Sankar P. et al. / Vol 4 / Issue 1 / 2014 / 5-11.

International Journal of

Innovative Drug Discovery

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

www.ijidd.com

FORMULATION AND EVALUATION OF LOSARTAN POTASIUM NIOSOMES

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ABSTRACT

Niosomes or nonionic surfactant vesicles are one of the many different carriers for transporting a drug molecule to its site of action. They can entrap both hydrophilic and hydrophobic drugs. Losartan is an angiotensin II type 1 receptor (AT1) antagonists drug used mainly to treat high blood pressure. Niosomes containing Losartan potassium were prepared by Thin film hydration technique using nonionic surfactants (span 60) and cholesterol at different concentrations. All the niosomes formulations were evaluated for entrapment efficiency, drug content, reproducibility, vesicular diameter, shape and size distribution microphotography, and *In vitro* release studies. The results suggest that in all the prepared niosomal formulations as the surfactant concentration increases the entrapment efficiency increases. The drug content was uniform in all the prepared niosomal formulations with low SD^3. The size of niosomes was found to be uniform and spherical in shape. The *In vitro* dissolution parameters were studied by using membrane diffusion cells. The formulation F6 shows better controlled released action then other formulation.

KEY WORDS: Cholesterol, Niosomes, Losartan potassium, Span 60.

INTRODUCTION

In recent years, pharmaceuticals modification by inclusion complexation has been extensively developed to improve drug absorption and bioavailability [1]. Non-ionic surfactant vesicles known as noisome are microscopic lamellar structures formed on admixture of a non ionic surfactant, cholesterol with subsequent hydration in aqous media [2]. They are akin to liposomes. Both Niosomes liposomes act as active carriers of both amphiphilic and lipophilic drugs. Difference in the niosomal and liposomal system is that niosomal bilayer is formed by non-ionic surfactant whereas liposomal bilayer made up of phospholipids [3]. Niosomes are formed by the self-assembly of non-ionic surfactants in aqueous media as spherical, unilamellar, bilayered, multilamellar system and polyhedral structures depending on the method used to prepare and the inverse structure in case of non-aqueous solvent [4].

The orientation of the surfactant in niosome in hydrophilic ends exposed outwards while hydrophobic ends face each other forming bilayer of the surfactant. The size of the niosomes ranges between 10 to 1000nm. Addition of cholesterol and a small quantity of anionic surfactant for instance dicetyl phosphate stabilizes the niosomal vesicles formed by the non-ionic surfactant. Niosomes are suggested to be better than liposomes because of the higher chemical stability of surfactants than phospholipids which are easily hydrolyzed due to the ester bond and cost effective [4].

Losartan is an angiotensin II type 1 receptor (AT_1) antagonists drug used for treatment of hypertension, either alone or combination with other anti-hypertensive drug such as thiazide diuretics [2]. It is reported that bioavailability of Losartan was 25-35 % and half life was 1.5- 2 hours. Niosomes are way to improve the bioavaiblity and minimize dose.

MATERIALS AND METHODS

Materials Losartan potassium was obtained from Hetro Lab, Hyderabad, India as a gift sample. Span 60, Cholesterol, chloroform, methanol were purchased from

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Rankem Chemicals, Secunderadad of analytical grade.

Methods

Niosomes were prepared by the thin-film hydration method. Accurately weighed quantities of drug, surfactant (Span-60), and CHOL were dissolved in chloroform and methanol (1:1 ratio) in a round-bottom flask. The organic solvent was evaporated at 60°C under reduced pressure using a rotary flash evaporator (Superfit, India). After chloroform and methanol evaporation, the flask was kept under vacuum overnight in a nitrogen atmosphere to remove residual solvent. The thin films were hydrated with 10 ml of phosphate buffered, pH 7.4, and the flask was kept rotating at 60°C at various revolutions per minute (rpms). Formulations were sonicated three times in a bath-sonicator (Ralsonics model RP 120, Mumbai, India) for 15 min with 5-min interval between successive time intervals.. Vesicle suspensions were also sonicated [6-11].

