DEVELOPMENT AND EVALUATION OF STAVUDINE NIOSOME BY THIN FILM HYDRATION METHOD

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ABSTRACT

Aim: The aim of the present study is an attempt to formulate and evaluate niosomal drug delivery of Stavudine by using cholesterol and span 60 as a surface active agent for potentially treating HIV infection. **Method:** The formulations FS1-FS5 were prepared by varying the concentration of cholesterol and surfactant (span 60) by thin film hydration method. The formulation variable like concentration of surfactant and cholesterol significantly affected the *in vitro* drug release from the prepared formulations. The *in vitro* drug release studies were performed in phosphate buffer pH 7.4. **Results:** Drug and physical mixture were characterised by FTIR and DSC, the result of IR and DSC study showed that no interaction between drug and polymers and other formulation parameters like vesicle size, drug content, entrapment efficiency and *in vitro* release of formulated niosomes were evaluated which showed better results. Formulation prepared by thin film hydration method showed proper controlleddrug release after 24 hrs of dissolution studies. **Conclusion**: The main objective of this study was to design suitable niosome encapsulated drug delivery for antiretroviral drugs like stavudine, to study the *in vitro* behaviour of the prepared system and to investigate the niosome encapsulated drug for its activity. Finding of all this investigation conclusively demonstrate prolongation of drug release at a controlled rate, after encapsulation of stavudine. It shows that niosomal drug delivery system may be a promising carrier for the novel drug delivery system.

KEYWORDS

Stavudine, Niosome, Controlled delivery, Surfactant and Cholesterol.

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INTRODUCTION

The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Ehrlich, who proposed drug delivery to be as a "magic bullet"[1].

During the past decade formulation of vesicles as a tool to improve drug delivery has created a lot of interest amongst the scientist working in the area of drug delivery systems. Vesicular system such as

liposomes, niosomes, transferosomes, pharmacosomes and ethosomes provide an alternative to improve the drug delivery. Design and development of novel drug delivery system (NDDS) has two prerequisites. First, it should deliver the drug in accordance with a predetermined rate and second it should release therapeutically effective amount of drug at the site of action. Conventional dosage forms are unable to meet this requisites[2].

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[3].

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[4].

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[5].The therapeutic efficacy of the drugs is improved by reducing the clearance rate, targeting to the specific site and by protecting the encapsulated drug[6].

Niosomes provides the usages through various routes like oral, parenteral, topical, ocular *etc*. The bi-layer of the niosomes act as protector for enclosed active pharmaceutical ingredient at both inside and outside the body from heterogeneous factors. So it can be used for labile and sensitive drugs for the delivery[7].

They can entrap solutes and drugs and are osmotically active and stable. In addition, handling and storage of the surfactant requires no special conditions. Niosomes behave *in vivo* like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability[8].

Niosomes are microscopic lamellar structure of size range between 10-1000 nm and consists of biodegradable, non-immunogenic and biocompatible surfactants. The niosomes are amphiphilic in nature, which allows entrapment of hydrophilic drug in the core cavity and hydrophobic drugs in the non-polar region present within the bilayer, hence, both hydrophilic and hydrophobic drugs can be incorporated into niosomes[9]. The parts of niosomes are given in below Figure No.1.

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[10].

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[11].

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[12].

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.

MATERIAL AND METHODS
Thin Film Hydration Technique[13]

Niosome was prepared by Thin Film Hydration Technique. Accurately weighed quantity of cholesterol and span 60 were dissolved in chloroform – methanol mixture (1:1 v/v) in 100ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 15ml distilled water at a temperature of $60 \pm 2°$ C for a period of 1hour until the formation of Niosomes. The ratios of the formulations were 1:1, 1:2, 1:3, 1:4, and 1:5 of drug: span-60.

RESULTS AND DISCUSSION
Characterization of prepared niosomes
FTIR

The compatibility of the drug and polymer were studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to a potassium bromide disc was mixed with dry KBr. The mixture was grind into a fine powder using an agate mortar and then compressed into a KBr disc in a hydraulic press at a pressure of 1000 psi. The characteristic peaks were recorded. FT-IR spectrum of Stavudine was compared with FT-IR spectra of drug and polymers are shown in Figure No. 2 to 5.

DSC

Niosomal pellets were lyophilized. Differential scanning calorimetry (DSC) thermo grams for individual components, Span 60, Cholesterol as well as the drug powder, were investigated. A heating rate of 5°C/min was employed over a temperature range (30–250) °C are shown in Figure No.6 and 7.

Evaluation of niosomes[14,15]
Vesicle size analysis

The particle size of niosomes was determined by using motic digital microscope. All the prepared batches of niosomes were viewed under microscope to study their size. Size of niosomal vesicles from each batch was measured at different location on slide by taking a small drop of niosomal dispersion on it and average size of niosomal vesicles were determined are shown in Table No.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 are shown in Figure No.9.

Zeta potential

Zeta potential of the niosomes was measured by zeta potential 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 are shown in Figure No.10.

Entrapment efficiency

For the determination of percent entrapment efficiency (EE %) the unentraped drug is first separated using centrifugation method. The resulting solution is then separated and supernatant liquid is collected. 1 ml of supernatant was taken and diluted with phosphate buffer upto 10 ml and absorbance was recorded at 266 nm using UV spectrophotometer. The % entrapment efficiency was determined by following formula:

%EE=Total amount of drug-unentraped drug/Total amount added×100

Drug content

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 266 nm and drug content was calculated are shown in Table No.3.

***In vitro* release 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 ℃ temperature.

Periodically 1ml of samples were withdrawn and same volume of medium was replaced. The samples were assayed by UV Spectrophotometer at 266 nm using phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and plotted against time are shown in Table No.4 and Figure No.11.

Release kinetics studies[16-18]

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 No.5.

