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Formulation and Evaluation of Atorvastatin Calcium Liposomes

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ABSTRACT

By improving the ether injection technique, a prolonged release drug delivery system for atorvastatin was created. The formation of unilamellar vesicles involved the usage of cholesterol and egg phosphatidylcholine. Methanol and ether were added to the vesicles to study the effects of charges on them. Numerous variables that might impact the size, shape, encapsulation effectiveness, and release rate were investigated. By streamlining the procedure, liposomes with sizes between 0.5 and 0.9 m were produced. It was discovered that neutral, positive and negatively charged liposomes had encapsulation efficiencies of 48, 64, 72, 64, 40, and 32 percent, respectively. On a specially created model, the *in vitro* drug release rate was investigated. Stability tests were carried out on lyophilized atorvastatin liposomes.

INTRODUCTION:

Liposomes' significance as a means of medication delivery is now well established. This is especially true when it comes to liposomes' capacity to reduce the toxicity of drugs they contain without compromising their efficacy [1]. Other applications for liposomes that show therapeutic promise include serving as carriers for anticancer agents [2-4], antiparasitic [5], antibacterial [6], antifungal [7], antiviral [8,9] and ocular liposomes[10–12]. These findings increase the need for liposome development to meet pharmaceutical needs. The intrinsic requirements include low-cost material, an easy and quick way to make liposomes, a homogenous and repeatable size distribution, and an effective way to load liposomes. The final liposomal formulation must also be extremely stable to ensure that the medicine is retained and that the liposomes themselves maintain their chemical and dimensional stability.

The vesicle size, lipid content, and lipid dosage all have a significant impact on how liposomes behave *in vivo* [13]. When administered intravenously without cholesterol, liposomes often leak significantly. The phagocytic cells of the reticuloendothelial system (RES) eventually remove the majority of liposomes administered intravenously, which often causes localization in the liver and spleen [14]. The size of the liposomes can have an impact on the pace of such clearance as well as the dispersion that results. For the same lipid dosage, smaller vesicles are removed more slowly than their bigger counterparts and are less quickly sequestered by the liver.

The lipid dosage affects the circulation time as well; greater doses result in longer circulation periods. These outcomes are probably connected to RES saturation at higher doses [15].The charge of the vesicle is another aspect that affects how long it stays in the body; vesicles with negatively charged lipids are eliminated more quickly than those with neutral or positively charged systems.

Ether injection was used to create unilamellar vesicles (ULVs). The size, shape, and effectiveness of liposome integration may be influenced by several variables, including the ratio of phosphatidylcholine to cholesterol, the ratio of lipid to medication, the incorporation of charged species, pH, and others that were examined. Both optical and transmission electron microscopes were used to investigate liposomes. An UV spectrophotometer was used to analyze the drug content. On a specifically created in vitro liposome model, research on the release rate were carried out.

MATERIALS AND METHODS:

Atorvastatin was a gift sample from Yarrow Chem Pvt., Ltd., Mumbai, India. Phosphatidylcholine, Methanol, cholesterol, ether were purchased from Sigma chemicals U.S. All other chemicals and reagents used were of analytical grade.

FTIR Spectroscopy: The physical characteristics of the physical combination and those of the pure drug were contrasted. 100 mg of potassium bromide IR powder was completely combined with the samples before being compressed for three minutes under vacuum at a pressure of around 12 psi. The final disc was placed in an appropriate holder within an IR spectrophotometer by Perkin Elmer, and the IR spectrum was recorded between 3500 cm and 500 cm. Comparing the resulting spectrum for any spectrum changes.

Determination of Absorption maxima: A solution containing the concentration 10 µg/ ml drug was prepared in methanol UV spectrum was taken using Double beam UV/VIS spectrophotometer. The solution was scanned in the range of 200 – 600 nm. [16]

COMPATIBILITY STUDIES: Using an IR spectrophotometer made by Perkin Elmer, investigations of the drug's compatibility with the polymers were conducted. Three parts dried potassium bromide and one part sample were completely combined before being compacted into clear, thin pellets. The IR area is then used to scan the pellets, and the spectra are recorded and explained in the next section. [17]

FORMULATION DEVELOPMENT:

Selection of the Best Method for Liposome Preparation: At a temperature between 55 and 65 degrees Celsius or under decreased pressure, a solution of lipids dissolved in diethyl ether or ether methanol combination is progressively injected into an aqueous solution of the substance to be encapsulated. In the process of removing ether under vacuum, liposomes are produced. The elimination of the solvent from the product, which enables the process to be run for longer periods and create a concentrated liposomal product with high entrapment efficiencies, is a benefit of the ether injection approach as opposed to the ethanol injection method.

