Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2013, Volume 1, Issue 4
ISSN : 2321–3647(online)

Formulation, Optimization and Evaluation of Niosomal Gel of Alitretinoin

Geeta M. Patel [*1] Darshan R. Parmar[1]
1.Department of Industrial Pharmacy, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva-384012, Gujarat, India

ABSTRACT

Alitretinoin is used in treatment of AIDS related Kaposi's sarcoma in concentrations of 0.1%. Alitretinoin is very effective but it causes skin erythema on the applied area. The Niosomes seems to be promising drug delivery in modern drug delivery systems. The main benefit over liposome is that the lipids are replaced by non-ionic vesicles and hence the preparation is totally non-antigenic. The non-ionic surfactants like SPANs and TWEENs are obtained from synthetic sources and hence the quality is maintained same all the time. The Alitretinoin was incorporated into niosomes using SPAN 60 and cholesterol. Various ratios of SPAN 60 and cholesterol were tried and optimized for the preparation of niosomes. Various process parameters were also optimized for the rotary flask evaporation method. The niosomal dispersion was incorporated in to carbopol 971NF gel. The gel was kept for 6 weeks accelerated stability studies. The niosomal dispersion was evaluated for various parameters like vesicle size, shape and morphology by Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). *In-vitro* and *ex-vivo* studies were carried out. The drug release pattern from gel was evaluated on the basis of *in-vitro* studies and skin irritation studies on rat skin. The *in-vitro* study shows sustained release gel effects whereas the *ex-vivo study* shows no signs of irritation on the applied skin area.

Keywords: Niosome, Alitretinoin, Span 60, Factorial design

*Corresponding Author Email: geekhappy2002@yahoo.co.in
Received 1 April 2013, Accepted 15 May 2013

Please cite this article in press as: Patel GM *et al.*, Formulation, Optimization and Evaluation of Niosomal Gel of Alitretinoin. American Journal of Pharmacy& Health Research 2013.

INTRODUCTION

Kaposi's sarcoma (KS) is a cancerous tumor of the connective tissue and is often associated with AIDS. It is a frequently encountered malignancy in HIV-positive patients. The most common manifestation of KS is purplish-red or brown skin lesions that appear on any part of the skin, head, and neck. The lesions can also be found on the outer surfaces of the penis and the inside of the mouth. As the disease becomes more advanced, the skin lesions can develop on internal organs like the lungs and intestines.

According new cognitions the transcellular route seems to be implausible, because of molecules have to penetrate alternating lipophilic and hydrophilic layers. Moreover, it seems that small polar molecules are penetrating through the corneodesmosomale route considering the high number of corneodesmosoes and procorneocytes. The intercellular pathway is possible for lipophilic polar molecules through lateral diffusion along the lipophilic hydrocarbon chains of the stratum corneum lipids. In recent years, the trans appendage route has become interesting for research. It seems to play a more important role as so far assumed.[1-2]

The Niosomes seems to be promising drug delivery in modern drug delivery systems. Niosomes are unilamellar or multilamellar vesicles that are made up of nonionic surfactant and can entrap amphiphilic and hydrophobic solutes.[3-4] Because of a liposome's instability, alternative nonionic surfactants have been investigated. Using similar techniques, nonionic surfactant vesicles or "niosomes" have been synthesized.[5] These formulations use alternative materials to phospholipids such as Span 60, Span 40 and Span 20, which are inexpensive and widely available permitted food additives. Niosomes has been used to encapsulate colchicines, estradiol, tretinoin, dithranol , enoxacin and for application such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine.[6]

Alitretinoin (9-cis-retinoic acid) is a naturally-occurring endogenous retinoid indicated for topical treatment of cutaneous lesions in patients with AIDS-related Kaposi's sarcoma. Alitretinoin (ALT) inhibits the growth of Kaposi's sarcoma (KS) cells in vitro. In the present study thin film hydration method was used for the preparation and optimization of ALT niosome as this method is simple and easy to scale up. Various process parameters were also optimized for the thin film hydration method. It is hypothesized that incorporation of into niosomes will improve the amount niosomal formulations over the plain drug formulations was indicated with regard to higher skin permeation and retention. Hence the present work was envisaged to develop a stable, novel, and aesthetic niosomal drug delivery system with improved efficacy.

MATERIALS AND METHODS

Alitretinoin was generous gift from Cadila Healthcare Ltd. (Ahmedabad, India) Span 60 and Tocopherol acetate was purchased from *Croda* Chemicals Pvt Ltd., (India). Cholesterol was generous gift from Merck chemicals, Virginia, USA. Dichloromethane, Ammonium acetate Glacial acetic acid and Sodium hydroxide were purchased from Finar Chemicals Ltd, (Ahmedabad, India). Ethanol, Chloform and Potassium hydroxide were purchased from S.D. Fine Chemicals Ltd., (Mumbai, India). A dialysis tube (DM-70; capacity 2.41 mL/cm, width 29.31mm, average diameter 17.5mm, and molecular weighted cutoff 12 000 to 14 000) was purchased from Himedia Laboratories, (Mumbai).

