ABSTRACT
The objective of present research work was to formulate niosomal gel of ACV and to evaluate it for enhancements of skin permeation and skin retention characteristics. ACV niosomes were prepared by classical thin layer hydration method. The niosomal vesicles were separated by centrifugation method and further evaluated for percentage drug entrapment (PDE) and vesicle mean geometric diameter. The separated niosomal vesicles were incorporated into the Carbopol® 971 gel base. The niosomal gel was further evaluated for skin retention characteristics, in vitro diffusion study and stability studies at accelerated and non-accelerated conditions. The batch F12 was considered as optimized batch as it showed minimum geometric mean diameter of vesicles (1.23 ± 0.19 μm) and maximum PDE (58.71 ± 0.89 %). The in vitro diffusion study using human cadaver skin (HCS) showed 1.57 times higher flux as compared to conventional ACV gel. The skin retention study reveals that the percentage drug retained was 4.92 times higher as compared to conventional ACV gel. The stability studies at 2-8 °C showed PDE 94.23% after 12 weeks whereas at accelerated condition it was found to be 90.11%.

Keywords: Acyclovir, Niosomes, Thin layer evaporation, Skin retention, Skin permeation.

INTRODUCTION
The herpes labialis is usually known as cold sore or fever blister. The infection of herpes labialis occurs when the herpes virus type 1 (HSV type 1) comes in contact with oral mucosa or abraded skin. The HSV type 1 can travel through micro breaks in the skin or mucous membranes to infect the new individual. The major symptoms are skin lesions around the lips, mouth and small blisters containing clear yellowish colored fluid. Viral culture, viral DNA test, or Tzanck test of the skin lesion can detect the infection of herpes virus 1, 2. The replication of herpes virus takes place at the basal dermis and hence the improved skin penetration of anti-viral drugs is required to shorten the treatment time 3.

Acyclovir (ACV) is chemically 2-amin o-1, 9- [(2-hydroxy ethoxy) methyl]-6h-purine-6-one, a guanosine analogue antiviral drug commonly prescribed in the treatment of herpes simplex viral infection. The ACV has very poor aqueous solubility and hence very poor skin penetration 4. Niosomes are basically non ionic surfactant based vesicles, formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures. The assembly into closed bilayers usually involves some input of energy such as physical agitation or heat 5, 6. The low cost, greater stability and resultant ease of storage of non-ionic surfactants has lead to the development of these compounds as alternatives to phospholipids. The niosomal vesicle acts by modifying the stratum cornea structure and hence improves drug penetration. The altered adsorption and fusion of niosome vesicle with skin surface leads to high thermodynamic activity and hence the drug adsorption gets improved. The retention characteristic is also improved due to fusion process between drug loaded niosome vesicle and skin 6.

The present study demonstrates the encapsulation of ACV in niosome vesicles using sorbitan monostearate as non ionic surfactant and its further incorporation into Carbopol® 971 gel base intended for the topical application. The detailed aims of the present study were, (1) To formulate ACV niosomes by thin layer evaporation technique by applying 33 full factorial design, (2) To carry out stability studies at accelerated and non-accelerated conditions, (3) To investigate the improvement in skin penetration and skin retention characteristics of ACV due to niosomal dosage form based dosage form as compared to the conventional topical dosage form.

MATERIALS AND METHODS
Materials
ACV was obtained as a gift sample from Matrix laboratories Ltd, Hydrabad. Sorbitan monostearate was obtained as a gift sample from Croda chemicals (India) Pvt Ltd. Cholesterol was purchased from Merck. The human cadaver skin (HCS) was obtained from the Autopsy department of civil hospital. All other chemicals and solvents were of analytical reagent grade. Phosphate buffer saline pH 7.4 (PBS pH 7.4) was prepared as described in Indian pharmacopoeia 1996. All necessary permissions were obtained from ethical committee before conducting this study.

Formulation of ACV niosomes
The vesicles were prepared by the thin layer hydration technique 7. The non ionic surfactant and cholesterol were dissolved in dichloromethane (DCM) in 250 ml round bottom flask. The rotary flask evaporator (Buchi rotavapor R215) was used for the formation of film. The ACV was dissolved in PBS solution (1mg/ml) and used for the hydration purpose. The prepared niosomal dispersion was stored at 2-4 °C in refrigerator (Samsumg) for vesicle annealing purpose. 33 full factorial design 8 was applied in the formulations by varying drug:sorbitan monostearate ratio, hydration temperature and hydration volume. The amount of cholesterol was kept constant through out all the experiments. For accuracy point of view each experiment was performed thrice and their mean values were considered in calculations.

