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FORMULATION, OPTIMIZATION AND EVALUATION OF NIOSOMAL DELIVERY SYSTEMS FOR ZIDOVUDINE

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ABSTRACT

To design and optimize Zidovudine niosomes, the Thin Film Hydration Technique was employed. Niosomes have shown potential in drug release studies and may serve as an effective option for drug delivery systems. The drug is incorporated into niosomes to enhance targeting at the appropriate tissue locations. **Materials and Methods:** Zidovudine niosomes were prepared using the film hydration technique, with Span 20 and Tween 20 as the main components. Various parameters such as zeta potential, entrapment efficiency, and drug release profile were analyzed to identify the optimal formulation. The physicochemical characteristics were assessed using FTIR and SEM. The niosomes were further characterized for pH, viscosity, spreadability, drug content, in vitro drug diffusion, stability studies, and sterility tests. Results: The Zidovudine niosomes demonstrated high drug encapsulation efficiency and compatibility with the polymer. The formulation exhibited significant in vitro drug diffusion at 90.86% and proved to be stable in sterility tests.

KEYWORDS: Zidovudine, Thin Film Hydration Technique, Niosomes, Novel drug Delivery System, Scanning Electron Microscopy.

INTRODUCTION

In the past few decades, considerable attention has been focused on the development of

novel drug delivery system (NDDS). The NDDS should ideally fulfil two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment.[1] Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery.[2]

Niosomes, also known as non-ionic surfactant vesicles, are microscopic lamellar structures created by mixing non-ionic surfactants from the alkyl or dialkyl polyglycerol ether class with cholesterol, followed by hydration in an aqueous media.[22, 23] These niosomes serve as a promising vehicle for drug delivery.[3] Being non-ionic, they can form either unilamellar or multilamellar vesicles from synthetic non-ionic surfactants, closely resembling liposomes.[24] The application of niosomal drug delivery is potentially beneficial for various pharmacological agents targeting different diseases. Niosomes have demonstrated effectiveness in release studies, making them a superior option for drug delivery systems. The drug is encapsulated within niosomes to enhance targeting to the appropriate tissue destination.[4]

Thin film hydration technique involves dissolving the components required for vesicle formation, such as surfactant and cholesterol, in a volatile organic solvent (like diethyl ether, chloroform, or methanol) within a round bottom flask.[25] The organic solvent is subsequently evaporated at room temperature (20°C) using a rotary evaporator, resulting in a thin layer of solid mixture adhering to the flask's wall. This dried surfactant film can then be rehydrated with an aqueous phase at temperatures ranging from 0-60°C with gentle agitation, leading to the formation of typical multilamellar niosomes.[5]

MATERIALS AND METHODS

MATERIALS

Zidovudine was acquired from Bafna Pharmaceuticals in Mumbai, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, sorbitan monolaurate (Span 20), polysorbate 20 (Tween 20), chloroform, methanol, dicetyl phosphate, and Triton X-100 were sourced from S.D. Fine Chem Ltd in Boisar, India. Cholesterol was obtained from Qualigens Chem Ltd, also located in Boisar, Mumbai, India. All other chemicals used were of analytical grade.

METHODS

DRUG-EXCIPIENT COMPATIBILITY STUDY[6, 7]

FTIR spectra of Zidovudine and a physical mixture of excipients (1:1) were obtained utilizing an Attenuated Total Reflectance (ATR) accessory with FTIR equipment. Individual spectra were recorded over a wavenumber range of 4000 to 400 cm^{-1} by averaging 10 scans at a resolution of 4 cm^{-1}.