Drug-Excipients compatibility study

To check the compatibility of ALT with other formulation excipients, the FTIR and DSC studies were performed.

3^2 full factorial design

On the basis of the preliminary trials in the present study a 3^2 full factorial design was employed to study the effect of independent variables, i.e. parts of SPAN 60 (X_1) and parts of Cholesterol (X_2) on dependent variables like % drug entrapment and particle size. The results as summarized in below table-1 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the nine batches (A_1 to A_9).

Table - 1: Effect on dependent variable 3^2 full factorial design layouts for Alitretinoin niosomes.

Batch No	Variables levels in coded form		Drug entrapment(%)	Particle size (nm)
	X_1	X_2		
A1	1.00	-1.00	57.32	1356.8
A2	0.00	-1.00	51.78	1789.6
A3	1.00	1.00	35.54	1128.7
A4	-1.00	-1.00	53.35	1790.2
A5	0.00	1.00	37.42	1578.6
A6	-1.00	0.00	59.43	1650.5
A7	**0.00**	**0.00**	**63.48**	**1237.4**
A8	-1.00	1.00	38.21	1887.4
A9	1.00	0.00	49.63	1134.7

Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (+1)
Parts of SPAN 60	15	20	25
Parts of Cholesterol	0.5	1	1.5

Note: All the batches contained the constant amount of drug and rest of the process parameters were kept constant and optimum.

The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The high values of correlation coefficient for the dependent variables indicate a good fit.

Preparation of niosomes

Niosomes were prepared by thin film hydration method.[7] Span 60: cholesterol at a ratio of 20:1 by weight and 50 mg of ALT were dissolved in 20 ml of Dichloromethane, in a 250 ml round-bottomed-flask. The flask was fitted onto a rotary flask evaporator (Buchi Rotavapor instruments Pvt Ltd., Mumbai, India) and connected to a vacuum pump. The solvent system was evaporated under vacuum at 50 °C using water bath for 10 minutes. The dried surfactant film was hydrated with 50 ml distilled water for 30 minutes at 50 °C using bath sonicator (PCI Pvt Ltd, U.K). The hydrated dispersion was kept in refrigerator for 2 hours for sealing of vesicles.

Vesicle Size Determination

The vesicle sizes of niosomes were determined using a particle size analyzer (Malvern Zetasizer, ZS 90, U.K). The apparatus consisted of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 mW using a Fourier lens (R-5) to a point at the center of a multielement detector and a small-volume sample holding cell (Su cell) The sample was stirred before determining the particle size as Mean Vesicle Diameter (MVD). [8]

Scanning Electron Microscopy

The surface characteristics of the niosome were studied by scanning electron microscopy (SEM). The samples were observed for morphological characterization using a gaseous secondary electron detector (working pressure: 0.8 torr, acceleration voltage: 30.00 kV) XL 30, Philips (Eindhoven, Netherlands). The particles were observed for surface characteristics.

PDE (percentage drug entrapped)

The PDE of ALT niosomes was calculated after determining the amount of unentrapped drug by dialysis.[9] The dialysis was performed by adding the niosomal dispersion to a dialysis tube (donor compartment) and then dipping the tube into a beaker containing 400 ml of phosphate buffer pH 4.5 (receptor compartment) on a magnetic stirrer, rotated at a speed of 80 to 120 rpm for 4 hours. After 4 hours, the solution in the receptor compartment was estimated for unentrapped drug at 265 nm using a UV spectrophotometer (UV 1601, Shimadzu, Kyoto, Japan). The PDE of the niosomes was calculated by:

$$PDE = \frac{Wt\text{-}We}{Wt} \qquad \textbf{(1)}$$

where, Wt - total amount of drug added and We - amount of unentrapped drug.

Optimum Formula

After developing the polynomial equations for the responses PDE and mean vesicle diameter (MVD) with the independent variables, the formulation was optimized for the response PDE. Optimization was performed to find out the level of independent variables (X_1, X_2,) that would yield a maximum value of PDE with constraints on MVD.

Formulation of niosome based gel (NBG) of ALT

The niosomal dispersion was incorporated in to carbopol 940NF gel. The gel was kept for 6 weeks accelerated stability studies.

Characterization of the ALT-NBG

Determination of drug content, pH, spreadability and consistency, viscosity

For determination of drug content, about 1 g of the gel was weighed in a 100-ml volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed by the UV spectroscopy method described earlier. The pH of the various gel formulations was determined using digital pH meter; pH was adjusted by means of 0.1 N NaoH.