Formulation of ACV niosomal gel
Carbopol® 971 gel base was used in the present study. Adequate amount of gel base was sprinkled on warm PBS solution with continuous stirring to prepare 1% w/w gel. The propylene glycol (10% w/w) and Glycerol (20% w/w) were added to improve the consistency of the gel. The niosomal
dispersion (batch F12) was added into the above gel base and kept for sonication in bath sonicator to remove the air bubbles over night. The prepared gel was packed in aluminum tubes and used for further studies. The conventional ACV gel (5% w/w) was prepared by incorporating ACV into gel base for the comparative in vitro studies.

**Evaluation of niosome vesicles**

**Microscopic evaluation**
The prepared niosome gel was diluted with PBS solution and examined under microscope (100X magnification) to observe vesicle shape and lamellarity (Olympus B 201 Microscope). The observed vesicles are shown in figure 1.

**Percentage drug entrapment (PDE)**
Centrifugation method was employed to estimate the percentage entrapment of the ACV within niosome vesicles. The niosomal dispersion was centrifuged at 15000 RPM for 30 minutes to separate the supernant and pellets at the bottom 9. The unentrapped drug present in the supernant liquid was detected at λ<sub>max</sub> 250 nm using Uv-Visible spectrophotometer (UV 1700, Shimadzu). The PDE was calculated using equation 1. The results of PDE are listed in table 1.

\[
PDE = \frac{\text{Total amount of loaded drug} - \text{amount of drug detected in supernant layer}}{\text{Total amount of loaded drug}} \times 100 \quad (1)
\]

**Niosome vesicle size determination**
All the batches were evaluated for vesicle size using Malvern particle size analyzer. The samples were prepared by applying suitable dilution 10. The data of geometric mean diameter are shown in table 1.

**In vitro diffusion study**
The obtained human cadaver skin (HCS) was stored at 0±2 °C and it was completely shaved before use. The excess fat tissues were removed and it was then carefully evaluated using a magnifying glass to ensure absence of surface abnormalities like cracks or crevices. It was properly cut of 4 cm² size using sterile surgical knife. The cut piece was soaked in phosphate buffer saline (PBS) pH 6.8 overnight before use. A modified Franz diffusion cell was used to carry out the study. 50 mg of gel and niosomal gel was applied separately on the epidermal skin of mounted HCS which served as donor compartment 11-14. The cell was assembled in such a way that receptor compartment fluid remained in contact with the bottom surface of HCS 15-18. The volume of the receptor compartment was 100 ml and the magnetic stirrer was kept rotating at 50 RPM. At the regular time interval adequate amount of sample was withdrawn and replaced with fresh PBS solution. The samples were analyzed at λ<sub>max</sub> 250 nm by UV-Visible spectrophotometry. Figure 3 shows the graphical representation of diffusion study data.

**Skin retention study**
After completion of 12 hours of in vitro diffusion study, the mounted skin was removed and scrapped off gently to remove the applied overlying niosomal gel 19-20. The skin tissue was smashed in a tissue homogenizer with required amount of PBS and was evaluated for ACV concentration.

**Stability study**
The prepared ACV niosomal gel was further kept at non-accelerated (2-8 °C) and accelerated conditions (25±2 °C) to carry out stability studies for 12 weeks 21. The figure 5 represents the stability study results in graphical form.

**RESULT AND DISCUSSION**
For the niosome preparation, various methods have been reported, among them thin layer hydration (TLH) method was selected. The sorbitan monostearate was selected for experimental purpose as its HLB values is 4.7 and highest phase transition temperature (50-55 °C) among all the non-ionic surfactants. The figure 1 indicates that the ACV niosomal vesicles are unilamellar. The ACV is BCS class III drug and hence the solubility is higher. Thus, the entrapped drug will be surrounded by the non-ionic surfactant bilayers. Among all the prepared batches the Batch F12 showed highest PDE (58.71 ± 0.89 %) and lowest vesicle size (1.23 ± 0.19 μm). The selected casual factors showed to have direct effects on PDE and vesicle size. The major component in niosome vesicle preparation is the non-ionic surfactant. The bilayer vesicle formation occurs due to non-ionic surfactant. The cholesterol serves as a vesicle stabilizer component and hence it improves the firmness of vesicles. The ayclovir is slightly water soluble and hence in vesicle structure it should be located in vesicle as shown in figure 2.