FORMULATION OF ZIDOVUDINE NIOSOMES[8, 9]

Zidovudine-loaded niosomes were prepared using the thin film hydration technique. A precise quantity of cholesterol and surfactant was dissolved in a chloroform-methanol mixture at a volume ratio of 2:1 in a 100 ml volumetric flask. The corresponding amounts of drug and dicetyl phosphate were subsequently added to the solvent mixture. The solvent was evaporated using a rotary evaporator at 60°C with a rotation speed of 150 rpm to obtain a thin film on the flask's wall. Complete solvent removal was ensured through the application of vacuum.[26] The dry lipid film was then hydrated with 5 ml of phosphate buffer saline at pH 7.4, maintained at a temperature of 60±2°C for a duration of 2 hours, allowing for the formation of niosomes. All batches underwent sonication for 2 minutes using a probe sonicator. The ratios of cholesterol and surfactant employed in the formulation were noted.[27]

EVALUATION OF ZIDOVUDINE NIOSOMES

Removal of unentrapped drug from niosomes

The unentrapped drug from the niosomal formulation was separated using a centrifugation method. The niosomal suspension was placed in a centrifuge tube and centrifuged at 15,000 rpm for 30 minutes in a cooling centrifuge, maintaining a temperature of 5°C. The supernatant was then collected; it contained the unentrapped drug, while the pellet consisted of the drug-encapsulated vesicles.[28] The pellet was subsequently resuspended in phosphate buffer saline at pH 7.4 to yield a niosomal suspension free of unentrapped drug.[10]

Encapsulation efficiency

Drug-entrapped vesicles were separated from unentrapped drug using centrifugation. A 0.5 ml sample of the zidovudine-loaded niosome preparation was combined with 0.5 ml of 10% Triton X-100 and mixed thoroughly before being incubated for one hour. Triton X-100 was utilized to lyse the vesicles to release the encapsulated zidovudine.[29] The resulting solution was diluted with phosphate-buffered saline at pH 7.4 and filtered through Whatman filter

paper. The filtrate was analyzed spectrophotometrically at 267 nm, using a mixture of phosphate-buffered saline at pH 7.4 and Triton X-100 as a blank.[11, 12]

$$\text{Percent entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

***In-vitro* release study for niosomal preparation**

The niosomal formulation was placed in a 5 cm dialysis membrane and suspended in a beaker containing 100 ml of phosphate buffer saline at pH 7.4. The temperature of the medium was kept at 37±0.5°C. The medium was stirred at a constant speed using a magnetic stirrer.[30] At hourly intervals, 1 ml of the sample was taken out and replaced with 1 ml of fresh buffer to maintain the volume of the diffusion medium at 100 ml. The withdrawn samples were diluted to 10 ml using phosphate buffer saline at pH 7.4. The samples were then analyzed spectrophotometrically at a wavelength of 267 nm.[13, 14]

Zeta potential

The zeta potential of optimized zidovudine niosomal formulation was measured using Malvern zeta potential analyser.[15]

Scanning electron microscopy

The optimized formulation was characterized morphologically using scanning electron microscopy (SEM). The sample for SEM analysis was attached to the specimen stub with adhesive, and a small sample was mounted directly on double-sided adhesive tape.[31, 32] The analysis was conducted using a Hitachi scanning electron microscope operated at 15 kV, and photographs were taken.[16, 17]

Sterility testing

The sterility of the prepared and optimized zidovudine niosomal formulation was assessed through a sterility test in accordance with the Indian Pharmacopoeia (IP). The testing method utilized is Method I – Membrane Filtration Method.[18,19]

Stability study of zidovudine niosomes

The zidovudine niosome formulation was assessed for stability. The formulations were placed in a 20 ml sealed glass vial and stored in three distinct environments: 4°C, room temperature, and 45°C with 75% relative humidity for three months.[33] Samples from each batch were taken at one-month intervals and analyzed for entrapment efficiency and in vitro

drug release.[20, 21]

RESULTS AND DISCUSSION

Development of zidovudine niosomes

In this study, zidovudine loaded niosomes were prepared by Thin film hydration technique using cholesterol and non ionic surfactants such as span 20 and tween 20. Chloroform methanol mixture (2:1v/v) was used as solvent.

After evaporation of solvent from the formulation, thin film was formed. The thin film was hydrated and removed by phosphate buffer saline pH 7.4. Size of the vesicles in formulation was reduced by sonicating the formulation in Probe sonicator.

Formulations with different ratios of surfactant and cholesterol were prepared. Several physicochemical characteristics of niosomes such as morphology, vesicle size determination, drug release profile were investigated. And stability of optimized formulation at various temperatures was evaluated.

Dicetyl phosphate (DCP) also included in the formulation as charge inducing agent. The inclusion of charge inducing agent (DCP) prevented the aggregation and fusion of vesicles. Integrity and uniformity also maintained by dicetyl phosphate.