Characterization of niosomes:

Niosome formulations characterized for the following parameters,

Measurement of angle of repose

The angle of repose of dry niosomes powder was measured by funnel method. The niosomes powder was poured into a funnel which was fixed at a position so that the outlet orifice of the funnel is 10 cm above a level black surface. The powder flowed down from the funnel to form a cone on the surface, and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base [12].

Angle of repose is calculated using the following equation:

$$\theta = \tan^{-1}(h/r)$$

Drug content

Weighed amount of LP niosomes equivalent to 100 mg of LP was dissolved in 100 ml of water. This solution was filtered and further diluted to make a conc. of 10 µg/ml solution[12]. The absorbance of the solutions was measured at 234 nm using double beam UV-Visible spectrophotometer against distilled water as blank and calculated for the percentage of drug present in the sample [13].

Entrapment efficiency

The entrapment efficiency can be calculated by using following equation.

$$\%\ Entrapment\ Efficiency = \frac{Actual\ Drug\ loading\,(mg) \times 100}{Theoreatical\ Drug\ loading\,(mg)}$$

Determination of Vesicle Diameter

The size, shape, and lamellar nature of vesicles in nonsonicated formulations were observed by optical microscopy using a calibrated eyepiece micrometer, and photographs were taken at ×400 magnification with a digital camera (Olympus, 8.1 megapixel, Japan) [14].

SEM

Particle size of niosomes is a factor of prime importance. The surface morphology and size distribution of niosomes were studied by SEM. A double-sided tape that was affixed on aluminum stubs and the niosomal powder was spread on it. The aluminum stub was placed in a vacuum chamber of scanning electron microscope (XL 30 ESEM with EDAX, Philips, Netherlands). The morphological characterization of the samples was observed using a gaseous secondary electron detector (working pressure of 0.8 torr, acceleration voltage-30.00 KV) XL 30, (Philips, Netherlands) [15].

***In vitro* release studies**

The release of LP from niosomal formulations were determined using membrane diffusion technique. The niosomal formulation equivalent to 10 mg of LP was converted to niosomal suspension and taken in a glass tube having a diameter 2.5 cm with an effective length of 8 cm that was previously covered with soaked osmosis cellulose membrane, which acts as donor compartment. The glass tube was placed in a beaker containing 100 ml of saline buffer pH 7.4, which acts as receptor compartment. The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touched (1-2mm deep) the surface of diffusion medium [16]. The temperature of receptor medium maintained at 37±1ºC and the medium was agitated at 100 rpm speed using magnetic stirrer. Aliquots of 5ml 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 using phosphate buffer 7.4 as blank.

Stability studies

The stability studies for best niosomes formulation were carried out as per ICH guides for 3 months. Formulated niosomes were divided into batches [17,18]. One batch was kept at refrigeration (4°±2ºC) 78. The second batch was kept at room temperature (25°±2°C). The third batch was kept at 40±2ºC and 60±5% RH. Every month 1 ml of formulation was withdrawn and analyzed for drug content. The drug release studies for formulation F-6 was also performed before and after 3 months at various temperatures.

RESULTS AND DISCUSSION

Angle of repose

The angle of repose of dry niosome powder measured by funnel method is shown in table 2. The angle of repose of dry niosomes was between 25.32±0.25 to 28.13±0.46.

Entrapment efficiency

The entrapment efficiency of niosomes prepared at varied concentration of surfactants (Span 60) are shown in table 2 the drug entrapped in the range of 49.35±0.14,

52.02±0.54, 54.93±0.47, 57.36±0.81, 58.35±0.63, 60.32±0.71, 62.03±0.38,and 63.43±0.27 for F1,F2,F3, F4, F5, F6, F7, and F8 respectively. The entrapment efficiency increases with increasing surfactant concentration.