Preparation of Drug Loaded Liposome by Ether Injection Method: After dissolving the necessary quantity of phospholipid and cholesterol in ether, the lipophilic medication was added to the organic one. With magnetic stirring and decreased pressure, the resultant organic

phase was injected into a predetermined amount of distilled water using a syringe pump at a temperature between 55 and 65 °C. When the ether comes into contact with the aqueous phase, it evaporates, and the distributed lipid mostly takes the form of unilamellar liposomes. The formulation for 50 ml was made. [18]

Table No. 1: Formulation design for preparation of drug loaded liposome by ether injection method

Formulation	Drug	Lipid	Cholesterol	Ether	methanol	Water
LFA1	10 mg	100 mg	100 mg	7 ml	3 ml	50 ml
FFA2	10 mg	200 mg	100 mg	7 ml	3 ml	50 ml
LFA3	10 mg	300 mg	100 mg	7 ml	3 ml	50 ml
LFA4	10 mg	100 mg	200 mg	7 ml	3 ml	50 ml
LFA5	10 mg	200 mg	200 mg	7 ml	3 ml	50 ml
LFA6	10 mg	300 mg	200 mg	7 ml	3 ml	50 ml
LFA7	10 mg	100 mg	300 mg	7 ml	3 ml	50 ml
LFA8	10 mg	200 mg	300 mg	7 ml	3 ml	50 ml
LFA9	10 mg	300 mg	300 mg	7 ml	3 ml	50 ml

PHYSICAL CHARACTERIZATION OF LIPOSOMES: All the liposomal formulation was evaluated by studying their physicochemical properties like [19, 20].

Morphology Analysis: To detect vesicle production and the discreteness of scattered vesicles, the produced Atorvastatin calcium liposomes for all formulations were observed under. A slide was created by adding a drop of liposome dispersion to a glass slide, covering it with a cover slip, then viewing the slide using a 40X optical microscope. Digital cameras were used to capture images for prepared slides.

Determination of Particle Size Distribution: Determination of average vesicle size of Atorvastatin calcium liposomes with carrier was very important characteristic. It was carried out by using Malvern Instruments, Startech Labs Pvt. Ltd.

Polydispersity Index: Polydispersity was determined according to the equation,

$$\text{Polydespersity} = D(0.9) - D(0.1) / D(0.5)$$

Where, D (0.9) corresponds to particle size immediately above 90% of the sample.

D (0.5) corresponds to particle size immediately above 50% of the sample.

D (0.1) corresponds to particle size immediately above 10% of the sample.

Zeta Potential Analysis: Any particle in a formulation exhibits zeta potential, a physical characteristic. Zeta potential is significant because its value can be connected to the stability of colloidal dispersions. Therefore, electrical stabilization occurs in colloids with high zeta potentials, whether they are negative or positive, whereas flocculation or coagulation occurs in colloids with low zeta potentials. The arbitrary value that distinguishes between low-charged surfaces and high-charged surfaces is 25 mV (positive or negative). Malvern Zetasizer at the Central Drug Research Institute in Lucknow conducted the zeta potential analysis.

Scanning Electron Microscopy: Analysis of the surface morphology (roundness, smoothness, and aggregate formation) of calcium atorvastatin Scanning electron microscopy was used to study liposomes with carriers (SEM). SEM samples were placed on metal studs and amplified by a factor of 2000.

IN-VITRO CHARACTERIZATION OF LIPOSOME [21-23]

pH: Every injectable preparation has to have a pH value that is compatible with the relevant bodily fluid being injected. Thus, after dispersing the final formulation in water for injection, the pH of each formulation was assessed using a pH meter that had been previously calibrated.

