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DESIGN AND CHARACTERIZATION OF LIPOSOMAL LOADED GELS FOR TRANSDERMAL DRUG DELIVERY OF FLUVASTATIN SODIUM

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Abstract:

In the present study, an attempt was made to develop the transdermal drug delivery systems of Fluvastatin sodium using Liposomes incorporated in a gel, which will control the release of drug, increasing the bioavailability of the drug and thus decreasing the dosing frequency of the drug. It was designed by encapsulating the drug in various Liposomal formulations composed of various ratios of Soya Lecithin: Span 80 or Tween 80 or sodium deoxycholate. The Liposomes were prepared by rotary evaporation sonication method. Lipid: surfactant ratio of 90:10 is found to be more effective when compared to other ratios. Experimental results of the present study showed that deformable lipid vesicles improve the transdermal delivery, prolong the release, and improve the site specificity of the Fluvastatin sodium. The drug diffusion studies showed that the prepared liposome vesicles followed zero order kinetics and mechanism of drug diffusion followed peppas model.

Key Words: *Liposomes, Anti-hyperlipidemic, Controlled release, Lipid, Surfactant.*

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INTRODUCTION:

The percutaneous route for drug administration has many advantages over other pathways, including avoidance of first pass effect, delivering drugs continuously, having fewer side effects, and improving patient compliance. The barrier nature of skin inhibits the penetration of most drugs. The use of lipid vesicles as delivery system for skin treatment has gained attention in recent years, but it has certain disadvantages. To overcome those disadvantages, a novel type of highly deformable lipid vesicles called Liposomes has been reported recently to penetrate intact skin, if applied nonocclusively.[1] Liposomes are enclosed vesicles containing a lipid bi layer composed of unimers that usually have a hydrophilic head and a hydrophobic tail and are oriented so that the hydrophobic head groups are inside the bi layer. Liposomes are highly biocompatible with low toxicity that helps in conniving drug delivery system with improved bioavailability [2].

Hydrogels are 3-dimensional networks consisting of hydrophilic polymers that swell in aqueous solution retaining large amount of water without dissolving. Hydrogels have biodegradable properties, high permeation of active materials with high degree of swelling and no associated toxicity or irritation makes them as ideal polymers for delivery of drugs through transdermal route as delivery vehicles [3] .

Fluvastatin sodium is an antilipemic agent that competitively inhibits HMG-CoA reductase. It belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular diseases. Its short biological Half life (3 hours) and low bioavailability(24%-29%) makes it appropriate candidate for transdermal drug delivery system.[4-5] The maintenance of a constant plasma drug concentration of a anti lipidemic drug is important in ensuring the desired therapeutic response and to improve patient compliance, hence the objective of the study was made to develop controlled release transdermal drug delivery system of Fluvastatin sodium using Liposomes incorporated in a gel, which will control the release of drug, increasing the bioavailability of the drug and thus decreasing the dosing frequency of the drug.

MATERIALS AND METHODS:

Fluvastatin sodium was received as gift sample from Dr.Reddy's Laboratories, Hyderabad. Soya lecithin, sodium deoxycholate, triton X-100 was purchased from Himedia Laboratories, Hyderabad. Span 80, tween 80 was purchased from sigma Aldrich laboratories.

Preparation of Liposomes by thin film hydration technique:

Liposomes were prepared by rotary evaporation-sonication method [6-7]**.** Different edge activators in different molar ratios were used for the formulation of Liposomes, the composition of these formulations is shown in Table 1.The lipid mixture (500mg) consist of phospholipid (Soya Lecithin), edge activator (Span 80, or sodium deoxycholate or Tween 80) and drug (10 mg/ml) in different ratios was dissolved in an organic solvent mixture consist of chloroform and methanol (2:1, v/v) then placed in a clean, dry round bottom flask. The organic solvent was carefully evaporated by rotary evaporation (Buchi rotavapor R-3000, Switzerland) under reduced pressure above the lipid transition temperature (at 60^0c for 1 hr) to form a lipid film on the wall of the flask. The final traces of the solvents were removed by subjecting the flask to vacuum over night. The dried thin lipid film deposited on the wall of the flask was hydrated with a phosphate buffer solution (pH 6.4) by rotation for 1hr at room temperature at 60 rpm. The resulting vesicles were swollen for 2 hrs at room temperature to get large multilamellar vesicles. To prepare small Liposome vesicles, the resulting vesicles were sonicated at 100 kHz, 80 Amp for 30 minutes at pulse on 30sec and pulse off 50 sec using a probe Sonicator (Orchid Scientifics, Nasik). The obtained suspension was passed through a series of 0.45µ and0.22µ polycarbonate filters and then stored at 4ºC.

