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IMPACT OF SURFACTANT TO CHOLESTEROL RATIO ON THE CHARACTERISTICS OF ZIDOVUDINE NIOSOMES

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ABSTRACT

The aim of the present work was to develop Niosomes drug delivery system with zidovudine as model drug. The surfactant selected was Span 60 and lipid forming agent was cholesterol. It was planned to have 6 formulations falling into two categories based on the method of preparation namely Ether injection method and Film hydration method. Under each category, 3 formulations were designed containing different span 60: cholesterol ratio. Formulation E1, E2 and E3 contain span 60: Cholesterol ratio of 1:1, 2:1,3:1 respectively and prepared by Ether injection method. Similarly, formulations F1, F2 & F3 contain span 60: cholesterol in the ratio of 1:1, 2:1, 3:1 and prepared by film by hydration technique. The prepared formulations were evaluated. It was found that the Entrapment efficiency of the Niosomes increased on increasing the amount of span 60. The trend was demonstrated in both categories of Niosomes prepared by ether injection & film hydration technique. The invitro drug release studies were done for 12 hours. The characteristics of the formulations were compared and found that the drug release was increase in the formulation containing more amount of surfactant. The formulation with 1:1ratio of span:cholesterol showed slow release of drug when compared to other formulations containinghigh amount of surfactant.

KEYWORDS:- Niosomes, Ether injection Method and Film hydration method.

1. INTRODUCTION[1-4]

In the recent years, nonionic surfactants vesicles known as Niosomes received a great

attention as an alternative potential drug delivery system to conventional liposomes.

Niosomes are vesicular systems similar to liposomes that can be used as carrier to ampiphilc and lipophilic drugs. Niosomes are promising vesicles for drug delivery and being nonionic it is less toxic and improves the therapeutic index of drug by restricting itsaction to target cells. Nonionic surfactant-based vesicles (Niosomes) are formed from the self- assembly of nonionic amphiphiles in aqueous media resulting in closed bi-layer structures which can entrap both hydrophilic and lipophilic drugs either in aqueous layer or in vesicular membrane (vesicles can entrap water soluble substances in the inner aqueous phase and oil soluble substances in the vesicular membrane). Niosomes can encapsulate both hydrophilic and lipophilic drugs and can be protected them against acidic andenzymatic effects in vivo.

Niosomes are biodegradable, bio compatible, no toxic capable of encapsulating large quantities in relatively small volumes of vesicles. Encapsulation of a drug in vesicularstructure can be predicted to prolong the existence of the dug in systemic circulation and enhance the penetration into target tissue and reduce toxicity.

