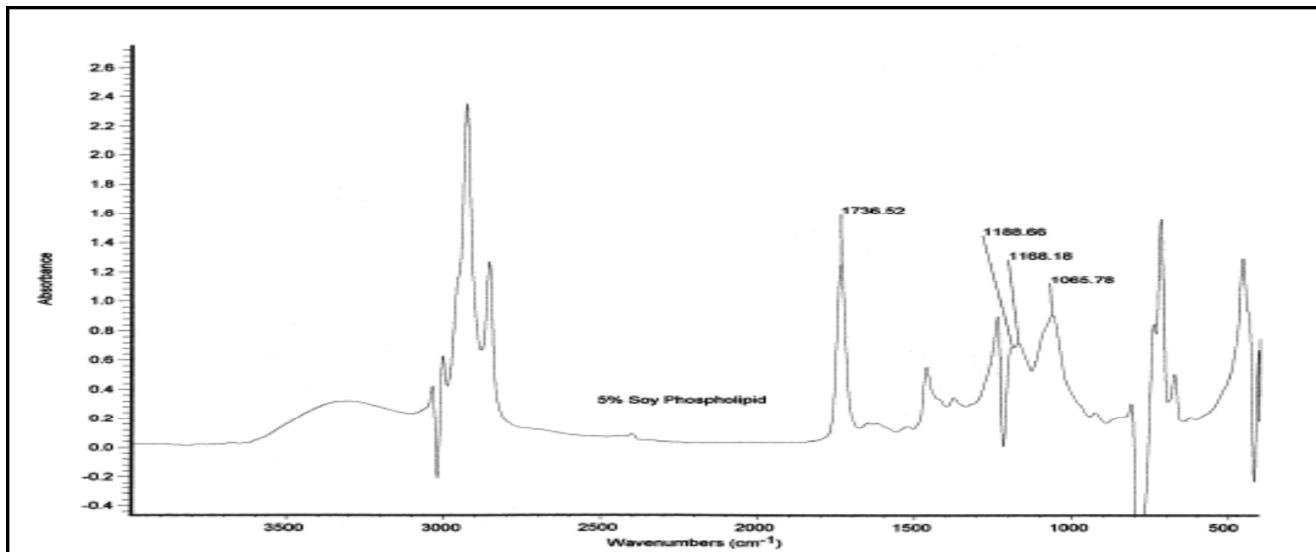
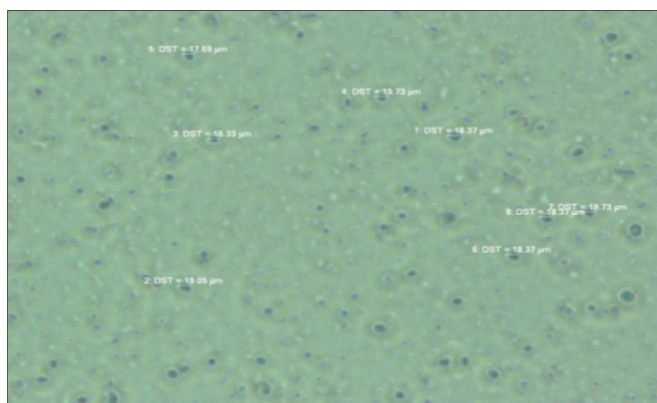


Figure No.3: FTIR spectrum of Cholesterol (Functional group region)