Drug Entrapment: All formulations underwent testing to identify drug entrapment. Pipetting 10 ml of the liposome formulation into a 100 ml volumetric flask with 20 ml of 0.1N NaOH solution, sonicating it, and then filtering it with Whatman filter paper. After diluting the filtrate with 0.1N NaOH, the concentration of atorvastatin calcium was measured at 249.98 nm using a UV-Vis spectrophotometer.

***In vitro* Release Studies:** Utilizing the dialysis membrane approach, in vitro release tests were carried out. 100 ml of phosphate buffer with a pH of 7.4 was put in a 250 ml beaker. The medium was equilibrated at 37.5oC, and the beaker was put together on a magnetic stirrer. One end of the dialysis membrane was sealed after it was removed. The dialysis membrane was filled with liposome formulation, and the other end was sealed. The sample

was suspended in the medium using the dialysis membrane. At predetermined intervals, aliquots (5ml) were removed, filtered, and the medium was immediately replenished with the same amount of brand-new buffer solution. Using a UV spectrophotometer set to 249.98 nm, the quantity of the medication in the aliquots was determined.

STABILITY STUDIES: By keeping the liposome at two distinct temperatures, namely 4°C (refrigerator RF), and 25°C2°C for a month, the liposome's behavior to maintain the medication was examined. In sealed vials, the liposomal formulations were stored. Following the same methodology stated in percent drug encapsulation efficiency and in vitro drug release, the samples were tested for drug content on the 30th day. The shape of the liposomes was also examined.

RESULTS AND DISCUSSION

FTIR Spectroscopy: The IR spectrum of the atorvastatin calcium pure sample recorded by FTIR spectrometer is shown in Fig 1, which was compared with standard functional group frequencies of Atorvastatin.

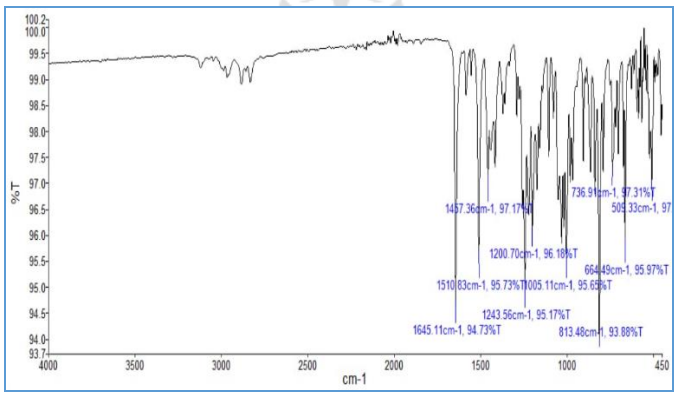

Figure No. 1: FTIR Spectra of Atorvastatin

UV Spectroscopy: λmax for the given sample of the drug was determined by using UV-Vis spectrophotometer. Λmax for atorvastatin calcium monohydrate was found to be 249.98 nm in methanol.

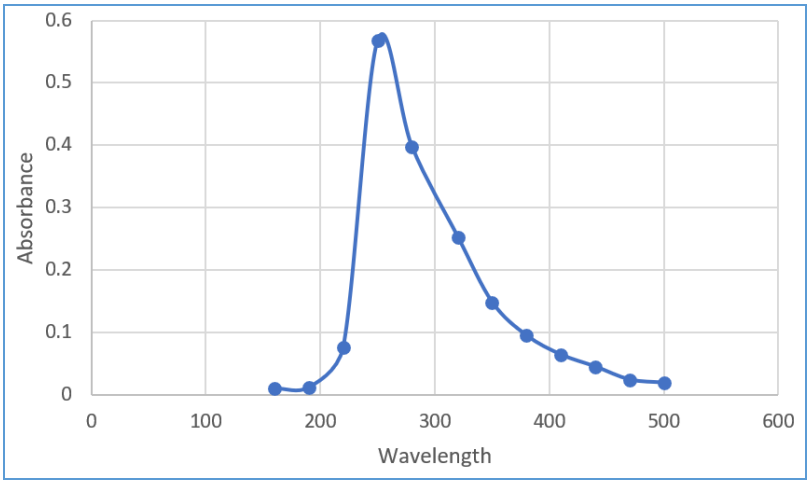

Figure No. 2: UV Spectra of Atorvastatin in methanol

Calibration Curve of Atorvastatin: Measured the absorbance of the above prepared standard solution at 249.98 nm, plotted a graph of concentration (in µg/ml) on X axis and absorbance (in nm) on Y axis.