Spreadability was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time were noted for upper slide (movable) to separate completely from the fixed slides. Spreadability was then calculated using the formula:

$$S = M.L / T \qquad (3)$$

where, S – spreadability, M - weight tide to upper slide, L - length of glass slide, T - time taken to separate the slide completely from each other.

The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such a way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance traveled by cone was noted down after 60sec.The measurement set up for the determination of consistency is shown in figure-1.

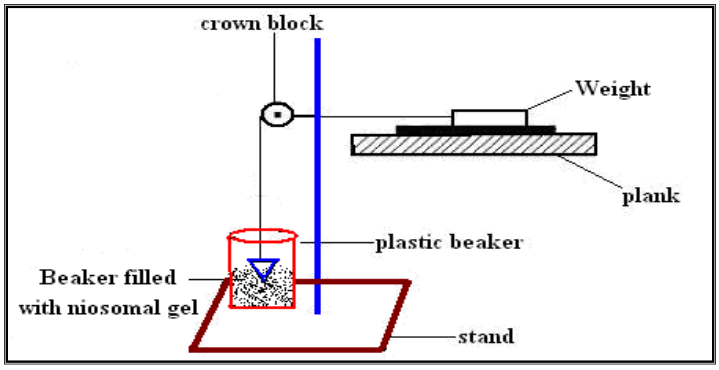


Figure - 1: Measurement set up for the determination of consistency.

Brookfield (Brookfield Pro-V II Viscometer, Japan) with helipath stand was used for rheological studies. The sample (30 g) was placed in a beaker and was allowed to equilibrate for 5min before measuring the dial reading using a T-C spindle at 0.5, 1, 2.5, and 5 rpm. At each speed, the corresponding dial reading on the viscometer was noted.

***In-vitro* drug release study**

The release of ALT from niosomal formulations were determined using membrane diffusion technique.[10] The niosomal formulation equivalent to 10mg of ALT was placed in a glass tube of diameter 2.5cm with an effective length of 8cm that was previously covered with soaked osmosis cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100ml of phosphate buffer (pH 6.4), which acted as receptor compartment. The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touching (1-2mm deep) the surface of diffusion medium. The temperature of receptor medium was maintained at 37±10°C and agitated at 100rpm 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 analyzed at 345nm in Double beam UV-VIS spectrophotometer using phosphate buffer (pH 6.4) as blank.

Accelerated stability study

The stability studies of niosomes and niosomal gel of ALT were carried out for six weeks at different accelerated conditions like 2-8 °C, 25 °C / 60 %RH and 40 °C / 75 %RH. The samples were analyzed for assay of the ALT. [11,12]

ex-vivo study

It is essential to carry out *ex-vivo* studies of niosomal formulations to see the difference of effect between plain drug formulation and niosomal formulation. The animal study was carried out by properly following CPCSEA guidelines and the project was approved by the IAEC/SKPCPER/2011-12.

RESULTS AND DISCUSSION

In preparation of niosomes, various ratios of cholesterol and surfactants were tried but due to homogeneity, drug entrapment and dispersion characteristics, some formulas were not selected. The surfactant: cholesterol ratio is very important for drug entrapment because the cholesterol acts as stabilizer and itself is lipophilic in nature so increased concentration of cholesterol may cause reduction in drug entrapment. The optimum concentration is needed otherwise vesicle stability may decrease. Various ratios were tried for the preparation and finally 20:1 surfactant:

cholesterol was selected to prepare further batches.

Drug-Excipients compatibility study

FTIR analysis

The FTIR spectrum of niosomal formulation is shown in figure-2. The niosomal dispersion was lyophilized for this purpose. The moisture content was checked by karl-fisher method and it was found to be less than 3 % thus it cannot interfere the FTIR studies. The figure shows the peak 1728.28 cm^{-1} which indicates the presence of acid group in the structure. The final formulation shows the presence of the characteristic peak of Alitretinoin and hence it proves that the excipients used in the formulation are not interacting with the drug and so the formulation is chemically stable.