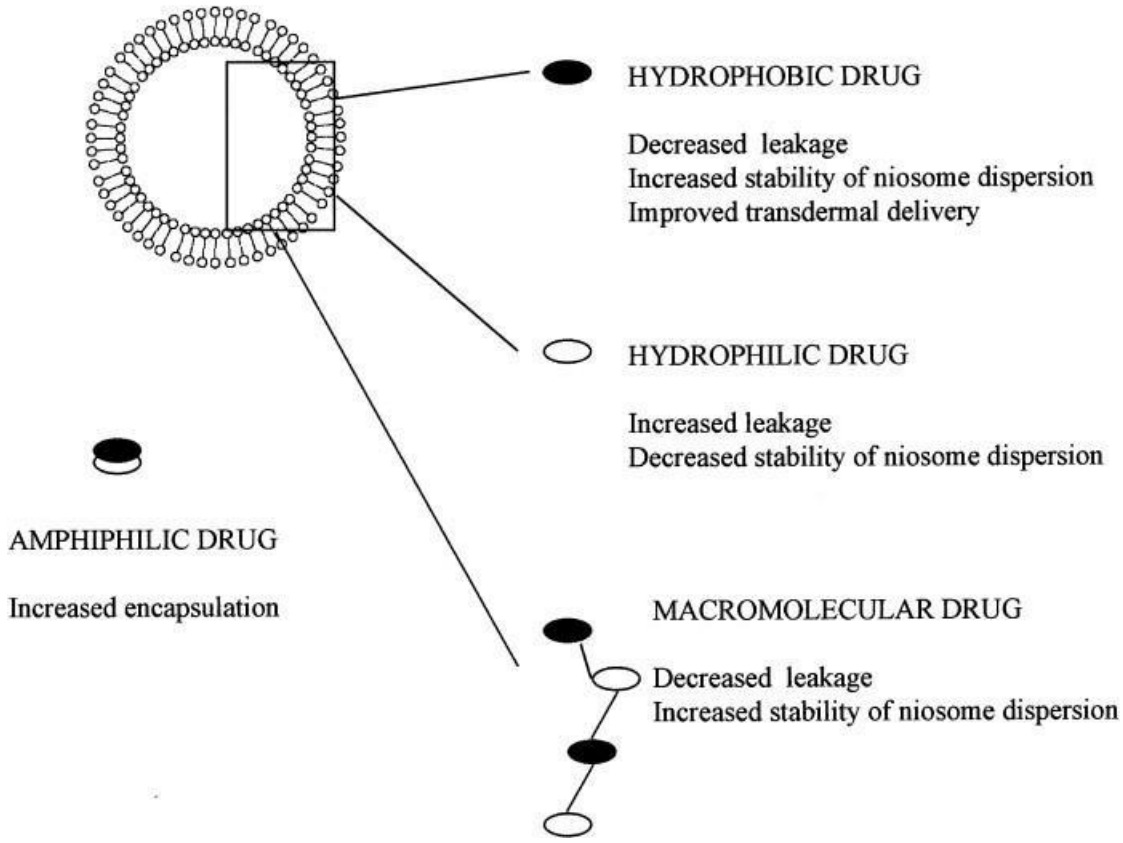


Fig. 1: Schematic diagram of niosomes.

2. MATERIALS AND METHODS

2.1 Materials used

S. no.	Name of the instrument /Chemical	Model /Batch Number	Manufacturer
1	Zidovudine	---	Cipla labs
2	Span60	MCR1309-080819	Moly chem
3	Cholesterol	27102142	Fina reagent
4	Diethyl ether	19014484	Fina reagent
5	Magnetic stirrer	--	REMI
6	Vortex mixer	SPINIX	TARSONS
7	UVS spectrophotometry	T600VV15	Analytical instruments
8	Dialysis membrane	---	HI-MEDIA

2.2 Methods used

Preparation of zidovudine loaded niosomes

Various methods available for preparing Niosomes, film hydration method andether injection method were taken for the present work

Film hydration method[5,6]

Accurately weighed amount of surfactant and cholesterol was taken anddissolved in 4 ml of diethyl ether. The solution was vortexed to form a thin film. The film was hydrated with 20ml aqueous solution containing zidovudine in the concentration of 2mg/ml maintained at 60ċand vortexed for1 hr.

Table 1: Composition of formulations prepared by film hydration method.

S. No	Formulation code	Span 60: Cholesterol ratio	Amount of Span 60	Amount Of cholesterol
1	F1	1:1	2.4 mg	2.4 mg
2	F2	2:1	4.8 mg	2.4 mg
3	F3	3:1	7.2 mg	2.4 mg

Ether injection method[7,8]

Specified quantity of Span 60 and cholesterol was dissolved in diethyl ether. It was then introduced into 20 ml aqueous phase containing zidovudine in concentration of 2mg/ml at 60^{o}C through 16 gauze needles. Vaporization of ether leads to the formation of vesicles.

Table 2: Composition of formulations prepared by ether injection method.