Stability studies

The purpose of stability studies is to provide evidence on the quality of a drug substance or drug product, which varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The optimized formulations were divided into 3 sets of samples and stored at 5º ± 3℃, 30º ± 2℃, 65% ± 5% RH and 40º ± 2℃, 75% ± 5% RH in humidity control ovens. After 90 days drug content and *in vitro* release studies of sample were carried out. Drug content and drug release were fixed as physical parameters for stability testing are shown in Table No.6 and Figure No.12 and 13.

Table No.1: Formulation design of Stavudine niosomes by thin film hydration method

S.No	Formulation Code	Drug (mg)	Span-60 (mg)	Cholesterol (mg)	Water (ml)
1	FS1	100	100	100	15
2	FS2	100	200	100	15
3	FS3	100	300	100	15
4	FS4	100	400	100	15
5	FS5	100	500	100	15

Table No.2: Average vesicle size of FS1-FS5 formulation

S.No	Formulation code	Average vesicle size in μm
1	FS1	0.55
2	FS2	0.59
3	FS3	0.71
4	FS4	0.91
5	FS5	1.15

Table No.3: Drug content and entrapment efficiency in niosomes prepared by thin film hydration method of formulation FS1-FS5

S.No	Formulation code	% Drug content	% Entrapment efficiency
1	FS1	57.60	55.82
2	FS2	64.10	61.52
3	FS3	78.20	65.43
4	FS4	89.60	81.24
5	FS5	84.31	80.52

Table No.4: *In vitro* release study of formulation FS6-FS10 in phosphate buffer pH 7.4

S.No	Time hrs	% Cumulative drug release				
		FS1	FS2	FS3	FS4	FS5
1	0	0	0	0	0	0
2	1	12.81	12.01	11.85	7.84	8.15
3	2	26.32	23.46	20.17	15.53	20.11
4	3	39.19	37.89	34.19	27.83	29.25
5	4	50.34	47.72	44.71	36.03	37.31
6	6	58.93	56.02	52.58	47.87	49.84
7	8	69.13	65.33	60.32	54.31	59.72
8	10	84.73	79.27	77.29	68.71	73.05
9	12	88.09	85.45	81.04	72.14	76.2
10	24	94.09	92.32	85.08	77.93	81.49

Table No.5: Results of model fitting for Stavudine niosomes

| S.No	Formulation code	Zero order	First order	Higuchi plot	Peppas plot	
					R^2 values	"n" values
1	FS1	0.727	0.915	0.976	0.962	0.710
2	FS2	0.750	0.929	0.979	0.964	0.727
3	FS3	0.732	0.838	0.970	0.967	0.731
4	FS4	0.755	0.846	0.966	0.966	0.842
5	FS5	0.746	0.843	0.969	0.963	0.819

Table No.6: Effect of storage condition on the stability of optimized formulation
FS4 at 5º ± 3ºC, 30º ± 2ºC, 65% ± 5% RH and 40º ± 2ºC, 75% ± 5% RH

