Development and evaluation of stavudine niosome by ether injection method.

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Abstract:

The main objective of the present study was to encapsulate Stavudine in niosomes for achieving prolonged release & longer duration of action. Niosome are now widely studied as an alternative delivery system to liposome. An increasing number of non ionic surfactant has been found to form vesicles, capable of entrapping hydrophilic and hydrophobic molecules. stavudine drug has shorter half life, Negligible protein binding. This drawback was decreased by formulating it as niosomes. In our present study we incorporated Stavudine into niosome by using ether injection method. In evaluation study the effect of the varying composition of non ionic surfactant and cholesterol on the properties such as zeta potential, drug content, vesicle size and drug release were studied. The result of the physiochemical characterisation and *in vitro* permeation studies of the prepared vesicles by dialysis membrane to get the idea of drug release. From the present investigation, it can be concluded that the developed niosome formulation of stavudine has shown great potential in the treatment of HIV by providing a prolonged release profile.

Keywords: Niosome, Cholesterol, Non ionic surfactant, Dialysis membrane,

Introduction:

Controlled drug delivery system release or deliver the drug at a rate dictated by the need of the body over a specified period of treatment. Greater attention is paid on development of controlled release drug delivery because it often prepared to permit the establishment and maintenance of any concentration at target site for longer period of time. Its utility is maximized through reduction in side effects and cure or control of disease condition in the shortest possible time by using smallest quantity of drug administered by most suitable route. The basic rationale of a controlled release drug delivery system is to optimize the bio-pharmaceutics, pharmacokinetics and pharmacodynamics properties of a drug. [1]

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bi-layer of non-ionic surface active agents and hence the name niosomes. [2]

In niosomes the drug is delivered directly to the body part where the therapeutic effect is required. So targeted drug delivery can also be achieved using niosomes. Thereby reducing the dose required to be administered to achieve the desired effect. It also leads to subsequent decrease in the side effects. [3]

The therapeutic efficacy of the drugs is improved by reducing the clearance rate, targeting to the specific site and by protecting the encapsulated drug. [4]

Niosomes provides the usages through various routes like oral, parenteral, topical, ocular ect. The bi-layer of the niosomes act as protector for enclosed active pharmaceutical ingredient at both inside and outside the body from deterogenous factors. So it can be used for labile and sensitive drugs for the delivery. [5]

Stavudine is used along with other medications to treat human immunodeficiency virus (HIV) infection. Stavudine is belongs to the class of medications called nucleoside reverse transcriptase inhibitors (NRTIs). It works by decreasing the amount of HIV in blood. Although stavudine does not cure HIV, it may decrease the chance of developing acquired immunodeficiency syndrome (AIDS) and HIV related illnesses such as serious infections or cancer. [6]

This drug has shorter half life, Negligible protein binding. The oral absorption rate of stavudine is over 80%. Stavudine is an analog of thymidine. It is phosphorylated by cellulose kinases into active triphosphate. Stavudine triphosphate inhibits the HIV reverse transcriptase by competing with natural substrate, thymidine triphosphate. It also causes termination of DNA replication by incorporating into the DNA strand. [7]

It is established that effective antiretroviral therapy requires long term treatment using higher dosage regimen to reduce and to maintain the viral suppression. In the case of stavudine the main limitations on the therapeutic effectiveness is very short biological half life. This necessitates frequent administration of large doses. Since it is crucial to maintain the systemic drug concentration within the therapeutic level throughout the treatment course.

In order to overcome these both disadvantages niosomes were selected as carrier in effective drug delivery system to deliver stavudine by which bio-distribution of drug can be altered to provide a greater degree of targeting of drugs to selected tissues in a controlled manner. [8]

Hence an attempt is made to provide niosomes containing stavudine with suitable surfactant by appropriate methods having the following advantages: Reduced the dose, decreased dosing frequency, overcoming the resistance of existing single drug regimen therapy, increased stability.

Materials and methods:

Stavudine used was a gift sample from Cipla Pvt Ltd., Mumbai and Span, Cholesterol, Chloroform and methanol was a gift samples from LR SD fine chemicals.

