Formulation of Niosome using simvastatin drug to increase bioavailability and release

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Abstract: Niosomes are a novel drug delivery system, where the medication is encapsulated in a vesicle, composed of a bilayer of non-ionic surface active agents, also call it as niosomes. Simvastatin is derived synthetically from fermentation products of *Aspergillus terreus*. It is used to treat hyperlipidaemia. Simvastatin when hydrolysed produces beta, delta, dihydroxy acid which is similar to HMG – CoA (Hydroxylmethyl glutaryl CoA) in structure. So hydrolysed simvastatin competes with HMG – CoA for HMG – CoA reductase. Simvastatin niosome to get better in vitro release and *in-vivo* better bioavailability. From the result of the experiment, it may be concluded that formulation F2 containing 2:1 (Span 60: Cholesterol) was found to be high % of entrapment efficiency and desired sustained release of simvastatin. The *in-vivo* study value it was found that the bioavailability of simvastatin niosome was greater than the plain simvastatin drug due to the decrease in particle size. Thus, we conclude that the niosomal drug delivery system of simvastatin is a simple and effective approach to produce nano particles of poorly water soluble drug to enhance the solubility and bioavailability.

Keywords: Niosomes, Simvastatin, Nano Particles, Drug Delivery.

Introduction

The novel drug delivery system is a most and approachable delivery system, which provides better therapeutic efficacy, controlled and sustained drug release of new as well as pre-existing drug [1]. Noisome are the vesicular systems which can enhance the bioavailability of the drug thus encapsulated drug provide therapeutic action in a controlled manner for a long period of time [2]. Niosome (non-lipoidal modified release system) also increase the pharmaceutical profile of API, also improves the patient compliance as well as reduce side effects of drug [3]. Niosomes composed of the bilayer of non- ionic surface active agent, in which medicament is encapsulated thus noisome act as a depot from which drug release in a controlled manner. The therapeutic activity of drug may improve [4, 5]. Delayed clearance of drug molecule from blood circulation. The bilayer protects the drug from gastric or biological or enzymatic environment. Niosome also restricting effect to target cells. Structurally noisome are similar to the liposome but in the case of noisome the bilayer is made up of non – ionic surfactant rather than the phospholipids [6]. Niosomes are lamellar in structure. They may be unilamellar or multilamellar

[7, 8]. Simvastatin is derived synthetically from fermentation products of *Aspergillus terreus* [9]. It is used to treat hyperlipidaemia. Simvastatin when hydrolyzed produces beta, delta,dihydroxy acid which is similar to HMG – CoA (Hydroxylmethyl glutaryl CoA) in structure. So hydrolyzed simvastatin competes with HMG – CoA for HMG – CoA reductase. Thus the quantity of mevalonic acid reduces which is precursor of cholesterol due to interference with this enzyme and level of cholesterol decreases [10]. Being a BCS class II drug simvastatin shows poor water solubility. First pass metabolism is a major problem which results in low bioavailability (5%) [11]. The biological half-life is 3 hrs so dose repetition required which may causes various adverse effects such as gastrointestinal complaints, fatigue headache and rash. Simvastatin may cause minor increase in serum creatine phosphokinase, which may be associated with myopathy [12]. On the other hand all the formulation of simvastatin are available in immediate release drug delivery system .this system shows instant drug release due to rapid disintegration. These formulations fluctuate the drug plasma level due to fast increase and decrease in dissolution which results loss of effect of drug and increase in effects [13].

2 MATERIALS AND METHODS

2.1 Materials

Simvastatin was obtained from Sun pharmaceuticals, Gurgaon. Cholesterol span 60 and span 20 were purchased from Sdfcl, fine chemical ltd. Mumbai. All other reagents and solvents used were of analytical grade. In house distilled water was used throughout the experiment.

Animal. The *in vivo* study was performed according to the protocol approved by the Institutional Animal Ethics Committee. Albino Wistar rats of either sex weighing 150-250 g were used for this study. Animal were kept in polypropylene cages under standard conditions of temperature (25°±1°C) with 12 h light and 12 h dark with a free access to a commercial pellet diet and water ad libitum. All the rats were transferred to the laboratory one week before the experiment to acclimatize.

2.2 Methods

The niosome of simvastatin were prepared by using hand shaking method. In this method, mixture of vesicles forming ingredients like surfactant and cholesterol were dissolved in a volatile organic solvent (diethyl either, chloroform or methanol) in a round bottom flask. The organic solvent was removed at room temperature (20°C) using rotary vacuum evaporator, with deposition of a thin layer of solid mixture on the wall of the flask. The dried surfactant film rehydrated with aqueous phase at 0-60°C with gentle agitation. This process formed typical multilamellar niosomes.

2.3 Characterisation of Simvastatin niosomes

2.3.1 Particle size, polydispersity index (PDI) and zeta potential

The simvastatin niosomes were characterized for morphology viz. particle size, zeta potential and PDI. The average particle size and PDI of niosomeses were evaluated by photon correlation spectroscopy zeta sizer nanoplus-3 (Japan). All the samples were suitably diluted and analysed using 1ml cuvette in a thermostatic chamber at 25^0C using a He-Ne laser.

2.3.2 Entrapment efficiency

Entrapment efficiency of niosomes was determined by centrifugation method**.** According to this method, 1.0 ml of niosomal formulation was taken and centrifuged at rpm. The supernatant was collected and diluted with 7.4 pH buffer. Then absorbance was taken at 234 nm and amount of unentraped drug was calculated. After that the percent of entrapment efficiency (EE %) was calculated by using following formula.