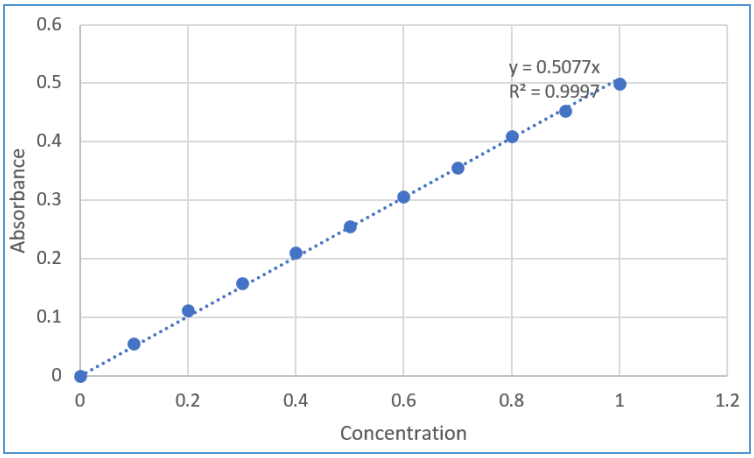

Figure No. 3: Calibration curve of Atorvastatin in HCl

COMPATIBILITY STUDY: The compatibility between the drug and the selected lipid and other excipients was evaluated using the FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-polymer mixture, which confirmed the absence of any chemical interaction between the drug, polymer and other chemicals. The results show Spectra 4.

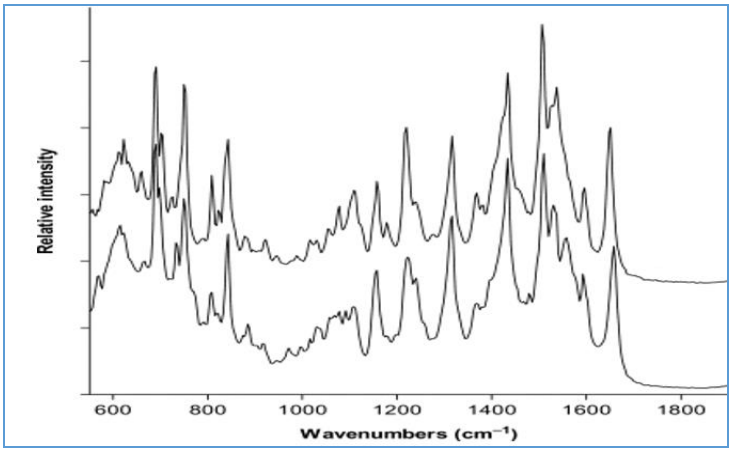

Figure No. 4: FTIR Spectra of Atorvastatin with Polymer

PHYSICAL CHARACTERIZATION OF LIPOSOMES

Morphology Analysis: The morphology characters of liposomes were analyzed by optical microscopy (Olympus Opto System, India) and the images were taken using digital camera. The formulation LFA6 microscopic images were showed in Figure 5.

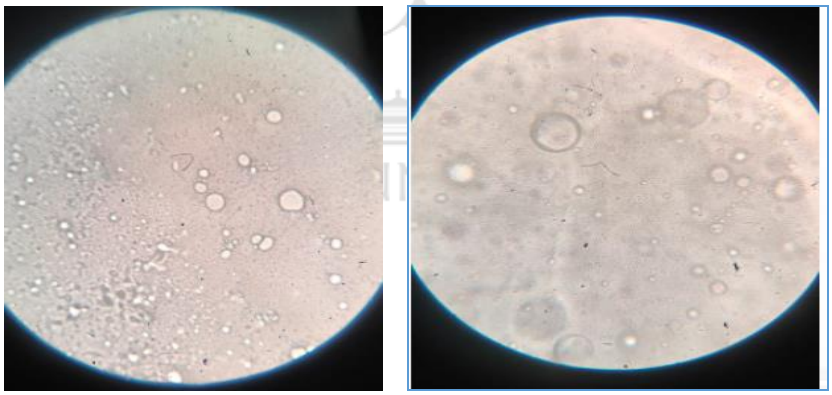

Figure No. 5: Morphology of Liposomal formulation LFA6

Particle Size Distribution: The particle size distribution was analyzed for LFA3, LFA6, LFA9 formulations of Atorvastatin calcium Liposomes by wet method. The particle size was optimum in LFA6 Formulation, when compared to LFA3 and LFA9. The results were shown in Table 2.

