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# PROVESICULAR BASED COLLOIDAL CARRIERS FOR TRANSDERMAL DRUG DELIVERY: EFFECT OF VARIOUS SORBITAN MONO ESTERS ON *EX VIVO* PERMEATION OF VALACYCLOVIR VIA PRONIOSOMAL GELS

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**ABSTRACT**

Valacyclovir [(S)-2- [(2-amino-6-oxo-1,6-dihydro 9H-purin-9-yl) methoxy] ethyl-2-amino-3methyl butanoate] is an antiviral drug used in the treatment of herpes simplex virus infections. The main objective of the present study is to investigate the effect of various sorbitan mo esters (non-ionic surfactants) like span-20, 40, 60 and 80 on the rate of permeation of valacyclovir loaded proniosome gels. Valacyclovir proniosomes formulated by co-acervation phase separation method with slight modification. The obtained gels present in yellowish semisolid in appearance and converts easily into niosomes upon addition of warm water with gentle agitation. Lecithin and cholesterol incorporated to improve the permeation of drug and to prevent the drug leakage from the formulated vesicles. The particle size was found to be between 10.21±2.02 µm to 13.24±1.56µm with high entrapment efficiency. SEM, studies performed to understand the particle shape, surface characteristics and skin permeation mechanism of the drug. Ex-vivo permeation studies carried out on excised rat abdominal skin using Franz-diffusion cell. The obtained permeation parameters data (flux, permeability co-efficient and enhancement ratio) reveals the rate of permeation across rat skin significantly higher for all the proniosomal formulations compared to control (drug suspension). The formulation containing span-40 has showed significant improvement ($p<0.005$) in enhancement ratio (1.681 fold) than all other formulations.

## INTRODUCTION

Multiple trials with antiviral medications for the treatment of recurrent herpes simplex virus (HSV) infections in immunocompetent patients have been undertaken in the last three decades, and the benefits of treatments to date have been modest [1]. Valacyclovir [(S)-2- [(2-amino-6-oxo-1, 6-dihydro 9H-purin-9-yl) methoxy] ethyl-2-amino-3methyl butanoate] is an antiviral drug used in the treatment of herpes simplex. Virus infections this should facilitate inhibition of HSV replication before the development of significant mucocutaneous damage [2&3]. Transdermal drug delivery system (TDDS) surrogate for the oral delivery, which upon application on skin surface will deliver the drug in to systemic circulation at sufficient concentration to ensure therapeutic efficacy. Limitations related to oral drug delivery, can be

avoided with transdermal administration. Provide suitability for self-administration [4]. The transdermal route has many advantages for the administration of drugs for local and systemic therapy. The outermost layer of skin, the stratum corneum (SC), forms a strong barrier to most exogenous substances including drugs. The barrier function of the SC is attributed to its multilayered wall-like structure, in which terminally differentiated keratin- rich epidermal cells (corneocytes) are embedded in an intercellular lipid-rich matrix. Various approaches were put forward for overcoming it. Of these, colloidal carrier is an efficient one as it acts as drug containing reservoirs and can loosen the stratum corneum, thereby modifying the barrier, and can adjust the release rate at the target site. Among the various colloidal carriers, liposome and niosome were the popular ones as they can efficiently encapsulate both hydrophilic and hydrophobic drugs [5]. Although the vesicular carriers are promising in providing the alternative routes of drug delivery and also provide a sustained action due to prolonged release yet on other hand these carriers also suffer from some shortcomings at industrial and clinical levels [6]. The main aim of present research work is to design a systematic approach for formulating proniosomal based gels are prepared by using different non-ionic surfactants and evaluation of proniosomes containing transdermal gel of VACV to achieve good physical stability and chemical stability of the dosage form. To study the effect of various surfactants on the drug release to achieve high success rate and patient compliance in the treatment of over active bladder. Evaluation of prepared proniosomal gels for percentage drug entrapment, particle shape, particle size. *Ex-vivo* drug release studies were performed using excised rat skin.

## MATERIALS AND METHODS

Valacyclovir was obtained as a gift from (Dr. Reddy's Labs, Hyderabad), Span -20, 40, 60 and 80 was procured from (Central Drug House Ltd., Delhi). Cholesterol (S.D. Fine-Chem. Ltd., Mumbai), lecithin (lipo. Germany), Ethanol (Merck Ltd., Mumbai), Potassium dihydrogen phosphate purchased from (Himedia Laboratories Pvt. Ltd), Mumbai. Sodium hydroxide (Merck Ltd., Mumbai) and other chemicals are analytical reagent grade.