%EE = [ED/TD] X 100

The EE% is the percent entrapment efficiency, ED is the amount of entrapped drug.

2.3.3 Surface Morphology TEM (Transmission electron microscopy)

To observe surface morphology CHNP were subjected to TEM (Hitachi H 7500). Samples were prepared by pouring a drop of previously diluted CHNP to laminate over 400 mesh copper grid, left to adhere on carbon substrate for about 1 min. Excess sample was removed by filter paper. Sample was air dried and assessed under 100 KV voltage and 2000 magnification over TEM (Hitachi H 7500).

***2.3.4 In vitro* release profile**

In-vitro release studies of niosomal formulation and API of simvastatin (0.25% CMC) through dialysis membrane were investigated using dissolution apparatus. Niosomal formulation (1ml) and API were placed in donor compartment of K.C cell and the acceptor compartment was filled with 0.1N HCL, pH 6.8 and pH 7.4 buffer. The temperature of receptor medium was maintained at 37± 5 ºC and agitated at 50 rpm speed using magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal, same volume of medium was replaced. The collected samples were analysed at 234 nm in Double beam UV-VIS spectrophotometer.

2.3.5 Drug release kinetics

The release kinetic was calculated with the help of zero order ($Q_t = K_0 .t$), first order $Q_t = Q_0 (1- e^{-k_1 t})$, Higuchi matrix model $Q_t = K_H . \sqrt{t}$ and Korsmeyer-peppas model $M_t/M_\infty = K.t^n$.

***2.3.6 In-vivo* study**

2.3.6.1 Animals and husbandry

The protocol for animal testing was approved by AEC No. (AEC/PHARM/1601/06/2016/R2). Albino Wistar rats of either sex weighing 150-250 g were used for this study. Animal were kept in polypropylene cages under

standard conditions of temperature (25°±1°C) with 12 h light and 12 h dark with a free access to a commercial pellet diet and water ad libitum. All the rats were transferred to the laboratory one week before the experiment to acclimatize.

2.3.6.2 Pharmacokinetic study

Procedure

18 Albino Wistar rats of either sex having weight of around 100-250 gm were housed in the laboratory with free access to food and water for 15 days. Animals were then kept on overnight fasting which were divided into three groups containg 6 animal each viz, A, B and C. The A group was treated with suspension of simvastatin in 0.25% of CMC solution orally. The B group was treated with formulation of niosome F2 (20 mg/kg) orally .The C group was treated with normal saline. Simultaneously the blood samples from A & B group were withdrawn by retro orbital venous plexus puncture at 5min, 15min, 30min, 1hr, 2hr, 4hr, 6hr, 16hr, 24hr, after oral administration of drug and formulation. All the samples were centrifuged at 10000 rpm for 15 min, serum was collected & stored at -20 °C for further analysis.

Sample preparation for standard curve

Firstly stock solution of 1000 µg/ml (5 mg in 5 ml ACN) of simvastatin was prepared. 100 µl solution was taken from the stock & diluted upto 1000 µl to obtain 100 µg/ml solution followed by the serial dilution (50, 10, 5, 2, 1, .5, and .1 µg/ml). 100 µl serum was added in each Eppendorf. After a thorough vortex mixing for 30 min, mixtures were centrifuged at 10000 rpm for 15 min. The supernatants were taken & dried at 60 ºC. Later the tubes were reconstituted with 100 µl ACN, vortex for 20 min. 20 µl sample was injected for HPLC analysis.

Sample preparation of API and formulation

100 µl ACN was added in each Eppendorf containing 100 µl serum (collected at different time interval from group A & B after oral administration of API & formulation F2). The samples were vortexed for 30 min, centrifuged at 10000 rpm for 5 min & dried at 60 ºC. The dried samples were further reconstituted with 100 µl ACN, vortexed for 20 min. Finally 20 µl sample was injected for HPLC analysis.

HPLC analysis for simvastatin

The HPLC method was used to determine the content of simvastatin & evaluate pharmacokinetic study of the formulation. The chromatographic system consist of Shimadzu 20 A HPLC, Tokyo, Japan equipped with Lichrospere reverse phase C18 analytical column. The temperature was maintained at 37 ºC. The flow rate was also maintained at 1 ml/min. The mobile phase consisted solvent A (acetonitrile) and solvent B (water). The HPLC was run through Empower software version 2 and absorbance was measured at 230 nm wavelength. The final data was calculated using Winnonlin version 1.5.3 software. The calibration curve was plotted between concentration and area of peak of individual concentration.

2.3.7 Stability study

The stability of a product may be defined as the extent, to which a product retains, within specified limits, throughout period of storage and use, the same properties and characteristics possessed at the time of its packing. The Characteristics include physical, chemical, microbiological, therapeutic and toxic properties and all the required to remains within acceptable limits till the use of the product by a patient.

The purpose of stability testing is to provide evidence on how the quality of an active substance or pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light.

2.3.7.1 Stability study of F2 formulation

The optimized batch that is F2 was stored in screw capped vials were kept at refrigerator (4 ± 1^0C), and room temperature (25 ± 1^0C). These studies were performed for the period of 3 month. The particle size and % drug content is appropriate mean to evaluate stability of formulations.