An effective niosomal drug delivery system should posses good physical and chemical stability on storage and should incorporate high drug loading with stable encapsulation.

Table 1: Composition of Zidovudine Niosomes.

Formulationcode	Zidovudine(mg)	Surfactant	Surfactant:Cholesterol (µM)
F_1	10	Span 20	100:100
F_2	10	Span 20	200:100
F_3	10	Span 20	300:100
F_4	10	Span 20	100:200
F_5	10	Span 20	200:200
F_6	10	Span 20	300:200
F_7	10	Span 20	400:200
F_8	10	Tween 20	100:100
F_9	10	Tween 20	200:100
F_{10}	10	Tween 20	300:100
F_{11}	10	Tween 20	100:200
F_{12}	10	Tween 20	200:200
F_{13}	10	Tween 20	300:200
F_{14}	10	Tween 20	400:200

FTIR studies

Pressed Pellet Technique was used to handle the sample in FTIR spectrometer. In this technique a required amount of sample was added and mixed well with potassium bromide and the mixture was pressed with special discs under high pressure into a transparent pellet and then inserted into special holder of IR spectrometer. The pellets were scanned from 4000 to 400 cm^{-1} in FTIR spectrophotometer and peaks obtained in both spectrums were identified. The wave number at which peaks appeared and peaks indicating functional groups.

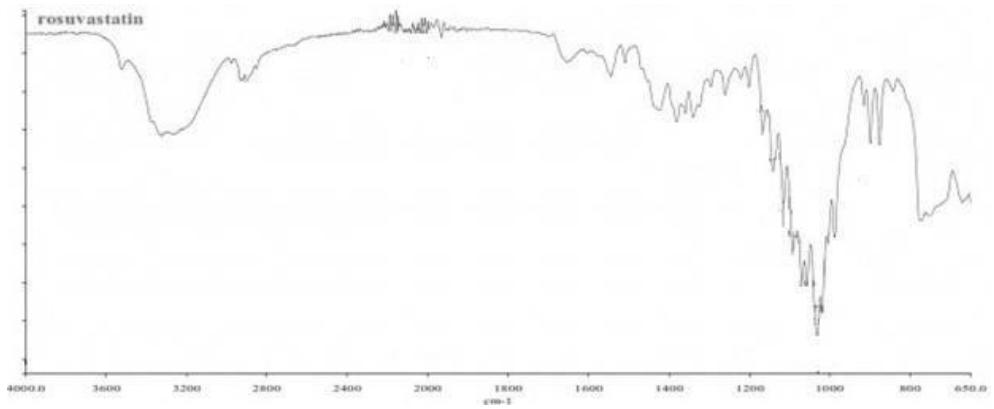

Fig.1: FT-IR Spectrum of Zidovudine.

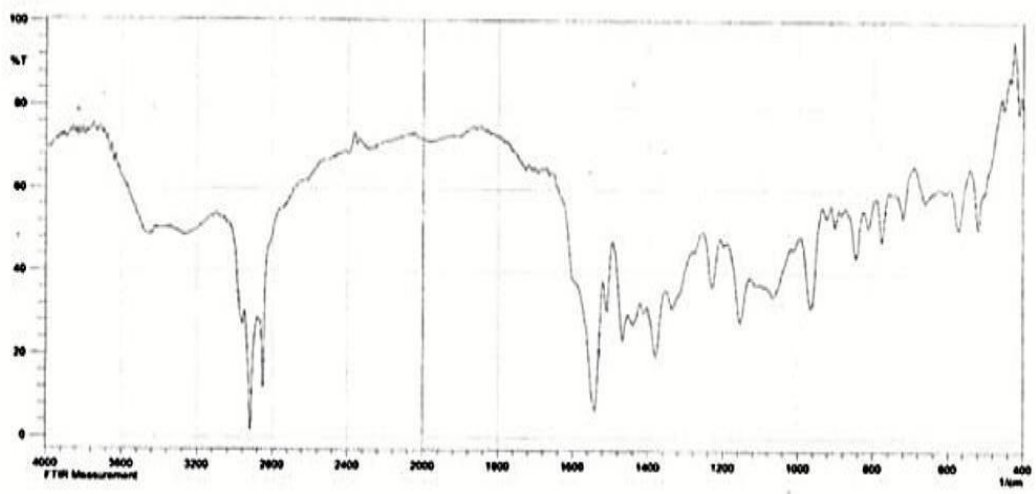

Fig.2: FT-IR Spectrum of Optimized batch (F13).