Zeta Potential Analysis: The zeta potential report of liposomal solution for LFA3, LFA6, LFA9 formulations are 7.45mV, 19.56mV, -36.98 which lies near to the arbitrary value. The report shows good stability value for a formulated liposomal solution, the results were shown in Table 2.

Table No. 2: Physicochemical characteristics of Atorvastatin calcium Liposomes for Optimized Batches

Formulation code	Average vesicular size (nm)	Zeta Potential (mV)	Poly dispersive index (Pdi)
LFA3	403	7.45	0.634
LFA6	574	19.56	0.712
LFA9	339	-36.98	0.569

Scanning Electron Microscopy: The Morphology and surface appearance of Liposomes were examined by using SEM. The SEM photographs of LFA3 formulation showed that the particles have a smooth surface. The SEM images were shown in Figure 6.

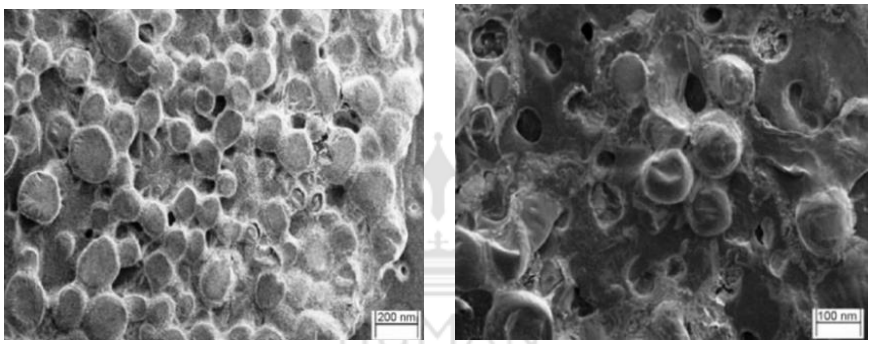

Figure No. 6: SEM image of liposome formulation LFA3

***IN-VITRO* CHARACTERIZATION OF LIPOSOME**

pH: The pH of the formulation was determined by dispersing the formulation in water for injection. The formulation pH was found to be under desirable limits, and thus was found to be appropriate for parenteral use.

Drug Entrapment: Table 3 shows the percent entrapment of drug for the formulations prepared by ether injection method. Entrapment for the formulations prepared by ether injection method LFA1, LFA2, LFA3, LFA4, LFA5, LFA6, LFA7, LFA8 and LFA9 were found to be 48%, 64%, 72%, 64%, 40%, and 32% respectively.

Table No. 3: pH and Entrapment Efficiency of Liposomal Formulation

Sr. No.	Formulation	pH	% drug entrapment
1	LFA1	6.7	87.63
2	LFA2	7.3	84.87
3	LFA3	6.6	90.87
4	LFA4	7.2	83.87
5	LFA5	7.5	89.98
6	LFA6	6.9	97.65
7	LFA7	7.3	92.30
8	LFA8	7.7	85.87
9	LFA9	7.4	94.56

***In Vitro* Release Studies:** The release profile for the formulation predicts how a delivery system might function and gives valuable insight into its in vivo behaviour. The various formulations of Atorvastatin calcium were subjected to in vitro release studies. These in vitro release studies were carried out using 0.1 N NaOH as the dissolution medium. The Cumulative release pattern concerning time for the formulations are shown in Fig. 7, 8 & 9.

The cumulative drug release for the formulations, prepared by ether injection method LFA1, LFA2, LFA3, LFA4, LFA5, LFA6, LFA7, LFA8 and LFA9 was found to be 73.985, 76.87%, 80.88%, 76.98%, 74.87%, 84.88%, 75.98%, 79.87%, and 81.88%, respectively.