3. RESULT AND DISCUSSION

3.1 Pre-formulation study

The physical appearance and melting point of the drug Simvastatin sample under investigation was found to be same as that of the official reports. UV- VIS estimation of simvastatin was done by UV- VIS spectrophotometer. The calibration curve was prepared in methanol. The data was regressed to obtain a straight line. The R^2 value was found to be 0.996 in methanol indicating good linearity. The calibration curve was found to obey Beer-Lambert's law in the concentration range studied. The solubility of simvastatin was determined in aqueous and organic solvents. It was found to be freely soluble in methanol, sparingly soluble in Phosphate buffer of pH 7.4, pH 6.8 and 1.2N HCl, insoluble in water.

S. No.	Solvent	Solubility
1	Methanol	Freely Soluble
2	Phosphate buffer (pH 7.4)	Sparingly Soluble
3	Phosphate buffer (pH 6.8)	Sparingly Soluble
4	1.2 N HCl	Sparingly Soluble
5	Water	Insoluble

Table 1.1 Solubility of simvastatin in different solvent system

3.2 Particle size, and polydispersity index

The particle size and poly dispersity index of the optimized formulation (F2) was found to be 225.40 nm and 0.153 respectively. The particle size are presented in table 1.2

S.No.	Formulation Code	Span60/span80:Cholesterol	Size (nm)	PDI
1	F1	Span 60 (1:1)	559.2	0.287
2	**F2**	**Span 60 (2:1)**	**225.40**	**0.153**
3	F3	Span 60 (3:1)	582.3	0.369
4	F4	Span 60 (1.3)	375.7	0.286
5	F5	Span 80 (1:1)	472.7	0.290
6	F6	Span 80 (2:1)	317.6	0.239
7	F7	Span 80 (3:1)	302.2	0.215
8	F8	Span 80 (1:3)	261.4	0.167

Table 1.2 Particle size and polydispersity index of niosomes

3.3 Entrapment efficiency

The entrapment efficiency of optimised formulation F2 was found to be 85.72**.**

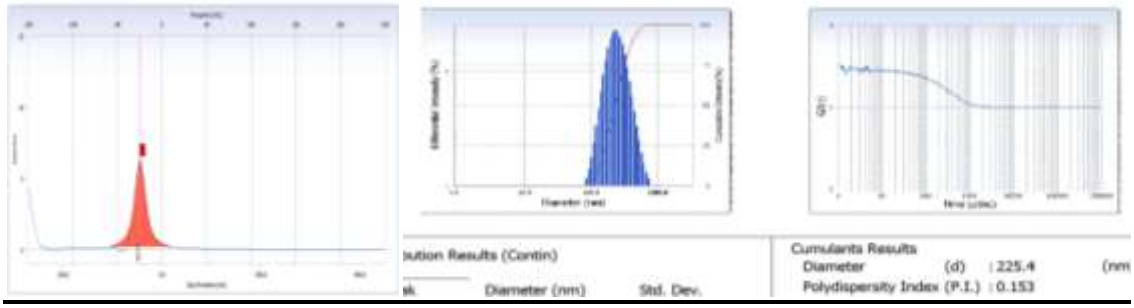
S. No.	Formulation Code	% Entrapment efficiency*
1	F1	71.17±2.91
2	**F2**	**85.72±2.10**
3	F3	77.69±2.05
4	F4	73.67±3.73
5	F5	73.93±4.31
6	F6	74.23±1.72
7	F7	81.44±3.83
8	F8	75.57±1.19

*Values expressed are mean ± SD where n=3

Table 1.3 % Entrapment efficiency of niosomes

3.4 Zeta potential, particle size and shape of F2

The zeta potential of optimized formulation was found to be 43.71. F2 formulation revealed absolute ZP values higher than 30 mV indicating long-term physically stable system.



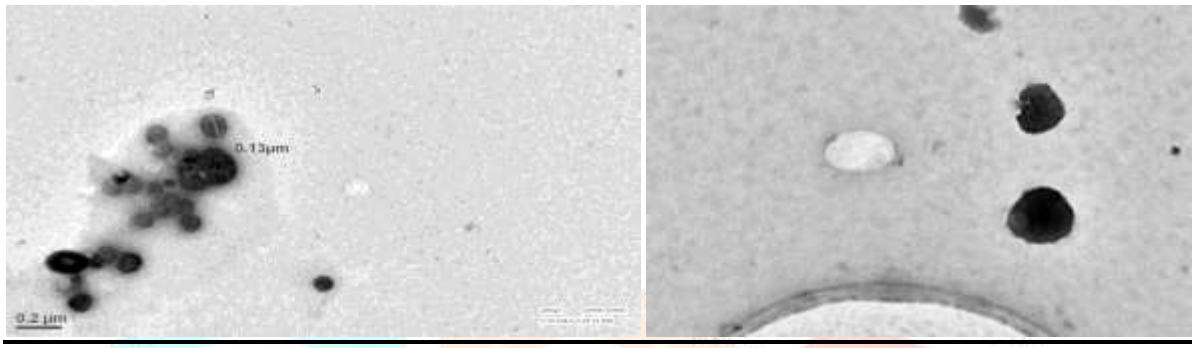


Fig. 1.1 Zeta potential, particle size and shape of F2

3.5 IN - *vitro* release

3.5.1 % Cumulative release of niosomal formulations at pH 6.8 buffer

In- vitro release of niosomal formulatios in pH 6.8 buffer are shown in Fig 1.2. The in- vitro release of F2 formulation were found to be 14.05± 4.39 at initial 15 min and 90.74±4.22 at 24 hr, which is better than the other formulation so F2 formulation considered as optimized formulation on the basis of better particle size, entrapment efficiency, and in vitro-release.