**Preparation of proniosome gel:**

Proniosomes were prepared using a modified literature method [7]. The compositions of different proniosomal formulations are listed in formulation chart (Table-1). Using a 5ml wide-mouth glass tube, 10mg of valacyclovir with surfactant, lecithin, and cholesterol was mixed with 0.5 ml of ethyl alcohol. Then the open end of the glass tube was covered with a lid and the tube was warmed in a water bath at 65±3°C for 5 min. Then 0.4 ml of pH 7.4 phosphate buffer was added and the mixture was further warmed in the water bath for about 2 min so that a clear solution was obtained. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. The proniosomal gel was then mixed with 2% polymeric carbopol gel to give a final concentration.

**Size and size distribution:**

Size and size distribution studies were done for niosomes prepared from proniosomes hydration. The proniosomal gel (100 mg) was hydrated in a small glass test tube using 10 ml of pH 7.4 phosphate buffer solution. The dispersion was observed under optical microscope at 40X magnification. Size and size distribution of 200–300 niosomes were noted using calibrated stage and ocular micrometers (Elico Instruments, Hyderabad). Similarly, size was noted for niosomes formed spontaneously from proniosomes after hydration without agitation in a cavity slide [8].

**Entrapment efficiency**

To 0.2 g of proniosome gel, weighed in a glass tube, 10 ml phosphate buffer pH 7.4 were added. The aqueous suspension was then sonicated. Niosomes containing valacyclovir were separated from untrapped drug by centrifugation at 9000rpm for 45 min at 4 ◦C. The supernatant was recovered and assayed spectrophotometrically using UVspectrophotometer (UV-1800 Shimadzu, Japan), at 213nm . The encapsulation percentage of drug (EP) was calculated by the following equation [9].

$$EP = [(C_t - C_r)/ C_t] * 100$$

where, Ct, concentration of total valacyclovir, Cr, concentration of free valacyclovir.

**Vesicle physical analysis**

The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy. Once again, 0.2 g of the proniosome gel in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The niosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage[10].

**Scanning Electron Microscopy**

The surface morphology and size distribution of proniosomes were studied by scanning electron microscopy (SEM). Proniosomes were sprinkled on to the double-sided tape that was affixed on aluminium stubs [5]. The aluminium stub was placed in vacuum chamber of a scanning electron microscope (Shimadzu, Germany.

***Ex Vivo* Permeation Study Using Excised Rat Abdominal Skin**

Male albino rats (150-200 g) were used for the experiment. The rats were sacrificed by using excess amount of anaesthetic ether. Before surgical removal of the skin, hair on dorsal side was removed with hair clipper taking extreme precautions not to damage the skin. The epidermis was prepared by a heat separation technique[11], which involved soaking of the entire abdominal skin in water at 60°C for 45 seconds, followed by careful removal of the epidermis. The epidermis was washed with water, wrapped in aluminium foil and stored at -20°C till further use (used with in 2weeks of preparation). Permeation of VACV through excised rat skin, from the selected proniosomal preparations was assessed. The abdominal hair of male albino rats (150±50 gm) was removed carefully. After the animals were sacrificed, the abdominal skin was excised and the adhering fat eliminated. The whole skin was equilibrated in phosphate buffer pH 7.4 solution for 1 h before the experiment. This membrane was mounted on a vertical Franz type diffusion cell with the dermis facing the receptor compartment. The donor side was charged with 500mg of the investigated preparation. The membrane surface area available for diffusion was 6.154cm$^2$. The receptor compartment was filled with the buffer. Temperature was maintained at 37±0.5C to simulate human blood temperature. The receptor compartment was constantly stirred at 300rpm. Samples from the receptor fluid (5 ml) were withdrawn at various time intervals (0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hrs respectively) and replaced immediately by fresh buffer solution to maintain the "sink" conditions constantly and a constant volume as well. The samples were then assayed spectrophotometrically at 213 nm. Flux was calculated from slope of the line obtained on plotting mean cumulative amount permeated per area versus time. Permeation rate was determined; being the slope of the line obtained on plotting cumulative amount of drug permeated versus time. Also, the enhancing ratio (ER: the relationship between the flux from a certain gel and that from the control gel) was calculated from the following equation [12].