S. No	Formulation code	Span 60: Cholesterol Ratio	Amount of Span 60	Amount Of cholesterol
1	E1	1:1	2.4 mg	2.4 mg
2	E2	2:1	4.8 mg	2.4 mg
3	E3	3:1	7.2 mg	2.4 mg

3. RESULTS AND DISCUSSION

The zidovudine loaded Niosomes were prepared by Film hydration method and Ether injection method. Under each method 3 formulations were made by altering the Surfactant: Cholesterol ratio in the order of1:1, 1:2, 1:3. The prepared formulations werethen evaluated for physical and drug release characteristics.[9]

Vesicle Size and Shape

The formulations prepared by ether injection method were subjected to optical microscopic studies to determine the vesicle size and shape. Thev vesicles were foundto be large vesicles with smooth exterior and uniform in size.

Table 3: Average vesicle size for E1 (Span: Cholesterol ratio 1:1) Ether injection.

Size Range (□m)	Mean size range(d) (□m)	No. of particles (N)	N.d	% of each range	% w/w undersize
10	5	33	165	33	30
20	15	35	525	35	65
30	25	24	600	24	89
40	35	5	175	5	94
50	45	3	135	3	97

Average vesicle size=16.0 □m

Table 4: Average vesicle size for E2 (Span: Cholesterol ratio 2:1) Etherinjection.

Size Range(□m)	Mean size range (d)(□m)	No. of particles(N)	N.d	% of each range	% w/w undersize
10	5	30	150	30	28
20	15	31	465	31	59
30	25	24	600	24	83
40	35	8	280	8	91
50	45	7	315	7	98

Average vesicle size=18.1 □m

Table 5: Average vesicle size for E3 (Span: Cholesterol ratio 3:1) Etherinjection.

Size Range(□m)	Mean size range (d)(□m)	No. of particles(N)	N.d	% of eachrange	% w/w undersize
10	5	29	145	29	28
20	15	27	405	27	55
30	25	31	775	31	86
40	35	7	245	7	93
50	45	6	270	6	99

Average vesicle size=18.4 □m

The average particle size of all the 3 formulations was determined by using eyepiece micrometer. The vesicle size found to be increased in the order of increasing content of surfactant. The vesicles size of **Formulation E3 (Span: Cholesterol ratio 3:1) >Formulation E2 (Span: Cholesterolratio2:1)>Formulation E1 (Span: Cholesterol ratio 1:1)**

The data for the determination of average vesicle size for the formulation

Prepared by film hydration technique were made.

Table 6: Average vesicle size for F1 (Span: Cholesterol ratio1:1) Film hydration.

Size Range(□m)	Mean size range (d)(□m)	No. of particles(N)	N.d	% of each range	% w/w under size
10	5	30	150	30	30
20	15	32	480	32	62
30	25	24	600	24	86
40	35	8	280	8	94
50	45	6	270	6	100

Average vesicle size=17.8 □m

Table 7: Average vesicle size for F2 (Span: Cholesterol ratio2:1) Film hydration.

Size Range(□m)	Mean size range (d)(□m)	No. of particles(N)	N.d	% of each range	% w/w undersize
10	5	28	140	28	30
20	15	31	465	31	61
30	25	24	600	24	85
40	35	10	350	10	95
50	45	7	315	7	102

Average vesicle size=18.7 □m

Table 8: Average vesicle size for F3 (Span: Cholesterol ratio3:1) Film hydration.

Size Range(□m)	Mean size range (d)(□m)	No. of particles(N)	N.d	% of eachrange	% w/w undersize
10	5	27	135	27	28
20	15	25	375	25	53
30	25	31	775	31	84
40	35	7	245	7	91
50	45	10	450	10	101

Average vesicle size=19.8 □m

The same trend with regard to the vesicle size was witnessed in case formulations prepared by Film hydration technique. The vesicle size found to be increased in the order of increasing content of surfactant. The vesicles size of **Formulation F3 (Span: Cholesterol ratio 3:1) > Formulation F2 (Span: Cholesterol ratio 2:1) > Formulation F1 (Span: Cholesterol ratio1:1)**.