Table 2: FT-IR Studies.

Frequency		Group assigned
Pure drug	Physical mixture	
3402	3401	OH & NH - stretching
2852	2924	CH – stretching
1660	1654	C=O – stretching
1094	1106	CO – stretching

Optimization of process related variables

The prepared niosomal vesicles were influenced by some factors like speed of rotation, hydration volume, hydration medium and vaccum. Before loading the drug, these factors should be optimized using empty vesicle.

The vaccum used for drying of thin film was 350 mmHg. Vaccum below 350mmHg was insufficient for complete removal of solvent from the formulation and resulted in aggregation of niosomes on hydration. The vaccum above 350 mmHg resulted rapid evaporation of solvent which leads to entrapment of air bubbles on the surface of film. This caused poor entrapment of drug in niosomes and the vaccum of 350 mmHg produced lipid film had appreciable drug entrapment in the niosomes. Hence 350 mmHg of vaccum was considered as optimum range.

Table 3: Optimization of process related variables.

Surfactant: Cholesterol	Speed of Rotation (rpm)	Hydration Time (min)	Chloroform: methanol	Hydration volume	VesicleSize (µM)
	75	30			10.29±1.48
100:100	100	60	2:1	5 ml	9.41±1.09
	125	120			9.11±1.88
	150	120			8.69±1.88

The time of hydration of lipid film was carried from 60 -120 min. When hydration allowed to 120 min, formed niosomes were spherical in shape and existed in desired size range. So hydration time 120 min and hydration volume 5 ml were selected as optimum.

Thickness and uniformity of thin film was influenced by speed rotation of round bottom flask. The optimum speed was selected to 150 rpm. At this speed of rotation, thin film formed was uniform. The bath temperature of rotary evaporator was maintained at 60±2°C as optimum.

Evaluation of Zidovudine Niosomes

Removal of unentrapped drug from Niosomes

The unentrapped drug from niosomes was removed by centrifugation technique. The results are presented in following table.

Percentage drug entrapment efficiency

The entrapment efficiency of the niosomes is governed by the ability of formulation to retain drug molecule in aqueous core or in bilayer membrane of vesicles. After removal of unentrapped drug, the entrapment of all formulation was studied. Entrapment efficiency was varied with varying the surfactant and cholesterolratio. Various factors like lipid concentration, drug to lipid ratio, and cholesterol content will change the entrapment efficiency.

Table 4: Percentage drug entrapment Efficacy.

Formulation code	Surfactant: Cholesterol(µM)	Surfactant used	Percentage offree Drug (%)	Percentage entrapment Efficiency (%)
F_1	100:100	Span 20	37	63
F_2	200:100	Span 20	26	74
F_3	300:100	Span 20	32	68
F_4	100:200	Span 20	38	62
F_5	200:200	Span 20	28	72
F_6	300:200	Span 20	16	84
F_7	400:200	Span 20	29	71
F_8	100:100	Tween 20	32	68
F_9	200:100	Tween 20	19	81
F_{10}	300:100	Tween 20	27	73
F_{11}	100:200	Tween 20	29	71
F_{12}	200:200	Tween 20	15	85
F_{13}	300:200	Tween 20	8	92
F_{14}	400:200	Tween 20	24	76

Entrapment efficiency of formulation F_1 was found to be 63%. In formulation F_2, increasing the surfactant concentration, entrapment efficiency was increased to 74%. Further increasing the surfactant concentration in F_3, the entrapment efficiency was decreased to 68%, due to very low concentration of cholesterol.