**Permeation Data Analysis**

The cumulative amount of drug permeated through a unit area of skin was plotted as a function of time. The Steady state Flux was calculated by using the slope of the graph where J=Flux(µg/cm$^2$/hr), A=Surface area, dQ/dt = Cummulative amount permeated per unit area per unit time.containing cumulative amount permeated through unit area (CAP) Vs Time.

$$\mathbf{J_{ss} = (dQ/dt)*(1/A)}$$

Permeability co efficient which represents the correlation between the flux and initial drug load was calculated using the following equation

$$\mathbf{Kp = J_{ss}/C}$$

Where, Kp=Permeability co efficient (cm/hr)

J= transdermal flux

C= Initial concentration of drug in the donor compartment.

The penetration enhancing effect of various formulations containing proniosomal gels were calculated in terms of Enhancement Ratio (ER) by using the following equation.

**ER = Jss of formulation/ Jss of reference**

**Skin irritancy test:** The skin irritancy potential of the proniosome formulations was evaluated in albino rats. The hair was removed on the back of the animal and the formulations were applied, and the animals were examined for any signs of skin irritation and erythema for a period of 1 week.

**Stability Studies:** The formulations stored in glass vials covered with aluminium foil were kept at room temperature and in refrigerator (4◦C) for a period of 30 days. At definite time intervals (10, 20, and 30 days), samples were withdrawn and hydrated with phosphate-buffered saline (pH 7.4) and observed for any sign of drug crystallization under optical microscope. Furthermore, the samples were also evaluated for particle size and percent retention of valacyclovir.

**Statistical Analysis:** Significance of difference between formulations was calculated by one way analysis of variance using Newman Keuls (compare all pairs) with Instant Graph Pad Prism software. The difference was considered to be statistically significant at $p<0.05$.

## RESULTS AND DISCUSSION

VACV content estimation in the proniosomal gel of different non ionic-surfactants, Lecithin, Carbopol ratio ( 1:1respectively) is carried out . This implies that more than 85% (90.65% and 97.01% respectively) of added VACV was recovered in the proniosomal gel. The rest of VACV not recovered might be adsorbed on to the surface of the test tube where the proniosomal gel was prepared. The order of drug content or percentage yield was F10> F14> F13> F3> F2> F1> F12> F5> F8> F7> F16> F11> F6> F9> F4> F15.

**Particle size analysis:** The particle size of Proniosomes was determined by optical microscopy. The prepared formulations were studied under 40x magnifications to observe the formation of vesicles. About 300 particles were measured and the results are shows in table 2. The Proniosomes were observed to be spherical vesicles with smooth surface. The vesicles were discrete and separate with no aggregation. Size range of the all formulations was found between 10.21±2.02µm to 13.24±1.56µm.

**Entrapment efficiency (EE)**

valacyclovir Proniosomal gel was prepared using non-ionic surfactant, Span 20, 40, 60, 80 in different proportions and cholesterol of same proportion by co-acervation method. Cholesterol used as a entrapping agent in the preparation of proniosomes. Increase in Span 60 concentration might increase the drug entrapment efficiency; Lecithin is used as a penetration enhancer. As shown in table no 2. Proniosomal gel formed from Span 60 exhibited very high encapsulation efficiency. This could be explained on the basis that the highly lipophilic portion of the drug expected to be housed almost completely without the lipid bilayer of the proniosome. Similar observations have been previously reported[13]. The result are also consistent with the high entrapment efficiency of valacyclovir proniosomal incorporation Span 60, most of the surfactant used to make non-ionic-surfactant vesicle have a low aqueous solubility. However, freely soluble non-ionic surfactant such as Span 60, can form micelles on hydration in the presence of Cholesterol. The Span 60 formulation in the present study was also able to entrap valacyclovir efficiently. Encapsulation studies were carried out on all formulations (F1-F16). The order of entrapment efficiency was F8> F6> F15> F10> F12> F14> F13> F7> F5> F16> F11>F9>F2>F1>F4>F3. In these studies the higher entrapment efficiency is for F8 formulation 96.51% and for F12 is 93.24 (Table 2). The effect of span 20 with different formulations were indicated in Table no 3. The permeation profile of span 20 figure shows that the drug releasing order indicates as below

$$F3 \geq F4 > F2 > F1 > RF$$

Span20 formulations release shows significant increase ( $P < 0.5$ ) in amount than reference drug. F2 is having greater permeation compared to F1 and reference.F3 and combination of lecithin and carbopol ( F4 ) shows equivalent release than other formulations. There is no major change in the drug release with the addition of carbopol