On comparison of vesicles size of Niosomes prepared by ether injection and film hydration technique it was determined that Niosomes prepared by film hydration techniqueshowed large sized vesicles than Niosomes formulated by ether injection.

Entrapment efficiency

The Niosomes were subjected to entrapment efficiency testing by using lysis method. The Niosomes which were separated from unentrapped drug was taken for entrapment efficiency studies. The formulations E1, E2 and E3 formulated by ether injection method containing span 60: Cholesterol in the ratio 1:1, 2:1 and 3:1 respectively showed Entrapment values of 58.9%, 71.6%and 82.5%.

Table 9: Table showing entrapment efficiency values of ether injectionformulations.

S. No	Formulation Code	Span 60: Cholesterol ratio	Absorbance*	Concentration (□g/ml)	Amount (mg/ml)	Total Amount	% Entrapped drug
1	E1	1:1	0.541	11.7843	1.1784	1.1784	58.92
2	E2	1:2	0.658	14.3328	1.4333	1.4333	71.66
3	E3	1:3	0.758	16.5111	1.6511	1.6511	82.56

*Average reading of 3 determinations

The formulations F1 (Span: Cholesterol ratio 1:1) had entrapment value of62.9% F2 and F3 prepared by film hydration technique with span: cholesterol in the ratio of 1:1, 2:1 and 3:1respectively showed drug entrapment of62.9%. Formulation F2 (Span: Cholesterol2:1)

showed drug entrapment of73.9%and formulationF3 (Span: Cholesterol3:1) demonstrated drug entrapment 81.4%.

Table 10: In vitro drug release data for formulation E1 (Span 60: cholesterol ratio1:1).

Time in hrs.	Absorbance*	Concentration (□g/ml)	Amount released (mg/ml)	Cumulative %Drug release
0	0.000	0.0000	0.0000	0.00
1	0.022	0.2087	0.0021	2.09
2	0.058	1.0952	0.0110	10.97
3	0.103	2.2032	0.0220	22.16
4	0.132	2.9173	0.0292	29.52
5	0.162	3.6560	0.0366	37.20
6	0.194	4.4440	0.0444	45.45
7	0.210	4.8380	0.0484	49.83
8	0.243	5.6505	0.0565	58.44
9	0.263	6.1430	0.0614	63.93
10	0.284	6.6601	0.0666	69.72
11	0.291	6.8325	0.0683	71.89
12	0.301	7.0787	0.0708	**75.25**

