Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [5] April 2021 : 34-46

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Provesicular Based Colloidal Carriers For Transdermal Drug Delivery. Formulation Aspects: Bioavailability Enhancement of Acyclovir through Proniosomal Gel Formulations.

Ashok Mateti[1*], Mohammed Habibuddin[2], Raju Jukanti[3]
Department of Pharmaceutics
[1] Trinity College of Pharmaceutical Sciences, Peddapalli, Telangana, India.
[2]Tou Pharma bio Pvt. Ltd., Hyderabad, Telangana, India.
[3]Drugs Inspector, Hyderabad, Telangana, India.
*Corresponding author E-mail: matetiashok2301@gmail.com

ABSTRACT
Acyclovir (ACV) a 2[1]-deoxy guanosine analogue effective against Herpes Simplex Virus (both type 1and 2) and other viral diseases, mainly acts by inhibiting the viral DNA polymerase. The limited oral bioavailability of ACV (5-10%) requires higher dosage (400-800 mg), results in neurotoxicity and renal failure. Topically, the lower efficacy of ACV attributed to inadequate drug permeation across the stratum corneum (SC). In present research, we formulated ACV containing proniosomes using various ratios of span 40 and cholesterol mixtures by co acervation phase separation method. The formulated vesicles having the acceptable particle size range (518-704 nm). Other characters like morphological behaviour, zeta potential, polydispersity index and entrapment efficiency were evaluated. In vitro release studies were revealed the controlled delivery of ACV from formed niosomes. Significant higher permeation parameter values such as flux (Jss), permeability coefficient (Kp) and enhancement ratio (ER)} evaluated from all the formulations by ex vivo permeation studies using male wistar rats. In vivo data evident that 3.07 fold increment bioavailability of optimized formulation than control (oral suspension). The formulated ACV proniosomal gels more stable when stored at 4 °C.
Key words: Proniosomes, Acyclovir, vesicles, Transdermal, Permeation.

Received 07.01.2021 Revised 03.04.2021 Accepted 20.04.2021

INTRODUCTION

Acyclovir (ACV) a Synthetic analogue of 21 deoxy guanosine (MW 225) a potent and reliable antiviral agent active against chicken pox, varicella zoster virus, herpes keratitis, cutaneous and genital herpes infections. The oral bioavailability of ACV between 10-30% with less half life (3.25 hr), hence repeated administration (5 times daily for 10 days) at high dose of 200 mg in effective management of HSV results in nausea, diarrhoea, rash or headache, neurotoxicity, renal failure and it shows local toxicity at injection site and renal precipitation in i.v. bolus rapid injections. Because of lower permeability (BCS Class III) of ACV cannot permeate across skin due to low efficiency in recurrent infections [1,2].

Non ionic surfactant vesicles regarded as an alternative to liposomes because of its physical structure similarity. The effective drug entrapment capacity of surfactants mainly depends on HLB and Phase transition temperature (T°c). Surfactant with optimum HLB value and greater phase transition temperature (Tc) are essential requirements for the efficient vesicle formation [3&4].

Sorbitan mono esters (Span 20, 40, 60& 80) having highest phase transition temperature (T°c) provides greater entrapment efficiency. Span 40 and 60 both are having highest phase transition temperatures (42 and 53 °C) and present in crystalline gel state at room temperature, where as span 20, 80 (T° C values 16& -12°C respectively) shows disordered liquid crystalline state[5].

HLB of surfactants play significant role in drug entrapment efficiency. Surfactants having HLB values between 14-17 are not suitable for niosomes. Decreasing HLB value from 8.6 to 1.7 decreases the entrapment efficiency proportionally. Surfactants with 8.6 HLB value provides greater drug entrapment efficiency [6]. The HLB values of Span 20, 40, 60 and 80 are 8.6, 6.7, 4.7 and 4.3 respectively. Even though span 20 having suitable HLB value (8.6), due to its lower phase transition temperature (16°C) results in formation of less ordered liquid with poor drug entrapment efficiency.

Span 80 having very less phase transition temperature (T°c=-12°C) and HLB (4.3) values. The phase transition temperature very high for Span 40& 60 (42 and 53 °C) with HLB values 6.7 and 4.7 respectively, capable to form more ordered liquid crystalline proniosomal gels.

Cholesterol, an amphiphilic molecule improves the microviscosity, rigidity of vesicle membrane results in formation of highly ordered gel structure. By the addition of cholesterol, HLB value of surfactant increases, thus helps in improved entrapment efficiency. Surfactants having less transition temperature (T°C), cholesterol made less ordered membrane structure, vice versa, in case of surfactants having greater T°C [7,8].

Span 40 (Sorbitan mono palmitate), a non ionic surfactant having greater transition temperature with less membrane permeability and entraps larger amounts of drug load. It fluidizes SC by Induced structural rearrangement, and alters its barrier function. Span 40 forms larger vesicles thereby lower surface area is provided to contact on the skin leads to offer controlled drug release [9,10]. Addition of cholesterol span 40 forms more ordered, stable crystalline proniosomal gels [11].

In present study, we formulated ACV containing proniosomes and evaluated for its morphological characters*. In vitro, ex vivo* permeation studies conducted to establish the drug release pattern and permeability parameters respectively. Bioavailability assessed by conducting pharmacokinetic studies in male wistar rats across the skin using optimized ACVPN gel.

MATERIAL AND METHODS

Materials

Acyclovir was a kind gift sample from Finoso Pharma, Hyderabad, India. Sorbitan monopalmitate (Span 40) was purchased from Sigma chemical co., St. Louis, MO, USA. Cholesterol was obtained from E merck, Mumbai, India. Dialysis Membrane (DM-70; MWCO 12 000) was purchased from Hi media, Mumbai, India. All other chemicals and solvents used were analytical and HPLC grade. Freshly prepared double distilled water was used in entire experiment.