Method for preparation:

The surfactants and lipid were first dissolved in suitable organic solvent. The prepared organic phase was then added drop wise into aqueous phase containing drug. Thus the dissolved organic solution containing surfactant were injected drop wise through 24 gauge needle into preheated 15 ml distilled water containing drug, which is magnetically stirred and maintained at 65^{0} C for 45 min. Stirring was continued until all ether evaporating to get drug loaded niosome. Vaporization of ether leads to formation of vesicles. Formulation design of stavudine niosomes were shown in table a. [9]

Table a: formulation design of stavudine niosomes

Formulation Code	Drug (mg)	Span-60 (mg)	Cholesterol (mg)	Water(ml)
FA1	100	100	100	15
FA2	100	200	100	15
FA3	100	300	100	15
FA4	100	400	100	15
FA5	100	500	100	15

Results and discussion:

Characterization of prepared niosomes:

Fourier transform infra red spectroscopy analysis:

Drug-excipients compatibility studies were carried out using FT-IR. The spectra of pure drug (Stavudine), Span 60, cholesterol and their physical mixture (1:1:1) were recorded by using the potassium bromide (KBr) disk technique. Infrared spectrum was measured over the range of between 500 to 4000 cm-1. IR spectrum of pure drug, polymers and mixture were shown in figure no 1-4.[10]