*Average reading of 3 determinations

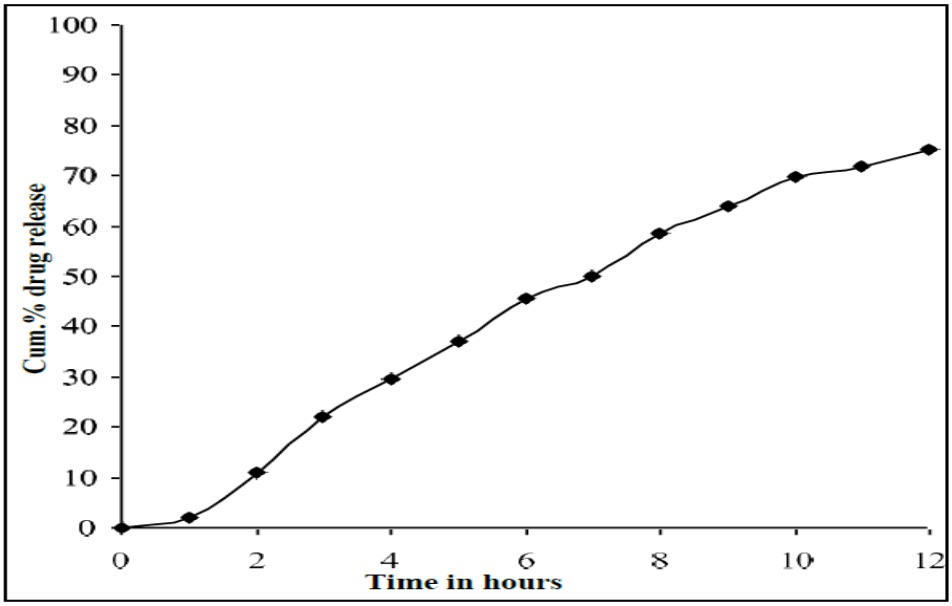


Fig. 2: Zero order plot forformulatione1 (Span 60: cholesterol ratio1:1).

Table 11: Invitro drug release data for formulation E2 (Span 60: cholesterol ratio 2:1).

Time in hrs.	Absorbance*	Concentration (□g/ml)	Amount released (mg/ml)	Cumulative %Drug release
0	0.000	0.0000	0.0000	0.00
1	0.028	0.3565	0.0036	3.56
2	0.067	1.3168	0.0132	13.20

3	0.118	2.5726	0.0257	25.89
4	0.143	3.1882	0.0319	32.31
5	0.173	3.9269	0.0393	40.01
6	0.201	4.6163	0.0462	47.30
7	0.234	5.4289	0.0543	55.89
8	0.252	5.8721	0.0587	60.86
9	0.275	6.4385	0.0644	67.11
10	0.301	7.0787	0.0708	74.16
11	0.315	7.4234	0.0742	78.06
12	0.329	7.7682	0.0777	**82.50**

*Average reading of 3 determinations

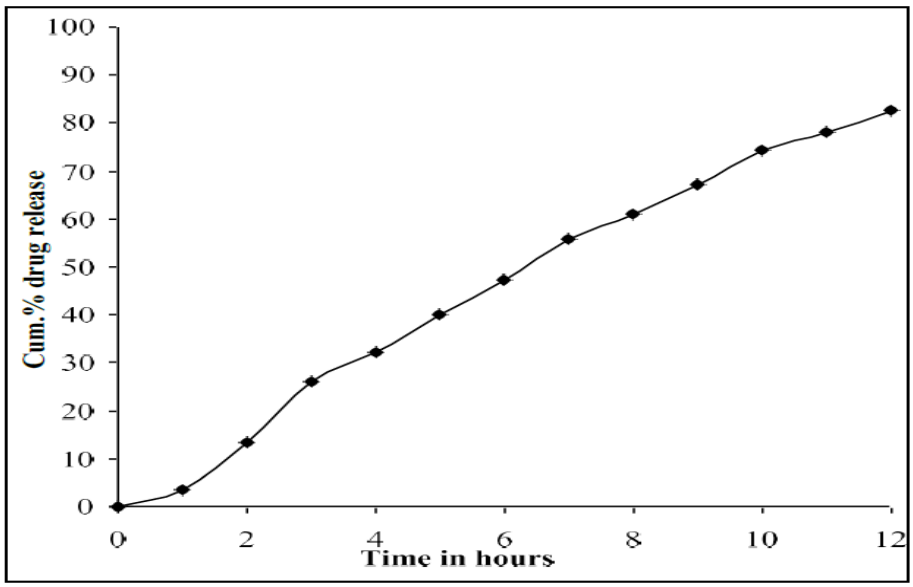
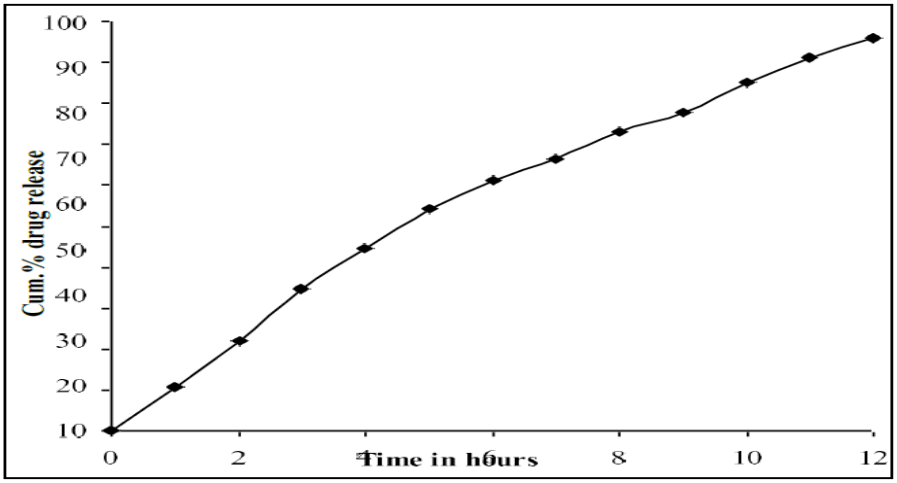