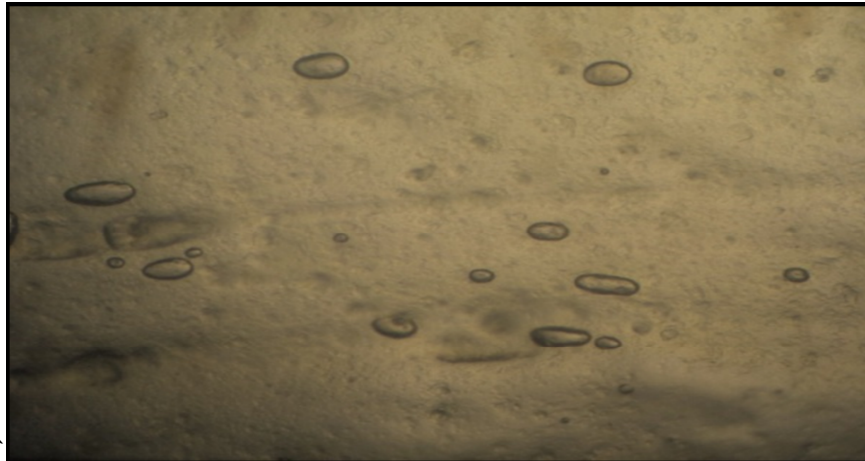


a. SEM image of the liposomes



b. Liposomes under phase contrast microscope





(c) Light microscopic image of liposomes  
Figure No.6: Morphology of liposomes

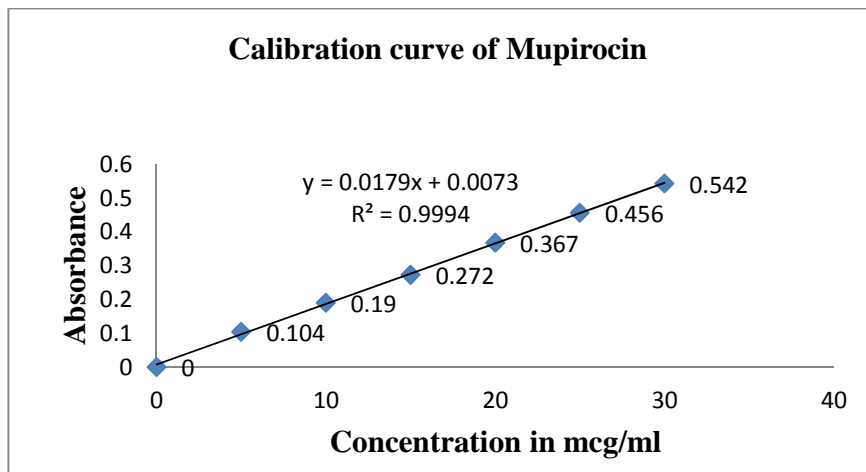


Figure No.7: Standard calibration curve of mupirocin

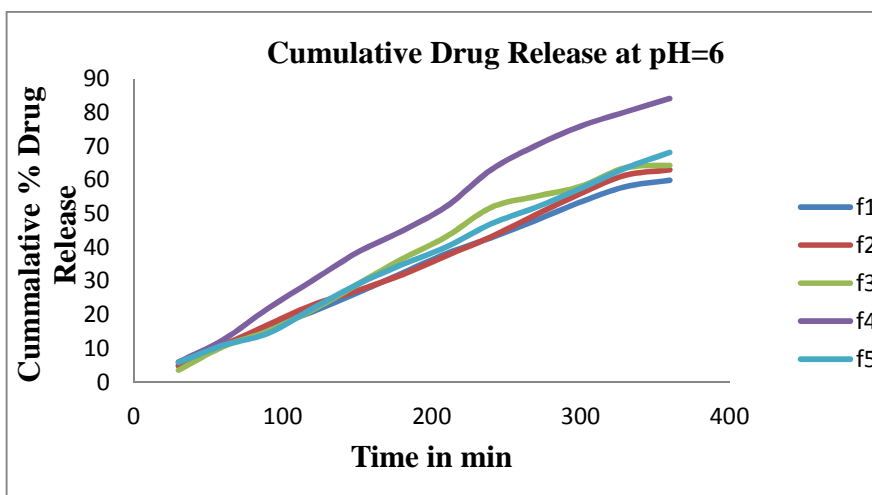


Figure No.8: Comparative drug release of all formulations at pH 6

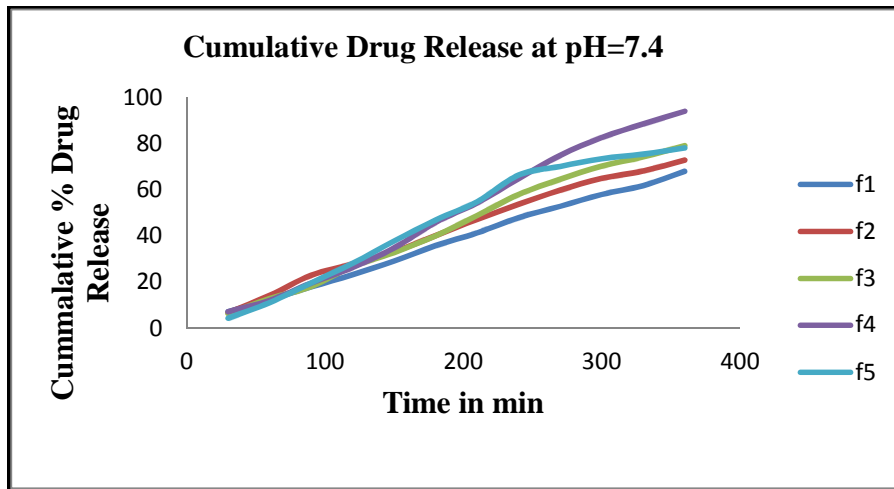


Figure No.9: Comparative drug release of all formulations at pH 7.4

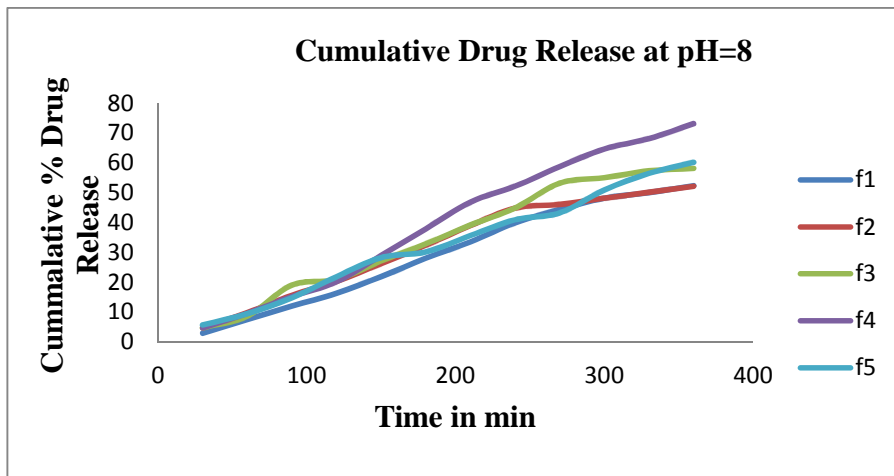


Figure No.10: Comparative drug release of all formulations at pH 8

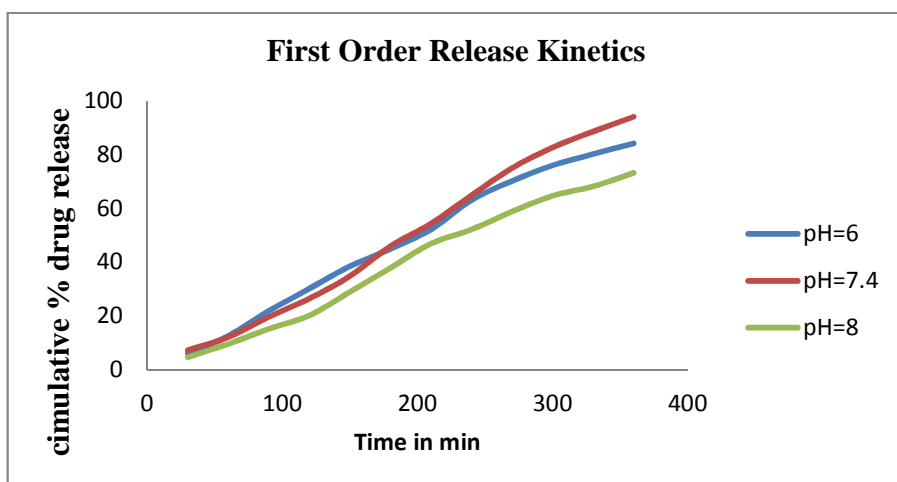


Figure No.11: First order release kinetics of F4 formulation