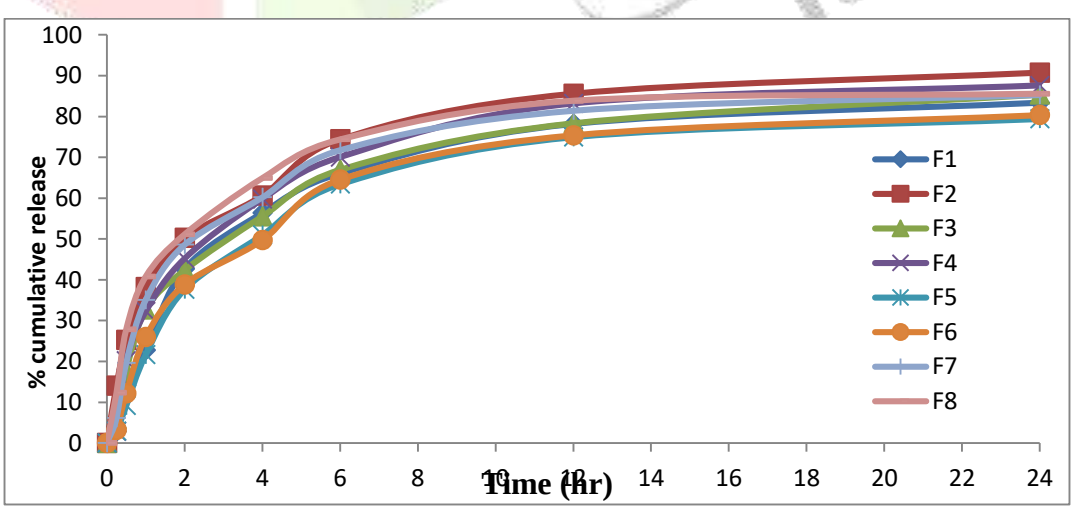


Fig. 1.2 % Cumulative release of niosome formulations at pH 6.8

3.5.2 % Cumulative release of F2 and API of simvastatin at pH 7.4

Dissolution profiles of the pure simvastatin powder, niosome formulation (F2) at pH 7.4 are shown in Fig.1.3. In pH 7.4, the percent drug dissolved of the formulation F2 (19.68±4.22 %) increased by 9 folds as compared to that of the simvastatin powder (2.23 ± 1.66%) at the first 15 min of dissolution.. Formulation F2 showed 93.23±3.64 % release within 24 hr which is better than the release of API i.e 48.76±7.59%.

S. NO.	Time (hr)	% cumulative release* of F2	% cumulative release* of API
1	0	0	0
2	0.25	19.68±4.22	2.23±1.66
3	0.5	30.43±8.43	10.42±5.04
4	1	42.83±7.67	12.22±5.97
5	2	54.40±3.09	16.25±2.99
6	4	64.33±4.22	28.33±4.61
7	6	77.56±1.17	33.12±7.22
8	12	88.30±3.51	38.54±8.65
9	24	93.27±4.05	48.76±7.59

*Values expressed are mean ± SD where n=3

Table 1.3% Cumulative release of F2 and API of simvastatin at pH 7.4

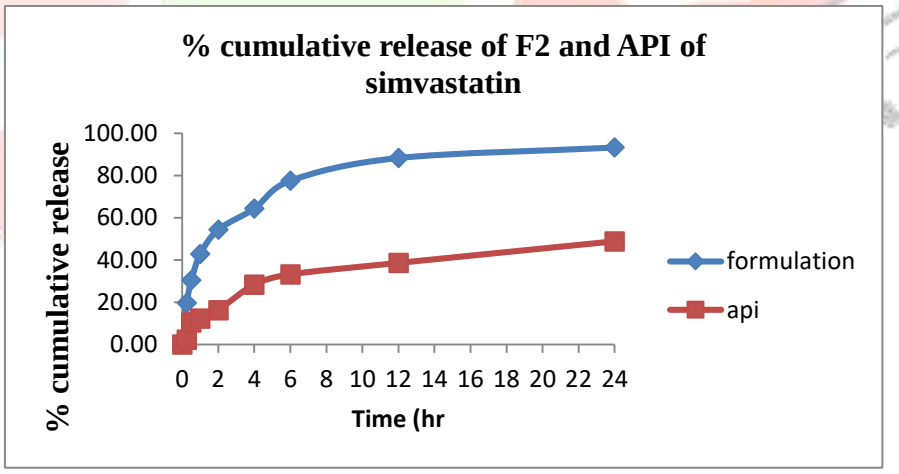


Fig. 1.3 Cumulative release of F2 and API of simvastatin at pH 7.4

3.5.4 % Cumulative release of F2 and API of simvastatin at 1.2N HCl

Dissolution profiles of the pure simvastatin powder, niosome formulation (F2) in 1.2N HCl buffer are shown in Fig.1.4. In 1.2N HCl, the percent drug dissolved of the formulation F2 (1.78±2.30 %) decreased as compared to the simvastatin powder (8.44 ± 1.57%) at the first 15 min of dissolution. Formulation F2 showed 78.23±3.64 % release within 24 hr which is better than the release of API i.e 50.78±2.58 %.

