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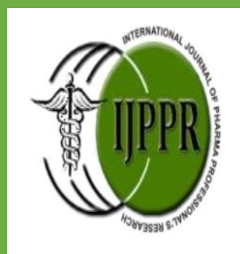

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RESEARCH

Formulation and evaluation of Liposomal gel of Atorvastatin for Diabetic Foot Ulcer

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ABSTRACT

Statins have applications that go beyond controlling cholesterol; when administered systemically, they help facilitate the healing of wounds. The current work aims to formulate and evaluate liposomal gel of Atorvastatin for treatment of diabetic foot ulcer. Thin-film hydration method was used in the formulation liposomes. Firstly, eight batches of liposomes with various ratios of surfactant and cholesterol were made and assessed according to various criteria. The following characteristics of prepared liposomes were extrapolated from the data: vesicle diameter (3.35–4.15), drug content (99.3%), and drug entrapment efficiency (82.2%). After 24 hours, the batch with the surfactant and cholesterol ratio 1:2:2 (B4) had the highest drug release (91.69%) and was chosen to prepare a liposomal gel utilizing HPMC as the gelling agent. The prepared gel was assessed for a number of physicochemical characteristics, with the following results: pH 7.4, spreadability (10.6±0.5), homogeneity (uniformly distributed), clarity (clear), and viscosity (8573cP). It was discovered that the produced gel's medication release percentage in a 24-hour period was 81.19%. The dissolution data was fitted into a number of kinetic models in attempt to determine the drug release mechanism, and it was discovered that the process was of zero order. From the result it is concluded that the prepared liposomal gel may produce transdermal drug delivery, enabling incorporated drugs to get retained for longer time period of time resulting in sustained effects for treatment of diabetic foot ulcer.

Keywords: *Liposomes, gel, atorvastatin, diabetic foot ulcer, transdermal, drug delivery*

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1. Introduction

Diabetes-related foot ulcers (DFUs), are a serious side effect that afflicts a large percentage of people with the disease and frequently result in infections, amputations, and higher death rates. Because diabetes patients' wound healing processes are complicated and impeded by things like poor blood circulation, neuropathy, and compromised immune response, managing DFUs is difficult. Novel therapeutic techniques are necessary, as the efficacy of conventional therapies such as antibiotics and wound dressings has been demonstrated to be limited.[1-3]

The medication atorvastatin, which is well-known for reducing cholesterol, has currently gained interest due to research suggesting that it may also improve wound healing. Beyond decreasing cholesterol, atorvastatin also has anti-inflammatory, antioxidant, and angiogenic actions, which makes it a good option for treating DFU. Because of the medication's capacity to increase vascularization and improve endothelial function, diabetic individuals could experience far better wound-healing results.[4-6]

On the other hand, problems with systemic atorvastatin delivery include side effects and inadequate medication concentrations at the wound site. An innovative formulation strategy that aims to resolve these problems is the incorporation of atorvastatin into a liposomal gel. Liposomes, which are nano-sized vesicles made of phospholipid bilayers, are a perfect drug delivery vehicle because they may increase medication stability and bioavailability, encapsulate both hydrophilic and hydrophobic medicines, and are biocompatible.[7-9]

In order to reduce systemic exposure and maximize therapeutic efficacy, the liposomal gel formulation attempts to deliver drug to the ulcer site in a targeted and sustained manner. With its tailored approach that accelerates wound healing, lowers inflammation, and encourages tissue regeneration, this localized delivery method may be able to overcome the drawbacks of oral or systemic approaches.[10-12]

This research explores the potential of a liposomal gel formulation of atorvastatin for DFU treatment. Liposomes with various ratios of surfactant and cholesterol were prepared and assessed for a number of physicochemical characteristics such as pH, spreadability, homogeneity, clarity and viscosity. Release percentage in 24-hour, dissolution study and accelerated stability study was also determined.

2. Materials and Methods

2.1 Materials

Drug sample and chemical reagents used in the formulation of liposomal gel of Atorvastatin were procured from different reputed companies.

2.2 Experimental work

2.2.1 Preparation of Standard Stock Solutions

2.2.1.1 Preparation of stock solution

To prepare the stock solution, precisely weigh out 10 mg and dissolve it in pH 7.4 phosphate buffer solution. Employ the same solvent to make the final volume up to 100 ml. This solution was then further diluted to yield a drug concentration of 10 µg/ml. To calculate the λ max value (compared to the blank PBS 7.4), a predefined sample concentration was measured using a UV spectrophotometer (200–400 nm). The result was recorded.

2.2.1.2 Estimation of λ max

The aforementioned solutions were produced in several dilutions (4, 8, 12, 16, 20, and 24 µg/ml), and spectra at 247 nm were acquired to check for repeatability and determine the validated λ max. Plotting a graph between concentration and absorbance allowed for the creation of a calibration curve using the same dilutions.[13]

2.2.2 Compatibility Study

2.2.2.1 Thin Layer Chromatography

Acetonitrile:Methanol (9:1; v/v) was used as the mobile phase and silica gel (F254) as the stationary phase to find drug spots and various excipients on TLC plates. At 356 nm, the visible spots were assessed densitometrically, and Rf values were determined using the following formula.[14]

Rf = Distance travelled by solute/ distance travelled by mobile phase x100

2.2.2.2 FTIR Technique

The KBr pallet approach was used to produce FTIR Spectrophotometry. The distinctive peaks obtained with the drug (alone) and those obtained with various combinations of the drug and excipient(s) were compared.[15]

2.2.3 Preparation of Liposomes of Atorvastatin

3.4.1 Ether injection method:

A pre-calculated and accurately weighed amounts of cholesterol and surfactant, in a definite ratio, were dissolved in di-ethyl ether thoroughly. The solution was injected into another solution, containing predetermined concentration of Atorvastatin in pH7.4 phosphate buffer saline. The temperature was maintained in the range of 55-65°C. Finally spontaneous liposomal vesicle formation occurred.[16] Table 1 summarizes the formulation design of Liposomes of Atorvastatin

Table 1: Formulation Design of Liposomes of Atorvastatin

Formulation Code	Drug (mg)	Span80 (mg)	Span60	Cholesterol (mg)	Di-ethyl ether (ml)	Methanol (ml)
A1	50	50	_	50	10	2
A2	50	100	_	50	10	2
A3	50	50	_	100	10	2
A4	50	100	_	100	10	2
B1	50	_	50	50	10	2
B2	50	_	100	50	10	2
B3	50	_	50	100	10	2
B4	50	_	100	100	10	2

2.2.4 Evaluation of Atorvastatin Liposome

2.2.4.1 Optical microscopy

For determination of vesicle size, a drop of prepared Liposomes (diluted with water) was placed on a clean glass slide and observed under optical microscope both under low and high magnifications. Vesicle counts were made and recorded.