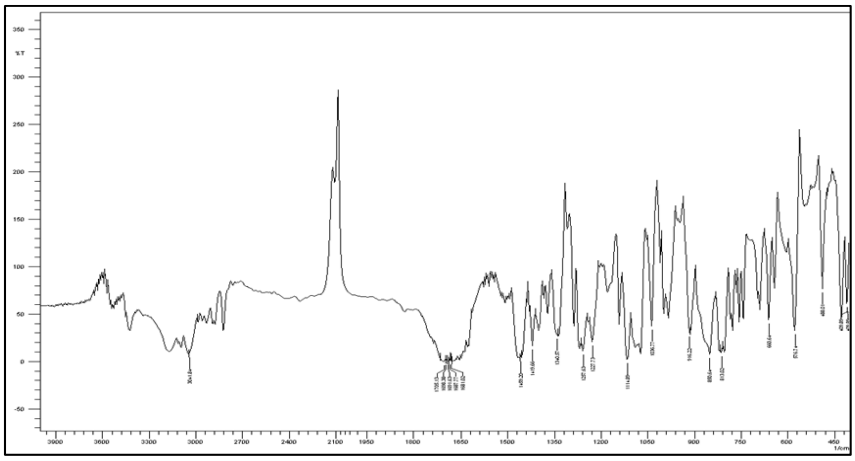


Figure no.1: IR spectrum of Stavudine

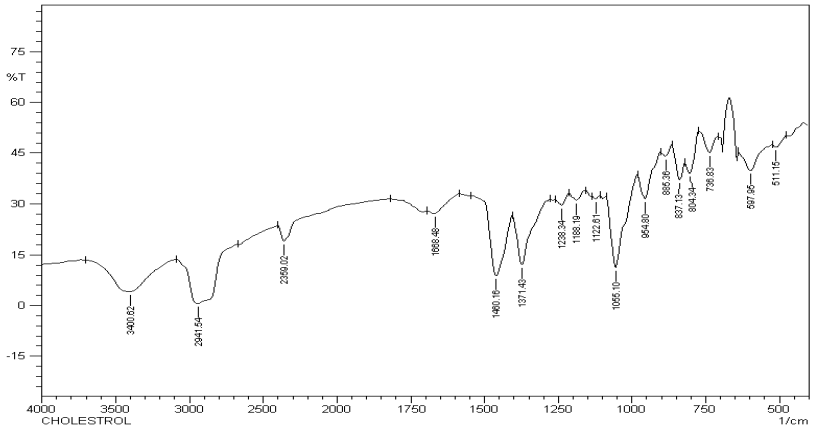


Figure no.2: IR spectrum of Cholestrol

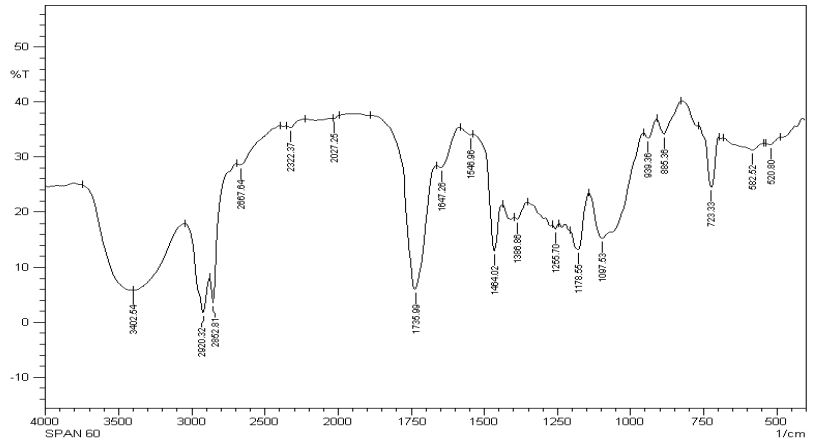


Figure no.3: IR spectrum of Span60

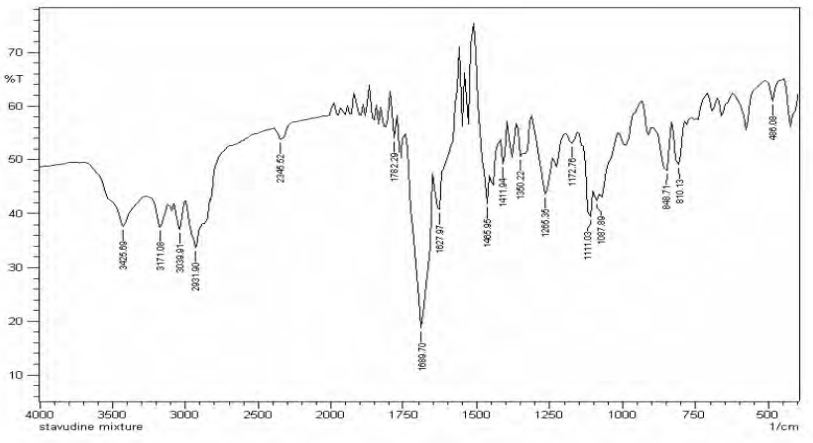


Figure no.4: IR spectrum of Stavudine mixture

Differential scanning calorimetry:

The DSC analysis of pure drug and drug-loaded niosomes were carried out using a diamond DSC (PerkinElmer, USA) to evaluate any possible drug-polymer interaction. The thermo grams of pure Stavudine and prepared niosomes were shown in figure no 5 and 6. [11]

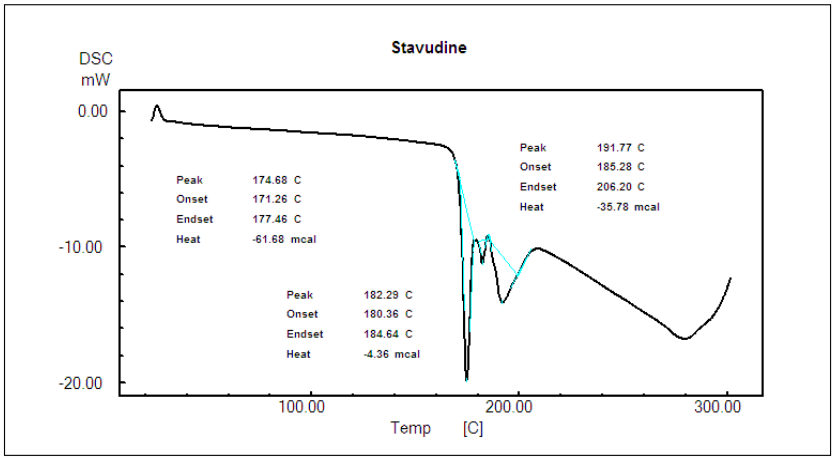


Figure no.5: DSC thermogram of Stavudine

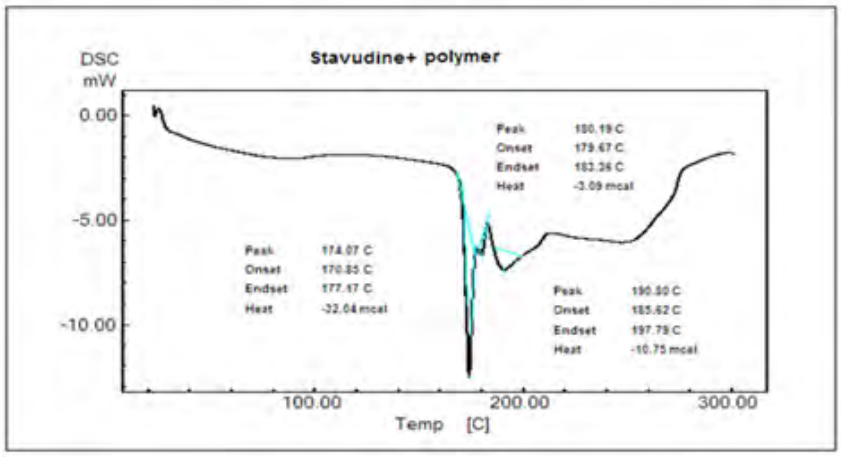


Figure no.6: DSC thermogram of Stavudine mixture

Drug content: [12], [13]

Stavudine content in niosomes was assayed by an UV spectrophotometric method.