Table 1: Composition of various Fluvastatin sodium Liposome formulations:

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg/ml)	10	10	10	10	10	10	10	10	10
Soya Lecithin (**%**)	95	92.5	90	95	92.5	90	95	92.5	90
Tween 80**(%)**	5	7.5	10			-	-	-	
Sodium deoxycholate **(%)**	-	-		5	7.5	10			
Span 80 **(%)**	-	-		-		-	5	7.5	10

Evaluation tests for Liposome formulations:
Liposomes were evaluated for eentrapment efficiency, invitro diffusion study, vesicles size and size distribution [8].

Entrapment Efficiency (EE%**):**
The entrapment efficiency of Liposomes was determined after separation of the non-entrapped drug. Entrapment efficiency of Fluvastatin sodium in Liposomal formulations can be done by Freeze thawing/centrifugation method [8]**..** 1 ml samples of Liposomal dispersion were frozen for 24 hrs at −20 °C in Eppendorf tubes. The frozen samples were removed from the freezer and let to thaw at room temperature, then centrifuged at 14,000 rpm for 50 min at 4 °C. Liposomal pellets were re-suspended in Phosphate buffer solution (pH 7.4) and then centrifuged again. This washing procedure was repeated two times to ensure that the un-entrapped drug was no longer present. The supernatant liquid was decanted. The drug remained entrapped in Liposomes was determined by complete vesicle disruption using 0.1% Triton X-100. For this, 2ml of 0.1% triton X 100 was added to 2ml of Liposomes suspension. This mixture was subjected to centrifugation 30 min and the resultant solution was analysed by UV-Visible spectrophotometer for the drug. The drug content was determined spectrophotometrically at 304 nm using Phosphate buffer solution (pH 7.4) as a blank. Each result was the mean of three determinations (±SD). The entrapment efficiency was defined as the percentage ratio of the entrapped drug concentration to the total drug concentration and calculated according to the following equation:
EE% = Total drug concentration – Free drug concentration X 100
Total drug concentration

Vesicles Size and Size Distribution:
The vesicles size and size distribution were determined by dynamic light scattering method (DLS), using a computerized inspection system (HORIBA NANOPARTICA SZ-100, U.K.). For vesicles size measurement, vesicular suspension was mixed with the appropriate medium and the measurements were conducted in triplicate [9].

Determination of zeta potential:
Zeta potential is a measure of the surface charge of dispersed particles in relation to dispersion medium. It was determined by using HORIBA zeta sizer. The zeta potential value is a measure of the physical stability of the Nanosponges [10].

Vesicles Shape:
Liposomes vesicles were visualized by Scanning Electron Microscopy (SEM). The sample for the SEM analysis were prepared by applying a monolayer of the Liposome dispersion on to one side of double adhesive stub and the stubs were then coated with platinum using the auto fine coater (JFC-1600, JEOL, Japan). The scanning electron microphotographs of Liposomes were taken using (JSM-6360, JEOL, Japan) scanning microscope [11].

Preparation of rate controlling membrane
Solvent evaporation technique was employed in the present work for the preparation of Cellulose acetate films. The polymer solutions were prepared by dissolving the polymer (2% w/w Cellulose acetate) in 50 ml of Ethyl Acetate-Methanol (8:2). Dibutyl phthalate at a concentration of 40% w/w of the polymer was used as a plasticizer. 20 ml of the polymer solution was poured in a Petri plate (9.4 cm diameter) placed on a horizontal flat surface. The rate of evaporation was controlled by inverting a funnel over the Petri plate. After 24 hours the dried films were taken out and stored in a desiccator [12].

Preparation of Liposomes loaded gels:
Accurately weighed quantity of 500 mg of carbopol 934 was dispersed in 5 ml of distilled water and was allowed for swelling over night. The swollen carbopol was stirred for 60 minutes at 800 rpm. The previously prepared required Fluvastatin sodium equivalent Liposomes, methylparaben and propylparaben were incorporated into the polymer dispersion with stirring at 500 rpm by a magnetic stirrer for 1 hour. The Ph of above mixture was adjusted to 7.4 with tri ethanolamine (0.5%). The gel was transferred in to a measuring cylinder and the volume was made up to 10ml with distilled water [13]. The Liposomes formulations (F1- F9) were incorporated into the carbopol gels (LTG1-LTG9). In order to achieve the more prolonged release LTG10, LTG11 and LTG12 were prepared by using 500 mgof Methyl cellulose, Sodium carboxy methyl cellulose and Hydroxy propyl methyl cellulose respectively instead of carbapol. Fluvastatin sodium Liposomes prepared with Soya Lecithin: Tween 80 in 90: 10 ratios were incorporated in those formulations.