The hydration temperature is the major contributing factor to form niosome vesicles. The bilayer vesicle form at the phase transition temperature. Thus, at 55 °C the vesicle formed with maximum PDE and minimum vesicle size. The energy produced at this temperature was highest and hence the vesicles were formed of small size (1.23 ± 0.19 μm). The application of sonication energy can further reduce the vesicle size. The sufficient hydration volume is also required to carry out the proper hydration. The table 1 shows the increase in hydration volume increases the percentage entrapment in vesicles. The hydration step allows the non-ionic surfactant to swell and form bilayer vesicles. Vesicle size is also reduced as the hydration volume is increased because the hydration can take place more conveniently.

**In vitro diffusion study**
The in vitro diffusion study shows that niosomal gel has more diffusivity than the conventional ACV gel. The diffusion of ACV was remarkably increased in niosomal dosage form. The ACV has very less solubility and hence the conventional ACV gel showed less diffusivity. The figure 3 indicates that the diffusion rate was reduced after 6 hours of application whereas the niosomal gel showed constant diffusion pattern. The niosome vesicles eased the penetration of ACV through skin and hence the percentage diffusion of ACV in niosomal dosage form is higher. The herpes virus replicates at the basal dermis and hence the improved penetration of ACV will surely reduce the treatment time as the ACV will be more available at the site of action.

**Skin retention study**
The percentage drug retained after for conventional ACV gel and niosomal gel were found to be 1.56% and 7.67% respectively. This clearly indicates that the retention of drug in skin has been remarkably improved (4.92 times) and hence the ACV remains available in affected area in higher concentration as compare to plain drug gel. The improved skin retention of ACV may lead to decrease dosage administration frequency and hence ultimately decreased total therapy time. The figure 4 represents the comparative skin retention data.

**Stability study**
The niosomal gel charged for stability at different accelerated (25±2 °C) and non-accelerated (2-8 °C) for 12 weeks. The
figure 5 shows the graphical representation of percentage drug retained in vesicles after various time points at different conditions. Percentage drug retained in niosomal gel stored at 2-8 °C and 25±2 °C were found to be 94.23% and 90.11% respectively after 12 weeks. The possible reason behind weak stability at 25±2 °C is the effect of temperature. Due to long term exposure at room temperature, the vesicles tend to leak and get burst. Thus the free drug concentration increases in gel. The increase in concentration of cholesterol can further impart the stability and firmness to the vesicles and hence the stability may get improved. But the increase in cholesterol concentration may also cause the decrease in PDE and hence the cholesterol:non-ionic surfactant ratio should be further optimized to balance the stability of vesicles and PDE.

**CONCLUSION**

The result of this research work concludes that the drug:non-ionic surfactant ratio, hydration temperature and hydration volume are very critical parameters for the quality of produced niosome vesicles. The ratio of drug:non-ionic surfactant and hydration volume should be properly selected for better percentage drug entrainment. The hydration temperature should be close to phase transition temperature for surfactant and hydration volume should be properly selected produced niosome vesicles. The ratio of drug:non-ionic surfactant should be further optimized to balance the stability of vesicles and PDE.

**REFERENCES**

1. Habif TP, Watts HPV. Herpes simplex and other viral infections. Elsevier Mosby; 2009
### Table 1: Experiment design of ACV niosome vesicles

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<tr>
<th>Formulation</th>
<th>x₁</th>
<th>x₂</th>
<th>x₃</th>
<th>PDE (%)</th>
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<td>-1</td>
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<tr>
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<td>1</td>
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<td>20.33 ± 1.27</td>
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<td>1.88 ± 0.21</td>
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<tr>
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</table>

Where, x₁ - drug: sorbitan monostearate ratio (mg), x₂ - hydration temperature (°C), x₃ - hydration volume (ml)

Figure 1: ACV niosome vesicles under 100X magnification
Figure 2: Illusion of ACV entrapment in niosome vesicles

Figure 3: Graphical representation of diffusion study data

Figure 4: Comparative skin retention study data
Figure 5: Stability study data of ACV niosome vesicles

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