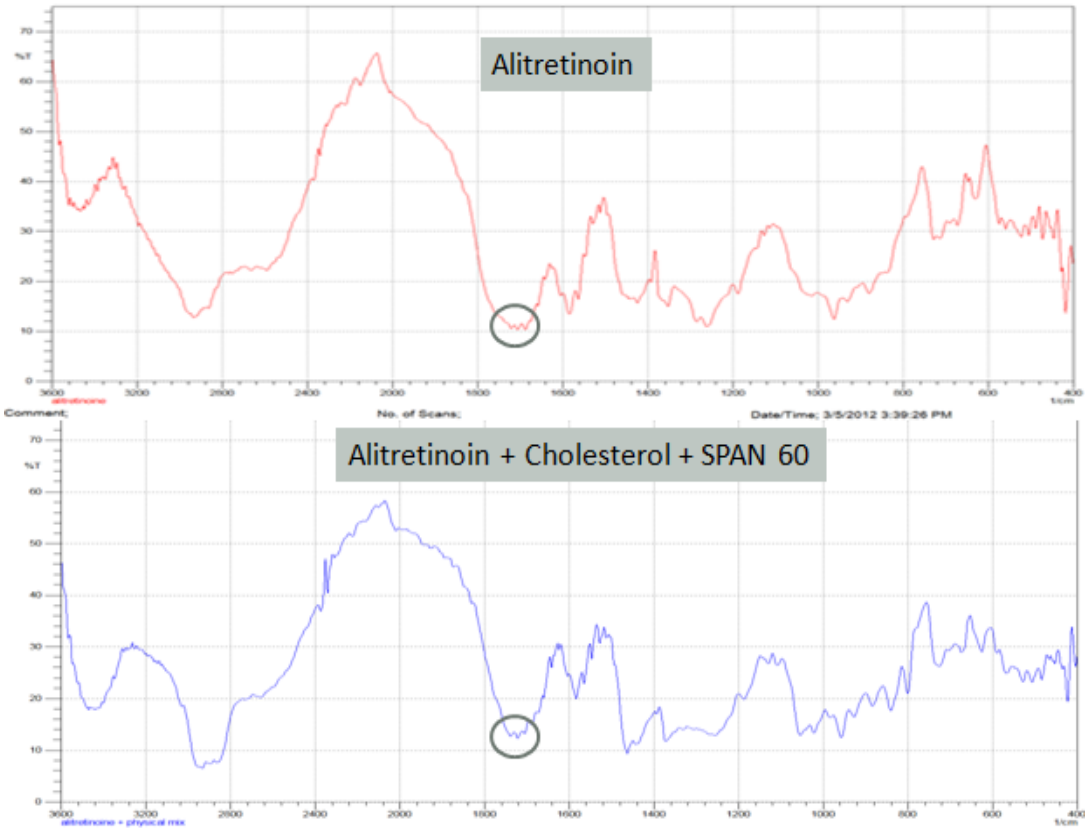


Figure-2: FTIR spectra of drug and excipients

DSC analysis

The figure-3 shows the combined peaks of cholesterol, SPAN 60 and ALT. The Span 60 shows peak at 57.27 ^{o}C, the cholesterol shows peak at 148.38 ˚C whereas the ALT shows its presence at 191.02 ˚C. All three peaks are present in graph and hence the ALT is not being interfered due to presence of other formulation excipients.

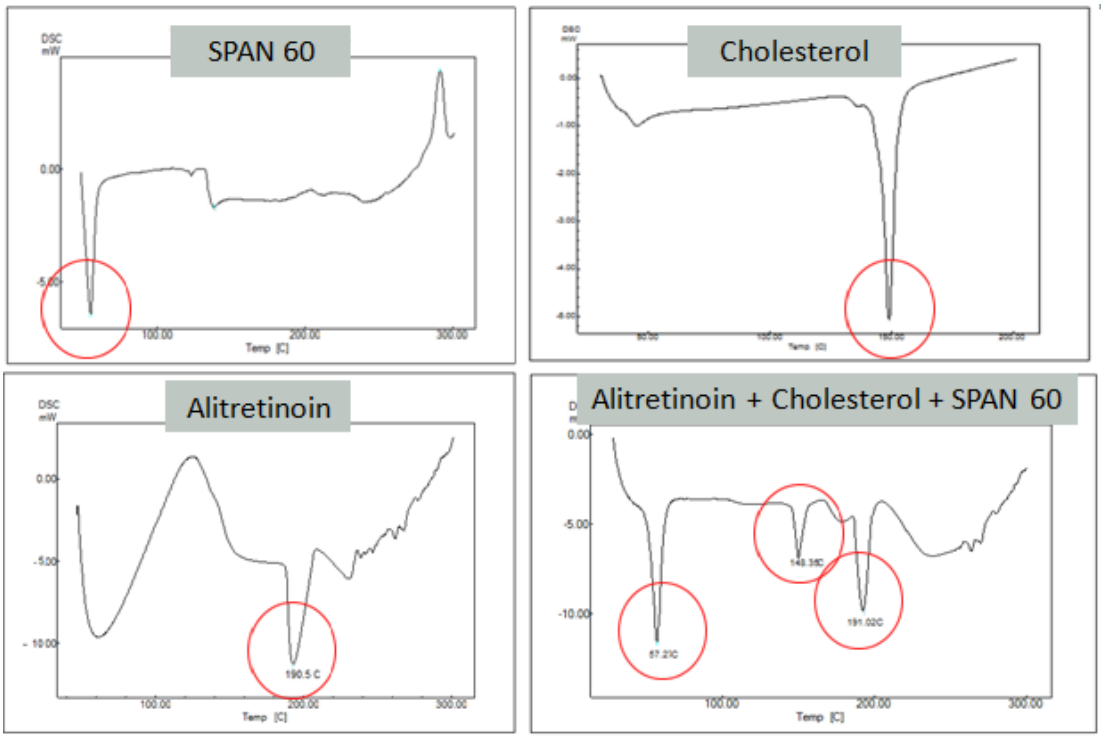


Figure-3: DSC spectra of drug and excipients

Data Analysis

Concerning PDE, the results of regression analysis showed that both the coefficients b_1 and b_2 bear a negative sign. So, factor have negative effect on the % drug entrapment, which revealed that as the concentration of X1 & X2 increases the % drug entrapment decrease.