Preparation of mouse skin:
Swiss albino mouse aged between 6 to 8 weeks were taken and sacrificed by cervical dislocation. The epidermal skin was carefully removed and rinsed with normal saline to remove any loose materials. The epidermal skin was cut into 5 cm length. The epidermal skin was stored in cold (5-8°C) normal saline solution [14].

Evaluation of drug reservoir gels:
The drug reservoir gels were evaluated for the Drug content, P^H, viscosity extrudability, and Spreadability[15].

Design of membrane moderated transdermal therapeutic system:
A circular silicon rubber ring with an internal diameter of 2.5 cm and a thickness of 3 mm was fixed on to a backing membrane (an imperforated adhesive strip was supplied by Johnson and Johnson Limited, Mumbai). This serves as a compartment for drug reservoir. Gel equivalent to 40 mg of Fluvastatin sodium was taken into the compartment as a drug reservoir. Cellulose acetate membrane of known thickness was fixed on the ring with glue to form a membrane moderated therapeutic systems. A double-sided adhesive strip was fixed on the rim of the ring above Cellulose acetate membrane [15].

***In vitro* diffusion study:**
Drug diffusion study was conducted using Franz diffusion cell. The receptor compartment was filled with 15 ml of phosphate buffer having pH 7.4 as diffusion media. The skin piece was mounted between the compartments of diffusion cells with the epidermis facing upward into the donor compartment. The membrane moderated therapeutic systems of Fluvastatin sodium was placed on the skin.Magnetic stirrer was set at 50 rpm and whole assembly was maintained at 32 $\pm$ 0.5 ˚C. The amount of drug released was determined by withdrawing 1 ml of sample at regular time intervals for 24 hours. The volume withdrawn was replaced with equal volume of fresh buffer solution. Samples were analyzed for drug content using a U V spectrophotometer at 304 nm for drug content [16].

RESULTS AND DISCUSSION:

The thin film hydration method or rotary evaporation-sonication method was used to prepare the Liposomal formulations. Formulations were prepared by using different types and concentrations of surfactants. However, a reduction of vesicle size was observed when surfactant concentration increased above 10% w/w. This is due to the formation of a micellar structure instead of the vesicles, which are relatively smaller in size. The entrapment efficiency increased significantly ($P<0.05$) with increasing edge activator concentration from 5 to 10% (w/w) for Liposomes prepared using Span 80 or sodium deoxycholate or Tween 80, Further increase in edge activator concentration to 15% (w/w).The surfactant concentration in the lipid components of vesicles had shown effect on the entrapment efficiency of Liposomes. The entrapment efficiency decreased with an increase in concentration of surfactant showed a significant decrease in entrapment efficiency**.** The entrapment efficiency of Liposomes prepared with three different ratios of phospholipid: edge activator formulations was decreased in the following order. (90:10) > (92.5:7.5) > (95:5). The phospholipid: edge activator ratio 90:10% (w/w) showed optimum entrapment efficiency**.** Upon incorporation of edge activatorin low concentration, growth in vesicle size occurred, whereas, further increase in the content of edge activator may have led to pore formation in the bilayers. When edge activator concentration exceeded 15%, mixed micelles coexisted with the Liposomes, with the consequence of lower drug entrapment due to the rigidity and smaller size of mixed micelles.

The maximum entrapment efficiency obtained was 90.44% for Liposomes formulation F9 shown in table 2. The surfactant concentration in the lipid components of vesicles had shown effect on the entrapment efficiency of Liposomes. The entrapment efficiency decreased with an increase in concentration of surfactant. The reduction in entrapment efficiency also depended on the surfactant type.