The formulation LFA3 and LFA9 showed quite similar pattern of release barring the first 5 hours. In this duration formulation, LFA6 showed a slight burst release in the first hour followed by a linear pattern of release while LFA3 showed a slow initial release, in the first hour, followed by a linear pattern, In the entire period of 3 hours LF9 showed significantly less drug release as compared to other formulation. on the other hand showed a slow initial release in the first 90 mins., followed by burst release till the 4th hour and this was followed by a linear release pattern. The formulation LFA3 and LFA9 showed an almost linear pattern of release. Formulation LFA6 also showed an almost linear pattern of release except for the period between 3rd to 5th hour in which it showed slight burst release. Overall these 9 formulations prepared by the ether injection method released lesser drug content within 6

hours and thus were found to be more suitable for sustained release, especially formulation LFA6.

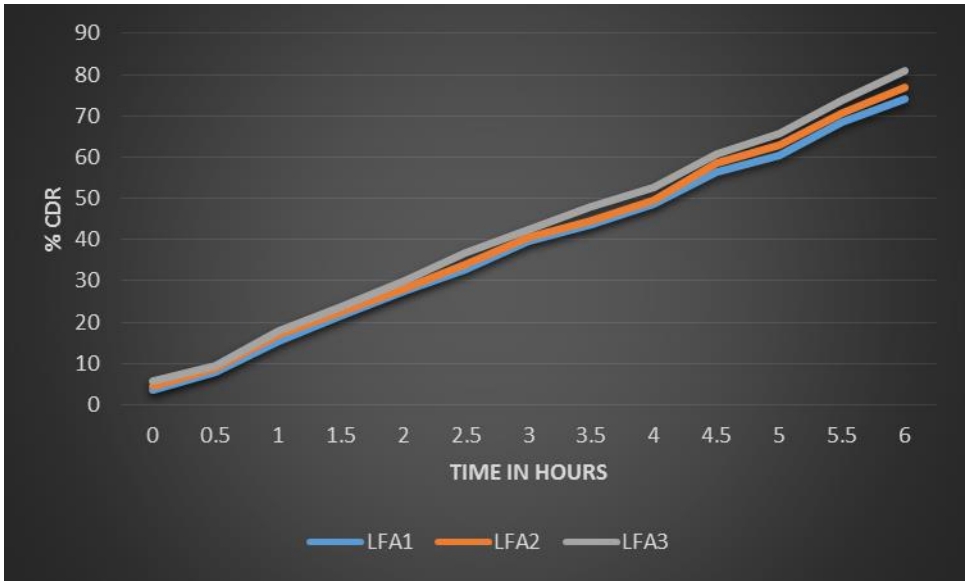

Figure No. 7: Cumulative % Drug Release of Formulation LFA1-LFA3

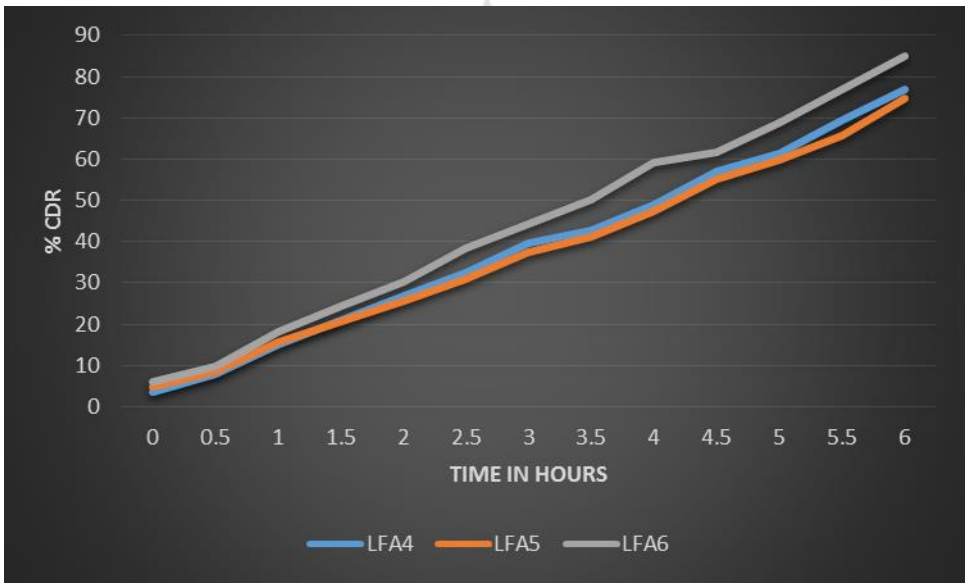

Figure No. 8: Cumulative % drug release of formulation LFA4-LFA6

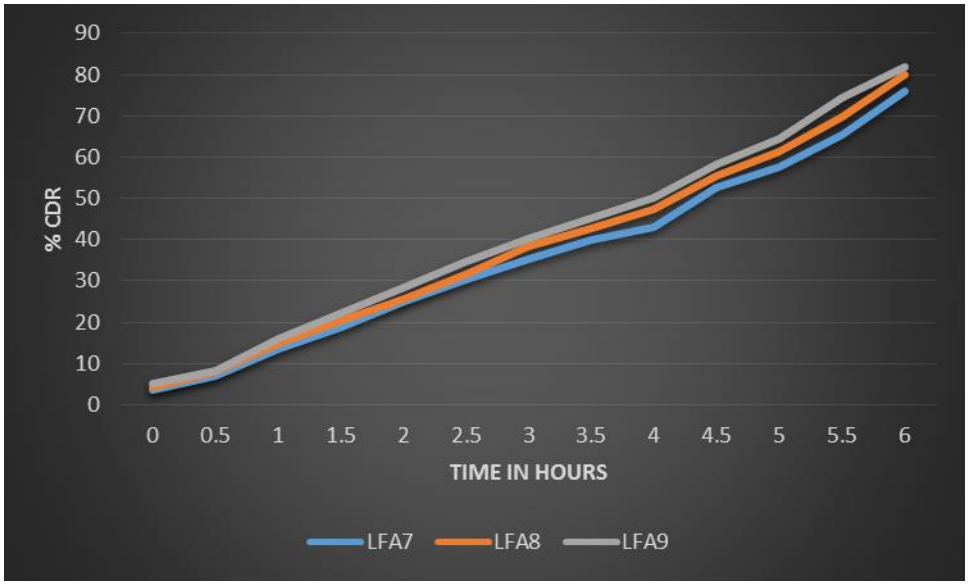

Figure No. 9: Cumulative % drug release of formulation LFA-7LFA9

Stability Studies: All the formulations of Atorvastatin Calcium liposomes were relatively stable at 4ºC storage condition. The drug leakage percent amounts of original entrapped in liposomes were very small and the amount retained in vesicle had no significant difference