Fig. 3: Zero order plot for formulation E2 (Span60: cholesterol ratio 2:1).

Table12: Invitro drug release data for formulation E3 (Span60: cholesterol ratio3:1)

Time in hrs.	Absorbance*	Concentration (□g/ml)	Amount released (mg/ml)	Cumulative %Drug release
0	0.000	0.0000	0.0000	0.00
1	0.043	1.0588	0.0106	10.59
2	0.089	2.1894	0.0219	22.00
3	0.139	3.4227	0.0342	34.55
4	0.178	4.3830	0.0438	44.50
5	0.215	5.2882	0.0529	53.99
6	0.242	5.9589	0.0596	61.22
7	0.261	6.4268	0.0643	66.50
8	0.285	7.0177	0.0702	73.05
9	0.301	7.4117	0.0741	77.69
10	0.328	8.0765	0.0808	85.08
11	0.351	8.6343	0.0863	91.12
12	0.365	8.9876	0.0899	95.86

*Average reading of 3 determinations

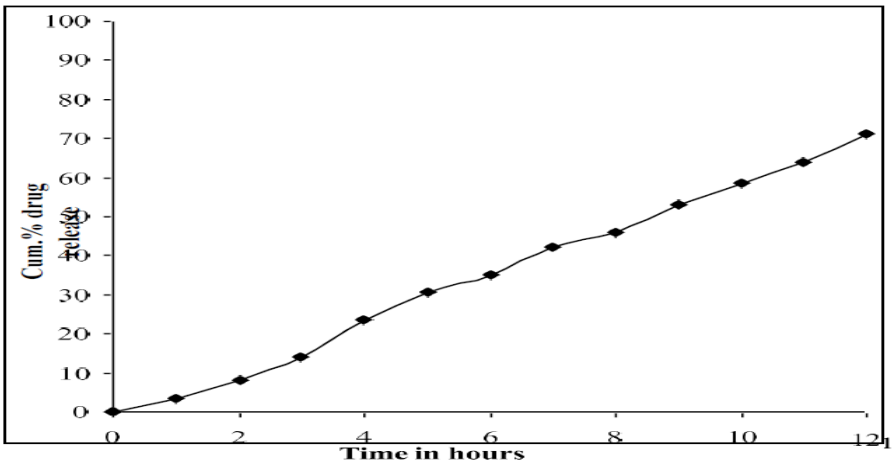


Zero order plot for formulation E3 (Span60: cholesterol ratio 3:1)

Table 13: In vitro drug release data for formulation F1 (Span60: cholesterol ratio1:1).