The Liposomes prepared with Span 80 showed the highest entrapment efficiency followed by Liposomes prepared with Tween 80and finally Liposomes prepared with sodium deoxycholate. These results are related to the HLB values of these edge activators. They are 4.3, 15, and 16 for Span 80, Tween 80 and sodium deoxycholate, respectively. Based on these HLB values, the affinity for lipids was expected to be in the order of Span 80 >Tween 80>sodium deoxycholate. This consideration explains the higher entrapment efficiency encountered with Span 80 when compared to Tween 80 and sodium deoxycholate. The entrapment efficiency of the Span 80 formulation was high because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The entrapment efficiency of Liposomes prepared with various surfactants was decreased in the following order. Tween 80 > sodium Deoxycholate > span 80

The mean particle size, poly dispersity index and zeta potential of the Liposomes were found to be good enough to maintain the physical stability of the Liposomes and shown in Table 2.

Table 2: Mean particle size, Poly dispersity Index and zeta potential of the Liposomes

S.No	Batch Code	Entrapment Efficency(%)	Average Particle size(nm±S.D)	Poly dispersity Index`(X±SD)	Zeta Potential (mV±SD)
1	F1	80.44 ± 0.6	296.4±2.75	0.274±0.13	-37.7±1.2
2	F2	84.68±2.20	279.3±2.69	0.266±0.09	-45.1±1.1
3	F3	88.77±2.09	265.3±3.9	0.253±0.12	-52.2±1.3
4	F4	74.34±1.03	287.2±2.57	0.268±0.07	-34.5 ±1.2
5	F5	78.47±2.57	273.3±2.75	0.252±0.06	-37.8 ±1.4
6	F6	81.59±1.37	256.5±1.65	0.238±0.09	-39.3±1.3
7	F7	82.26±2.27	258.6±2.7	0.245±0.07	-38.9±1.2
8	F8	86.33±2.84	247.1±2.26	0.229±0.13	-44.2 ±1.5
9	F9	90.44±2.29	238.5±2.25	0.219±0.06	-54.9±1.6

The mean particle sizes of all the formed Liposomes varied in the range 247.1±2.26to 296.4±2.75nm and 265.3±3.9nm for F3 .The poly dispersity index values of all the formed Liposomes varied in the range 0.238±0.09 to 0.274±0.13 and 0.253±0.12 for F12 .The zeta potential of all the formed Nanosponges varied in the range -34.5 ±1.2to --54.9±1.6 mV and -52.2±1.3mV for F3 .The Liposomes morphology was analyzed by scanning electron microscope. The Liposomes were found to be spherical with good structural composition having a definite boundary as shown in the figure 2. The Liposomes reservoir gels were evaluated for the Drug content, P^H , viscosity extrudability, and spreadability. The results were shown in table 3. The evaluation parameter values of gels shown good characteristic features of gel. Prepared Liposomes were incorporated into gels were subjected to In-vitro diffusion studies and the diffusion profiles were shown in figure3-6.

Table3: In-Vitro Drug Release Kinetic Data of Fluvastatin sodium Loaded Liposomes Prepared With Different Concentrations of surfactants:

Formulation	Correlation Coefficient Values				Diffusion Rate Constant (mg/hr)	Exponential Coefficient (n)	T_{50}	T_{90}
	Zero Order	First Order	Higuchi Model	Peppas Model				
FTG1	0.9915	0.8232	0.9532	0.9967	2.17	0.7585	9.2	16. 58
FTG2	0.9949	0.7399	0.9460	0.9969	1.88	0.8022	10. 3	18.5
FTG3	0.9996	0.8163	0.9272	0.9996	1.74	0.9668	11.4	20.4
FTG4	0.9971	0.7717	0.9207	0.9959	2.09	0.8765	9.56	17.22
FTG5	0.9990	0.6638	0.9269	0.9966	1.98	0.8709	10.10	18.18
FTG6	0.9983	0.6954	0.9349	0.9975	1.82	0.8545	10.98	19.78
FTG7	0.9903	0.8243	0.9570	0.9957	2.59	0.7347	7.7	13.8
FTG8	0.9889	0.8462	0.9582	0.9972	2.35	0.7495	8.5	15.30
FTG9	0.9924	0.8014	0.9511	0.9964	2.06	0.7368	9.7	17.5
FTG10	0.9915	0.7116	0.9527	0.9963	1.96	0.7548	9.7	17.4
FTG11	0.9950	0.6553	0.9448	0.9965	1.80	0.7973	10.8	19.5
FTG12	0.9995	0.7579	0.9275	0.9998	1.65	0.9772	11.7	21.0

Figure 1: Zeta Potential Peak of Liposome prepared with soya Lecithin: Tween 80 in 90:10.