Table 1. Formulation development of Niosomes

Formulation code	Drug (mg)	Cholesterol (mg)	Span-60 (mg)	Chloroform (ml)	Methanol (ml)	Phosphate buffer P^H 7.4 (ml)
F1	10	10	5	10	10	10
F2	10	10	10	10	10	10
F3	10	10	15	10	10	10
F4	10	10	20	10	10	10
F5	10	10	25	10	10	10
F6	10	10	30	10	10	10
F7	10	10	35	10	10	10
F8	10	10	40	10	10	10

Table 2. Angle of repose ,% encapsulation and %Drug content of formulation F1 to F8

Formulation code	Angle of repose(θ)*±SD	% Encapsulation* ±S.D	% Drug content* ±S.D
F1	26°85′±0.18	49.35±0.14	89.06±0.57
F2	27°12′±0.36	52.02±0.54	92.97±o.37
F3	26°95′±0.12	54.93±0.47	90.46±0.62
F4	28°10′±0.27	57.36±0.81	91.38±0.29
F5	27°50′±0.61	58.35±0.63	94.14±0.58
F6	25°.32′±0.25	60.32±0.71	98.35±0.51
F7	28°13′±0.46	62.03±0.38	93.53±0.27
F8	27°12′±0.32	63.43±0.27	94.15±0.41

*Average of three readings

Table 3. Particle size of niosomes

Formulation code	Mean Particle size*±S.D in (nm)	Mean Particle size*±S.D in (μm)
F1	275±0.97	0.275±0.097
F2	280±6.23	0.280±0.623
F3	254±3.15	0.254±0.315
F4	271±0.24	0.271±0.024
F5	245±5.10	0.245±0.051
F6	280±4.43	0.280±0.044
F7	258±4.54	0.312±0.025
F8	292±4.24	0.292±0.042

*Average of three readings

Table 4. *In vitro* drug release data

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	25.32	29.62	23.51	19.21	18.63	17.73	23.65	22.82
2	36.83	42.73	32.38	31.62	28.74	25.36	32.84	32.47
3	48.02	53.62	42.57	40.38	39.3	33.64	39.29	42.61
4	57.52	62.81	51.41	49.92	48.91	41.92	47.21	53.04
5	71.27	73.01	60.83	62.26	57.5	50.29	56.05	64.07
6	83.45	84.07	70.91	71.43	65.61	58.62	65.02	73.92
7	95.03	96.02	82.46	82.7	73.71	66.91	74.26	82.05
8			93.75	93.64	81.82	77.63	83.01	95.53
9					90.58	83.06	92.47	
10						92.6		

Table 5. *In vitro* drug release kinetic for F6 formulation

Time in hrs	square root of time	log time	cumulative percentage drug release	cumulative percentage drug remaning	log cumulative percentage drug release	log cumulative percentage drug remaning
0	0	-	0		-	-
1	1	0	17.73±0.65	82.27	1.248709	1.9152
2	1.414	0.30103	25.36±0.43	74.64	1.404149	1.87297
3	1.732	0.477121	33.64±0.25	66.36	1.526856	1.82191
4	2	0.60206	41.92±0.31	58.08	1.622421	1.76403
5	2.236	0.69897	50.293±0.65	49.707	1.701508	1.69642
6	2.449	0.778151	58.62±0.95	41.38	1.768046	1.61679
7	2.645	0.845098	66.91±0.74	33.09	1.825491	1.5197
8	2.828	0.90309	77.63±0.84	22.37	1.89003	1.34967
9	3	0.954243	83.06±0.63	16.94	1.919392	1.22891
10	3.162	1	92.65±0.32	7.35	1.966845	0.86629