2.2.4.2 Scanning Electron Microscopy (SEM)

Samples were examined for scanning Electron Microscope.[17]

2.2.4.3 Determination of vesicle size

Vesicle size of Liposomes was determined by using Zeta sizer (Malvern instrument).

2.2.4.4 Zeta potential

It signified the magnitude of repulsive force operational, between similar charged particles at their vicinity, in the prepared dispersion system. It was determined using Zeta sizer (Malvern instrument).

2.2.4.5 Drug content

It was determined by taking an equivalent amount (60mg) of prepared Liposomes of the drug (Atorvastatin) treated with 100ml of n-propanol solution with mechanical shaking. A unit volume of the solution was further diluted with pH 7.4 PBS solution and absorbance (at 247nm) was recorded. Finally with the help of the calibration curve, drug content of the Liposomal formulation was calculated.[18]

2.2.4.6 Drug entrapment efficiency

Entrapment efficiency was determined by centrifuging the Liposomal dispersion at 3500 rpm for 2hr. Thclear supernatant was separated, filtered and sufficiently diluted with ethanol and absorbance was recorded at respective wavelength by U.V visible spectrophotometer and % drug entrapment was calculated.

2.2.4.7 In-vitro drug release

In-vitro diffusion study was performed by Franz diffusion cell using cellophane membrane previously soaked in mixture of phosphate buffer pH7.4 and ethanol. A section of membrane was cut, measured and placed on receiver compartment. The donor compartment was filled with Liposomal formulation, mixture of ethanol and pH7.4 phosphate buffer (4:6 v/v) was used as receptor medium, maintained at 37ºC and stirred at 300rpm. About 0.5 ml receptor medium was withdrawn and replaced by equal volume of fresh medium at appropriate time interval up to 12 hr. The samples were diluted appropriately and observed spectrophotometrically at respective wavelength.[19] Based on outcomes of various evaluation parameters; the most promising Liposomal batch was selected for preparation of Liposomal gel.

2.2.5 Preparation of Atorvastatin Liposomal gel

The best Liposomal batch thus selected was converted in to Liposomal gel formulation using HPMC. The weighed quantity of polymer powder was sprinkled gently in 70ml boiling distilled water, which sprinkled gently in 70ml boiling distilled water, which was stirred magnetically at high speed until a thin hazy dispersion was formed devoid of lumps. Later on, 10gm glycerine and 1g PG were added as permeation enhances followed by addition of Methyl Parabens and Propyl Parabens as preservatives. Then, Liposomal suspension consisting of drug was added with continuous stirring to get Liposomal gel. Finally total weight was maintained up to 100gm with distilled water.[20] Table 2 summarizes the formulation design of liposomal gel of Atorvastatin

Table 2: Formulation design of Liposomal gel of Atorvastatin

S. No.	Ingredients	Quantity
1	HPMC	1gm
2	Glycerine	10gm
3	PG	1ml
4	Methyl parabens	.5gm

5	Propyl parabens	.5gm
6	Liposomal suspension	18
7	Distilled water	70ml

2.2.6 Evaluation of Atorvastatin Liposomal Gel

The formulated Liposomal gel was evaluated for various parameters such as pH, viscosity, spreadability, in vitro drug release, release kinetic and stability studies.

2.2.6.1 Physical examination

The prepared gel formulations were inspected visually for colour, homogenecity, consistency, grittiness and spreadability.

2.2.6.2 Clarity

Prepared gel was visualized against white and dark background to check the clarity of preparation.

2.2.6.3 Homogenicity

Hompogenicity of prepared Liposomal gel was tested by visual observation.

2.2.6.4 pH Measurements

It was done with digital pH meter.

2.2.6.5 Viscosity Measurements

The viscosity of the gel formulations was determined by Brookfield viscometer (DV1 prime). The gel (25g) was processed in a beaker agitated with dipped spindle, and was measured by rotating the spindle (50 rpm).

2.2.6.6 Spreadability

Spreading ability of the Liposomal gel was determined with the help of spread ability test apparatus consisting of two glass slide. The gel sample (1gm) was placed on the lower slide and the upper one was slided over the sample. It was calculated using the following formula:

$S = m \times L/t$

Where S = spreadability,

m = amount of gel taken on slide,

L= distance covered by the upper slide.

t = time (sec.).[21]

2.2.6.7 *In-vitro* release study

similar method as adopted for in-vitro release study of Liposomal vesicles was adopted for Liposomal gel also.[22]

2.2.6.8 Stability study

The prepared Liposomal gel was kept at non-accelerated (2-8°C) and accelerated (25±2°C) conditions to carry out stability analysis for 90 days.

3. Results and Discussion

3.1 Preformulation studies

3.1.1 Spectrophotometric scan of Atorvastatin

A sample solution of Atorvastatin scanned between 200-400nm which concluded λ max. of 247nm which is shown in figure 1.

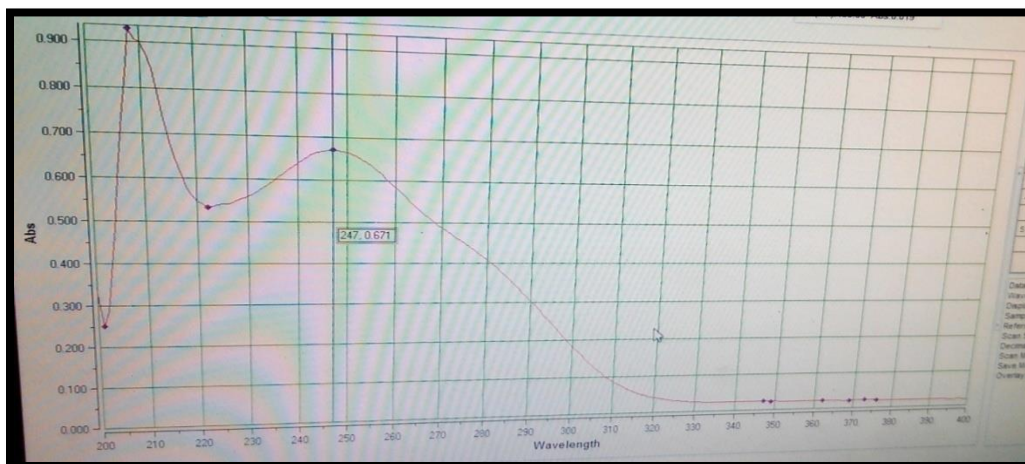

Figure 1: U.V. scan of Atorvastatin showing characteristic wavelength

3.1.2 Validation of max

The samples comprised of different concentration (4-24µg/ml) of the Atorvastatin were run and overlain spectra describing the reproducibility of λ max was obtained, that confirmed and validated the process.