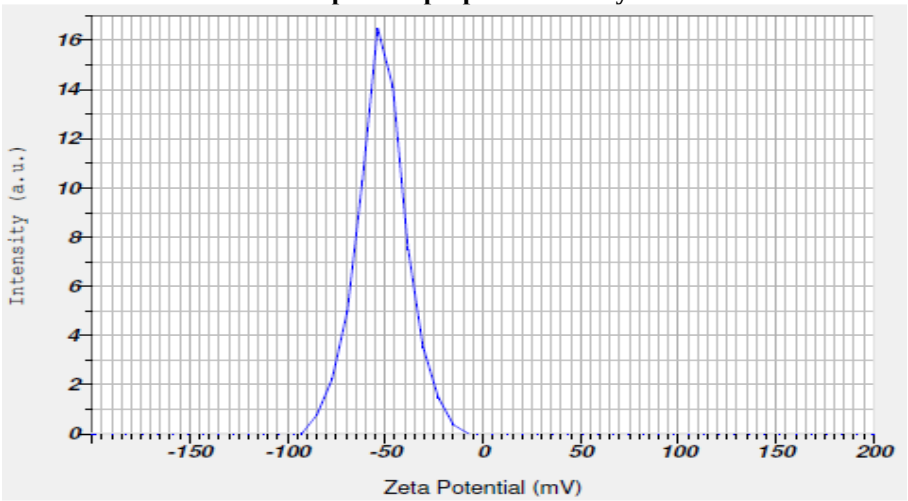


Figure 2: SEM Photographs of Tween 80 (90:10) Liposome Formulation

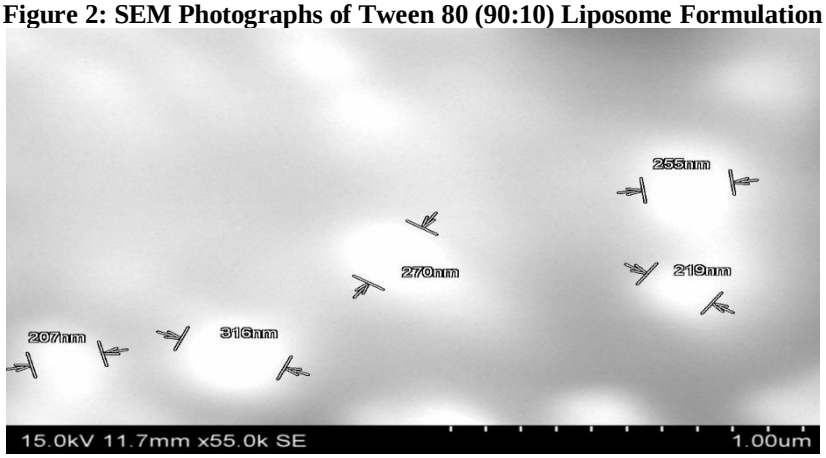
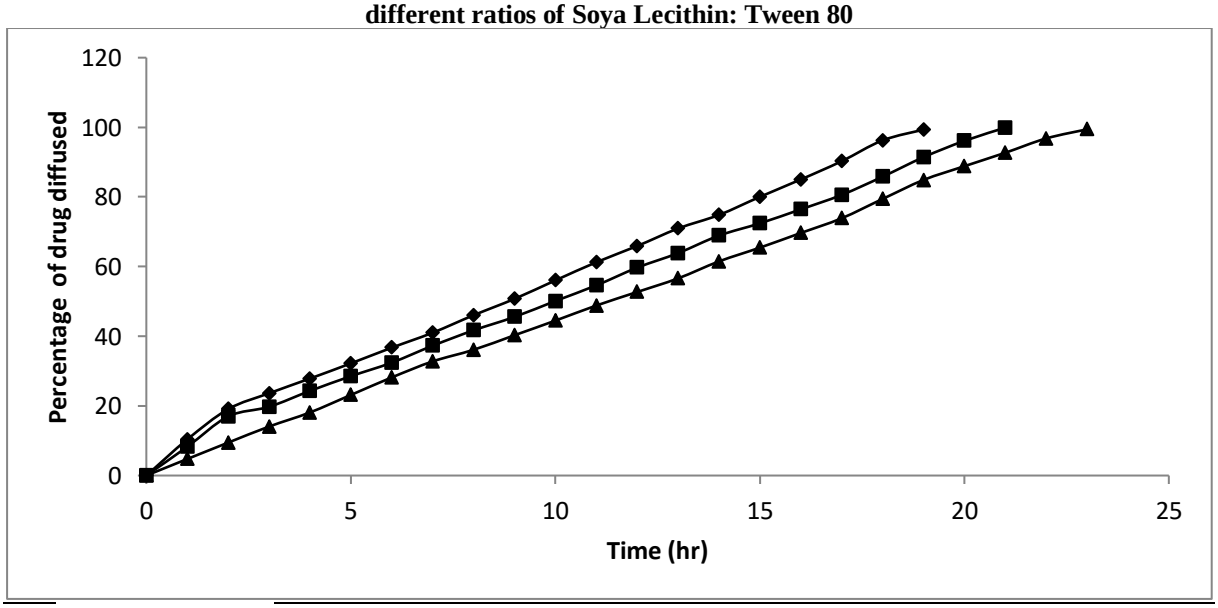