So, to improve the entrapment efficiency cholesterol concentration was increased to 200µM in formulations F_4, F_5, F_6, and F_7. Because, increase the amount of cholesterol will improve the entrapment efficiency. But entrapment efficiency achieved in formulations F_4, F_5, F_6, and F_7 were 62%, 72%, 84%, and 71%. The drug entrapment was not improved satisfactorily. This is due to, surfactant used in those formulations was span 20. The span 20 is more hydrophobic, hence the hydrophilic drug gets encapsulated in the aqueous core only.

The formulations were tried with tween 20 in formulations F_8, F_9, and F_{10} contained 100 µM of cholesterol and 100, 200, 300 µM of tween 20 respectively. The releases were accordingly, 68%, 81%, and 73%, due to low level of cholesterol concentration.

So quantity of cholesterol was increased 200 µM in formulations F_{11}, F_{12}, F_{13} and F_{14}, entrapment efficiency was improved to 71%, 85%, 92% and 76% respectively. The increase in the entrapment efficiency is attributed to the ability of surfactant and cholesterol to cement the leaking space in the bilayer membrane, which in turn allows enhanced drug level in niosomes. Compared to span 20, the better entrapment efficiency was achieved in tween 20. This can be explained as the tweensare more water soluble, the hydrophilic Zidovudine drug, gets encapsulated as well aspartitions into vesicle membrane. Hence formulation F_{13} was optimized one.

***In vitro* release study**

The release of Zidovudine from niosomes was determined using the membrane diffusion technique. Release study was carried for 24 hours and results arenoted in following tables.

In vitro drug release was carried out for 24 hours using phosphate buffer as diffusion medium. It was found to be biphasic, and the release was controlled by lipid bilayer and dialysis membrane. Incorporation of cholesterol affected the release rate of encapsulated drug. *In vitro* drug release characteristics for formulations containing two different surfactants were compared. Zidovudine niosomes were tried with two different surfactant and cholesterol concentrations.

Table 5: *In vitro* drug release studies for formulations containing Span 20.

Time (Hrs)	Cumulative percentage drug release (%)						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
1	3.0	8.0	3.0	2.0	2.0	5.0	6.0
2	8.03	10.08	6.03	4.02	5.02	7.05	8.06
3	15.08	11.10	14.06	9.04	7.05	10.07	15.08
4	16.15	15.11	16.14	10.09	8.07	15.10	17.15
5	22.16	22.15	23.16	13.10	13.08	16.15	20.17
6	24.22	26.22	27.23	14.13	15.13	18.16	21.20
7	28.24	30.26	29.27	18.14	19.15	25.18	26.21
8	33.28	32.30	34.29	22.18	25.19	28.25	33.26
9	35.33	33.22	48.34	25.22	26.25	33.28	34.33
10	41.35	37.33	49.48	26.25	30.26	34.33	40.34
11	43.41	42.37	51.49	30.26	31.30	39.34	49.40
12	47.43	48.42	54.51	31.30	33.31	41.39	55.49

13	49.47	54.48	55.54	32.31	36.33	45.41	63.55
14	53.48	56.51	57.55	35.32	38.36	47.45	70.63
15	58.53	68.56	64.57	36.35	42.38	55.47	-
16	61.58	69.68	67.64	38.36	43.42	57.55	-
17	-	72.69	-	39.38	45.43	61.57	-
18	-	-	-	40.39	48.45	66.61	-
19	-	-	-	43.40	51.48	69.66	-
20	-	-	-	47.43	55.51	72.69	-
21	-	-	-	49.47	60.55	74.72	-
22	-	-	-	50.49	63.60	76.74	-
23	-	-	-	57.50	65.63	79.76	-
24	-	-	-	59.57	71.65	81.97	-

Drug release from formulations F_1, F_2 and F_3 were found to be 61.58%, 72.69%, and 67.64% in 16 hrs, 17 hrs and 16 hrs respectively. The release was not extended upto 24 hrs, because those formulations contained low cholesterol concentration.

Quantity of cholesterol was increased to 200µM in formulations F_4, F_5, F_6 and F_7 and release was achieved to 59.57% in 24 hrs, 71.65% in 24 hrs, 81.97% in 24 hours and 70.63% in 14 hrs. Except F_7, release from other formulation was extended to 24 hrs. This is due to higher concentration of surfactant in formulation F_7. Extended release was achieved but those formulations were not satisfied with percentage drug release. Higher release was found to be 81.83%. This is due to the water insoluble nature of span 20.