Niosomes containing equivalent to 10 mg of drug were dissolved in a 10 ml of methanol. After suitable dilution absorbance was measured by UV spectrophotometer against blank at λmax 267 nm and drug content was calculated. Drug content of stavudine niosomes was shown in table b.

Table b: drug content of stavudine niosomes

Formulation code	%Drug content
FA1	**55.68**
FA2	**60.98**
FA3	**69.41**
FA4	**79.8**
FA5	**87.2**

Surface morphology:

Surface morphology of the optimized niosomal formulation will be determined by using a Scanning electron microscope.

Procedure: The samples are dried thoroughly in vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold palladium alloy of 120 °A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 °C with plasma voltage

about 20mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. SEM of ideal formulation FA5 was shown in figure no 7.

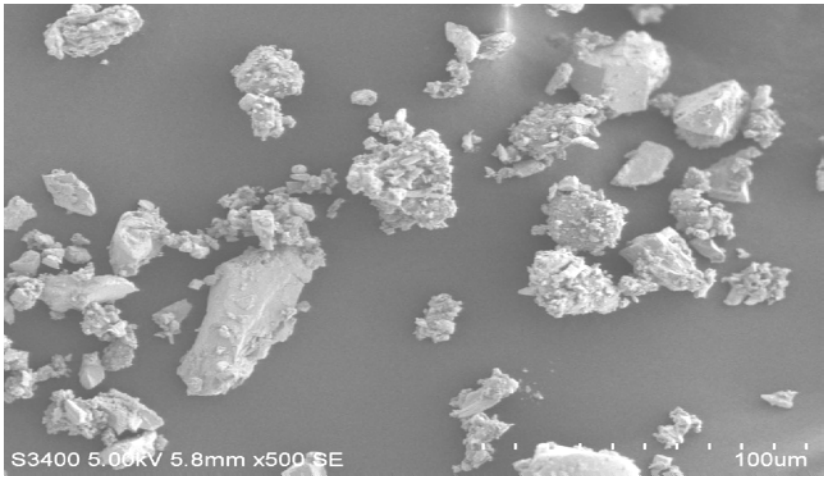


Figure no.7: SEM of formulation FA5

Vesicle size analysis:

Vesicle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. About 300 niosomes were measured individually, average was taken and their size distribution range and mean diameter were calculated and shown in table c.

Table c: vesicle size analysis of stavudine niosomes

Formulation code	Vesicle size analysis(µm)
FA1	**0.664**
FA2	**0.711**
FA3	**1.379**
FA4	**1.519**
FA5	**1.988**

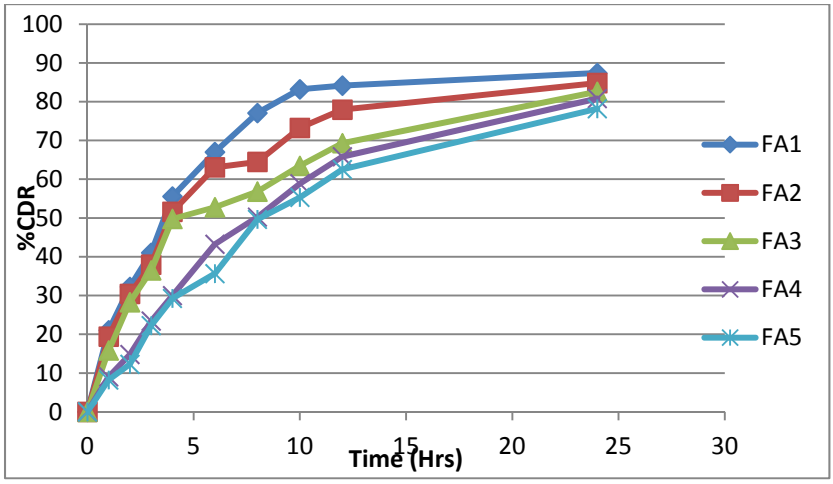
Zeta potential:

Zeta potential of the niosomes was measured by PALS zeta analyser. The zeta analyser mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the centre of the sample cell, and the scattered light at an angle of about 13º is detected. when an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed and this information is passed to the digital signal possessor and then to a computer. Zeta analyser software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potentials calculated.