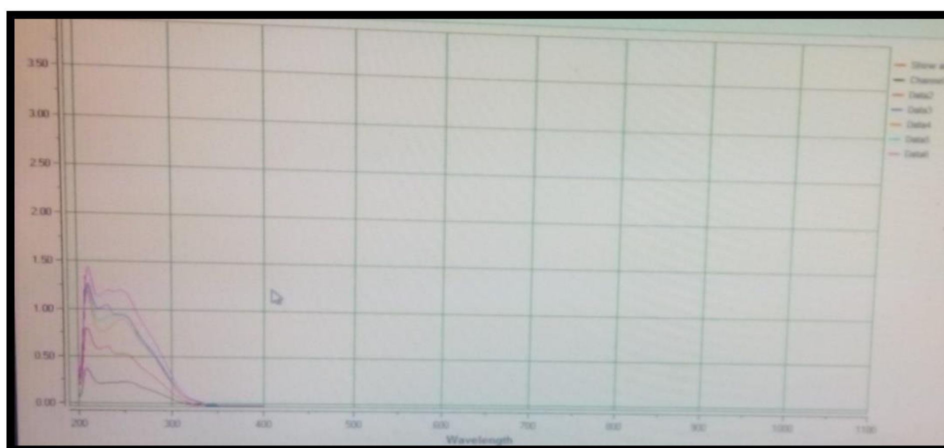

Figure 2: Overlain spectra of Atorvastatin

3.1.3 Preparation of standard curve of Atorvastatin in pH 7.4 phosphate buffer

A standard curve of Atorvastatin was obtained by measuring absorbance of various aliquots at 247nm and plotting the graph [Concentration (µg/ml) vs Absorbance]

Table 3: Concentration vs Absorbance data of Atorvastatin in pH 7.4 phosphate buffer at 247nm

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.00
2	4	0.18
3	8	0.31
4	12	0.49
5	16	0.62
6	20	0.79
7	24	0.94

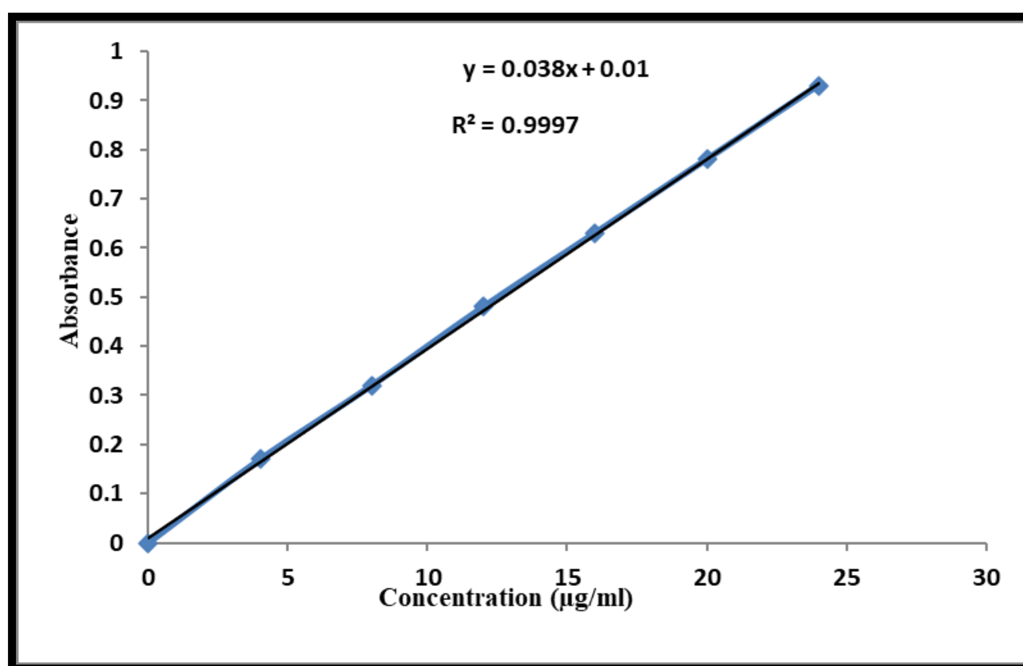

Figure 3: Regression curve of Atorvastatin in pH 7.4 phosphate buffer

3.2 Compatibility studies

3.2.1 Thin Layer Chromatographic method

The RF value of Liposomes of Atorvastatin with various excipients were found nearly similar with that of the pure drug and thus showed compatibility between drug and different excipients used.

Table 4: Rf value of different combination of Drug and Excipients

S. No.	Ingredient	Rf value
1	Drug	0.83
2	Drug + cholesterol	0.79
3	Drug + cholesterol + span60	0.45
4	Drug + cholesterol + span80	0.76

Figure 4: Photographic representation of TLC with different Drug-Polymer combination

3.2.2 FTIR Technique

The FTIR spectral analysis revealed that the characteristic peak of the pure drug was retained in its combination with different excipients (polymers). Conclusively, the drug was found compatible with all other excipients used in the formulations.

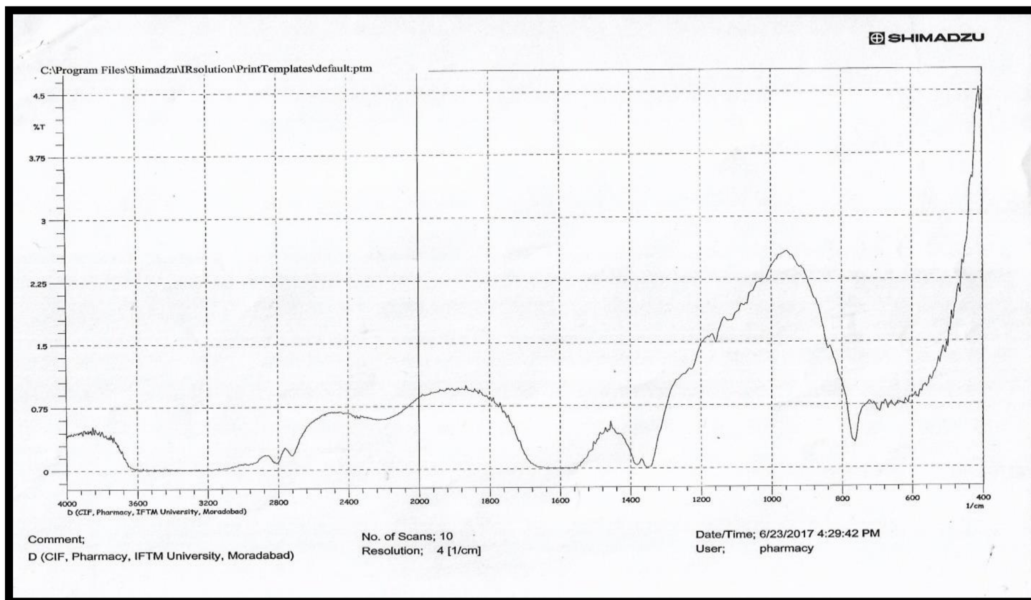

Figure 5: FTIR spectra of Atorvastatin (pure drug)