Methods

Preparation of ACVPN gel

ACVPN gels prepared by using co acervation phase separation technique with slight modification mentioned elsewhere. Transfer predetermined proprtions (1mM) of span 40 and cholesterol (1:0, 2:1, 1:1.5, 1.5:1 and 1:1) mixtures into a wide glass vial. Add 5mg of ACV followed by the addition of 400 mg of distilled ethanol. Close the vial appropriately to prevent the loss of alcohol. Boil the mixture on thermostat maintaining temperature between 60-65°C with intermittent shaking until all the contents dissolved. Add 160µl of 7.4 pH phosphate buffer saline (PBS) maintaining same temperature in streamline manner and shake gently to get homogenous dispersion. Store the vials over night in dark place at room temperature.

Formation and morphological evaluation

Optical microscopy used to evaluate formation and morphology of niosomes. The morphological evaluation of ACVPN gels were carried out by hydrating with 4ml of PBS (pH 7.4), mixed gently, adjust the volume upto 10 ml with same buffer. The niosomes formed later hydration was evaluated by observing at magnification of 450X through optical microscope (Coslab micro, India) and transmission electron microscope (JEOL-200 CX; Jeol, Tokyo, Japan).

Table 1: Composition and appearance of Acyclovir loaded proniosome gels.

Formulation Code	Molar Ratio (Span40:CHOL)	Span 40 (mg)	CHOL (mg)	Ethanol (mg)	Water (mg)	Appearance
ACV PN-1	1:0	402.57	-	400	160	Yellow liquid
ACV PN-2	2:1	268.38	127	400	160	White translucent gel
ACV PN-3	1.5:1	241.55	154	400	160	White semisolid gel
ACV PN-4	1:1	201.29	193	400	160	White semisolid gel
ACV PN-5	1:1.5	161.03	231	400	160	White semi solid gel

CHOL indicates cholesterol

Total 1mM lipid mixture was used in all the preparations

Characterization of ACV Proniosomes

Vesicle size, surface charge and entrapment efficiency

The prepared ACVPN gels hydrated using PBS (7.4 pH), then subjected to sonication for 5min (Sonica, Milano. Italy). The obtained dispersion was used for the evaluation of vesicle size, surface charge and entrapment efficiency (EE)[12]. The mean size and size distribution of niosomes was determined by

photon correlation spectroscopy using Zetasizer 3000 HSA (Malvern Instruments, Malvern, UK). polydispersity index of niosomes were obtained from the instrument.

Determination of spreadibility

The spreadability of gel and marketed formulations was determined as described in literature [13]. In brief, 0.5 g of different formulations was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The spreadability was noted down by measuring the increase in diameter.

Rheological behaviour

The rheological properties of ACVPN gels was measured using Brookfield programmable DV111 + Digital rheometer applying a controlled stress rheometer with the cone (24 mm) and plate geometry. Before the study the sample was equilibrated for 5 minutes and the torque sweep was in the range of 10- 110%. All the studies were performed in triplicate at an ambient temperature and the rheological behaviour was measured using Rheolac 32 software.

Number of vesicles per cubic mm

The copious formation of niosomal vesicles from proniosomes is one of the significant parameter to optimize the composition. Priorly, the proniosomal gels were hydrated using phosphate buffered saline (pH 7.4) and mounted on haemocytometer and using optical microscope. The number of niosomes converted from proniosomes per cubic mm was counted and calculated by using the following formula.

Total number of niosomes formed per cubic mm = total number of niosomes counted X dilution factor X 4000/ total number of squares counted.

Differential Scanning Colorimetry

Thermal properties of optimized proniosome gel (ACVPN2), Span 40, cholesterol and ACV was studied by using differential scanning calorimeter (Mettler DSC 821e, Mettler-Toledo, Switzerland). 5 mg of sample was heated in hermitically sealed aluminum pan over a temperature range of 20°C to 350°C under a constant nitrogen gas flow of 30 mL/min at a heating rate of 10°C/min.

Fourier transform infrared spectroscopy

The optimized proniosomal gel equivalent to 5mg of acyclovir treated on to the prepared rat skin for 6 hours. Later treatment, the skin sample was washed with water and blotted dry where untreated skin was used as control. The FTIR - spectrum of the above treated skin sample using FTIR -multipurpose spectrophotometer (Shimadzu, Japan) against untreated normal rat skin which served as the control was recorded in the range of 4000 - 400cm^{-1}[14].

In vitro release study

In vitro release study performed to determine the ACV release pattern across the cellophane membrane (0.45 µm pore size). Initially, the cellophane membrane equilibrated by soaking in the 7.4 pH PBS for 1 hr. After, it was mounted between the two halves of the Franz diffusion cell. The formulation was uniformly applied on to the membrane facing towards donor compartment. Receiver compartment filled with the 7.4 pH PBS. The donor compartment and sampling port covered using parafilm to prevent evaporation. The buffer stirred constantly 400 rpm maintaining at 37±2 °C. Carefully withdrawn 1 ml of sample at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hrs), replaced with equal volume of fresh buffer. Estimated the quantity of ACV released from all the prepared proniosomal gel and control using HPLC.