**Table 1: Composition of proniosomal valacyclovir gel (F1 to F16)**

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 | F14 | F15 | F16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Valacyclovir | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Span 20 | 360 | 360 | 180 | 180 | - | - | - | - | - | - | - | - | - | - | - | - |
| Span 40 | - | - | - | - | 360 | 360 | 180 | 180 | - | - | - | - | - | - | - | - |
| Span 60 | - | - | - | - | - | - | - | - | 360 | 360 | 180 | 180 | - | - | - | - |
| Span 80 | - | - | - | - | - | - | - | - | - | - | - | - | 360 | 360 | 180 | 180 |
| Lecithin | - | - | 180 | 180 | - | - | 180 | 180 | - | - | 180 | 180 | - | - | 180 | 180 |
| Cholesterol | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Carbopol 934 | - | 1:1 | - | 1:1 | - | 1:1 | - | 1:1 | - | 1:1 | - | 1:1 | - | 1:1 | - | 1:1 |

**Table2. Percentage of drug content, entrapment efficiency and particle size of valacyclovir proniosome gel, mean ± S.D. (n=3) (F1-F16)**

| Formulation code | % Drug Content | Entrapment Efficiency (%) | Particle Size (μm) |
|---|---|---|---|
| F1 | 94.84±3.55 | 89.88±7.42 | 11.10±2.11 |
| F2 | 95.02±2.68 | 91.10±3.88 | 10.21±2.02 |
| F3 | 95.68±2.08 | 87.50±8.32 | 12.31±1.85 |
| F4 | 92.05±5.52 | 89.21±7.34 | 10.23±2.34 |
| F5 | 94.05±1.66 | 91.90±5.21 | 10.57±3.11 |
| F6 | 93.14±3.29 | 94.61±6.23 | 12.45±1.44 |
| F7 | 93.79±4.52 | 92.10±4.33 | 13.02±1.11 |
| F8 | 93.81±5.39 | 96.51±1.11 | 11.45±0.56 |
| F9 | 92.53±4.33 | 91.22±5.21 | 12.11±2.22 |
| F10 | 97.01±2.32 | 93.45±2.45 | 10.21±4.12 |
| F11 | 93.47±3.22 | 91.50±4.34 | 10.31±3.23 |
| F12 | 94.94±4.56 | 93.24±3.33 | 11.52±2.21 |
| F13 | 95.36±3.21 | 92.70±2.56 | 11.65±2.45 |
| F14 | 96.35±1.21 | 93.05±3.24 | 12.31±2.01 |
| F15 | 90.65±4.33 | 94.21±3.33 | 13.24±1.56 |
| F16 | 93.68±3.46 | 91.88±4.34 | 10.67±3.67 |

***Ex vivo* permeation study.**

**Table 3. Effect of Span 20 on transcutaneous permeation of valacyclovir across rat abdominal skin from different formulations (mean±SD; n=3).**

| Permeation parameters | | | | |
|---|---|---|---|---|
| FORMULATION CODE | Q24 (μg) | Flux ($J_{ss}$) (μg/cm$^2$/hr) | Permeability coefficient Kp (cm/hr)x10$^{-3}$ | Enhancement ratio (ER) |
| RF | 61.82±1.36 | 0.134 | 0.027 | - |
| F1 | 66.77±1,42 | 0.158 | 0.030 | 1.128 |
| F2 | 66.84±1.32 | 0.153 | 0.030 | 1.123 |
| F3 | 72.51±2.48 | 0.170 | 0.33 | 1.219 |
| F4 | 72.10±0.96 | 0.170 | 0.34 | 1.249 |