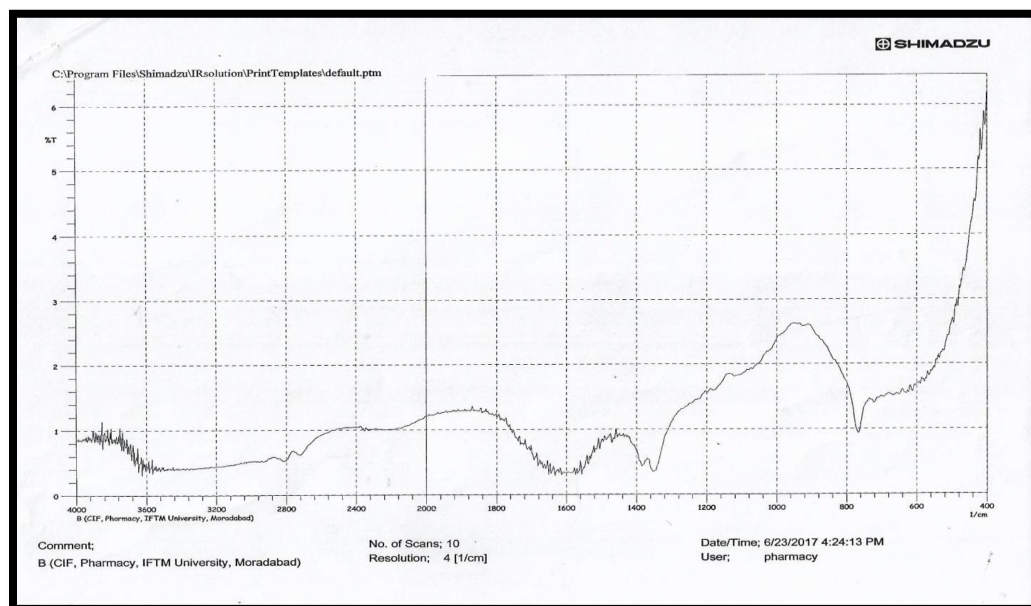

Figure 6: FTIR spectra of Atorvastatin with Surfactant (span60)