Ex vivo permeation study

Male wistar rats (180 – 200 grams) were used in ex vivo skin permeation study with prior approval of Institutional Animal Ethical Committee, Trinity college of Pharmaceutical sciences. Euthanasia and disposal of carcass was in accordance of the guidelines. The animals had free access to food and water until euthanasia. The rats were sacrificed with excess ether inhalation. Depilation can be carried out on the rat abdominal skin with hair clipper, taking extreme precautions not to damage skin. The skin was excised and obliterate from adhering matter such as fat tissue, absterge capillaries with a pair of scissors. The rat epidermis was prepared by heat separation technique[15]. which involves soaking of the excised abdominal skin in water at 60°C for 45 seconds. The epidermis was washed properly using water, wrapped in aluminium foil and taxidermy can be done at -20°C (Used within two weeks). The rat skin was brought to room temperature and placed between two halves of fabricated vertical Franz diffusion cell (effective diffusional area 4.59cm^2) stratum corneum facing towards donor compartment. The receptor compartment filled using 17 ml of 7.4pH PBS and equilibrated the skin for 1 hour. The permeation study was carried out by replacing with fresh 7.4 pH PBS containing 0.003% W/V of sodium azide as a microbial retardant. The prepared ACVPN gel and control (5 mg ACV suspended in same solvents) uniformly applied on the skin. The donor compartment and sampling port covered with glass lid and parafilm respectively to prevent from evaporation of the contents. Receptor compartment was maintained under constant stirring (400 rpm) at 37±2°C for 24 hrs. 1 mL of sample was withdrawn at

predetermined time intervals and replenished with equal volume of fresh buffer. The obtained samples were suitably diluted and quantified by HPLC.

Drug content remained/retained in the skin

The amount of ACV deposited in the epidermal skin layers was estimated after 24 hours permeation study. The skin was separated from the diffusion cell and the adhering matter was removed by brief washing /ablution with methanol followed by drying at room temperature for 10 minutes. The skin was chopped into small pieces; homogenization carried out using phosphate buffered saline (pH 7.4) and subjected to sonication for 30 minutes (bathsonicator, Sonica, Italy) to leach out the ACV from skin layers. The samples were filtered through membrane filter (0.45µm) and estimated by HPLC.

Permeation data analysis

The cumulative amount of drug permeated through unit area was plotted against time. From the linear portion of the cumulative amount permeated per unit area against time steady state flux was obtained. The permeability co efficient (kp) was calculated by correlating steady state flux with initial drug concentration. From the transdermal flux ratio between proniosomal gel and control ER (Enhancement Ratio) was calculated.

The permeation parameters were subjected one way analysis of variance (ANOVA) to get the statistical significance. Significance of difference between formulations was calculated by student-Newman-Keuls (compare all pairs) with Instant Graph pad prism software. The difference was considered to be statistically significant at $p < 0.001$.

HPLC assay of ACV

The proportion of ACV in blood plasma estimated by the method mentioned by Sanjay et al,.17.. HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with LC-10 AT VP Liquid Chromatograph pump, SPD-10A AVP UV-Spectrophotometric detector, Spinchrom software and injector (Rheodyne) fitted with a 20 µL capacity was used for the analysis. An octadecylsilane (C-18) reverse phase stainless steel analytical column (250 x 4.6 mm) with 5 µm particle size (Lichrospher 100) was employed for chromatographic separation. Mobile phase: 0.02M Potassium dihydrogen phosphate, pH 3.5 and Acetonitrile (95:5). Flow rate: one mL per minute, UV detection at 254 nm and separation was at ambient temperature and the sensitivity was set at 0.005 AUFS. The plasma proteins precipitated by treating 0.1 mL of blood plasma with equal volume of 10% (v/v) trichloro acetic acid and 50µL of acetonitrile followed by vortexing for 30 seconds.

Pharmaco kinetic study and estimation of pharmaco kinetic parameters

Male wistar rats weighing between 180-200g were used to conduct the pharmaco kinetic study with the prior approval of Institutional Animal Ethical Committee, Trinity College of pharmaceutical sciences, Peddapalli, India. The rats were divided in to test and control groups containing six in each. Before the study the rats were prepared by careful shaving of abdominal hair with electric shaver followed by clean with water. The optimized proniosomal gel equivalent to 25 mg/Kg applied to test group and the control group received ACV oral suspension (5 mg/ml in 0.5% w/v of HPMC). After uniform application of proniosomal gel on to the shaved area (3.14 cm^2) was covered with impermeable backing membrane followed by adhesive membrane.

The rats under testing maintained carefully in separate cages throughout the study. Blood samples (500 µl) were collected at predetermined time intervals from the retro orbital plexus in to the heparinised micro centrifuge tubes. Blood plasma separated through centrifugation at 10000 rpm for 10 min in a micro-centrifuge (Remi equipments, India) and stored in freeze at -20°C for further study. From the blood plasma concentration Vs time profile maximum concentration (C max) and Maximum Time (T max) estimated directly.

Trapezoidal rule used for calculation of AUC (AUC_{0-t}).

$AUC_{t-\infty}$ was calculated by using the following formula.

$$AUC_{t-\infty} = \frac{\text{Plasma concentration at last time point}}{\text{Elimination rate constant (K)}}$$

and

$$\text{Relative bioavailability (F)} = \frac{AUC_{0-\infty} \text{ of ACV containing proniosomal gel}}{AUC_{0-\infty} \text{ of control (Oral suspension)}}$$

Skin irritation test

The skin irritation test was conducted on the male wistar rats. The proniosomal gel formulations were applied on to the dorsal side of the cleanly shaved rats and observed for any sign of irritation or reddening for a period of one week.

Stability studies

All the prepared gel formulations were sealed in the glass vials and stored at 4°C and room temperature, observed for a period of 6 months for any sign of aggregation or sedimentation or leakage of drug from the vesicles. The samples were withdrawn periodically (30, 60, 120 and 180 days), hydrated using phosphate buffered saline (pH 7.4) and analysed. The mean size of formed niosomes were measured by diluting each sample and analysed change in particle size by optical microscope and Entrapment efficiency was quantified by HPLC.