S.No	Parameters	Duration of months			
		0	1	2	3
1	% Drug content	89.6	89.47	88.1	87.9
		89.6	89.6	88.5	88.1
		89.6	84.3	78.7	70.14
2	*In vitro* Drug release	72.14	72.01	70.91	70.65
		72.14	72.09	71.56	71.01
		72.14	70.91	67.87	67.23
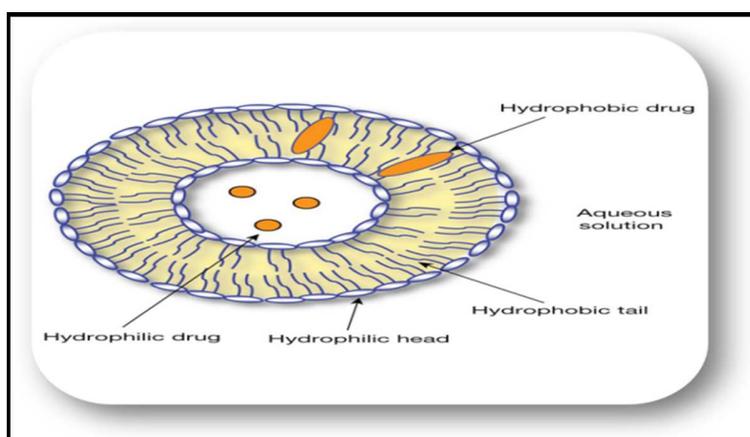
Figure No.1: Parts of niosomes
FTIR studies to find out the compatibility of drug with the excipients
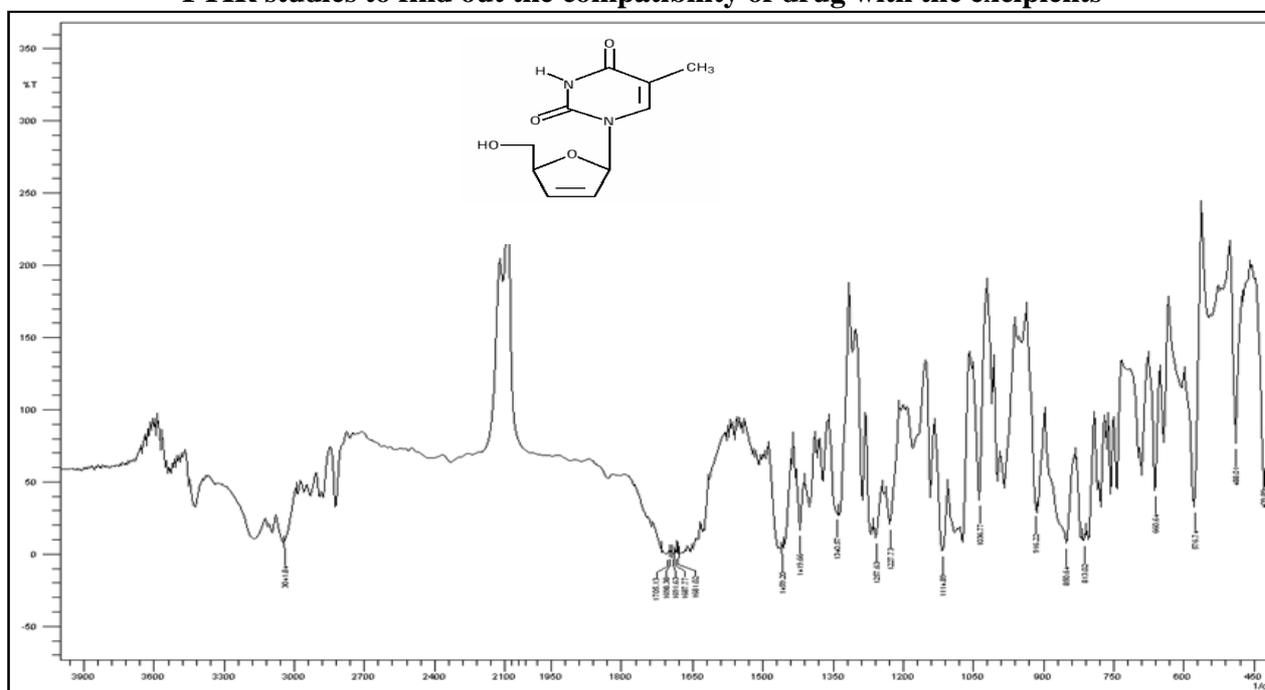
Figure No.2: FTIR spectrum of pure drug Stavudine
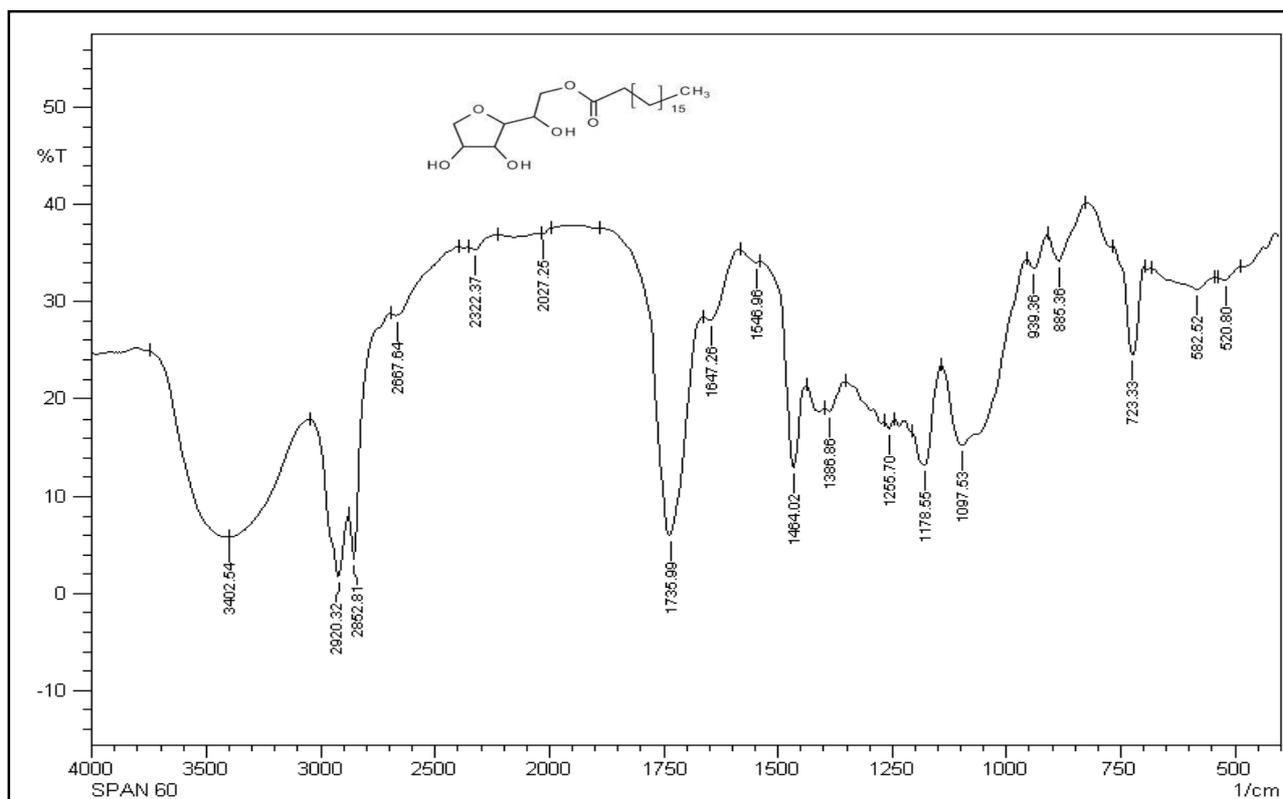