Table 6. Stability study for formulation F6 in Different storage condition

4°C ± 1°C		25°C ± 5°C		30°C ± 2°C and 60% RH ± 5% RH	
Time period	**% Drug content**	**Time period**	**% Drug content**	**Time period**	**% Drug content**
15 Days	98.34±0.81	15 Days	98.30±0.55	15 Days	97.83±0.75
30 Days	98.03±0.35	30 Days	97.91±0.72	30 Days	96.94±0.73
60 Days	97.61±0.42	60 Days	97.05±0.41	60 Days	95.63±0.73
90 Days	97.07±0.84	90 Days	96.43±0.67	90 Days	95.01±0.64

Table 7. *In vitro* studies of formulation F6 stored at refrigeration condition

Time in hrs	Cumulative percentage drug release			
	15 Days	**30 Days**	**60 Days**	**90 Days**
0	0	0	0	0
1	17.13	17.02	16.62	16.21
2	24.43	26.52	23.82	23.93
3	31.13	35.54	33.92	31.32
4	38.43	44.64	40.82	39.71
5	45.95	52.57	47.03	48.63
6	53.04	60.62	56.92	57.01
7	62.67	68.61	65.91	64.31
8	71.51	76.73	74.02	71.75
9	82.45	84.04	83.94	80.04
10	92.04	91.95	91.03	90.43

Table 8. *In vitro* studies of formulation F6 stored at room temperature

Time in hrs	Cumulative percentage drug release			
	15 Days	**30 Days**	**60 Days**	**90 Days**
0	0	0	0	0
1	17.03	16.92	16.02	15.21
2	23.43	24.52	23.82	23.93
3	30.13	32.54	31.92	30.32
4	37.43	40.64	39.82	38.71
5	44.95	48.57	47.03	45.63
6	52.04	57.62	54.92	54.01
7	61.67	66.61	63.91	63.31
8	70.51	74.73	71.02	70.75
9	81.45	82.04	80.94	79.04
10	91.64	89.95	88.83	88.03

Table 9. *In vitro* studies of formulation F6 stored at Accelerated condition

Time in hrs	Cumulative percentage drug release			
	15 Days	30 Days	60 Days	90 Days
0	0	0	0	0
1	17.64	16.03	15.96	15.05
2	25.13	23.86	22.53	23.95
3	33.04	32.85	30.16	33.04
4	41.83	40.06	38.42	42.13
5	49.51	47.41	47.85	50.63
6	57.64	54.52	52.42	57.32
7	66.85	62.61	61.32	65.25
8	74.61	71.53	71.14	72.04
9	83.09	80.15	80.61	79.31
10	90.64	89.05	88.13	87.03