RESULTS AND DISCUSSION

The passage of drug molecules across the skin after releasing from vesicle follows multiple step process to reach systemic circulation this includes partitioning of drug into the aqueous epidermal layer and rate limiting diffusion process, eventually reaches to the capillary network. Hence, selected drug possess adequate lipophilicity and hydrophilicity. As per old dragma rule the drug molecular weight not exceed 500 g/mol., logp between 1-3and M.P. <200°C. Lipophilic moiety partitions into the subcutaneous layer where as hydrophilic moeity moves down by diffusion[18].

ACVPN gels were prepared by simple idea of co-acervation phase separation technique which involves decrease in the solubility of colloidal components by addition of non solvents that leads to formation of semi solid liquid crystalline compact proniosomal gel suitable for transdermal application. After topical application under in situ occlusive conditions, the liquid crystalline proniosomal gel absorbs moisture from skin layers get converts into niosomes itself spontaneously and possess versatile drug delivery into the skin[19].

Formulation and morphological characterization of ACVPN gels:

The formation of niosomes upon hydration with slight agitation ruptures the bilayer resulting in formation of small size vesicles (Fig. 1). All the prepared ACVPN gel formulations were bearing particle size in nano-range (518-713 nm). Proportional increment in the particle size has been observed with the increased addition of cholesterol concentration. Ethanol acts as an excellent permeation enhancer for many drugs. Ethanol miscible both hydrophilic and hydrophobic moieties, thereby decreasing in repulsion causes delaying in phase separation at lesser extent results in little increase in particle size.

The formulation ACVPN1 (without cholesterol) present in yellow colour liquid with poor entrapment efficiency (69.56%). Cholesterol abolishes gel to sol transition, intercalates into the vesicle bilayer thus imparts rigidity. Increase in cholesterol concentration gel consistency also increases proportionally and drug permeation lowers across the bilayer film[20]. White translucent gel was obtained in the formulation ACVPN2 with the highest entrapment efficiency (86.32%) by the addition small amount of cholesterol. It was observed that further increase in cholesterol concentration (ACVPL3-ACVPL5) the formulations turn into white semisolid gel. Cholesterol imparts viscosity and due to its lipid nature the formulations appear in white colour.

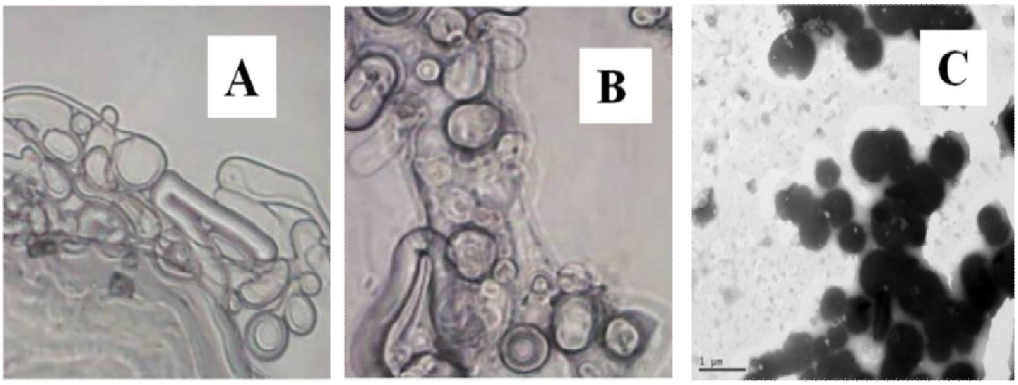


Fig. 1: Microscopic photo graphs (at magnification of 450X) showing the formation of niosomes from proniosome formulation upon hydration with 7.4 pH PBS. (A). Up on contact with water tubular structures are forming B). Conversion in to small size spherical shaped acyclovir niosome vesicles after ruptured by complete hydration. C). TEM image of spherical vesicles of acyclovir niosomes from proniosomes.

Table 2: Size, Zeta Potential,% Entrapment Efficiency, Thixotropic Degree, Spreadability and Number of vesicles formed from acyclovir proniosome gel formulations (n=6).

Formulation code	Size (nm)	Zeta Potential (mV)	PDI	Entrapment Efficiency (%)	n	Spreadability (cm)	Number of vesicles per cubic mm. X10^4
ACVPN-1	518±15	-12.5±1.6	0.189	69.56±1.22	0.981	6.45±1.08	2.9
ACVPN-2	562±20	-21.25±2.3	0.194	86.32±1.84	0.983	6.40±0.86	4.6
ACVPN-3	649±20	-19.27±1.1	0.255	78.12±2.14	0.992	6.25±0.59	4.1
ACVPN-4	713±45	-20.14±2.6	0.249	74.58±1.18	0.986	6.13±0.62	3.8
ACVPN-5	704±22	-18.66±1.8	0.269	71.52±1.36	0.996	5.89±0.32	3.4

n, PDI indicates thixotropic degree, Poly Dispersity Index respectively.

However, the reduction in entrapment efficiency from ACVPN3-ACVPN5 was observed. This could be explained that the cholesterol concentration restricted up to certain extent, the increased addition of cholesterol competes with the drug molecules partitioned in the bilayer membrane and leaches out from the vesicle results in reduced drug entrapment efficiency.