Figure No.3: FTIR spectrum of span-60
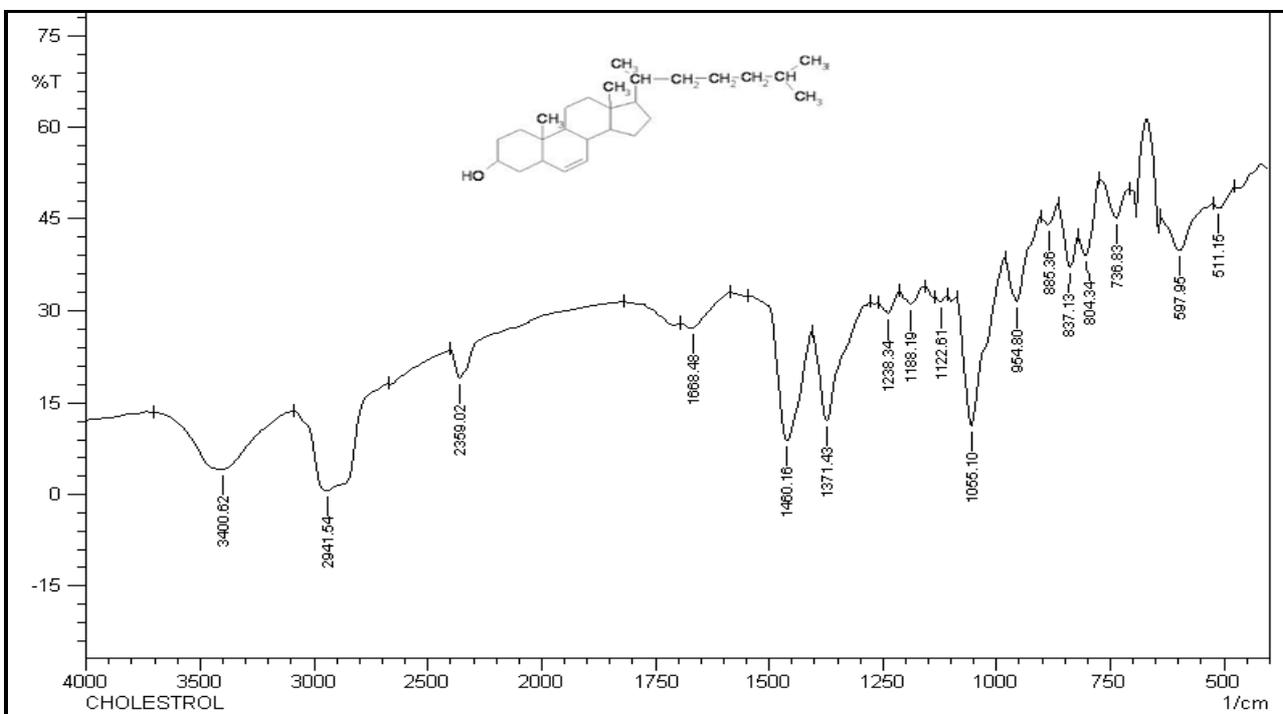
Figure No.4: FTIR spectrum of Cholesterol
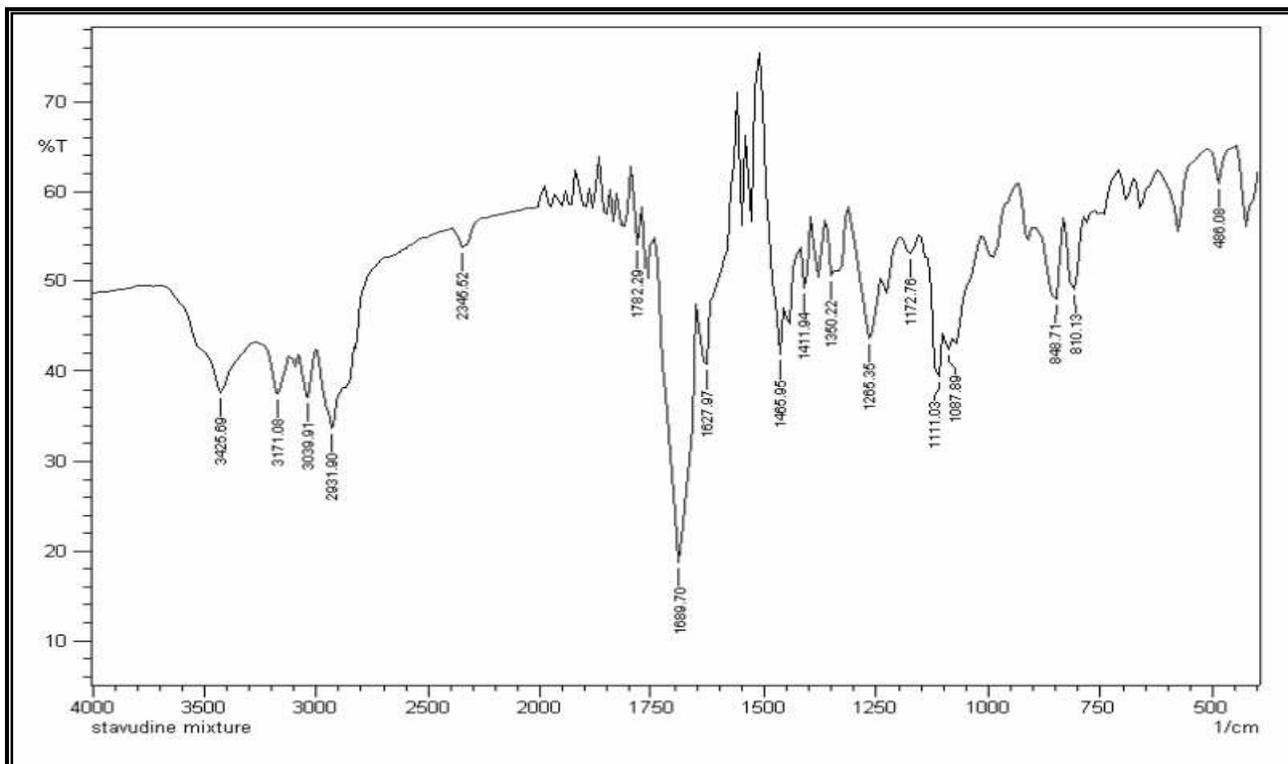
Figure No.5: FTIR spectrum of physical mixture of Stavudine, Span-60 and Cholesterol Differential scanning calorimetry