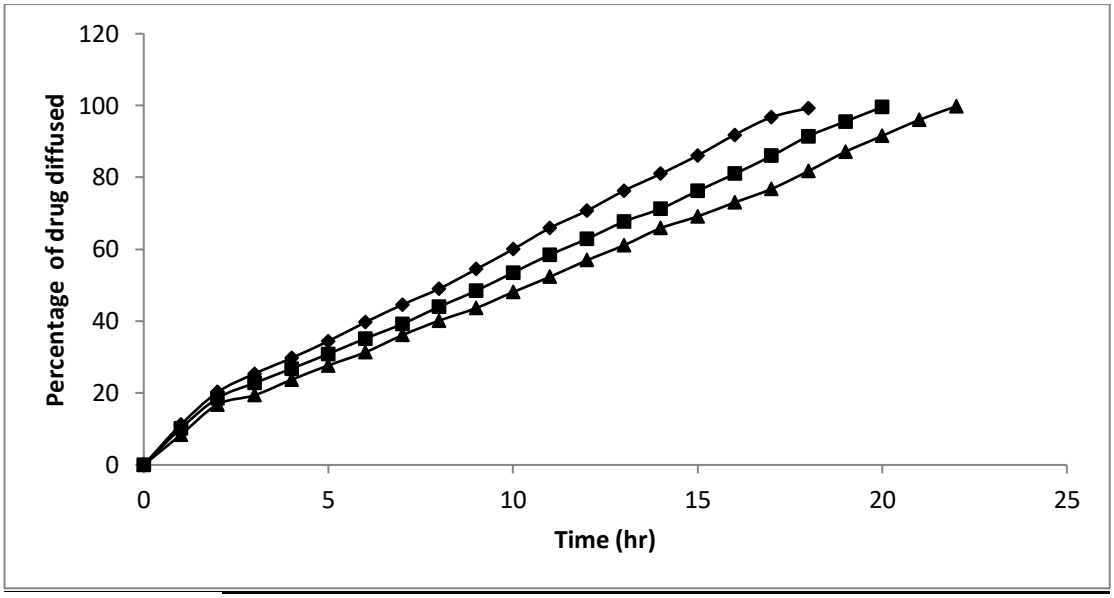


Figure 3: Comparative *in vitro* drug diffusion profiles of Fluvastatin sodium Liposomes prepared with different ratios of Soya Lecithin: Tween 80