Spreadability is an important parameter for acceptance of topical formulation. The observed spreadability values were higher for gel formulations which suggest the ease of application onto the skin. The formulated gels were translucent, pseudoplastic in nature and spreadable with good skin feel. Zetapotential values were foud to be between -12 to -21 mV. Smaller values of polydispersity index (PDI) was observed in the acceptable limits (0.189-0.269) indicates the higher heterogeneity (Table 2).

The rheological behaviour of the gel formulations play pivotal role meant for transdermal route. Viscosity of the formulations decreased with increased shear rate which reveals the shear thinning property of prepared proniosomal gels (Fig. 2) suitable for topical application.

Thixotropic degree (n) is an important rheological property of gels which deals with the thinning of gel when constant force is applied. It is clearly evident from Table 2, that the thixotropic degree mainly depends on the cholesterol concentration that higher the cholesterol concentration lowers the thixotropic degree.

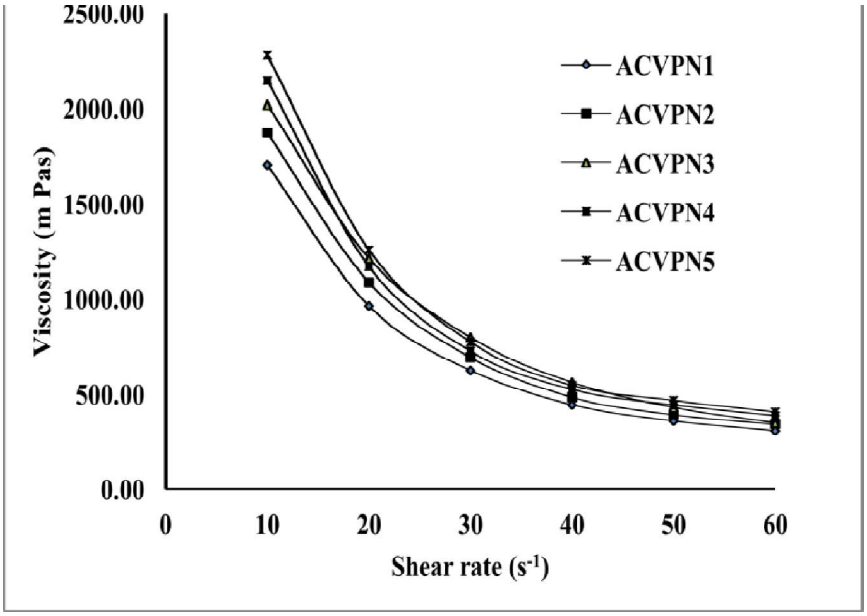


Fig. 2: Rheograms of various ACV proniosomal gel formulations

In vitro drug release study

The main objective of in vitro drug release study is not only to determine the drug release pattern and also to know the stability of niosomes on the cellophane membrane. Around 60% of ACV was released from control within 12 hrs due to the concentration gradient between receptor medium and drug on the cellophane membrane reveals that establishment of sink conditions. ACVPN formulations shows release of ACV in constant amounts after 4 hrs suggests that the initial increment of ACV concentration is due to release of unentrapped ACV across cellophane membrane followed by release from niosomal vesicle

bilayer establishment of biphasic release (Fig. 3). ACVPN2 showed greater R^2 value (Table 3) because of its appropriate linear crystalline structure formation in the vesicle bilayer.

Table 3: *In vitro* release kinetics of Acyclovir from proniosome gels across cellophane membrane (n=3).

Formulation code	Zero order		First order		Higuchi
	K_0 (h^{-1})	R^2	K (h^{-1})	R^2	R^2
Control	0.440	0.797	0.486	0.549	0.901
ACVPN-1	0.228	0.887	0.574	0.608	0.941
ACVPN-2	0.163	0.936	0.679	0.663	0.937
ACVPN-3	0.225	0.921	0.493	0.631	0.952
ACVPN-4	0.243	0.818	0.540	0.592	0.820
ACVPN-5	0.237	0.900	0.729	0.642	0.939

K_0 and K represent Zero and First order release rate constants respectively.

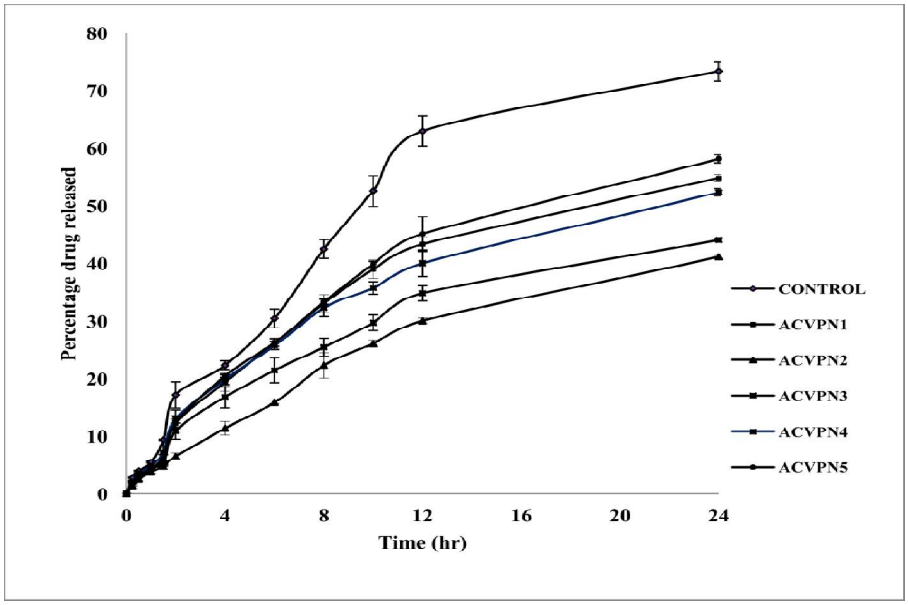


Fig. 3: In vitro Percentage of ACV released across the cellophane membrane from different proniosome gels (mean±SD; n=3).