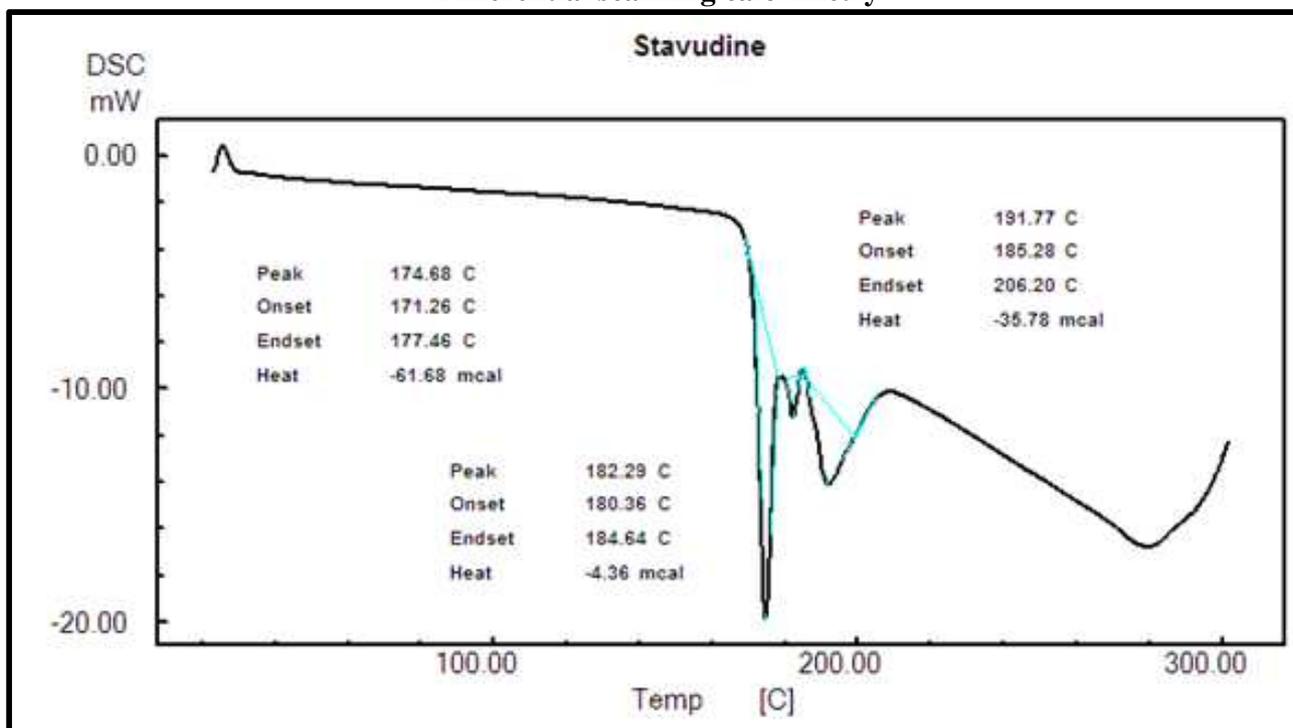
Figure No.6: DSC Thermogram of Stavudine
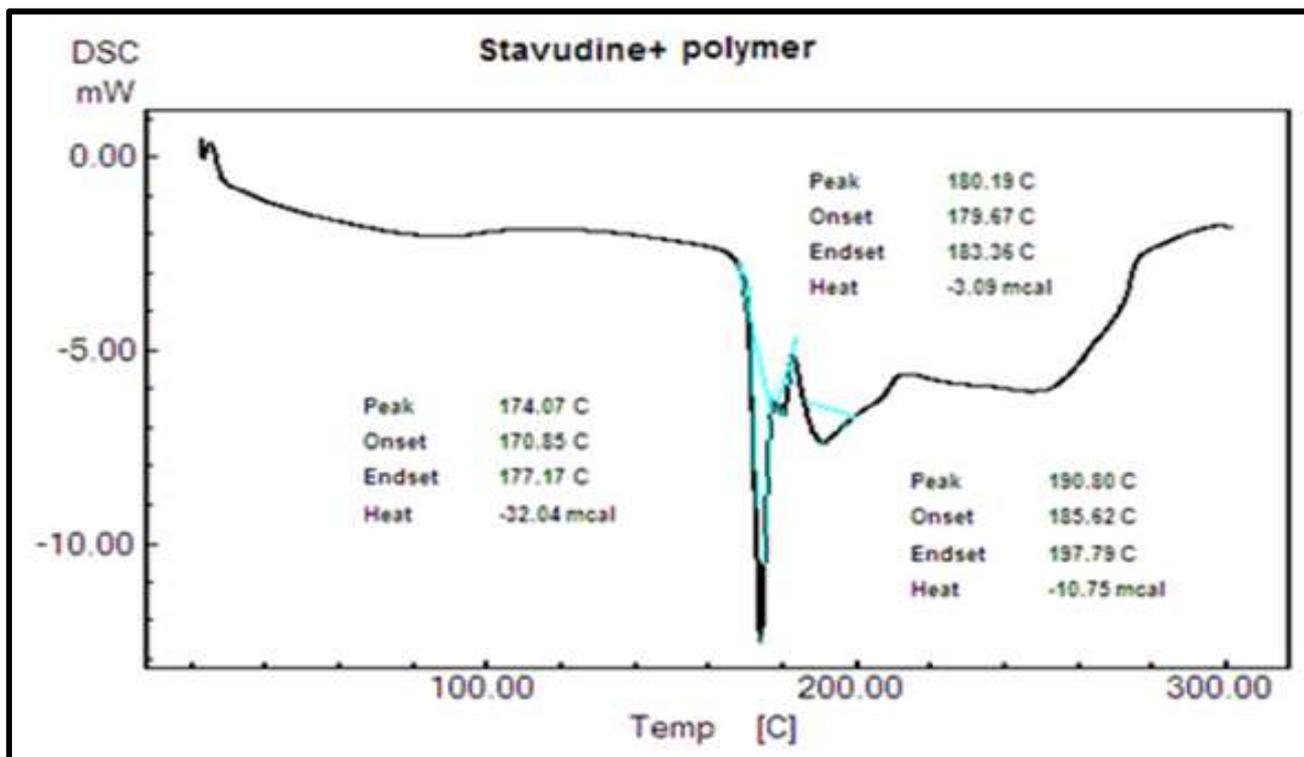
**Figure No.7: DSC Thermo gram of Stavudine mixture**
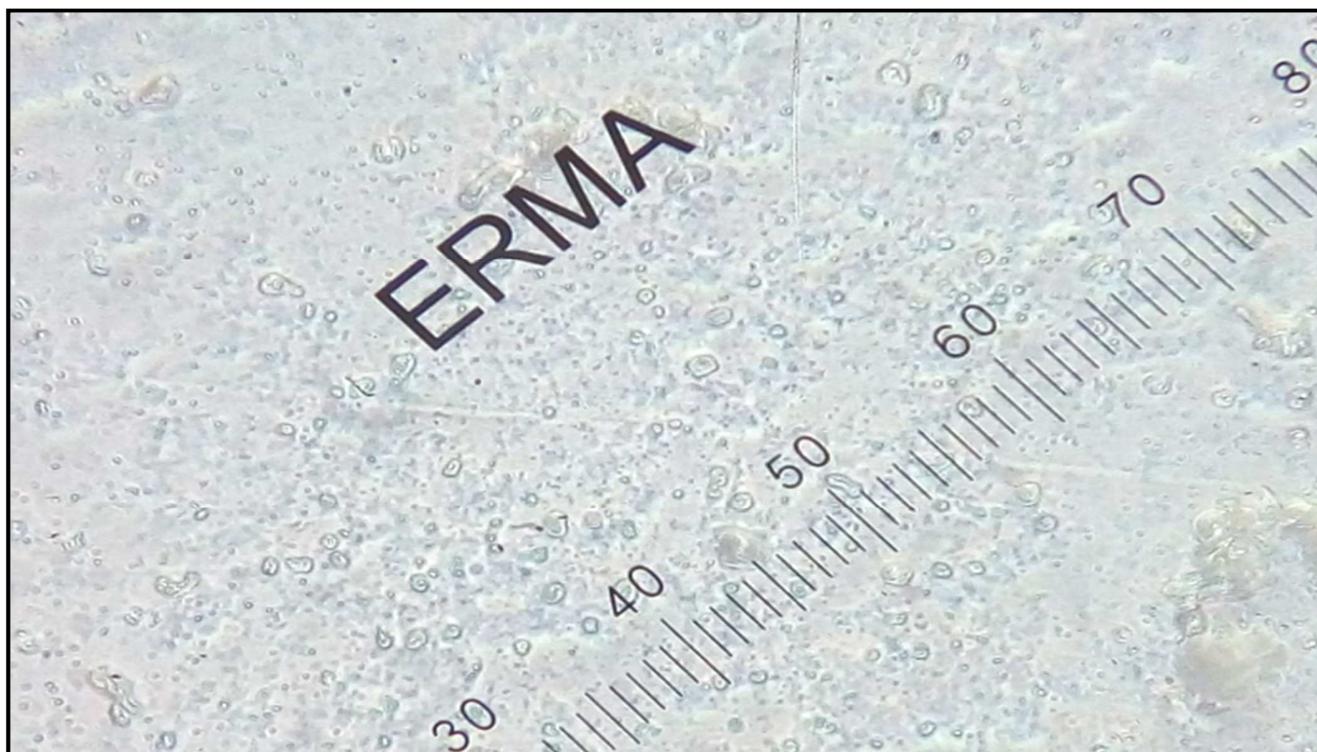