Due to lower concentration of cholesterol in formulations F_8, F_9 and F_{10}, the release were 65.61% 16 hrs, 79.48% at 18 hrs and 71.68% at 18 hrs respectively. Formulations F_{11}, F_{12}, F_{13} and F_{14} contained 200 µM of cholesterol showed 69.61% of drug release in 24 hrs, 83.78% of drug release in 24 hrs, 90.86% of drug release in 24 hrs and 73.69% of drug release in 20 hrs. Higher release of 90.86% was found in formulation contained 300:200 µmol ratio of surfactant and cholesterol. So formulation F_{13} (300:200 µmol) was considered as optimized formulation.

Table 6: *In vitro* drug release studies for formulations containing Tween 20.

Time (Hrs)	Cumulative percentage drug release (%)						
	F_8	**F_9**	**F_{10}**	**F_{11}**	**F_{12}**	**F_{13}**	**F_{14}**
1	4.0	9.0	4.0	4.0	8.0	12.0	5.0
2	8.04	14.09	15.04	05.04	10.08	19.12	10.05
3	11.08	15.14	18.15	08.05	15.10	21.19	16.10
ta4	19.11	19.15	21.18	11.08	16.15	23.21	18.16
5	22.19	24.19	28.21	12.11	18.16	24.23	23.18
6	34.22	29.24	30.28	15.12	23.18	29.24	29.23

7	36.34	32.29	34.30	19.15	26.23	31.29	31.29
8	39.36	35.32	39.34	24.19	28.26	39.31	32.31
9	41.39	36.35	42.39	25.24	33.28	41.39	35.32
10	45.41	41.36	48.42	30.25	35.33	44.41	39.35
11	47.45	48.41	49.48	31.30	39.35	48.44	42.39
12	50.47	51.48	53.49	33.31	40.39	50.48	44.42
13	53.5	53.51	58.53	39.33	44.40	52.50	49.44
14	59.53	58.53	59.58	40.39	46.44	54.52	50.49
15	61.59	61.58	63.59	42.40	51.46	57.54	52.50
16	65.61	68.61	66.63	46.42	55.51	58.57	57.52
17	-	74.68	68.66	47.46	60.55	64.58	60.57
18	-	79.74	71.68	50.47	66.60	68.64	62.60
19	-	-	-	51.50	67.66	69.68	69.62
20	-	-	-	53.51	69.67	75.69	73.69
21	-	-	-	57.53	71.69	79.75	-
22	-	-	-	58.57	72.71	84.79	-
23	-	-	-	61.58	78.72	86.84	-
24	-	-	-	69.61	83.78	90.86	-

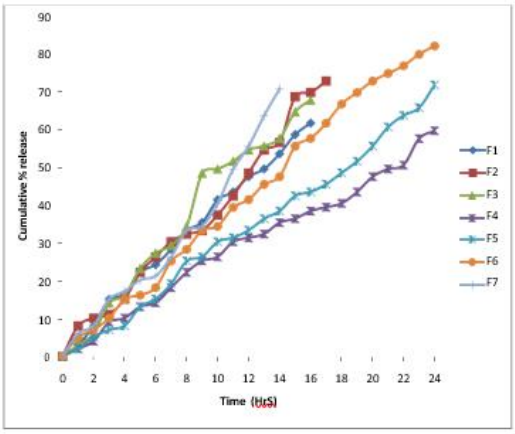

Fig: 3 *In-vitro* drug release of Zidovudine F1-F7.

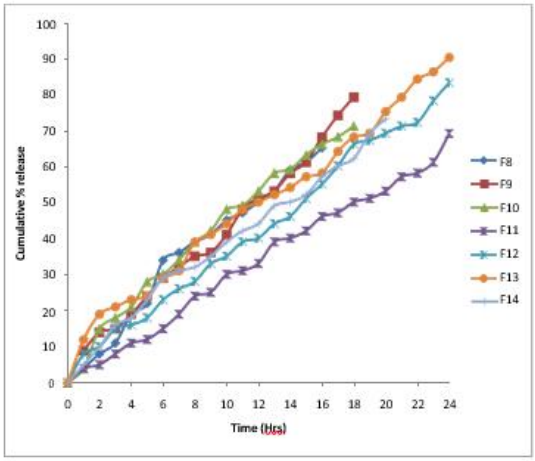

Fig: 4 *In-vitro* drug release of Zidovudine F8-F14.