$$\mathbf{PDE = 60.49 - 1.42\ X_1 - 8.55X_2 - 1.66\ X_1X_2 - 2.98X_1^2 - 12.91X_2^2}$$

(R^2 = 0.8973) **(2)**

The PDE for all the batches A_1 to A_{10} varied from 37.42 % to 63.68 % showed good correlation coefficient as 0.8973. Results of the equation (2) indicated that both the concentration of the X_1 and X_2 were responsible for the PDE.

(b) Factorial equation for Size

On the basis of regression analysis, factor X1 and X2 has negative effect on size of niosome. So, factors have negative effect on particle size, which revealed that as the concentration of X1 and X2 increases the particle size decreases.

$$\mathbf{Size = 1322.69 - 284.65X_1 - 56.98X_2 + 81.32\ X_1X_2 - 15.39\ X_1^2 + 276.11\ X_2^2}$$

(R^2= 0.9221) **(3)**

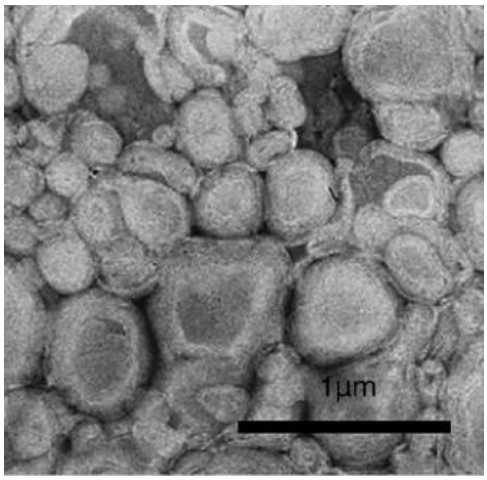
The size of niosome came in the range of 1128.7nm to 1887.4 nm showed good correlation coefficient as 0.9221. Results of the equation (3) indicated that both the concentration of the X1 and X2 were responsible for the size of niosome.

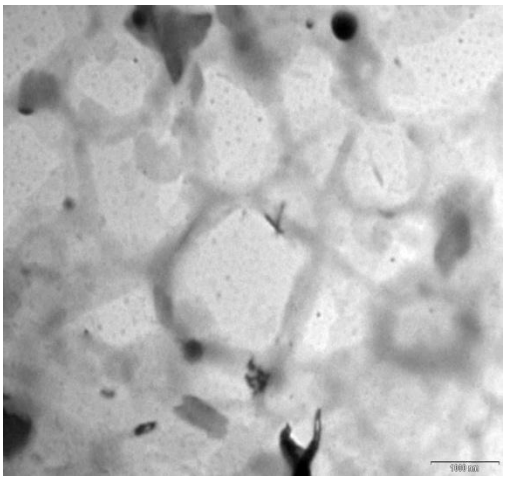
Particle size analysis

The average particle size was determined for the various batches having the different ratios of span 60: cholesterol by SEM analysis (figure-4). However, the particular interest is to find the formulation which is having the least particle size at the same time negative repultative zeta potential, which is the good sign of stability of colloidal system which is in the desired range for the niosomal formulation, too.

The particle size range for the batch having SPAN 60: Cholesterol of 20:1 was found to be minimal, viz. 1162 nm. So the batch was considered as suitable for the formulation. However the batches 15:1 and 25:1 showed good results but considering the size and zeta deviation and polydispesity index, the 20:1 was found to be most suitable.

Zeta potential was found to be in the range of -12.8 mV to -24.7 mV. Here in surfactant: cholesterol of 20:1 zeta potential was found to be -24.7mV which indicates high negative surface charge on niosomes indicate higher stability because of the anticipated surface repulsion between similar charged particles hence inhibiting aggregation of the colloidal niosomal particles.





a) **b)**

Figure - 4: a) Scanning Electron Micrographs and b) Transmission Electron Micrographs of Optimized Batch

Characterization of ALT-NBG

The ALT content of the NBG was found to be 97.8 ± 4.2 % of the theoretical value. The pH of ALT-NBG was 4.5 ± 0.2. Spreadability is an important property of topical formulation from patient compliance point of view. The spreadability was found to be 5.1 ± 0.8 g.cm/sec which is indicative of good spreadability. The ALT-NBG showed pseudo-plastic behavior and the viscosity of ALT-NBG at 5 rpm was 61237 Cps (table-2).