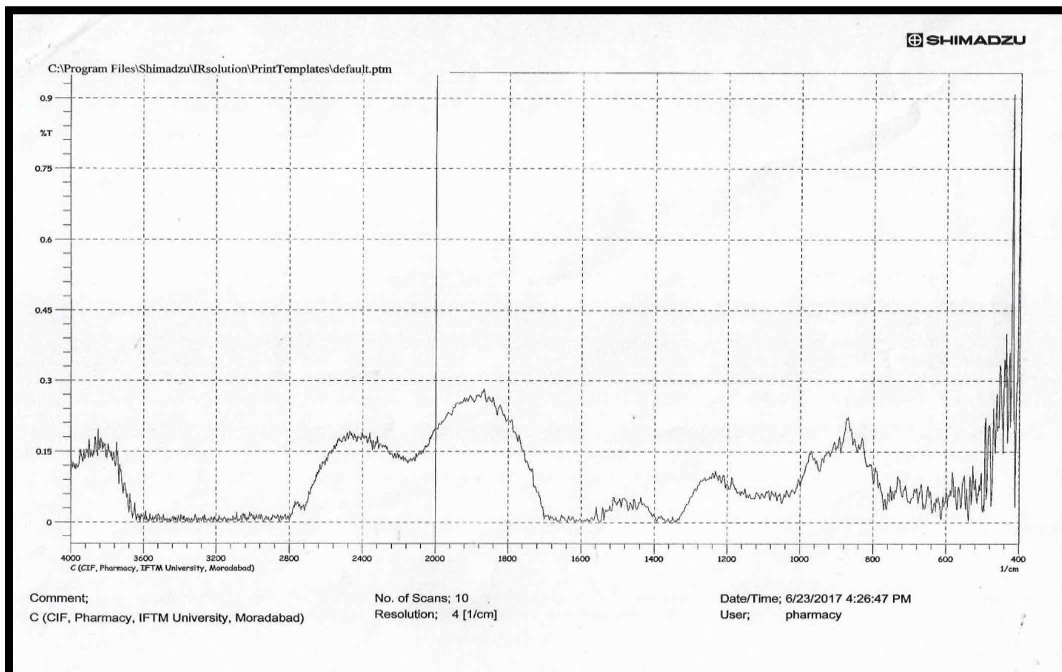

Figure 7: FTIR spectra of Atorvastatin with Hydroxyl Propyl Methyl Cellulose

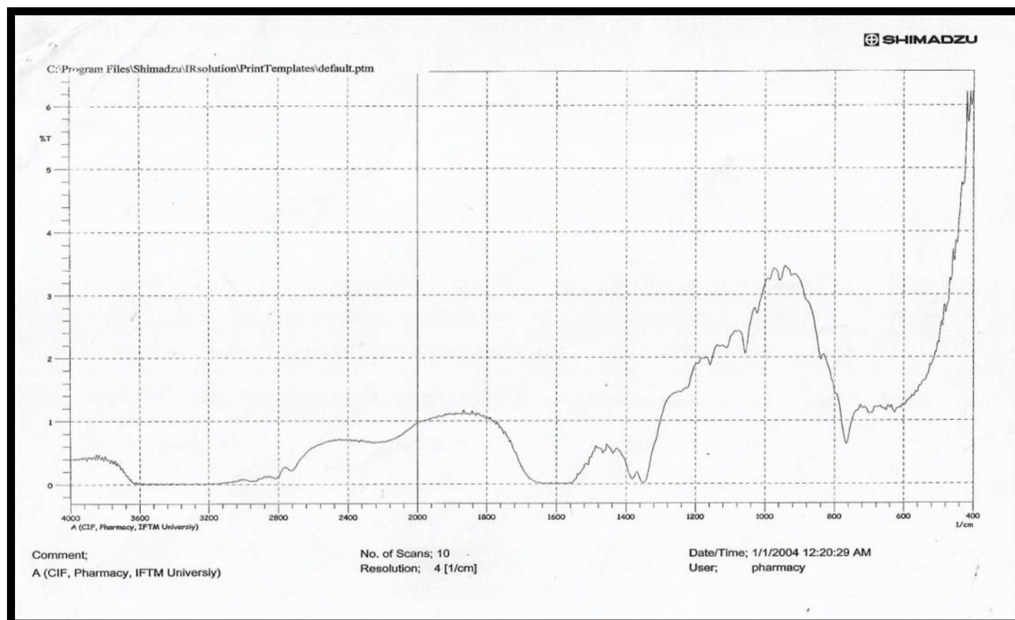

Figure 8: FTIR spectra of Atorvastatin with cholesterol

3.3 Evaluation parameter for Atorvastatin Liposomes

3.3.1 Optical microscopy

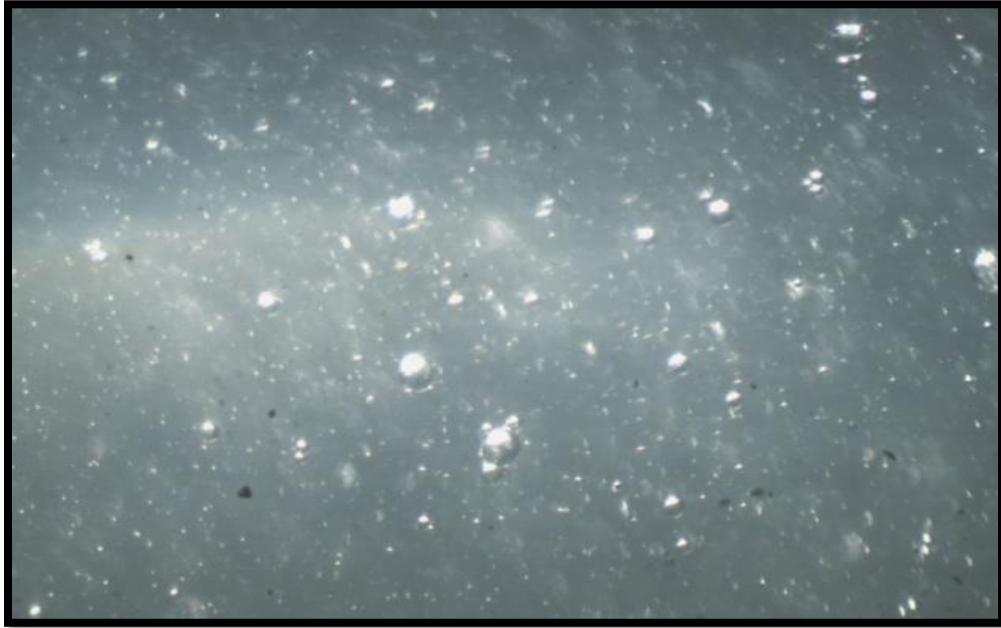

Figure 9: Optical microscopy of Liposomes

3.3.2 Scanning Electron Microscopy (SEM)

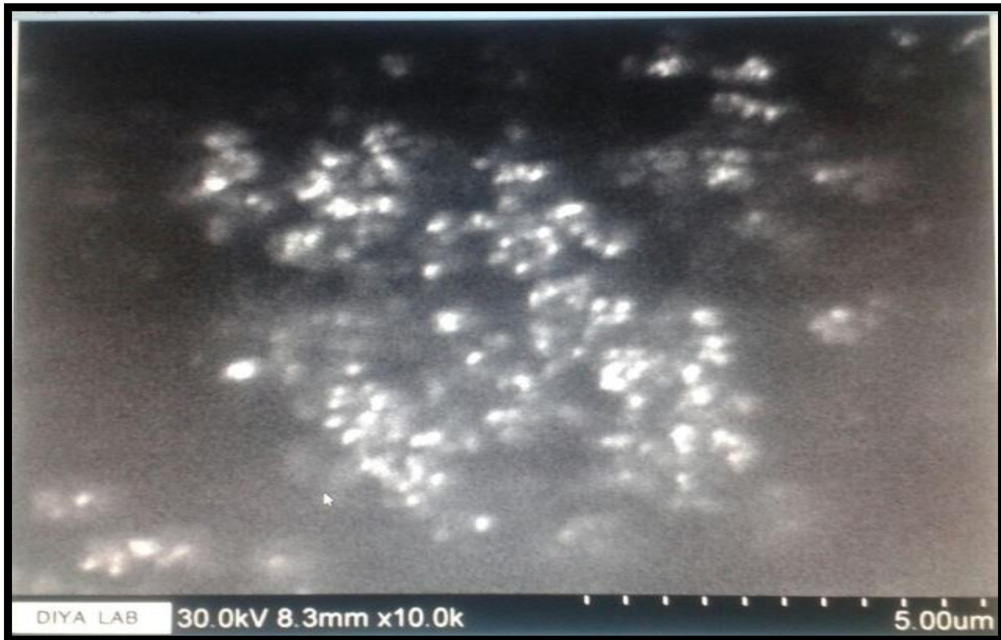

Figure 10: SEM Image of Liposomes obtained with best batch (B4)

3.3.3 Determination of Vesicle Size

Prepared Liposomes were spherical in shape and their size ranged between 3.35- 4.15.

Table 5: Particle size analysis of prepared Liposomes of Atorvastatin (batch A1-B4)

S. No.	Formulation	Mean particle size (µm)
1	A1	3.35
2	A2	3.55
3	A3	3.42
4	A4	3.70
5	B1	3.64
6	B2	3.79
7	B3	3.92
8	B4	4.15