Figure 1. Optical microscopy image of formulation F6

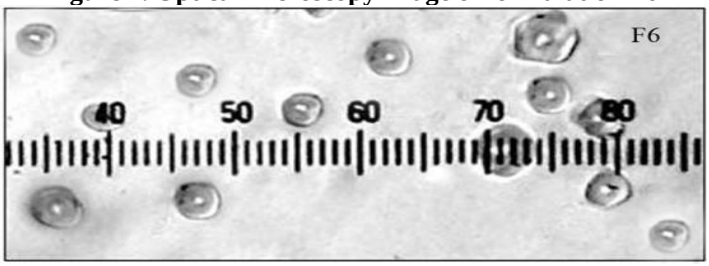


Figure 2. SEM image of formulation F6

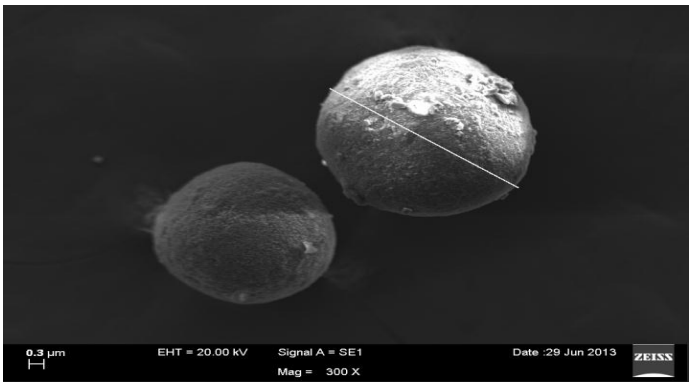


Figure 3. % Drug release of niosomal formulations F1 to F8

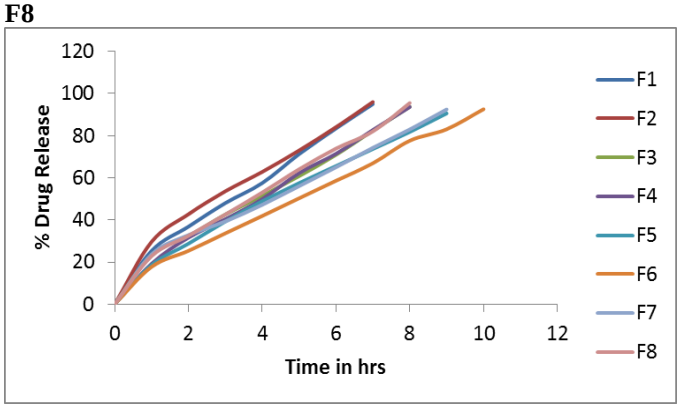


Figure 4. Zero order order kinetic for F6 formulation

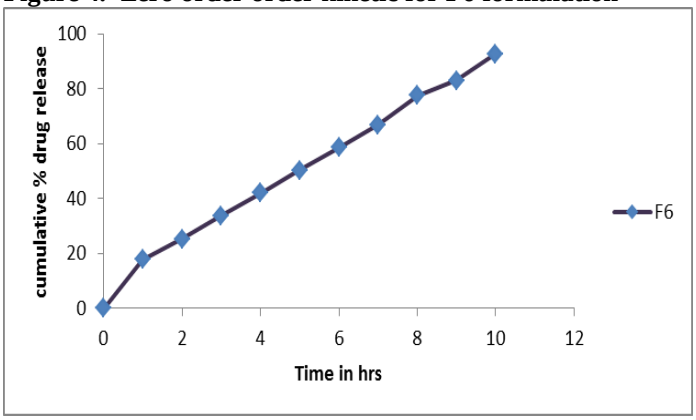


Figure 5. First order order kinetic for F6 formulation

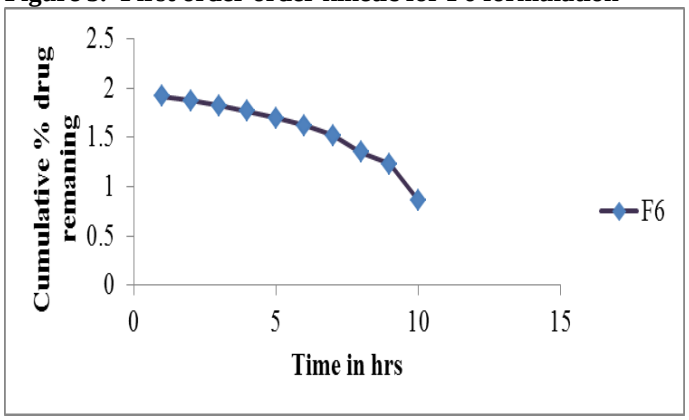