Figure No.8: Microphotographs of formulation FS4
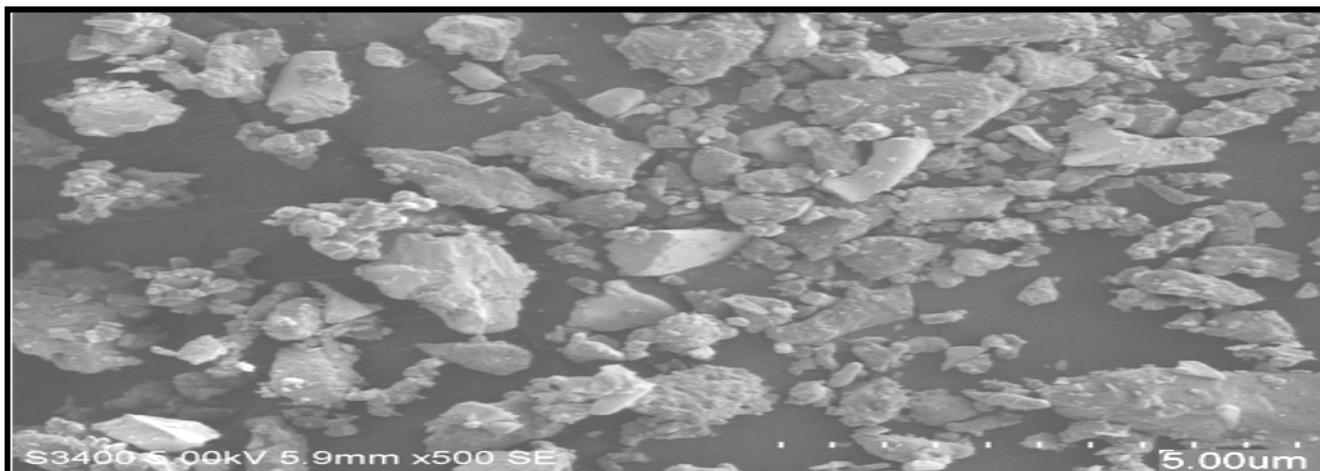
Figure No.9: SEM images of formulation FS4
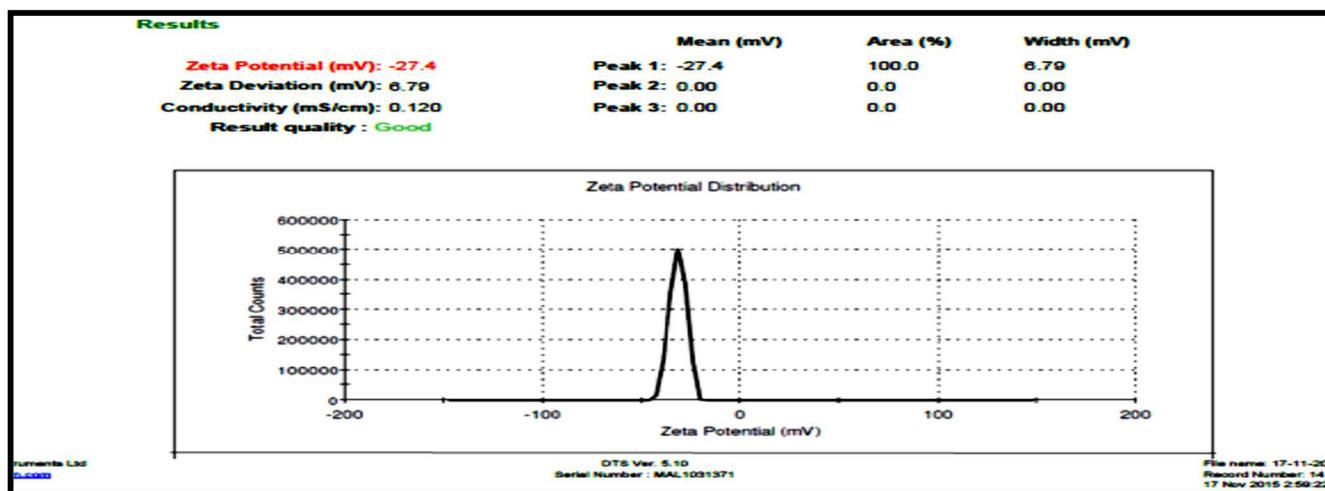
**Figure No.10: Zeta potential analysis of formulation FS4**
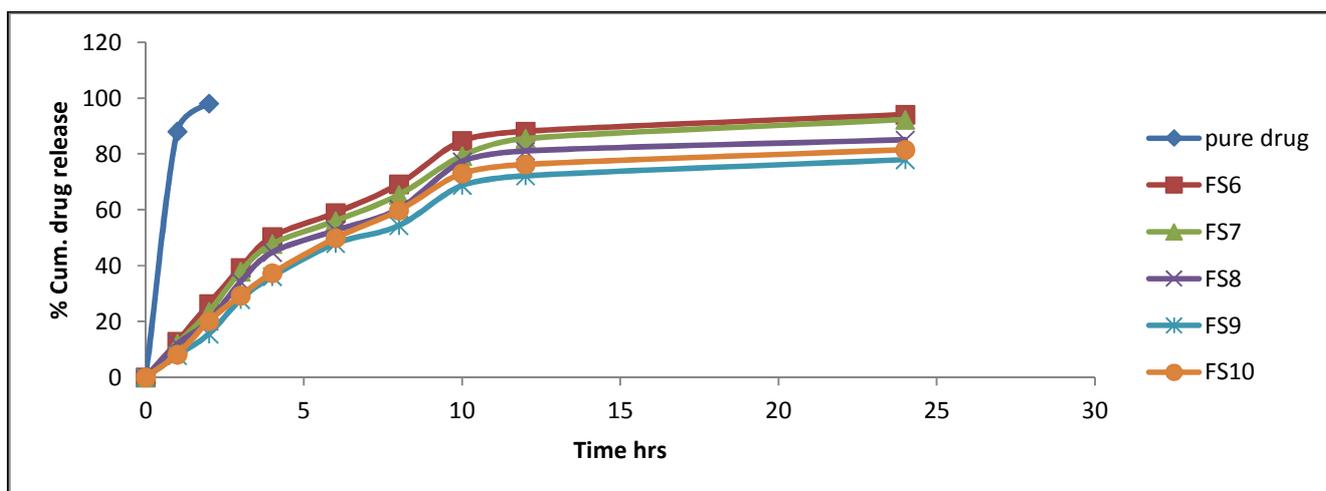
**Figure No.11:** *In vitro* **release profile of Stavudine niosomes from FS1-FS5**
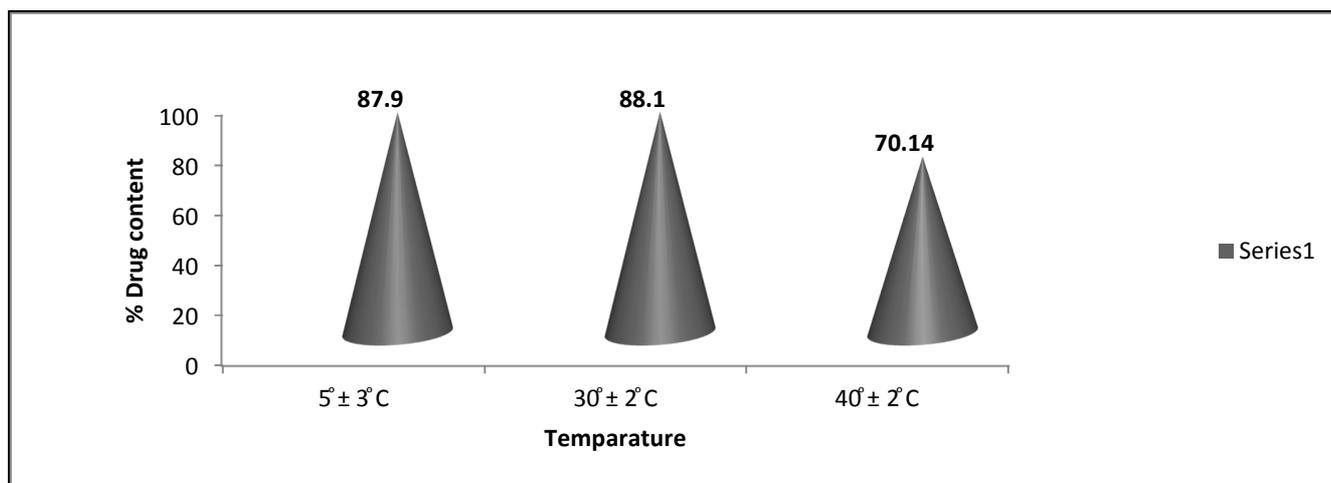
**Figure No.12: Stability studies - Drug content of ideal formulation FS9**
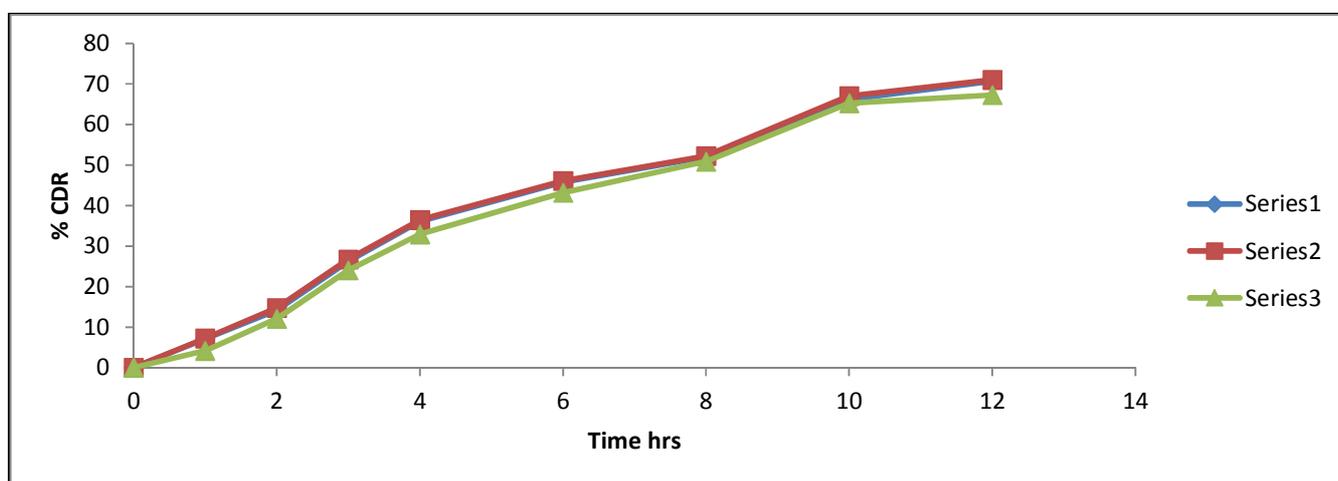
Figure No.13: Stability studies - *In vitro* drug release of ideal formulation FS9