3.3.4 Zeta potential

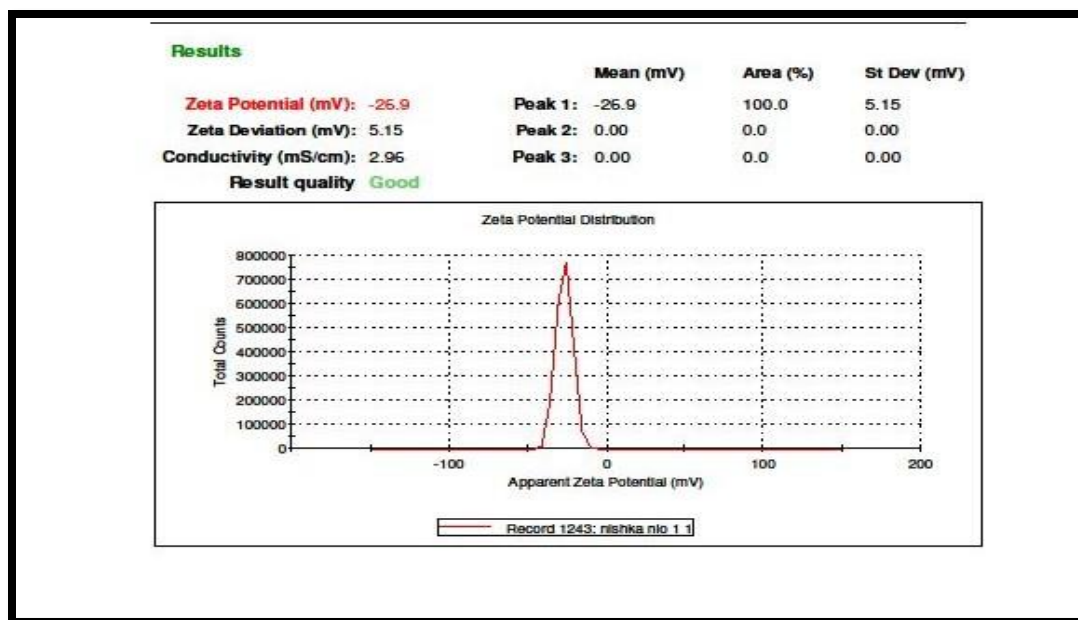

Figure 11: Zeta potential of Liposomes obtained with best batch (B4)

3.3.5 Drug content

The maximum drug content was found to be 99.3% with batch B4 and minimum of batch A1.

Table 6. Drug content of prepared Liposomes of Atorvastatin (batch A1-b4)

S. No.	Formulation code	Drug content (%)
1	A1	95
2	A2	96

3	A3	97
4	A4	98.12
5	B1	96.8
6	B2	97.68
7	B3	98.21
8	B4	99.3

3.3.6 Drug Entrapment Efficiency

The maximum drug entrapment was found to be 82.2% for batch B4 and minimum entrapment of 50.64% obtained with batch A1.

Table 7. Drug Entrapment Efficiency of prepared Liposomes of Atorvastatin (batch A1-B4)

S. No.	Formulation code	% Entrapment efficiency
1	A1	50.64
2	A2	50.12
3	A3	66.15
4	A4	71.9
5	B1	51.55
6	B2	63.82
7	B3	75.54
8	B4	82.2

3.3.7 In- vitro diffusion study of the Atorvastatin Liposome

Table 8. In -vitro diffusion profile of Atorvastatin Liposomes (batch A1-B4) in pH 7.4 phosphate buffer

Time (hrs.)	A1	A2	A3	A4	B1	B2	B3	B4
0	0	0	0	0	0	0	0	0
2	1.33	1.405	1.97	1.53	2.71	3.11	4.05	6.15
4	3.434	3.93	4.95	4.86	6.55	8.88	10.57	12.6
6	8.171	9.16	9.77	9.87	11.97	13.7	15.06	18.82
8	13.94	14.53	14.96	15.70	19.11	21.13	23.46	23.69
10	18.353	19.17	19.88	20.08	23.54	25.63	27.88	29.52
12	25.58	22.82	22.99	27.49	29.5	31.03	33.02	35.66
14	34.74	34.76	36.18	38.11	40.70	42.86	44.85	46.26

16	41.94	42.65	42.84	42.51	44.68	47.44	49.64	52.98
18	55.52	56.63	56.88	58.94	60.99	62.46	64.52	66.84
20	64.378	65.813	64.11	66.09	68.57	70	72.53	74.16
22	75.68	76.54	77.68	78.97	81.96	83.03	85.15	87.20
24	77.295	79.89	81.28	83.56	85.2	87.92	89.93	91.69

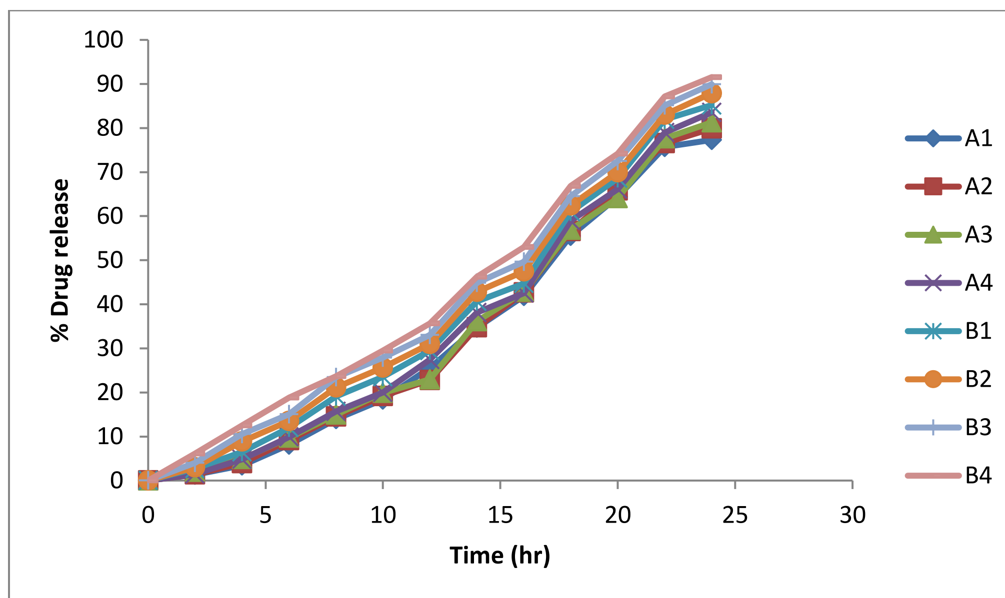

Figure 12: Percentage Drug release profile of Liposomes of Atorvastatin (Batch A1-B4) in pH 7.4 phosphate buffer

3.3.8 Evaluation parameters for best batch (B4)

Table 9. Evaluation parameters for best batch (B4)

S. No.	Parameter	Results
1	Vesicle diameter	4.15
2	Drug content	99.3
3	Dug entrapment efficiency	82.2
4	In-vitro diffusion study	91.69
5	Release kinetic model	Zero-order
6	Surface morphology	Almost spherical in shape

3.4 Evaluation of Liposomal gel of Atorvastatin

Liposomal gel of Atorvastatin thus prepared were subjected to various evaluation parameters such as; clarity, homogeneity, spreadability, extrudability, pH, viscosity, and in-vitro diffusion study

which revealed that gel prepared with HPMC showed good results and considered as best Liposomal gel. % drug release from this formulation was found to be 81.19%.