**Q24-** cumulative drug release for 24 hours

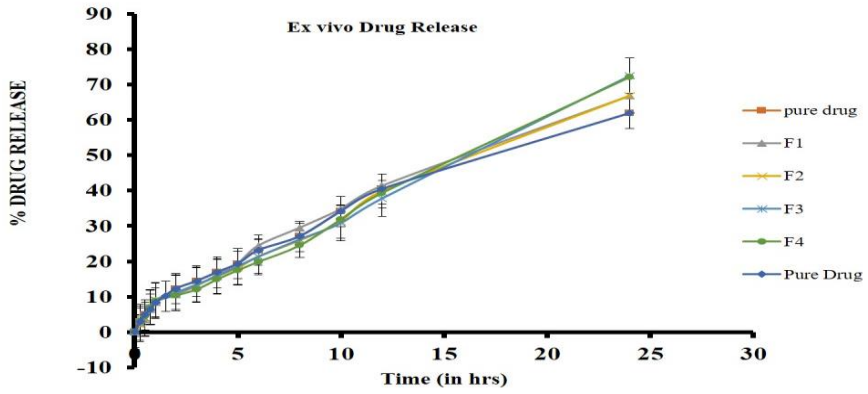


**Fig. 1.** Effect of Span 20 on transcutaneous permeation of valacyclovir across rat abdominal skin from F1 to F4 formulations (mean±SD; n=3).

**Table 4. Effect of Span 40 on transcutaneous permeation of valacyclovir across rat abdominal skin from different formulations (mean±SD; n=3).**

| Permeation parameters | | | | |
|---|---|---|---|---|
| FORMULATION CODE | Q24 (µg) | Flux ($J_{ss}$) (µg/cm$^2$/hr) | Permeability coefficient Kp (cm/hr)x10$^{-3}$ | Enhancement ratio (ER) |
| RF | 61.82±1.36 | 0.134 | 0.027 | - |
| F5 | 74.06±0.42 | 0.174 | 0.035 | 1.296 |
| F6 | 74.50±1.56 | 0.181 | 0.035 | 1.309 |
| F7 | 93.44±1.25 | 0.217 | 0.044 | 1.632 |
| F8 | 96.131±1.86 | 0.232 | 0.045 | 1.681 |

**Q24-** cumulative drug release for 24 hours

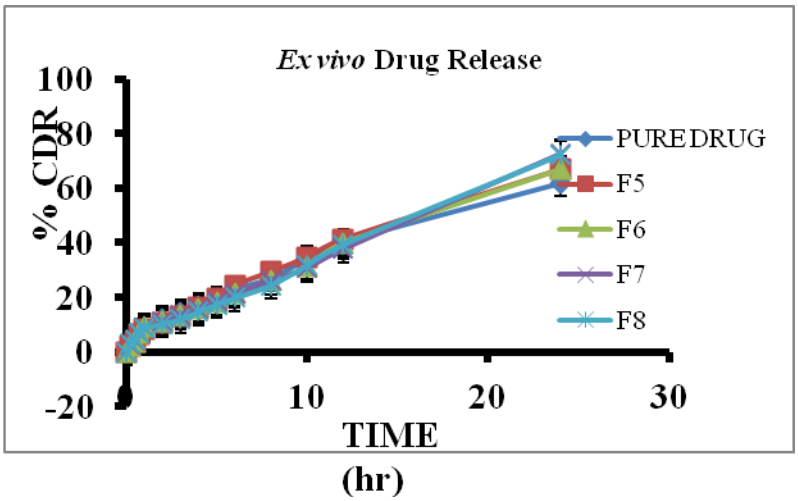


**Fig. 2.** Effect of Span 40 on transcutaneous permeation of valacyclovir across rat abdominal skin from F5 to F8 formulations (mean±SD; n=3).

**Table 5. Effect of Span 60 on transcutaneous permeation of valacyclovir across rat abdominal skin from different formulations (mean±SD; n=3).**

| Permeation parameters | | | | |
|---|---|---|---|---|
| FORMULATION CODE | Q24 (µg) | Flux ($J_{ss}$) (µg/cm$^2$/hr) | Permeability coefficient Kp (cm/hr)x10$^{-3}$ | Enhancement ratio (ER) |
| RF | 61.82±1.36 | 0.134 | 0.027 | - |
| F9 | 72.84±0.65 | 0.191 | 0.033 | 1.258 |
| F10 | 73.58±1.32 | 0.175 | 0.035 | 1.314 |
| F11 | 91.45±0.48 | 0.219 | 90.044 | 1.637 |
| F12 | 92.86±1.22 | 0.257 | 0.045 | 1.660 |