CONCLUSION

Niosomes prepared by ether thin film hydration 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 FS4 registered highest entrapment of 81.24%. 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 thermo gram of pure stavudine showed a sharp endothermic peak at

174 °C. The thermo grams of formulations FS4 of Figure No.6, showed the same endothermic peak at the similar temperature. This further confirmed that there is no drug to polymer interaction.

Cumulative percentage drug released for FS1, FS2, FS3, FS4 and FS5 after 24 hrs were found to be 94.09%, 92.32%, 85.08%, 77.93% and 81.49% respectively. Zeta potential for FS4 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 FS4 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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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Jain N K. Advances in controlled and novel drug delivery, *CBS Publishers, New Delhi,* 1st edition, 2001.
2. Rampal Rajera, Kalpana Nagpal, Shailendra Kumar Singh, Dina Nath Mishra. Niosomes: A controlled and novel drug delivery system, *Biol Pharm Bull,* 34(7), 2001, 949-53.
3. Raj K Keservani, Anil K Sharma, Md Ayaz, Rajesh K Kesesvani. Novel drug delivery system for the vesicular delivery of the drug by niosomes, *Int J Res Control Release,* 1(1), 2011, 1-8.
4. Kumar Abhinav, Pal Jogender Lal, Jaiswal Amit, Singh Vishwabhan. Review on niosomes as novel drug delivery system, *Int Res J P,* 2(5), 2011, 61-65.
5. Anchal Sankhyan, Pravin Pawar. Recent trends in noisome as vesicular drug delivery system, *J Appl Pharm Sci,* 02(06), 2012, 20-32.
6. Mohamed S El Ridy, Alia A Badawi, Marwa M Safar, Amira M Mohsen. Niosomes as a novel pharmaceutical formulation encapsulating the hepatoprotective drug Silymarin, *Int J Pharm Sci,* 4(1), 2012, 550-58.
7. Pola Chandu V, Arunachalam A, Jagadish S, Yamini K, Tharangini K, Chaitanya G. Niosomes a novel drug delivery system, *Int J Novel Trends Pharm Sci,* 2(1), 2012, 25-31.
8. Jain NK. Advances in controlled and novel drug delivery, *CBS publishers and distributors,* 1st edition, 2003.
9. Lohumi Ashutosh, Rawat Suman, Sarkar Sidhyartha, Sipai Altaf bhai, Yadav M Vandana. A novel drug delivery system: niosomes review, *J Drug Dev and Therapeutics,* 2(5), 2012, 129-35.
10. Medlineplus.com. National Institutes of Health; updated 03 December 2014. Available from:www.nlm.nih.gov/medlineplus/druginfo/meds/a694033html.
11. Wikipedia the free encyclopedia.com. Updated 22 November 2014, Available from:en.wikipedia.org/wiki/stavudine.
12. Yasmin Begum M, Sankar Dasari, Sudhakar M, Lakshmi B V S, Manga K. Development and evaluation of co-encapsulated Stavudine and Lamuvudine niosomes for the controlled delivery, *Der Pharmacia Sinica,* 5(1), 2012, 1-10.
13. Jaya Vaishnavi Chennam, Sirisha Velchuri, Babu Rao Chandu, Amulya Ala. Niosomes–A Novel Drug Carrier Approach, *Int J Res Pharm Biomed Sci,* 3(2), 2012, 722-29.
14. Srinivas S, Anand Kumar Y, Hemanth A, Anitha M. Preparation and evaluation of niosomes containing Aceclofenac, *Digest J Nanomater Biostruct,* 5(1), 2010, 249-54.

15. Salagado H R N, Oliveira C L C G. Development and validation of an UV Spectrometric method for determination of Gatifloxacin in tablets, *Pharmazie*, 60(4), 2005, 263-64.

16. Saparia B, Murthy R S R, Solanki A. Preparation and evaluation of chloroquine phosphate microspheres using cross-linked gelatin for long term drug delivery, *Indian J Pharm Sci,* 64(1), 2002, 48-52.

17. Haznedar S, Dortunc B. Preparation and evaluation of Eudragit microspheres containing acetazolamide, *Int J Pharm,* 269(1), 2004, 131-140.

18. Higuchi T. Mechanism of sustained action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *J Pharm Sci,* 52(12), 1963, 1145-1149.