Ex Vivo permeation studies

The exvivo permeation studies were conducted across the excised male wistar rat abdominal skin and the results were represented in Table 4 and the cumulative amount of ACV permeated Vs time profile, mean flux depicted in Fig. 4 &5. The main objective of performing *ex vivo* permeation study is to investigate the feasibility and to improve the bioavailability of ACV from ACVPN gels. All the ACVPN gel formulations detected with in 0.5 hrs indicates the formation of niosomes from Proniosomes spontaneously by hydration with skin fluids followed by release of ACV from the formed vesicles. However, control shows greater release of ACV from cellophane membrane, due to the barrier properties of skin and lower permeability of ACV, the exvivo permeation was significantly decreased compared to ACVPN formulations.

Table 4: Permeation parameters of acyclovir from different proniosomal formulations across rat skin (mean±SD; n=3).

Formulation code	Q_{24} (µg)	Jss (µg/cm^2/h)	Kp (cm/h)x10^{-3}	ER	DCS
Control	1489.24±21.92	13.69±0.87	2.74±0.17	-	481.54±28.73
ACVPN-1	2026.62±24.43	17.85±1.48[1]	3.57±0.30	1.30	1289.42±39.40
ACVPN-2	2981.38±52.01	26.79±0.76[1,2,4,5,6]	5.37±0.15	1.96	1083.89±33.26
ACVPN-3	2655.04±117.50	23.65±0.64[1,2,5,6]	4.74±0.13	1.73	1000.33±37.01
ACVPN-4	2274.69±42.17	19.77±1.84[1,2,3]	3.96±0.37	1.44	849.12±21.92
ACVPN-5	2053.16±31.31	18.23±1.70[1]	3.65±0.34	1.33	772.53±53.01

$p<0.001$; Q_{24}, Jss, Kp, ER, DCS represents amount permeated in 24h, steady state Flux, Permeability coefficient, Enhancement Ratio, Drug Content retained in the Skin layers respectively. [1,2,3,4,5,6] represents Control, ACVPN1, ACVPN2, ACVPN3, ACVPN4 and ACVPN5 respectively.

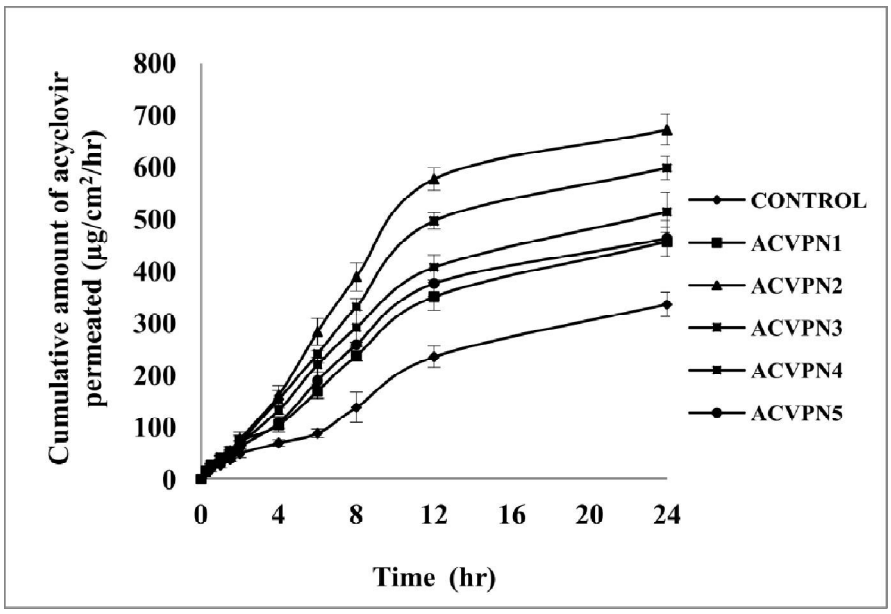


Fig. 4: Cumulative amount of ACV permeated from proniosomal gel formulations through the rat skin (n=3).