S. No.	Time (hr)	% cumulative release* of F2	% cumulative release* of API
1	0	0	0
2	0.25	1.78±2.30	8.44±1.57
3	0.5	7.37±6.31	12.15±4.47
4	1	24.89±2.67	30.69±1.89
5	2	41.89±5.08	45.87±3.60
6	4	56.79±5.47	47.47±3.88
7	6	69.34±1.92	50.02±1.36
8	12	72.22±0.37	50.19±0.26
9	24	78.23±3.64	50.78±2.58

*Values expressed are mean ± SD where n=3

Table 1.4 % Cumulative release of F2 and API of simvastatin at 1.2N HCl

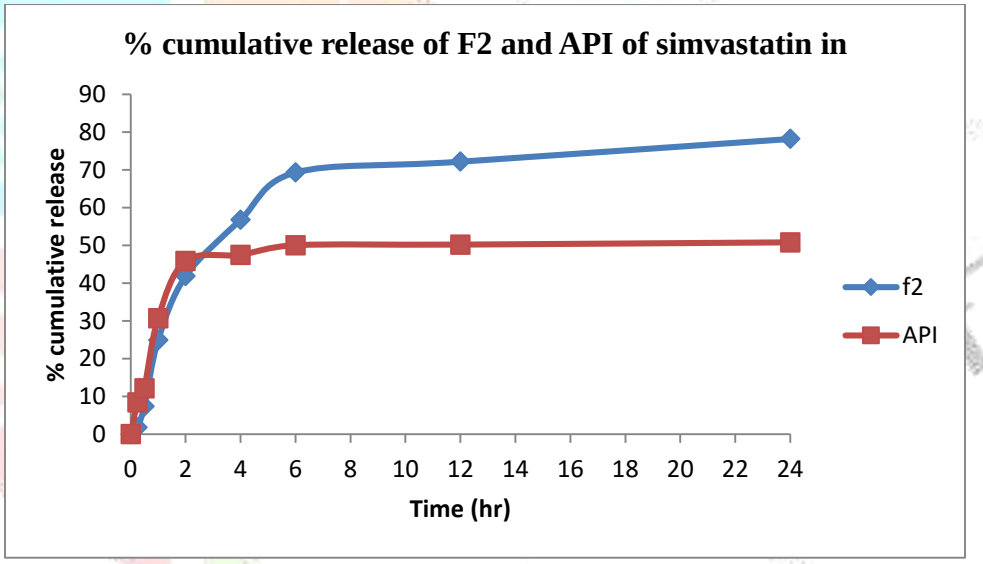


Fig. 1.4 % Cumulative release of F2 and API of simvastatin at 1.2N HCl

3.5.4 % Cumulative release of F2 and API of simvastatin at pH 6.8 buffer

Dissolution profiles of the pure simvastatin powder, niosome formulation (F2) at pH 6.8 buffer are shown in Fig. 1.5. In pH 6.8, the percent drug dissolved of the formulation F2 (14.05±4.39%) increased as compared to the simvastatin powder (9.95±3.11%) at the first 15 min of dissolution. Formulation F2 showed 90.74±4.22 % release within 24 hr which is better than the release of API i.e 64.27±2.99%.

S. No.	Time (hr)	% Cumulative release* of F2	% Cumulative release* of API
1	0	0	0
2	0.25	14.05±4.39	9.95±3.11

3	0.5	25.25±8.79	29.04±6.22
4	1	38.18±7.99	42.99±5.66
5	2	50.24±3.22	48.19±2.28
6	4	60.58±4.39	62.61±3.11
7	6	74.37±1.22	63.24±0.86
8	12	85.57±3.66	63.44±2.59
9	24	90.74±4.22	64.27±2.99

*Values expressed are mean ± SD where n=3

Table 1.5 % Cumulative release of F2 and API of simvastatin at pH 6.8

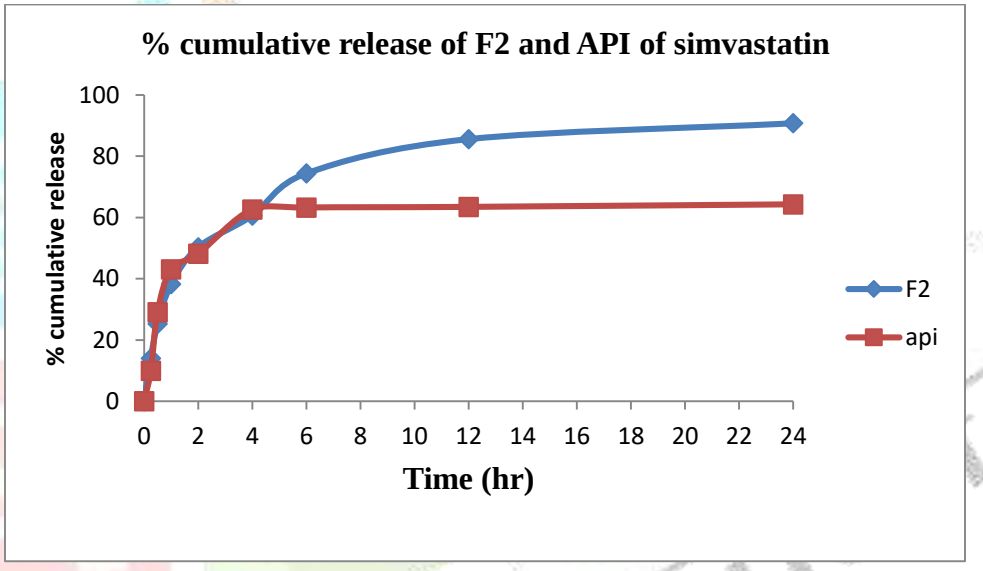


Fig. 1.5% Cumulative release of F2 and API of simvastatin at pH 6.8

3.6 Drug release kinetics

It is well documented that drug release from carrier shows a typical time-dependent profile (i.e. increased drug release with time because of increased diffusion path length). The release mechanism of simvastatin from optimized formulation (F2) was determined by comparing their respective correlation coefficient. The release pattern was fit into korsmeyer-peppas model and r^2 value was found to be 0.9288. The n value was found to be 0.398 which shows that the release followed Fickian diffusion.