***In vitro* drug release studies: diffusion study:**

The *in vitro* releases of Stavudine niosomes were studied by open ended cylinder

method. The diffusion cell apparatus consist of a glass tube with an inner diameter of 2.5 cm, open at both ends. One end of the tube tied with dialysis membrane which serves as a donar compartment. The niosomes equivalent to 50 mg of drug was taken in this compartment and placed in a beaker containing 100 ml of phosphate buffer pH 7.4 stirred at a moderate speed maintaining the 37 ºC temperature. Periodically 1ml of samples was withdrawn and same volume of medium was replaced. The samples were assayed by UV Spectrophotometer at 267 nm using phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and plotted against time (t) shown in figure no 8.



TIME(Hrs)	FA1	FA2	FA3	FA4	FA5
0	0	0	0	0	0
1	21.05	19.41	15.84	9.05	8.23
2	32.24	30.42	28.27	14.79	12.27
3	41.03	37.98	36.47	23.41	22.25
4	55.56	51.61	49.77	29.99	29.25
6	67.01	63.06	52.77	43.23	35.65
8	77.07	64.46	56.82	50.24	49.65
10	83.19	73.24	63.45	58.85	55.31
12	84.15	77.95	69.29	65.81	62.51
24	87.39	84.81	82.56	80.8	78.18

Figure no.8: Cumulative % release of stavudine niosomes.

Release kinetics studies: [14], [15], [16]

In order to understand the kinetic and mechanism of drug release, the result of in vitro drug release study of niosomes were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), Peppas plot (log of cumulative % drug release vs. log time). R^2 and k values were calculated for the linear curve obtained by regression analysis shown in table d.

Table d: Release kinetics of stavudine niosomes.

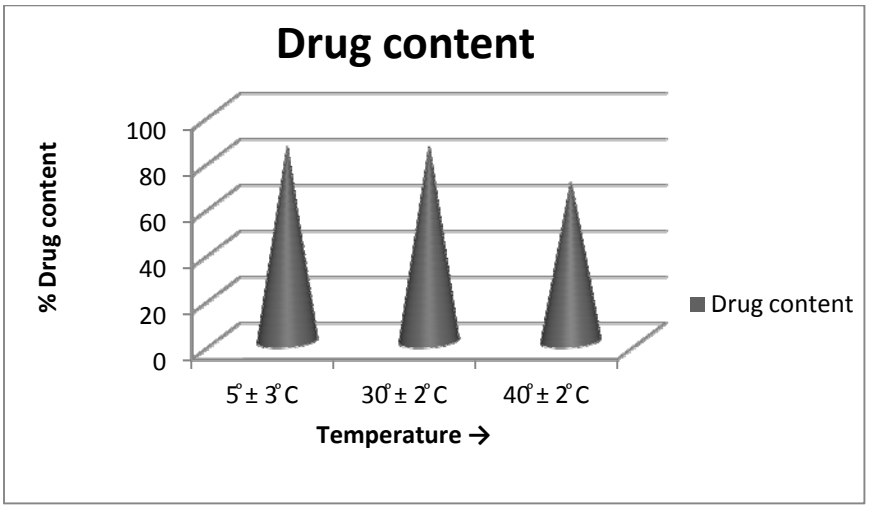
Formulaton code	%CDR	Zero order	First order	Higuchi plot	Peppas plot	'n' value
FA1	**87.39**	**0.633**	**0.771**	**0.962**	**0.962**	**0.542**
FA2	**84.81**	**0.689**	**0.875**	**0.978**	**0.97**	**0.536**
FA3	**82.56**	**0.749**	**0.948**	**0.98**	**0.957**	**0.553**
FA4	**80.8**	**0.853**	**0.97**	**0.97**	**0.993**	**0.804**
FA5	**78.18**	**0.866**	**0.969**	**0.961**	**0.986**	**0.834**

Stability study: [17]

The stability study was carried out using the batch FA-5. Formulation FA-5 was divided into 3 sets of samples and stored at 4ºC in refrigerator, room temperature 45ºC ± 2 ºC, 75% RH in humidity control ovens. After 90 days drug content of all samples were determined by the method as in drug content and result was shown in figure no 9. In vitro release study of formulation FA5 was also carried out after 90 days of storage was shown in table e and figure no 10.