Time inhrs	Absorbance*	Concentration (□g/ml)	Amount released (mg/ml)	Cumulative %Drug release
0	0.000	0.0000	0.0000	0.00
1	0.017	0.0856	0.0009	0.86
2	0.047	0.8243	0.0082	8.25
3	0.070	1.3909	0.0139	14.00
4	0.108	2.3231	0.0232	23.46
5	0.136	3.0158	0.0302	30.62
6	0.152	3.4098	0.0341	34.86
7	0.180	4.0895	0.0409	42.00
8	0.194	4.4440	0.0444	45.95
9	0.221	5.1088	0.0511	53.05
10	0.241	5.6013	0.0560	58.48
11	0.262	6.1184	0.0612	64.07
12	0.288	6.7586	0.0676	**71.23**

*Average reading of 3 determinations

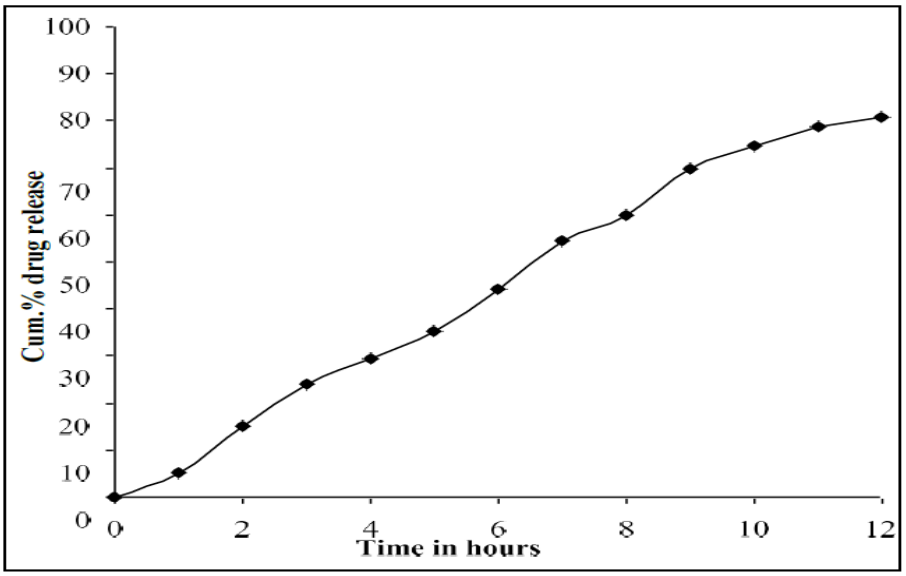


Zero order plot for formulationf1 (Span60: cholesterol ratio1:1).

Table 13: In vitro drug release data for formulation F2 (Span 60: cholesterol ratio 2:1).

Time inhrs	Absorbance*	Concentration (□g/ml)	Amount released (mg/ml)	Cumulative %Drug release
0	0.000	0.0000	0.0000	0.00
1	0.034	0.5042	0.0050	5.04
2	0.074	1.4891	0.0149	14.94
3	0.110	2.3756	0.0238	23.96
4	0.132	2.9173	0.0292	29.61
5	0.154	3.4590	0.0346	35.32
6	0.189	4.3209	0.0432	44.28
7	0.229	5.3058	0.0531	54.56
8	0.249	5.7983	0.0580	60.02
9	0.286	6.7093	0.0671	69.71
10	0.303	7.1279	0.0713	74.57
11	0.318	7.4973	0.0750	78.74
12	0.323	7.6204	0.0762	**80.95**