Table 10. Evaluation parameters for Liposomal gel of Atorvastatin

Formulation	Clarity	Homogeneity	pH	Viscosity (cPs)	Spreadability	In vitro release study
B4	Clear	Excellent	7.4	8573	10.6±0.5	81.19%

3.4.1 In-vitro diffusion study of the Atorvastatin Liposomal gel

Table 11. Percentage Drug release profile of Liposomal gel of Atorvastatin in pH 7.4 phosphate buffer

S. No.	Time (hrs.)	%Drug release of optimized batch B4
1	0	0
2	2	5.97
3	4	10.51
4	6	17.74
5	8	21.83
6	10	26.54
7	12	31.55
8	14	42.57
9	16	50.97
10	18	62.44
11	20	69.522
12	22	75.653
13	24	81.19

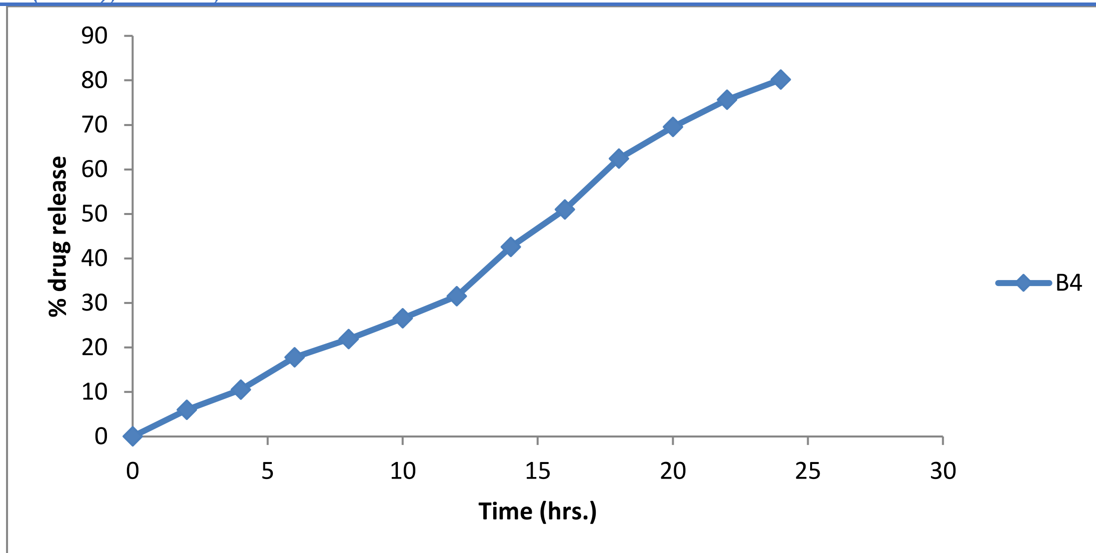

Figure 14: Percentage drug release profile of Liposomal gel (B4) in pH 7.4 phosphate buffer

3.4.2 Release kinetic studies

In-vitro release data of best batch was fitted into various kinetic models like Zero order, First order, Higuchi, Korsmeyer-peppas models in order to find out the mechanism of drug release from Liposomes.

Table 12: Estimated value of R^2 after fitting of dissolution data of best batch (B4) into various release kinetic models in pH 7.4 phosphate buffer

Formulation code	**Zero order**		**First order**		**Higuchi**		**Korsemeyer-peppas**	
pH 7.4 phosphate buffer								
B4	Y	R^2	Y	R^2	Y	R^2	Y	R^2
	0.232	0.99	0.022	0.58	1.292	0.92	0.65	0.97

Figure 16: Zero order drug release of Liposomal gel obtained with optimized batch (B4) in pH 7.4 phosphate buffer

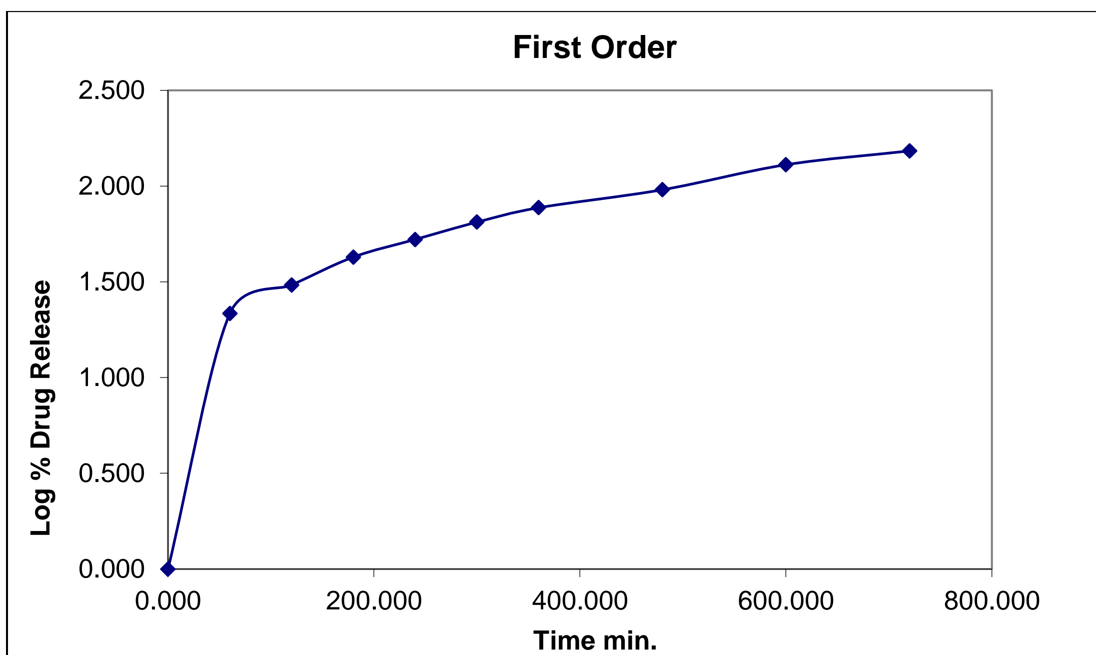

Figure 17: First order drug release of Liposomal gel obtained with optimized batch (B4) in pH 7.4