Zero Order		**First Order**		**Higuchi Model**		**Korsmeyer-peppas**	
R^2	**K_0**	**R^2**	**K_1**	**R^2**	**K_H**	**R^2**	**n**
0.676	3.886	0.4906	0.1890	0.871	9.657	0.9288	0.3983

Table 1.6 Release kinetics

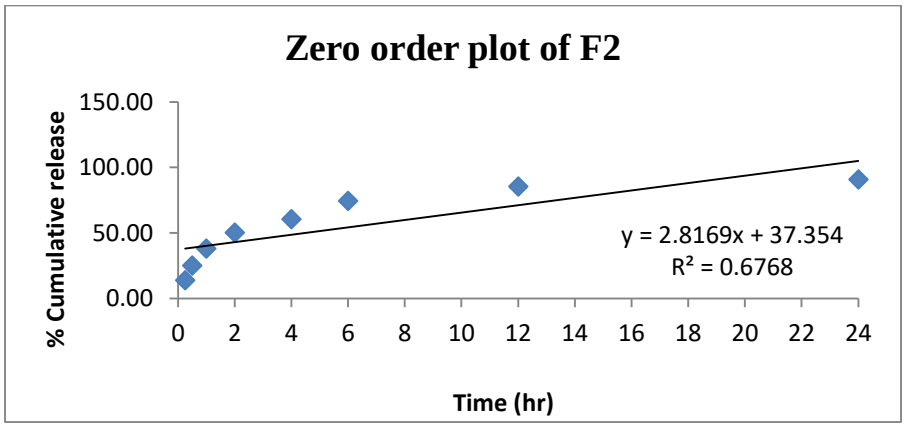


Fig. 1.6 Zero order plot of F2

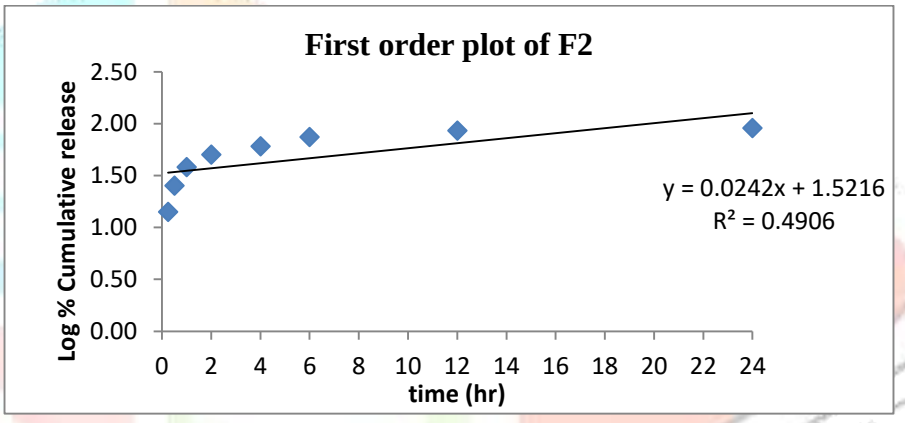


Fig. 1.7 First order plot of F2

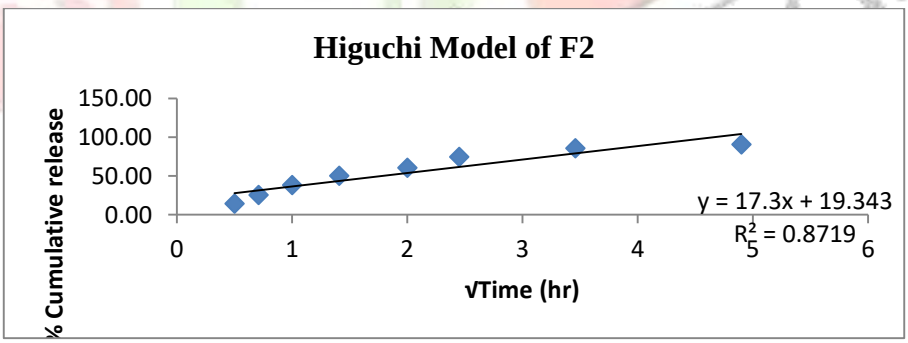


Fig. 1.8 Higuchi model of F2

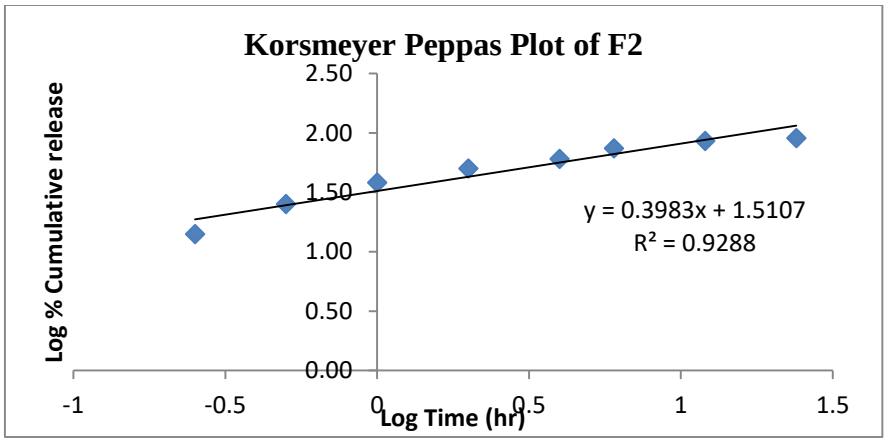


Fig. 1.9 Korsmeyer-peppas plot of F2

3.7 *In- Vivo* study

3.7.1 Chromatogram of API

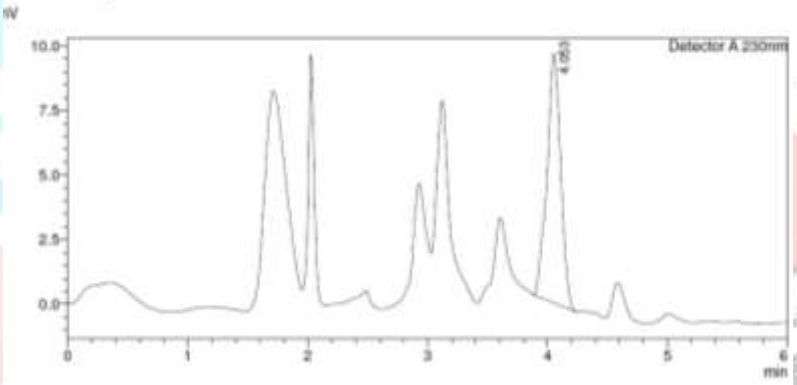


Fig. 1.10 Chromatogram of API

3.7.2 Calibration curve of simvastatin using HPLC

S. No.	Concentration	Area of peak
1	0	0
2	0.1	34124
3	0.5	74359
4	1	55171
5	2	41810
6	5	56896
7	10	174480
8	50	239276

9	100	466076

Table 1.8 Area of peak at different concentration

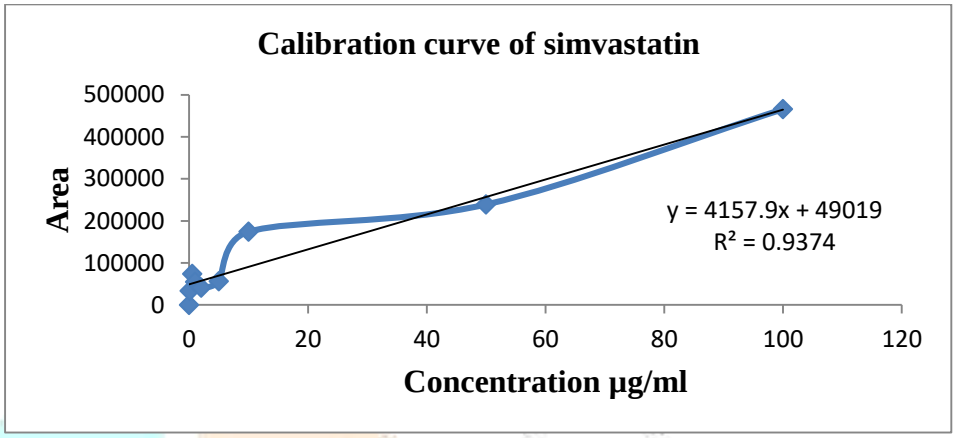


Fig. 1.11 Calibration curve of simvastatin using HPLC

3.7.3 Pharmacokinetic parameter of formulation F2 and API in Rats