*Average reading of 3 determinations



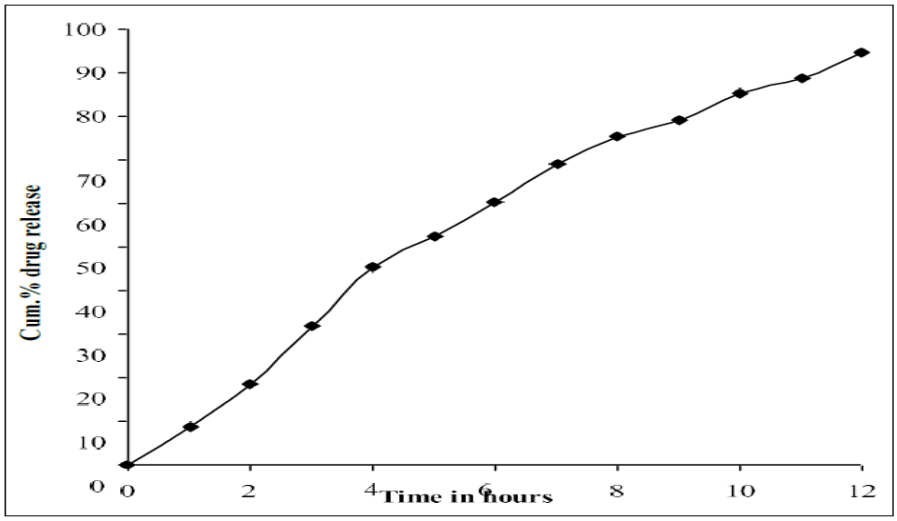
Zero order plot forformulation F2 (Span60: cholesterol ratio 2:1).

Table 14: In vitro drug release data for formulation F3 (Span60: cholesterol ratio 3:1).

Time in hrs.	Absorbance*	Concentration (□g/ml)	Amount released (mg/ml)	Cumulative %Drug release
0	0.000	0.0000	0.0000	0.00
1	0.035	0.8618	0.0086	8.62
2	0.075	1.8468	0.0185	18.55
3	0.128	3.1518	0.0315	31.79
4	0.182	4.4815	0.0448	45.40
5	0.209	5.1463	0.0515	52.50
6	0.239	5.8850	0.0589	60.40
7	0.272	6.6976	0.0670	69.11

8	0.294	7.2393	0.0724	75.20
9	0.307	7.5594	0.0756	79.13
10	0.329	8.1012	0.0810	85.30
11	0.341	8.3967	0.0840	88.75
12	0.361	8.8891	0.0889	**94.83**

*Average reading of 3 determinations



Zero order plot for formulation F3 (Span 60: cholesterol ratio 3:1).

4. SUMMARY AND CONCLUSION

The aim of the present work was to develop Niosomes drug delivery system with zidovudine as model drug. The surfactant selected was Span 60 and lipid forming agent was cholesterol. It was planned to have 6 formulations falling into two categories based on the method of preparation namely Ether injection method and Film hydration method.

Under each category,3 formulations were designed containing different span 60: cholesterol ratio. Formulation E1, E2 and E3 contain span 60: Cholesterol ratio of 1:1,2:1, 3:1 respectively and prepared by Ether injection method. Similarly, formulations F1, F2 & F3 contain span 60: cholesterol in the ratio of 1:1, 2:1, 3:1 and prepared by film by hydration technique.

The prepared formulations were evaluated. It was found that the Entrapment efficiency of the Niosomes increased on increasing the amount of span 60. The trend was demonstrated in both categories of Niosomes prepared by ether injection & film hydrationtechnique.

The vesicle size of the Niosomes was determined by optical microscopy. It was found to increase on increments of span 60. The Niosomes prepared from film hydration method had

larger vesicle size when compared to the vesicles of the Niosomes prepared from ether injection.

The impact of osmotic shock on the formulations was studied. All the formulationswere stable in isotonic medium whereas it undergoes swelling in hypotonic medium and shrinkage in hypertonic medium. It was clear that the Niosomes is stable with any isotonicvehicle and can be suitable candidate for parenteral preparations.

The invitro drug release studies were done for 12 hours. The characteristics of the formulations were compared and found that the drug release was increase in the formulation containing more amount of surfactant. The formulation with 1:1ratio of span cholesterol showed slow release of drug when compared to other formulations containinghigh amount of surfactant.

In this work, invitro studies for Niosomes were conducted and found to havebetter release of drug. In future, in vivo studies should be done for ensuring the effective ness of the formulation.

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