Scanning electron microscopy

The surface characteristics of zidovudine niosomal formulation were studied by scanning electron microscopy. SEM image of prepared niosome formulation shows that the coating of surfactant cholesterol mixture on drug particles. Some particles in the images are broken, which might be due to handling and processing. Most of the vesicles are spherical and discrete sharp boundaries. The appearance of niosome vesicles in scanning electron micrograph is smooth, which indicates a thin and uniform coating over the drug. Based on the scale of micrograph, no significant change in size of particles is seen. The observation clearly shows that, there is no aggregation between the particles, due to

surfactant coating.

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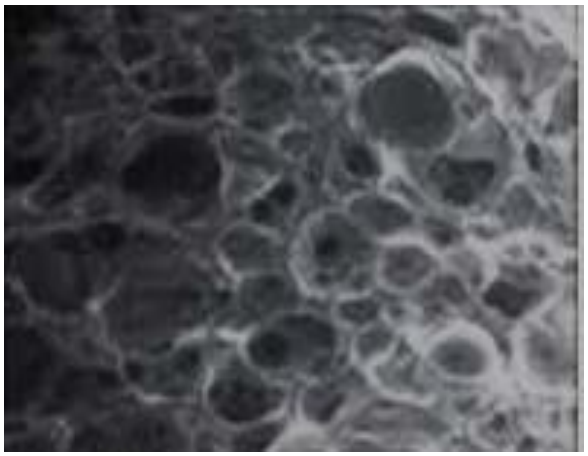


Fig. 5: SEM Image of optimized formulation F13.

Zeta potential

The addition of membrane additives affects zeta potential value depending onthe type of membrane additives. Zeta potential of optimized zidovudine niosome formulation was measured and found to -27.3 mv. The negative zeta potential observed with niosomes reflects the presence of negatively charged DCP on the surface of vesicles. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable. The particles being suspended. The formulation was found to be very effective for parenteral administration.

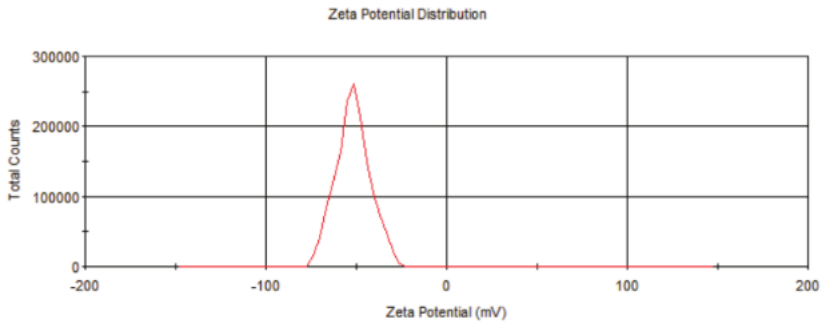


Fig. 6: Zeta potential distribution of optimized formulation F13.

Kinetics of drug release

The optimized formulation F_{13} was subjected to graphical treatment to assessthe kinetics of drug release.

ZERO ORDER PLOT

The optimized formulation F_{13} is most suitable parenteral administration as it founds to be good in the *in vitro* release kinetic study. The zero order plot obtained by plotting cumulative percentage drug release versus time. The regression value is 0.9887.

FIRST ORDER PLOT

The first order plot was obtained by plotting log cumulative percentage of drug remaining versus time. The regression value is 0.889.

HIGUCHI PLOT

The Higuchi plot was made by plotting cumulative percentage drug release versus square root of time. The regression value is 0.9539. It confirms that the release is diffusion mediated.

KORSEMEYER PLOT

The graph was obtained by log cumulative percentage drug release versus logtime. The n value is 0.6579. The n value ($0.45 < n < 0.89$) indicates that the drug release follows anomalous (non fickian) diffusion.