after one month as compared to the amount immediately after preparation. But at the storage condition of 25°C±2°C, all the formulations of Atorvastatin calcium liposomes were unstable. In addition, the result of drug entrapment studies showed higher leakage at higher temperatures. This may be due the higher fluidity of lipid bilayer at higher temperatures, resulting into higher drug leakage. The drug entrapment results were shown in table 4.

Table No. 4: Stability study of pH and drug entrapment of Atorvastatin calcium liposome compared with percentage drug entrapment of immediately after preparation

Sr. No.	Formulation	Immediately after preparation	After one month	
		% Drug Entrapment	**4 °C**	**25 °C ± 2 °C**
1	LFA3	90.87	90.85	90.45
2	LFA6	97.56	97.35	97.21
3	LFA9	94.65	94.45	94.34
		pH		
1	LFA3	6.6	6.5	6.5
2	LFA6	6.9	6.8	6.7
3	LFA9	7.4	7.2	7.1

At storage condition 4°C showed better stability than another condition. This may due to their elevated temperature reduce the stability. But in both storage conditions higher proportion of soya lecithin-containing formulations like LFA6 showed better stability than other their formulations.

CONCLUSION:

Preparing and testing the Atorvastatin calcium Liposomes was the major goal of this study. This formulation will formulate non-pegylated Liposomes to prevent side effects while targeting the site of action with the influence of different stabilizers on drug entrapment effectiveness. The less hazardous soya bean lecithin and cholesterol were used to create this liposomal mixture.

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