Table-2: Characterization parameters of niosomal gel

Batch code	pH	Spread ability (gm.cm/sec)	Consistency mm penetrated	Viscosity (cps)
A1	4.6	5.9	6.7	60435
A2	4.6	5.3	4.7	40234
A3	4.6	5.6	6.6	62090
A4	4.6	5.2	4.8	47987
A5	4.6	5.7	5.8	42090
A6	4.6	4.7	**6.8**	**61237**
A7	**4.6**	**5.9**	6.1	41258
A8	4.6	5.1	5.2	42345
A9	4.6	5.2	5.8	42348

***In-vitro* drug release study**

The release studies show that 95.88 % and 64.12 % drug diffusion occurred within 8 hours from drug solution and plain drug gel respectively, while 57.64 % and 49.58 % drug diffusion occurred from niosomal dispersion and niosomal gel respectively. The difference is significant between drug solution and niosomal dispersion as well as the drug gel and niosomal gel. This indicates the slow release properties of niosomal formulations. The release rate is retarded due to niosomal barrier. In niosomal gel, the drug has to pass from two barriers to reach to the receptor component. Thus, the release rate is slow as compare to conventional formulation.

The figure-5 shows the comparative release profiles of all the formulations together which makes easy for us to understand the release pattern. The drug solution is highly diffusible by nature thus the curve shows slight decrease in release rate after some hours of release, whereas in niosomal dispersion, the curve shows slight increase in release rate as the saturation in receptor component has not been attained yet.

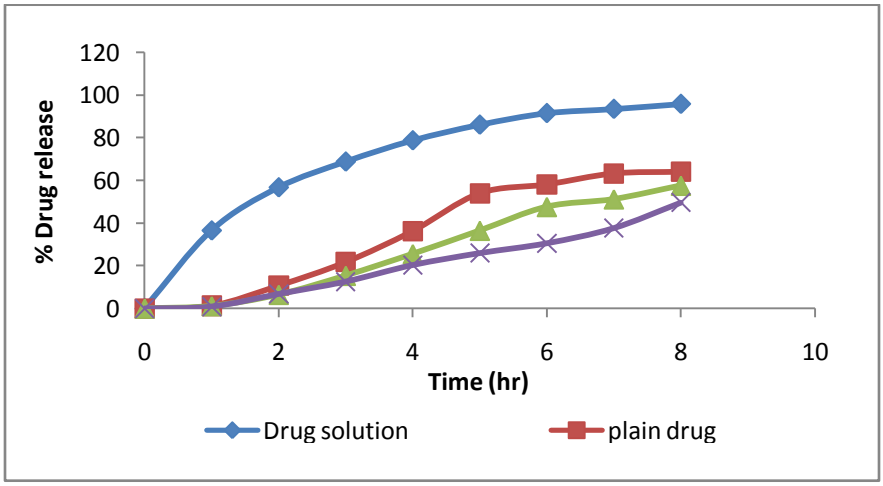


Figure-5: Graphical representations of release profiles

Since the ALT is entrapped within niosomes, the diffusion of drug will occur across two membranes, one the niosomal membrane and the other cellophane membrane. This may be the reason for the sustained release.

The diffusion coefficients for drug solution, drug gel, niosomal dispersion and niosomal gel were found to be 1.68×10^{-09}, 1.26×10^{-09}, 9.07×10^{-10} and 5.94×10^{-10} respectively. The diffusion co-efficient is low for the niosomal formulations that indicates the slow release pattern for the niosomal formulations. The diffusion co-efficient is highest for the drug solution which indicates the faster release of the drug across the semi-permeable membrane.

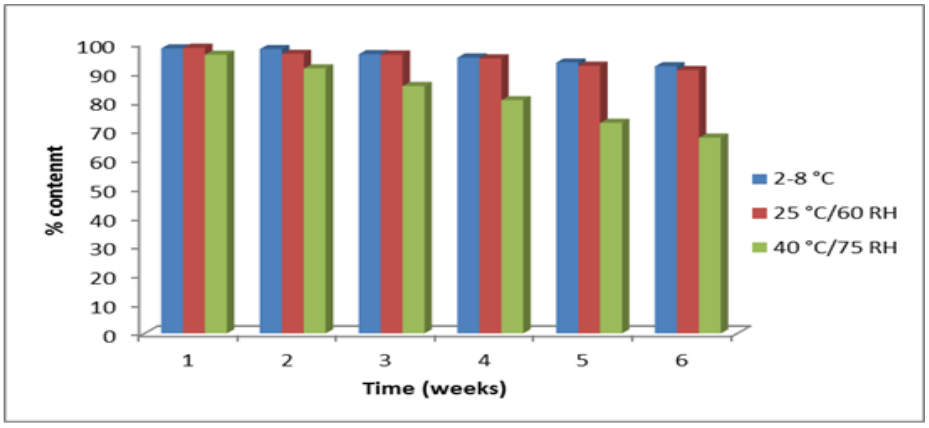
Accelerated stability study

Percentage drug retained in niosomes stored at 2-8 °C showed 98.11% at first week which was reduced gradually to 91.98 % at sixth week. In case of niosomes stored at 25 °C / 60 % RH showed 98.31% at first week which was reduced gradually to 90.62% at sixth week, whereas, in niosomes stored at 40 °C / 75 %RH showed 95.94% at first week which was reduced gradually to 67.36 % at sixth week.