Figure 6. Higuchi kinetic model module for F6 formulation

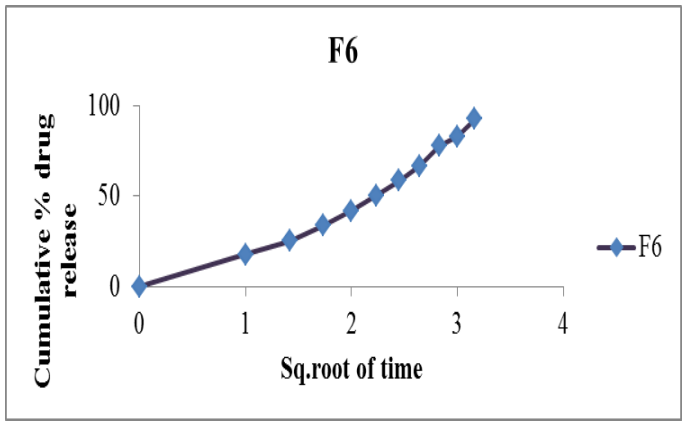


Figure 7. Korsmeyer-Peppas model module for F6 formulation

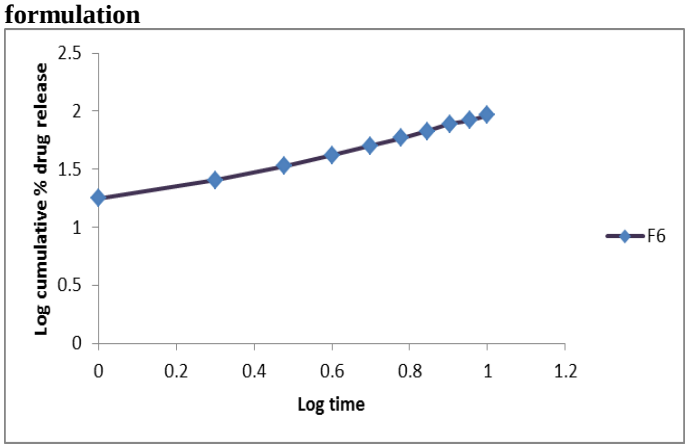


Figure 8. *In vitro* studies of formulation F6 stored at refrigeration condition

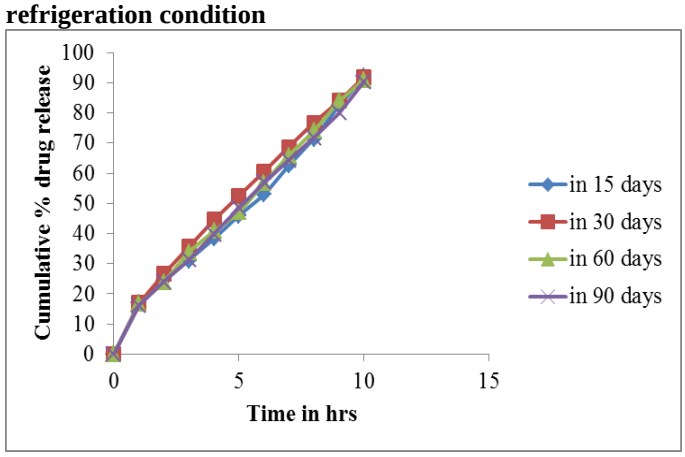


Figure 9. *In vitro* studies of formulation F6 stored at room temperature

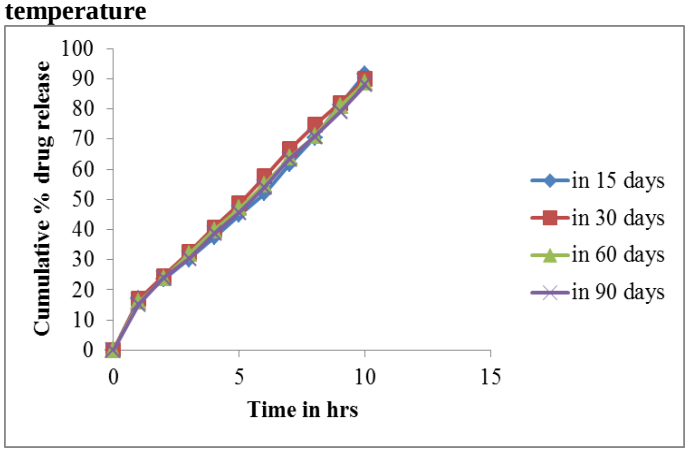
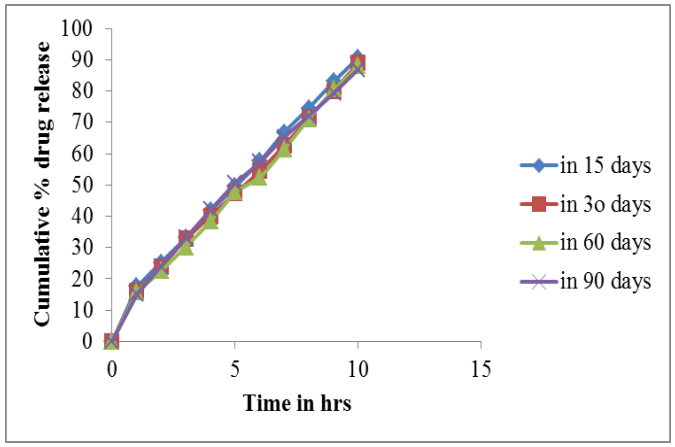