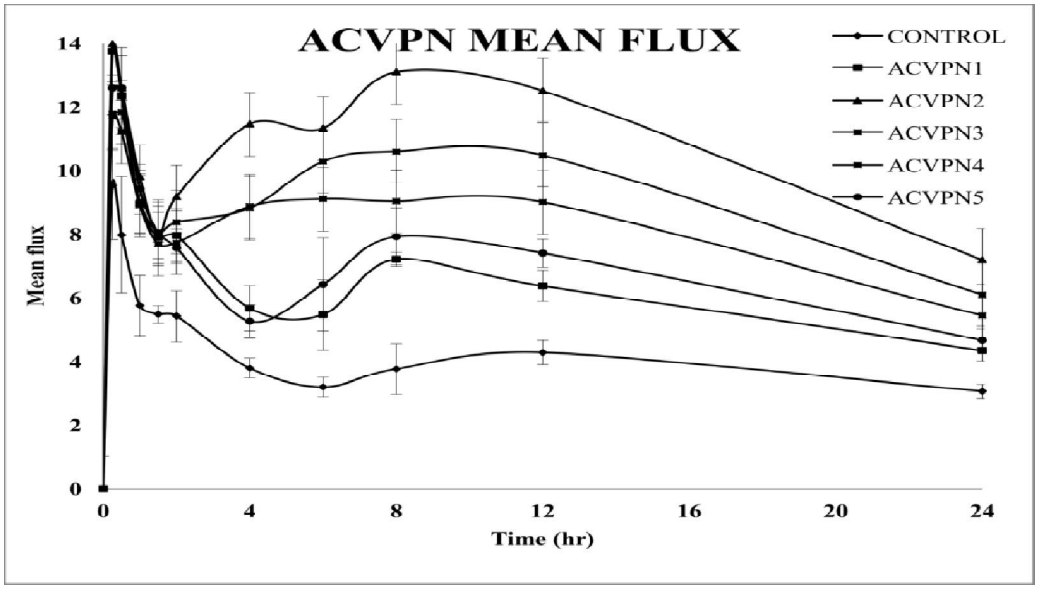
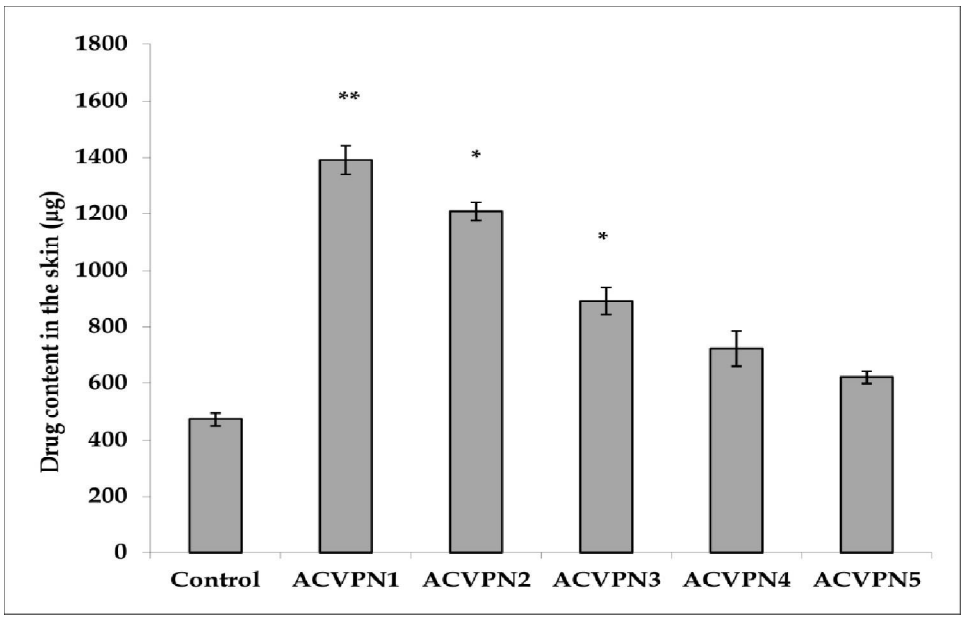


Fig. 5: Mean flux of ACV from different proniosomal gel formulations across rat skin (mean±SD; n=3).

The cumulative amount of ACV permeated from control and ACVPN1 (devoid of cholesterol in 24 hrs was found to be 1489.24±21.92 and 2026.62±24.43 µg respectively. Significant improvement in drug permeation was observed by the addition of cholesterol in ACVPN2 formulation 2981.38±52.01 µg. In contrast, we couldn't establish the linear relationship, further addition of cholesterol in ACVPN3-ACVPN5. This could be due to improved cholesterol concentration increases the vesicle bilayer lipophilicity thus drug solubility enhanced drug intercalation in bilayer leads to reduction of drug partition promotion in SC. Permeation parameters such as flux, permeability coefficient and enhancement ratio were calculated for all the ACVPN formulations and control. The increasing order of flux was ranked as Contro l< ACVPN1 < ACVPN5 < ACVPN4 < ACVPN3 < ACVPN2.

Drug content retained in the skin

Fig.6 depicts the extent of drug retained in the skin after 24 hrs drug permeation studies. In our findings all the proniosomal formulations showed significant higher drug retention than control. This may be due to the interaction between skin and vesicle which results in rupture of S.C. layer. Because of negative charge, increasing in cholesterol content repulsion generated with negatively charged skin lipids, hence inverse relation was established.



** &* indicates p<0.001 and p<0.05 significance.

Fig. 6: Drug deposited in the skin layers after 24 h following treatment with different proniosomal gel formulations (mean±SD; n=3).

DSC and FTIR Studies

The ACV and cholesterol has shown sharp endothermic peaks at 256.62 °C and 148.42 °C respectively. The disappearance in the corresponding peaks observed in the proniosomal formulation is obviously due to the transformation of the ACV from crystalline form to amorphous form in the span 40 and cholesterol mixture (Fig.7). The disappearance in the sharp endothermic peak of the cholesterol is due to the change in phase transition behaviour of the pure lipids to liquid crystalline state in the proniosomal formulation. FIG.8 illustrates the FTIR spectrum of both the treated and untreated skin and the peaks represents the molecular vibrations of lipids and proteins present in the skin. The characteristic peaks around 2800-3000 cm^{-1} is due to asymmetric and symmetric -CH_2 vibrations of hydrophobic alkyl chains of SC extra cellular lipids. Both the peak height and its area are proportional to the amount of SC lipids, if any extraction of lipids leads to decrease in peak height and area. In our findings we could observed that the reduction in the area of the peaks suggesting the fluidisation of SC by fusion of the proniosomal vesicle bilayer.