Percentage drug retained in niosomal gel stored at 2-8 °C showed 98.04% at first week which was reduced gradually to 90.23% at sixth week, in niosomes stored at 25 °C / 60 %RH showed 97.16% at first week which was reduced gradually to 88.32% at sixth week, whereas, in niosomes stored at 40 °C / 75 %RH showed 95.44% at first week which was reduced gradually to 62.81% at sixth week.

Percentage drug retained in plain drug gel stored at 2-8 °C showed 91.26% at first week which was reduced gradually to 52.34% at sixth week, in niosomes stored at 25 °C / 60 %RH showed 87.30% at first week which was reduced gradually to 41.62% at sixth week, whereas, in niosomes stored at 40 °C / 75 %RH showed 81.23% at first week which was reduced gradually to 32.49% at sixth week.

Here, the result in figure-6 shows that the stability of the drug is highest in niosomal gel. However it didn't show 100% stability but compare to the plain drug gel it shows better stability. Efforts has been made to improve the stability of the drug by encapsulating it into niosomal vesicles but still 100% results has not been achieved so a further efforts are required to improve the stability of the drug.



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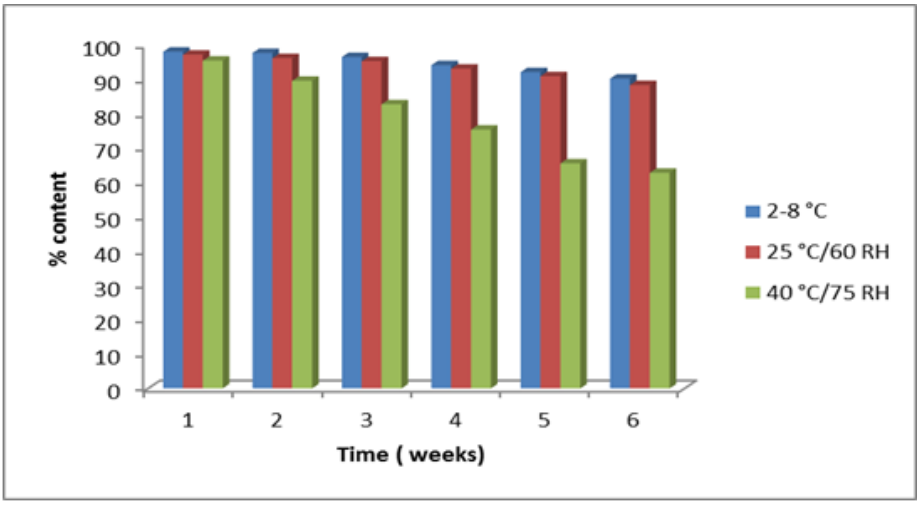

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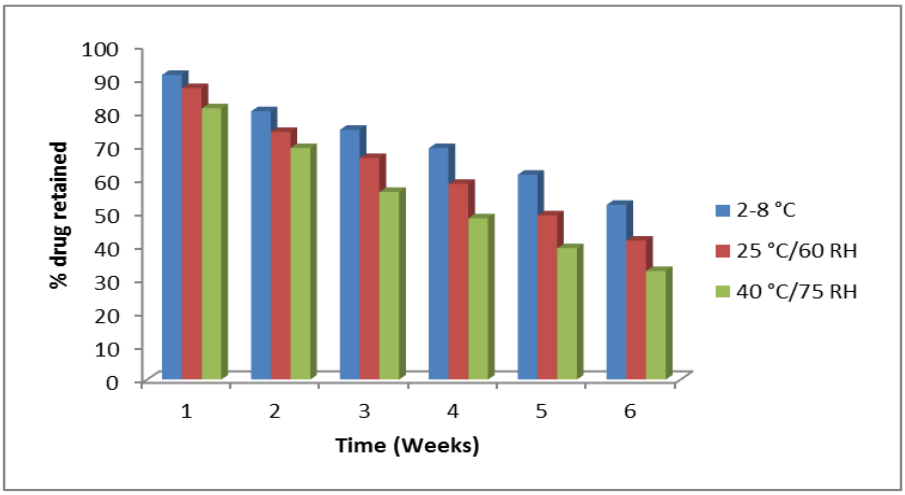