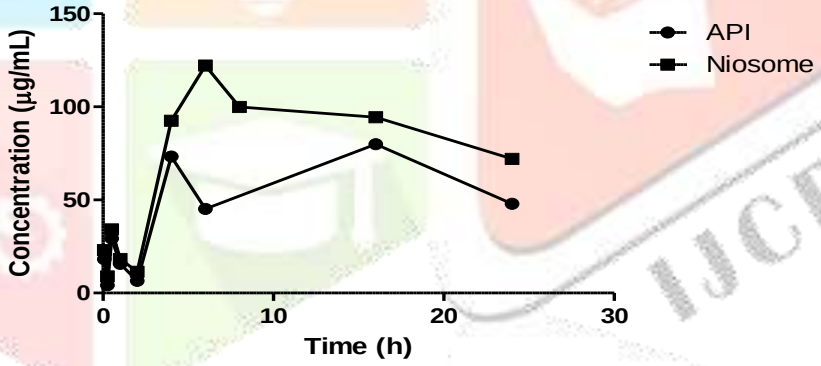


Fig. 1.12 Pharmacokinetic parameter of formulation F2 and API in Rats

Pharmacokinetic parameter	Standard drug	Formulation
C_{max} (ng/mL)	79.99	122
T_{max} (h)	16	6
$t_{1/2}$(h)	10	20
$AUC_{(0-\infty)}$ (ng.h/mL)	1837.25	4146.752
$AUMC_{(0-\infty)}$ ($ng.h^2/mL$)	45596.88	139025.14
MRT (h)	24.81	33.52

Table 1.9 Pharmacokinetic parameter of formulation F2 and API in Rats

From HPLC data, we observed that API and niosome had $T_{1/2}$ of 10 and 20 hr, respectively which signified that our prepared niosome had better oral bioavailability than API. In Fig. 6.11., it is shown that the niosonal formulation (F2) has better absorption than API after oral administration. However, AUC of niosome was three times higher than API, also indication good plasma distribution and oral bioavailability of prepared niosome. From our result, similar trends was found for mean residence time (MRT), and better distribution in plasma had been demonstrated for F2 formulation.