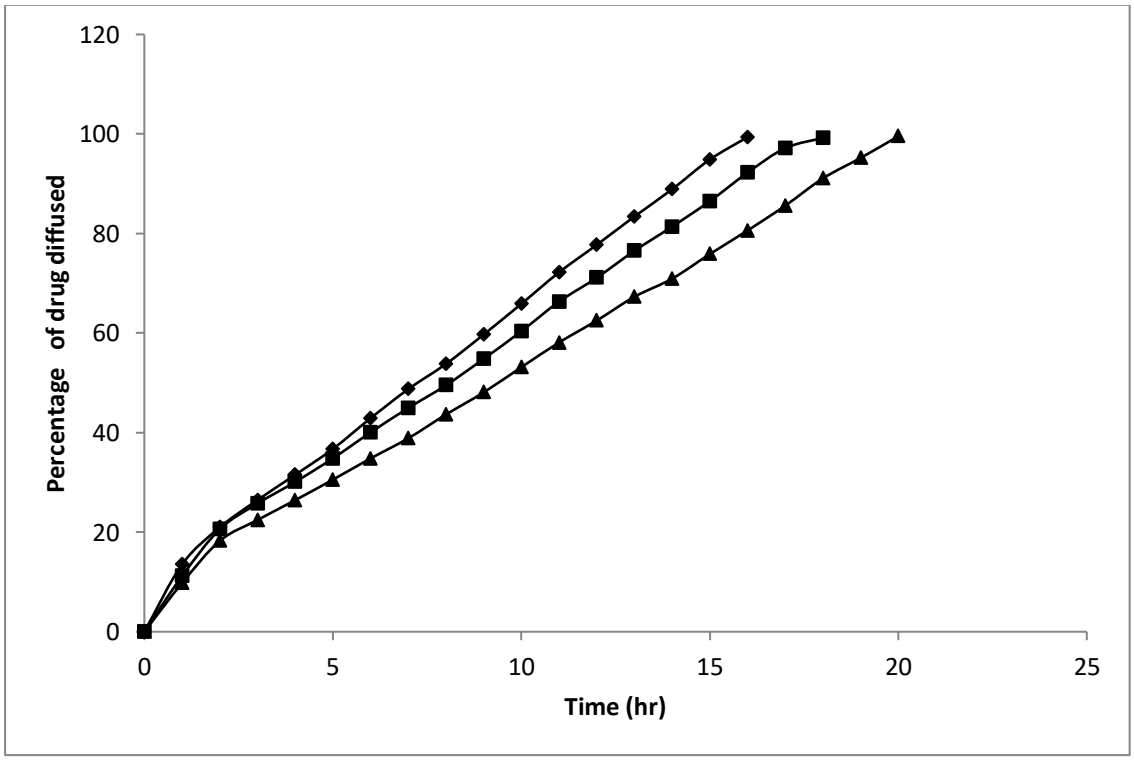
(-♦-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: Tween 80 in 95: 5 ratio
(-■-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: Tween 80 in 97.5: 2.5 ratio
(-▲-) Fluvastatin sodium Liposomes prepared with Soya Lecithin: Tween 80 in 90: 10 ratio

Figure 4: Comparative *in vitro* drug diffusion profiles of Fluvastatin sodium Liposomes prepared with different ratios of Soya Lecithin: sodium deoxycholate



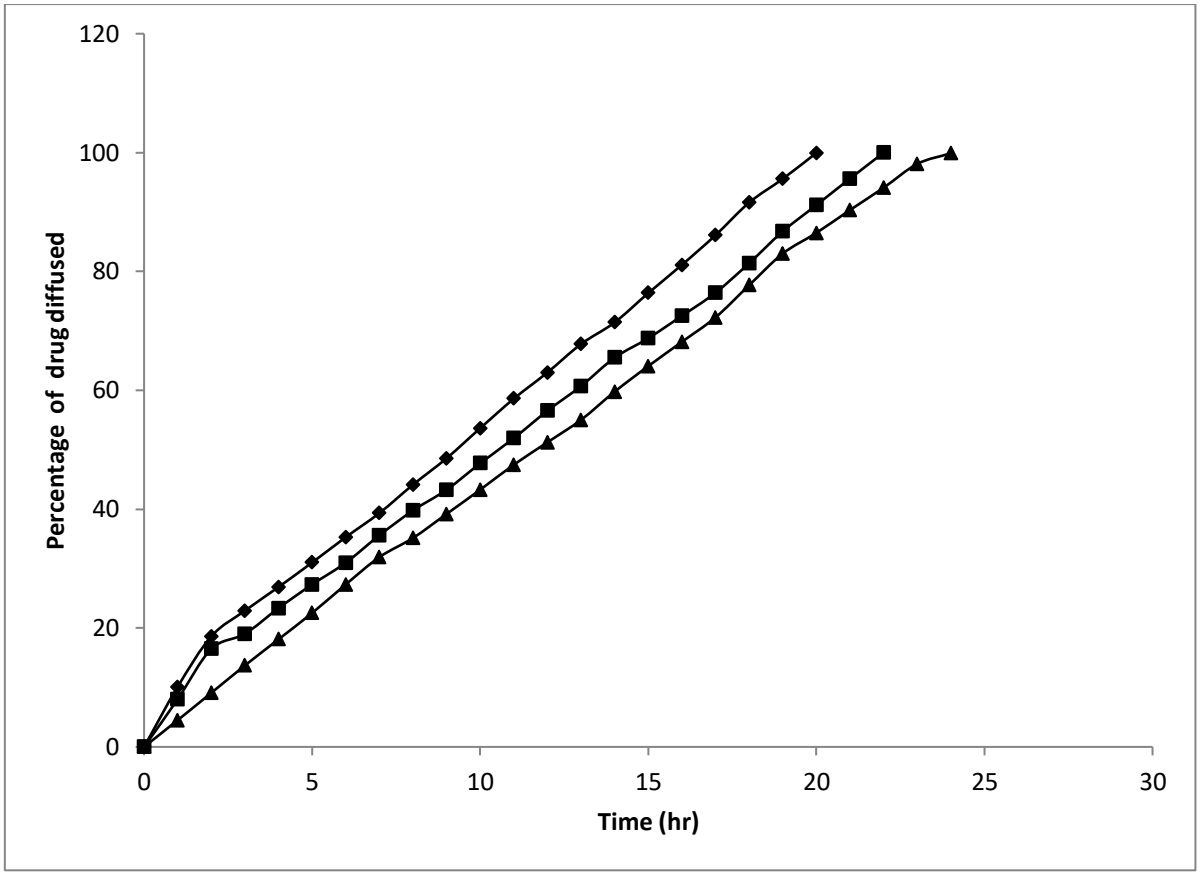
(-♦-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: sodium deoxycholate in 95: 5 ratio
(-■-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: sodium deoxycholate in 97.5: 2.5 ratio
(-▲-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: sodium deoxycholate in 90: 10 ratio