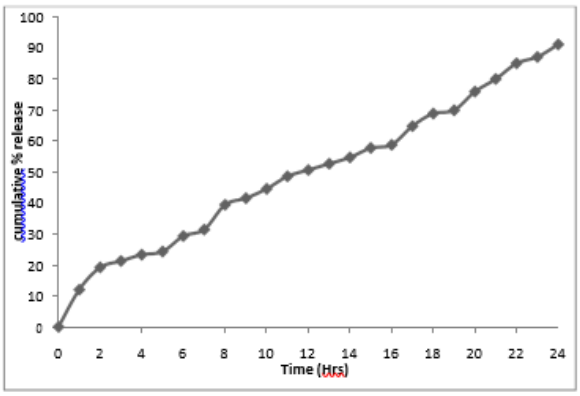


Fig: 7 Zero order plot for formulation F_{13}.

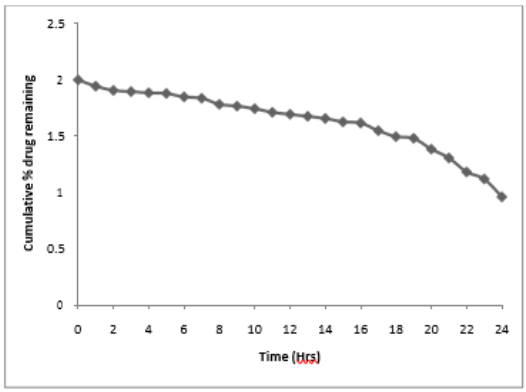
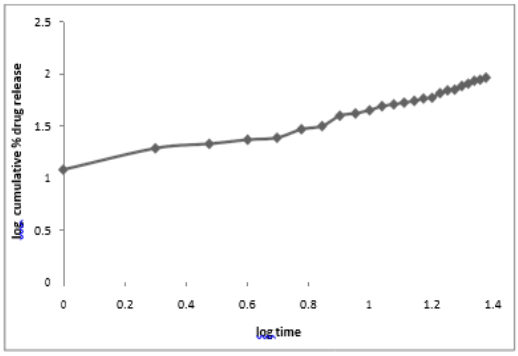


Fig: 8 First order plot for formulation F_{13}.



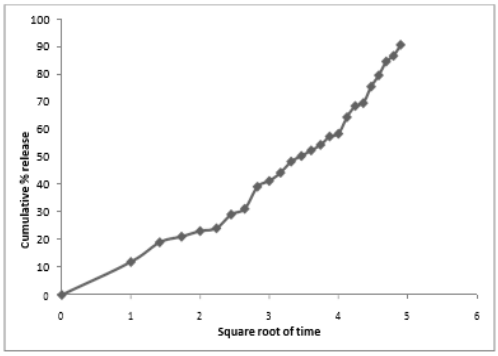


Fig: 9 Higuchi plot for formulation F_{13}. **Fig: 10 Korsemeyer plot for formulation F_{13}.**

CONCLUSION

In this study niosomal drug delivery system was developed using non-ionic surfactant incorporating Zidovudine by Thin film hydration technique. The prepared niosomal vesicles were quite stable. The formulation was subjected to Entrapment efficiency, Scanning electron microscopy, Invitro release, and Zeta potential analysis. From the results of experimental investigation, we concluded that, formulation F_{13} containing drug with 300:200 µmol (surfactant:cholesterol) ratio was showing higher percentage entrapment with desired sustained release of zidovudine. Hence formulation F_{13} was considered as optimized formulation. Invitro release from optimized zidovudine niosomal formulation (F_{13}) showedextended release for 24 hours. SEM image revealed the vesicles are exist spherical shape and uniform in size. Scanning electron micrograph shows there is no aggregation between the particles. Negative zeta potential value was observed in zeta potential analysis. This confirmed the presence of negative charge inducing agent in formulation. The formulation was checked for sterility as per I.P specification. The optimized formulation passes the sterility test. Stability study was carried out for the period of three months at various storage conditions. The results showed that the formulation remains stable at 4°c. The optimized formulation was found to follow zero order release pattern which was revealed by the linearity shown from the plot of Time Vs cumulative percentage drug release. From the drug release kinetic studies, we concluded that thedrug was released from niosome by a zero order diffusion controlled mechanism.

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