Figure 10. *In vitro* studies of formulation F6 stored at Accelerated condition



Drug content

Drug content was determined for all niosomal formulations. Average of three determinations was considered. The drug content was found to be in the range of 89.06±0.57 to 98.35±0.51%.

Vesicular size, shape and surface morphology

The niosomes were converted to niosomal suspension and the size of the niosomes was measured using an optical microscope with calibrated eyepiece micrometer. From every batch about 200 niosomes were measured for the diameter individually, the average was calculated and was computed in tables 12. The size distribution is shown in table 3. The SEM images (figure 2) shows niosomes are spherical in shape and smooth surface.

***In vitro* Drug Release Studies**

The various LP niosomal formulations prepared were subjected to *In vitro* drug release studies and data is shown in tables 4 and dissolution profiles are given in figures 19-50. The percentage drug release from niosomal formulations F1are 95.03, at 7 hrs, F2 are 96.02 at 7 hrs, F3 are 93.75 at 8 hrs,F4 are 93.64 at 8 hrs,F5 are 90.58 at 9 hrs, f6 are 92.65 at 10 hrs,F7 are 92.47 at 9 hrs and F8 are 95.53 at 8 hours. In all formulation, 17 % to 30% of drug is released in the first hours, due to initial bursting of improper niosomes in the formulations. However, after 3 hours, the release was steady because the stable niosomes retain the

drug and the release was extended up to 10 hours with sustained action. The niosomal formulations F6 shows better controlled release compared to other formulations. The kinetic models selected were Zero order, First order, Higuchi Matrix, and Korsemayer Peppas. Best formulation F6 the drug was released by Zero order kinetics.

Stability studies

Stability studies are done for best formulation (F6) as per ICH guide line in 3 different storage conditions for 3 months. The formulations are analyzed for the drug content, *In vitro* drug release studies. After 3 months studies it revolves that there slightly change in % of drug content but it was in accepted limit. Table- 6 shown % of drug content in different stability condition.

CONCLUSION

Niosomal formulations can be conveniently prepared by thin film hydration method using nonionic surfactants (span 60), and cholesterol at different concentrations. All the niosomal powders were found to be free flowing with repose angle in the range of 25.32±0.25 to 28.13±0.46 by funnel method. The entrapment efficiency of niosomal formulations increases with increases the concentration of span 60. Drug content of all niosomal formulations were in range of 89.06±0.57 to 98.35±0.51% with low SD and the results were reproducible. The average vesicular size of niosomes of all the batches was measured in the range of 0.271±0.024 µm to 0.312±0.025µm. The SEM image of niosomal formulations revealed that the surface of niosomes were smooth and spherical in shape.

The drug release from vesicles is dependent on concentrations of span 60. It is mainly due to the influence of phase transition temperature. Better dissolution profiles were found with niosomal formulations F6. The best fit model is peppas with 'n' value between between 0.768 to 0.917 and suggesting that the drug was released by Zero order kinetics.

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