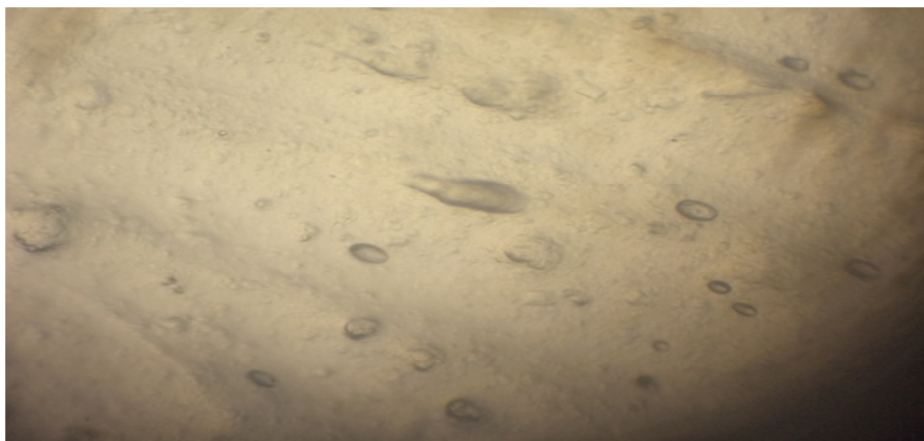


Figure No.12: Light microscopy image of liposome (after Stability studies)

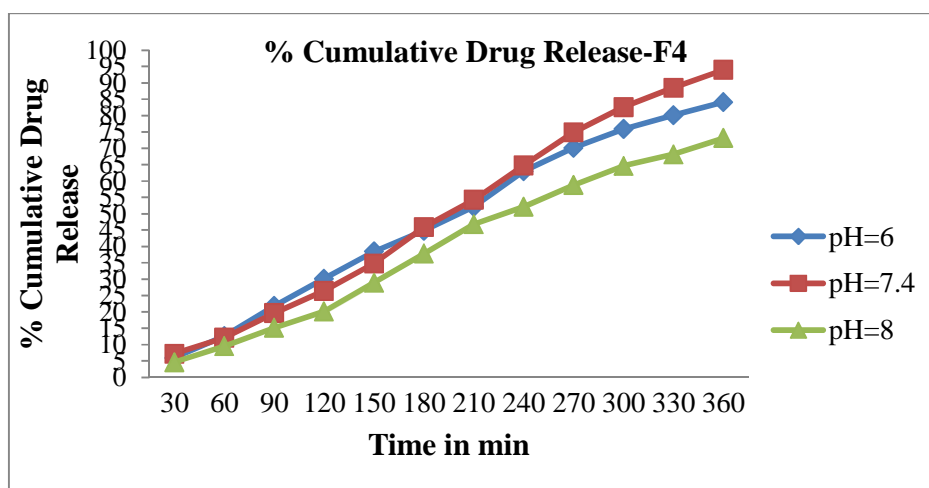


Figure No.13: Drug release studies of F4

## CONCLUSION

Among the various Novel Drug Delivery systems the Liposomal drug delivery system is one of the best advantageous carriers for the drug delivery because of its lipophilic moieties in the formulations. And also the Mupirocin being one of the best topically used broad spectrum antibiotic, we had made an attempt to formulate the Mupirocin in the liposomal carriers. We prepared five formulations of varying proportions and on optimization we found that the formulation F4 was effective in all the parameters when compared with the others viz., Good vesicle shape, Mean diameter, Entrapment efficiency and Drug release profile. The drug release pattern was in controlled manner which follows the First order kinetics. And also the

prepared liposomes showed good stability profile without any much declination in their properties. Thus we conclude that in addition to other Novel Drug Delivery carriers like nanoparticles, microspheres. The Mupirocin can also be formulated in the liposomal carrier which finds its best way for the topical administration for treating various bacterial infections.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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