**Q24-** cumulative drug release for 24 hours

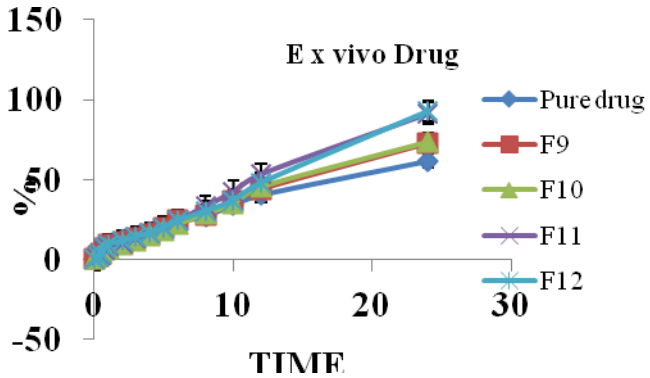


**Fig. 3.** Effect of Span 60 on transcutaneous permeation of valacyclovir across rat abdominal skin from F9 to F12 formulations (mean±SD; n=3).

**Table 6 . Effect of Span 80 on transcutaneous permeation of valacyclovir across rat abdominal skin from different formulations (mean±SD; n=3).**

| Permeation parameters | | | | |
|---|---|---|---|---|
| FORMULATION CODE | Q24 (µg) | Flux (Jss) (µg/cm$^2$/hr) | Permeability coefficient Kp (cm/hr)x10$^{-3}$ | Enhancement ratio (ER) |
| RF | 61.82±1.36 | 0.134 | 0.027 | - |
| F13 | 74.85±1.25 | 0.163 | 0.033 | 1.272 |
| F14 | 78.42±0.50 | 0.184 | 0.036 | 1.350 |
| F15 | 81.99±0.26 | 0.197 | 0.038 | 1.405 |
| F16 | 87.84±0.96 | 0.221 | 0.042 | 1.567 |

**Q24-** cumulative drug release for 24 hours

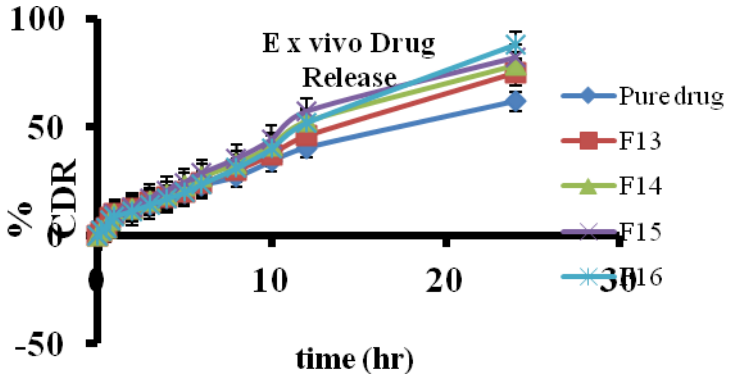


**Fig. 4. Effect of Span 80 on transcutaneous permeation of valacyclovir across rat abdominal skin from F13 to F16 formulations (mean±SD; n=3).**