Figure 5: Comparative *in vitro* drug diffusion profiles of Fluvastatin sodium Liposomes prepared with different ratios of Soya Lecithin: Span 80



(-♦-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: Span 80 in 95: 5 ratio
(-■-)Fluvastatin sodium Liposomes prepared with Soya Lecithin: Span 80in 97.5: 2.5 ratio
(-▲-) Fluvastatin sodium Liposomes prepared with Soya Lecithin: Span 80 in 90: 10 ratio

Figure 6: Comparative *in vitro* drug diffusion profiles of Fluvastatin sodium Liposomes prepared with Soya Lecithin: Span 80 in 90:10 ratio from gels prepared with different polymers



(-♦-) Fluvastatin sodium Liposomes loaded methyl cellulose gels
(-■-) Fluvastatin sodium Liposomes loaded sodium carboxy methyl cellulose gels
(-▲-) Fluvastatin sodium Liposomes loaded hydroxyl propyl methyl cellulose gels

The diffusion of drug from Liposomal formulations for a period of 24 hrs revealed that increasing edge activators concentration (from 5 to 10%, w/w) in the Liposomal formulations, further decreasing the drug diffusion. At high edge activator concentrations, the diffusion of the drug was low due to the loss of vesicular structure and formation of rigid mixed micelles.

Carbopol gels containing Fluvastatin sodium loaded Liposomes prepared with of Soya Lecithin: Tween 80 in 95:5, 92.5:7.5, 90:10 ratios shown drug diffusion for a period of 19 hours, 21 hours, and 23 hours respectively. Whereas the gels prepared with Soya Lecithin: Sodium deoxycholate in 95:5, 92.5:7.5, 90:10 ratios shown drug diffusion for a period of 18 hours, 20 hours, and 22 hours respectively and the gels prepared with Soya Lecithin: span80 in 95:5, 92.5:7.5, 90:10 ratios shown drug diffusion for a period of 16 hours, 18 hours, and 20 hours respectively. With a view to design a prolonged release dosage form, various types of gel formulations were prepared using polymers like methyl cellulose, sodium carboxymethyl cellulose and hydroxypropyl methylcellulose. Fluvastatin sodium Liposomes prepared with Soya Lecithin: Tween 80 in 90: 10 ratio were incorporated in those formulations and subjected to diffusion study. The gels prepared with the methyl cellulose, sodium carboxymethylcellulose, and hydroxy propyl methyl cellulose shown drug diffusion for a period of 20 hours, 22 hours and 24 hours respectively.

The hydroxyl propyl methyl cellulose gels prepared using the ratio of Soya Lecithin: Tween 80 in 90: 10 ratio were selected for the prolonged and controlled therapeutic efficacy.

The correlation coefficient values (r) were shown in table 3 and revealed that the diffusion profiles followed zero order kinetics and mechanism of drug diffusion followed by peppa's model.

The diffusional exponential coefficient (n)values were found to be in between 0.7347to 0.9722, indicating that the drug diffusion followed non fickian diffusion mechanism.

The hydroxyl propyl methyl cellulose gels prepared using the ratio of Soya Lecithin: Tween 80 in 90: 10 ratio were selected for the prolonged and controlled therapeutic efficacy.

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