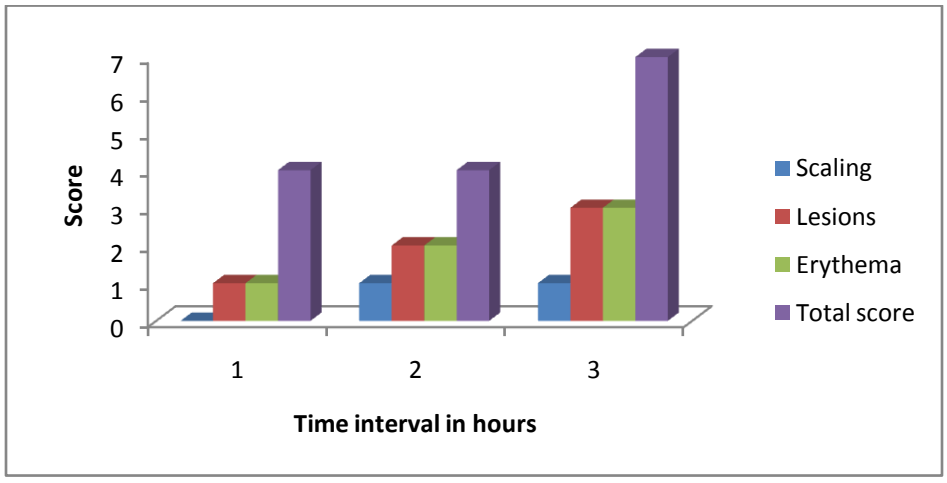
c)

Figure - 6: Accelerated stability studies of a) Niosomal gel, b) Niosomal dispersion c) plain drug gel

ex-vivo study

From the figure-7(a), it is seen that the rats treated with plain gel are showing more irritation characteristics. The erythema signs are continuously increasing at regular time intervals (initially it was marked as 1 but after 48 hours it was 3). The scaling was absent with plain gel initially but after 12 hours it appeared on applied skin portion. The lesions are also increasing as the therapy time increased.

From the figure-7(b), it is seen that the rats treated with niosomal gel are showing comparatively less irritation characteristics. The erythema signs were absent initially but after 48 hours it showed slight erythema signs. The scaling was completely absent with niosomal gel initially as well as even after 48 hours. The lesions were also completely absent initially and even after 48 hours. So, from the above comparisons of data it can be concluded that niosomal gel is more effective than plain gel.



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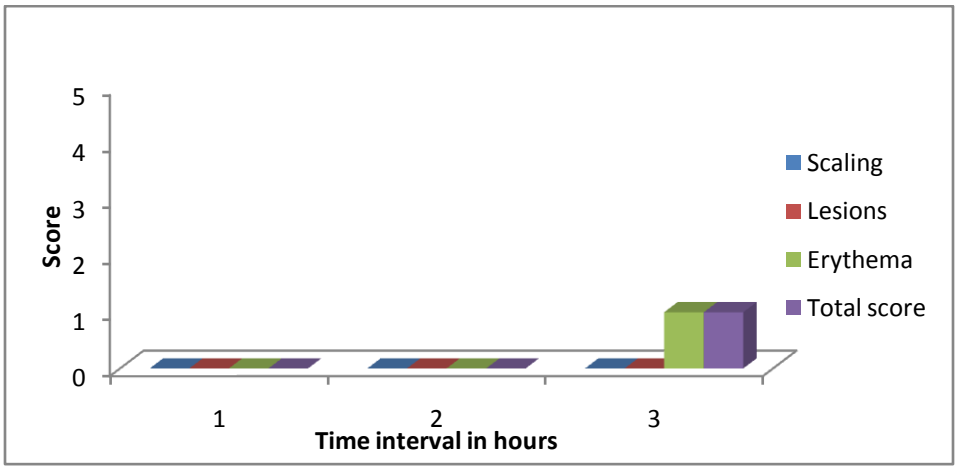

b)

Figure - 7: Graphical presentation of *ex-vivo* studies of a) plain drug gel b) Niosomal gel

CONCLUSION

ALT causes erythema when applied on skin for the treatment of AIDS related Kaposi's sarcoma as such which can overcome by its incorporation in niosomes. The thin film hydration technique using rotary flask evaporator shows good vesicle forming properties as well as better efficiency. ALT used in plain gel formation in strength of 0.1% showed higher release as compare to niosomal gel at the same time interval. The niosomal gel applied on the rat skin showed no signs of erythema whereas the plain drug gel showed it clearly. The results of the stability study show that the stability of the drug is highest in niosomal gel. However it didn't show 100% stability but compare to the plain drug gel it shows better stability. Efforts has been made to improve the stability of the drug by encapsulating it into niosomal vesicles but still 100% results has not been achieved so a further efforts are required to improve the stability of the drug. Thus, from this research work it can be concluded that the novel niosomal gel formulation is much better than the plain drug gel for topical purpose.

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