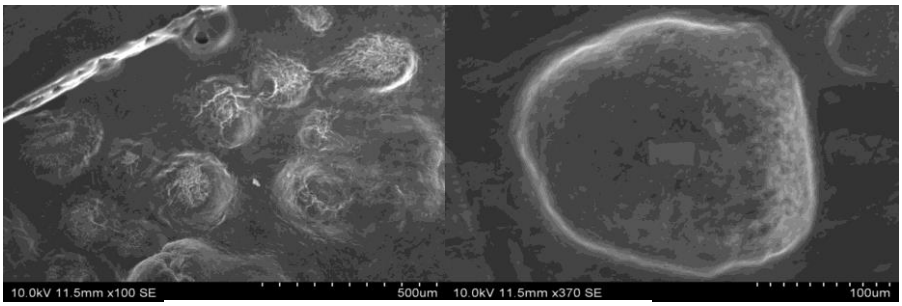


**Fig. 5. SEM of optimized formulation F8**

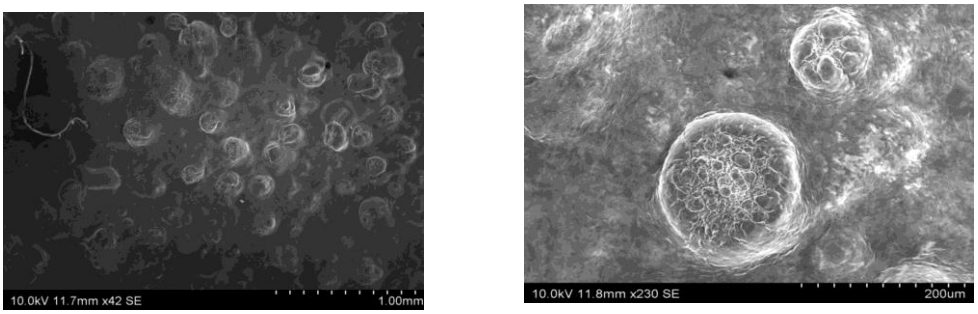


**Fig. 6. SEM of optimized formulation F12**

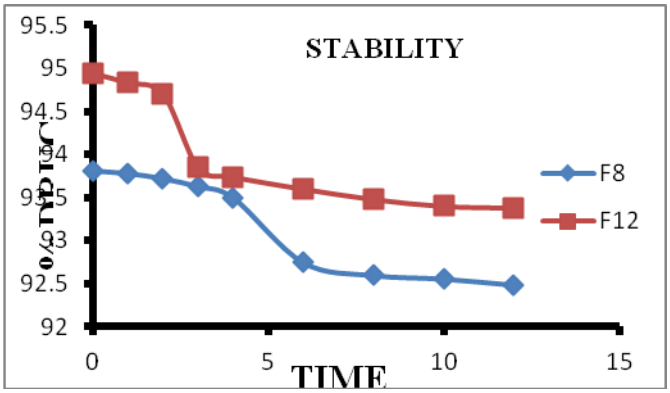


**Fig. 7. Stability Profile of Formulation F8, F12**

The effect of span 40 with different formulations were indicated in Table no 4 The permeation profile of span 40 figure shows that the drug releasing order indicates as below

F8 >F7> F6 > F5 > RF

Span40 formulations release shows significant increase ( $P < 0.5$ ) in amount than reference drug. F6 is having greater permeation compared to F5 and reference. F8 is having greater permeation than F7 and other formulations. There is a significant change in the drug release with the addition of carbopol

The effect of span 60 with different formulations was indicated in Table no 5. The permeation profile of span 60 figure shows that the drug releasing order indicates as below

F12 > F11 > F10 > F9 > RF

Span60 formulations release shows significant increase ( $P < 0.5$ ) in amount than reference drug. F10 is having greater permeation compared to F9 and reference. F12 is having greater permeation than F11 and other formulations. There is a significant change in the drug release with the addition of carbopol .

The effect of span 80 with different formulations was indicated in Table no 6. The permeation profile of span 80 figure shows that the drug releasing order indicates as below

F16 > F15> F14 > F13> RF

Span80 formulations release shows significant

increase ( $P < 0.5$ ) in amount than reference drug. F14 is having greater permeation compared to F13 and reference. F16 is having greater permeation than F15 and other formulations. There is a significant change in the drug release with the addition of carbopol.

**Scanning Electron Microscopy:**

The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy. Results were shown in figure no 5&6.

**Stability profile:**

Formulation F8 and Formulation F12: valacyclovir proniosomal gel content at different storage conditions

**Skin Irritancy Test:** When optimized formulations F8 and F12 gels are applied to the skin of rat, no possible skin irritations and no oedema are seen. Hence these are optimized.

## CONCLUSION

Prepared formulations of proniosomal gel using various non ionic surfactants like span 20, 40, 60, 80 and cholestrol used as a stabilizer and lecithin is used as a penetration enhancer. Carbopol is used as a gelling agent. By carrying out different evaluation parameters finally f8 and f12 are found as the optimized formulations. The percent drug content for f8 formulation is found to be 93.81 and for f12 formulation is 94.94. The entrapment efficiency of f8 is 96.51 and for f12 is 93.24. The particle size of f8 is found to be 11.45 and for f12 is 11.51. The *ex-vivo* drug release of f8 is found to be 96.13 and f12 is 92.86. The flux of f8 is found to be 0.232 and f12 is 0.257. By above parameters f8 and f12 are found to be the optimized formulations.

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