3.8 Stability study

Formulation F2	Particle size & Drug content					
	Initial	10 Days	20 Days	30 Days	45 Days	90 Days
Size	225.4	227.6	229.3	234.2	238.7	239.4
% residual drug content	100	99.54	99.14	98.84	98.55	97.9

Table 1.10 Effect of storage on the particle size & Drug content at 4±2 ºC

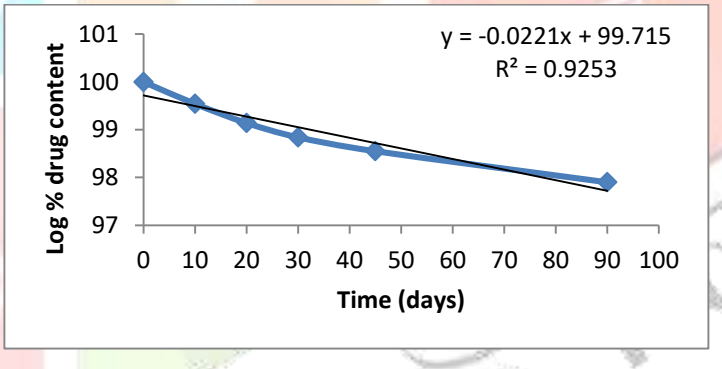


Fig. 1.13 Degradation kinetics at temperature (4±2ºC)

Formulation F2	Particle size & Drug content					
	Initial	10 Days	20 Days	30 Days	45 Days	90 Days
Size	225.4	231.5	237.1	246.3	255.7	269.2
%drug content	100	98.59	98	97.36	96.2	95.09

Table 1.11 Effect of storage on the particle size & Drug content at 30±2 ºC

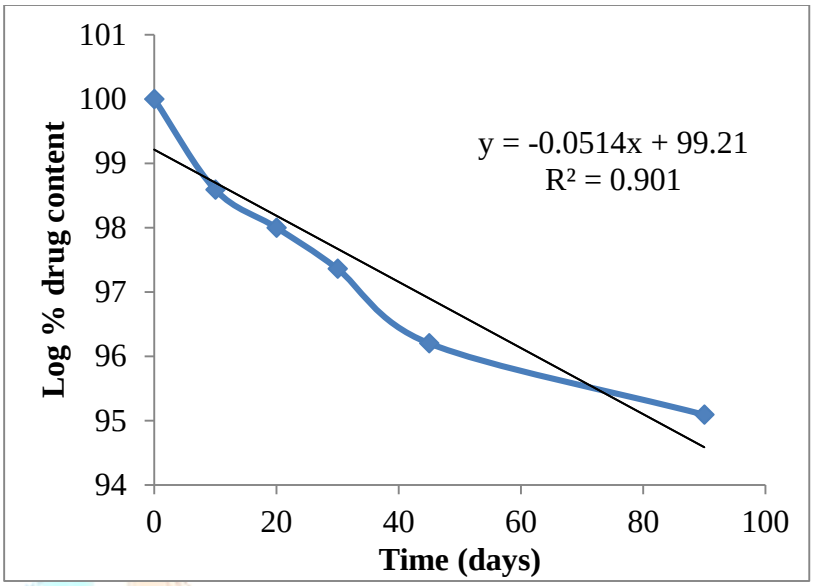


Fig. 1.14 Degradation kinetics at temperature (30±2^{0}C)

S.No	Parameter	At 4 ºC	At 30 ºC
1	K(day$^{-1)}$	2.2x10^{-4}	5.3 x10^{-4}
2	t1/2 (days)	3150.69	1305
3	t $_{10}$	472.72	196.22

Table 1.12 Shelf life, half-life of the optimized formulation (F2)

The formulation code F2 was used for stability study. Formulation was stored at 4±2ºC, and 30±2ºC, Variation in the particle size and drug content after time interval of 10, 20, 30, 45 and 90 days were analysed.

The average particle size of the niosomes was found to be increase on storage, which may be due to aggregation of particles. This effect was lower in the case of formulation stored at 4±2ºC than those stored at 30±2ºC. By keeping the initial% drug content 100 %, the drug significantly lost was 2-3% from the formulation within 90 days, which was stored at 4±2ºC and 4-5 % from those stored at 30±2ºC .which could be due to more leaching of the drug from the formulation stored at 30±2ºC. Data obtained from stability tests indicated that niosomes stored at 4±2 ºC was more stable than those stored at 30± 2 ºC.

4 CONCLUSION

An effort was made to formulate the simvastatin niosome to get better in vitro release and *in-vivo* better bioavailability. From the result of the experiment, it may be concluded that formulation F2 containing 2:1 (Span 60: Cholesterol) was found to be high % of entrapment efficiency and desired sustained release of simvastatin. The particle size of simvastatin niosome was obtained in nanosize ranges (225.40 nm), which demonstrated that

our prepared niosome are in optimum size. The dissolution of nanosized simvastatin was significantly enhanced when compared with the pure simvastatin. From the *in-vivo* study value it was found that the bioavailability of simvastatin niosome was greater than the plain simvastatin drug due to the decrease in particle size. Thus, we conclude that the niosomal drug delivery system of simvastatin is a simple and effective approach to produce nano particles of poorly water soluble drug to enhance the solubility and bioavailability.

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