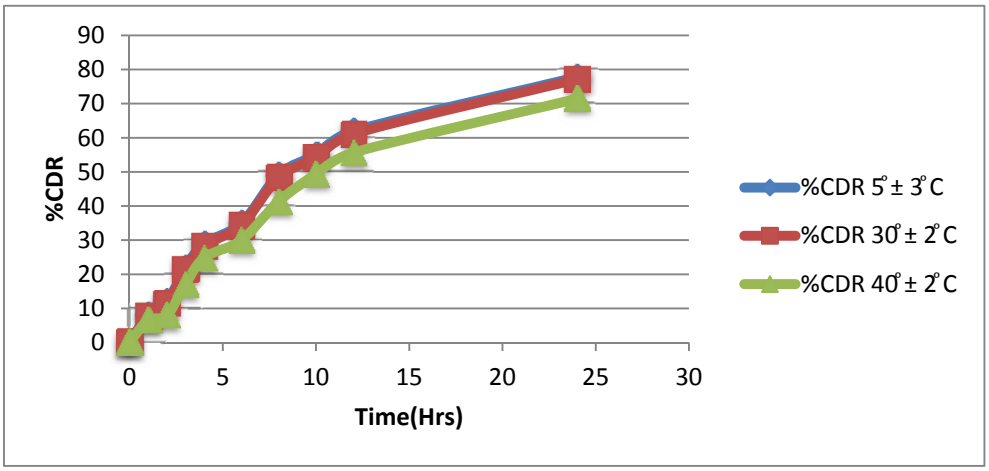
Table e: Selected formulation FA5 after three months Storage at 5º ± 3ºC, room Temperature 30º ± 2C and 40º ± 2ºC.

Time in hrs	%CDR	%CDR	%CDR
	5 ± 3 C	30 ± 2 C	40 ± 2 C
0	0	0	0
1	7.99	7.55	6.56
2	11.99	11.39	7.93
3	22.01	21.53	17.01
4	28.99	28.34	24.83
6	34.98	34.46	30.01
8	49.18	48.37	41.35
10	55.05	54.22	49.35
12	61.99	61.12	55.64
24	77.78	77.17	71.44



temperature in ºC	Drug content
5º± 3ºC	85.3
30º± 2ºC	84.9
40º± 2ºC	69.5

Figure no.9: Stability study: comparison of % drug content of formulation FA-5 at 5º ± 3ºC, room Temperature 30º ± 2C and 40º ± 2ºC.



Time in hrs	%CDR	%CDR	%CDR
	5° ± 3° C	30° ± 2° C	40° ± 2° C
0	0	0	0
1	7.99	7.55	6.56
2	11.99	11.39	7.93
3	22.01	21.53	17.01
4	28.99	28.34	24.83
6	34.98	34.46	30.01
8	49.18	48.37	41.35
10	55.05	54.22	49.35
12	61.99	61.12	55.64
24	77.78	77.17	71.44

Figure no.10: Stability study: comparison of in vitro drug release Profile for formulation FA5 at 5° ± 3° C, room Temperature 30° ± 2° C and 40° ± 2° C.

Conclusion:

Niosomes prepared by ether injection technique were found to be discrete and through SEM analysis. The drug content containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found. Thus there was a steady increase in the drug content on increasing the polymer concentration in the formulation. The formulation FA-5 registered highest entrapment of 87.2%. The interaction study between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of pure and drug loaded niosomes. Differential scanning calorimetry study thermogram of pure stavudine showed a sharp endothermic peak at 174 °C. The thermo grams of formulations FA-5 of Fig. 6, showed the same endothermic peak at the similar temperature. This further confirmed that there is no drug to polymer interaction. Zeta potential of all formulated niosomes was in the range of -24.8 to -29.54 mV, which indicates that they are moderately stable.

Cumulative percentage drug released for FA-1, FA-2, FA-3, FA-4 and FA-5 after 24 h were found to be 87.39, 84.81, 82.56, 80.80 and 78.18 respectively. Zeta potential for FA-5 was found to be -27.04 mV and it shows good stability. It was apparent that in vitro release of stavudine showed slow drug release. In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, Higuchi, and Peppas respectively. As indicated by higher R^2 values, the drug release from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled.

The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, non-Fickian diffusion or zero order. ‘n’ value could be used to characterize different release mechanisms. The ‘n’ values for all formulations were found to be more than 0.50. This indicates that the release approximates non-Fickian diffusion mechanism.

From the above study it can be concluded that Stavudine niosome can be used as promising delivery system. According to the above evaluation formulation F5 shows sustain release. This attempt is made to provide niosome containing stavudine with suitable surfactant by appropriate methods having the advantages in reducing the dose, decreases dosing frequency, overcome the resistance of existing single drug regimen therapy and increases stability.

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