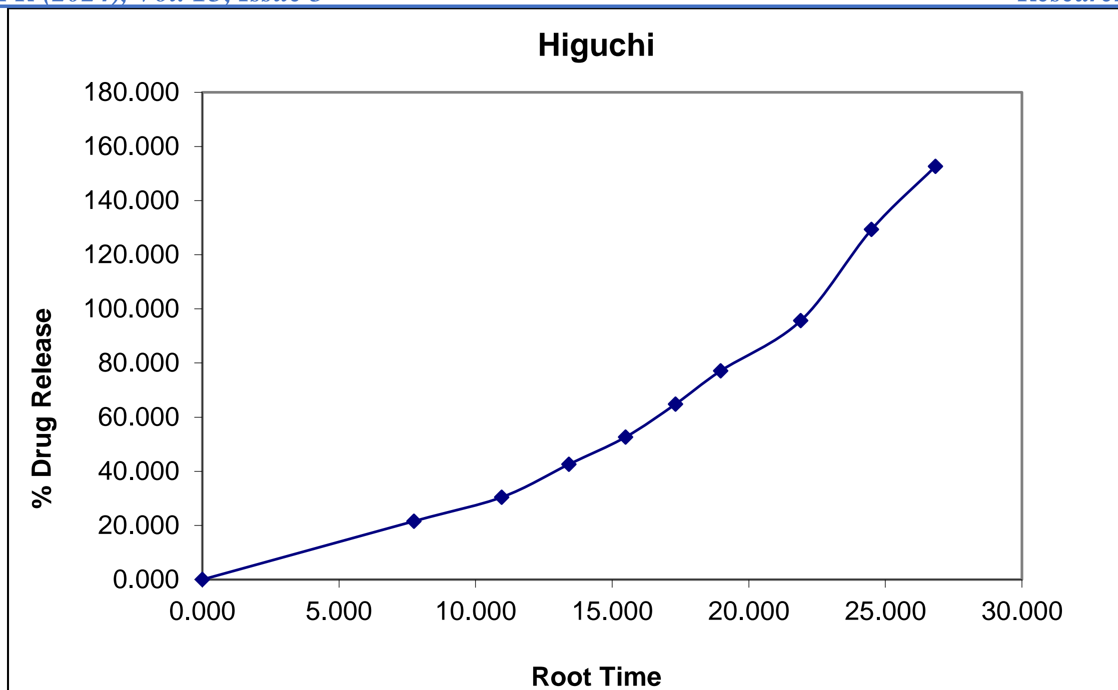

Figure 18: Higuchi drug release of Liposomal gel obtained with optimized batch B4 in pH 7.4 phosphate buffer

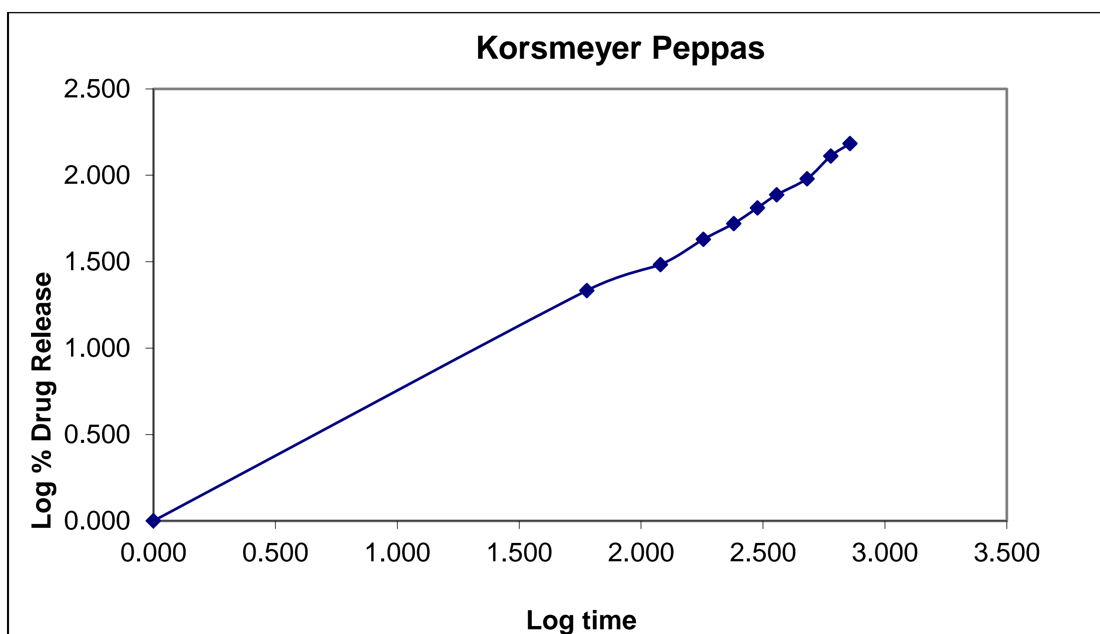

Figure 19: Korsmeyer peppas drug release of Liposomal gel obtained with optimized batch (B4) in pH 7.4 phosphate buffer

4. Conclusion

The present research work aiming at increasing the residence time of drug at the site of application and prevent severe gastrointestinal side effects associated with its oral administration. The selected drug (Atorvastatin), studied for physicochemical characteristics, was run on U.V-visible spectrophotometer (in different concentrations) and wavelength maxima was recorded which was further confirmed from overlain spectra thus obtained, Standard calibration curve was also prepared using pH 7.4 phosphate buffer. FTIR & densitometry TLC studies were carried out for testing the compatibility of the drug with selected excipients. The characteristic peaks of the pure drug were compared with that obtained with the drug-excipient combination, which remained nearly same. The thin layer chromatographs of pure drug and that of the Drug-Excipients combination, resulted approximately equivalent Rf values. Conclusively, Atorvastatin was found to be compatible with excipient used in gel formulations. Initially, eight batches of Liposomes containing different surfactant and cholesterol ratios were prepared and evaluated for different parameters. Prepared Liposomes were characterized for various parameters and the results inferred were: vesicle diameter (3.35-4.15), drug content (99.3%) and drug entrapment efficiency (82.2%). The (B4) batch showed maximum drug release (91.69%) at the end of 24 hrs. Prepared gel was evaluated for various physicochemical parameters and results were: clarity (clear), Homogeneity (uniformly dispersed), Spreadability (10.6±0.5), pH 7.4 and viscosity (8573cP). The percentage drug release from prepared gel was found to be 81.19% in 24 hrs. The dissolution data was fitted into various kinetic models to assess the mechanism of drug release which indicated zero order.

5. Conflict of interest

The authors have no conflict of interest.

6. Acknowledgement

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