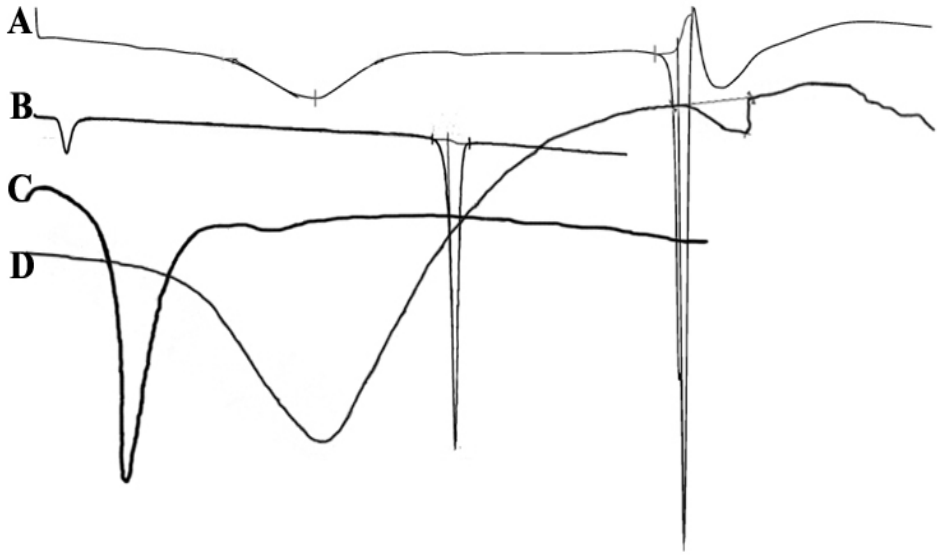


Fig. 7: DSC thermograms of (A) Acyclovir B) cholesterol C) span 40 D) optimized proniosome gel formulation (ACVPN2).

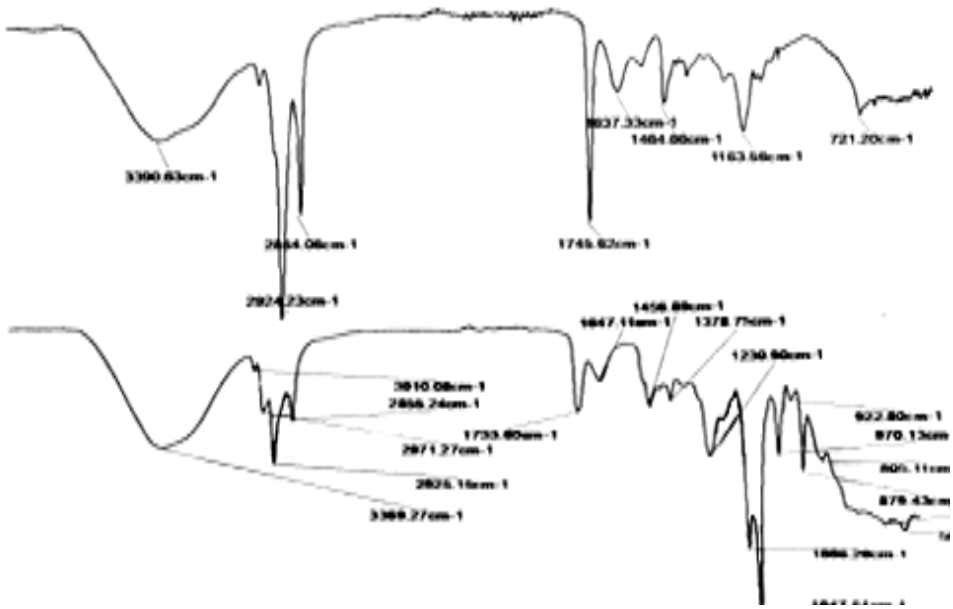


Fig. 8: FTIR spectra of **(A)** untreated rat skin (Control) **(B)** rat skin treated with optimized ACV containing proniosome gel formulation (ACVPN2).

Pharmacokinetic studies

ACV variable in BCS classification with oral dose, where it is BCS 3 at 400 mg and converts in to BCS 4 at 800 mg. These higher dose causes neurotoxicity and renal failure which needs to other alternative administration route[21]. In oral therapy ACV absorbs slowly and incompletely. The main objective of our research is to enhance the bioavailability using proniosomal transdermal gels and to check its feasibility. In vivo studies were performed for control and optimized formulations and its respective pharmacokinetic parameter values determined (Table 7). ACV shows rapid C_{max} from oral conventional dosage forms within 2 hrs. Our results also coincided with the reports[22]. From the Fig. 9, release of ACV from control shows rapid C_{max} within 2 hrs due to the ready availability in the well developed oral absorption mechanism. From the release profile of ACVPN gel we could notice maximum plasma drug concentration (C max) at 6 hrs followed by slow decreasing of ACV concentration in blood plasma due to sustained delivery from ACVPN gel. Significant ($p<0.001$) improvement in AUC was observed in ACVPN gel formulation. The *ex vivo* and *in vivo* correlation was performed between cumulative amount of drug permeated (µg) and AUC (µg/h/mL^{-1}). The higher regression coefficient value (R^2=0.9903) indicates the point to point correlation following the level A correlation.

Table 5: Pharmacokinetic parameters of acyclovir in Rats Following Administration of optimized Proniosome Gel (ACVPN gel) Proniosome gel (ACVPN gel) and Control (Oral Suspension) (n=6).

FORMULATION CODE	Cmax (µg/mL)	Tmax (hr)	AUC_{0-t} µghrmL	$AUC_{t-\infty}$ µghrmL	$AUC_{0-\infty}$ µghrmL	K (hr)	F
CONTROL	0.598±0.010	2.00±0.00	4.75±0.09	1.06±0.132	5.81±0.21	0.048±0.001	-
ACVPN GEL	0.376±0.009	6.00±0.00	6.37±0.12	11.47±0.87	17.84±0.98	0.016±0.001	3.07±0.15

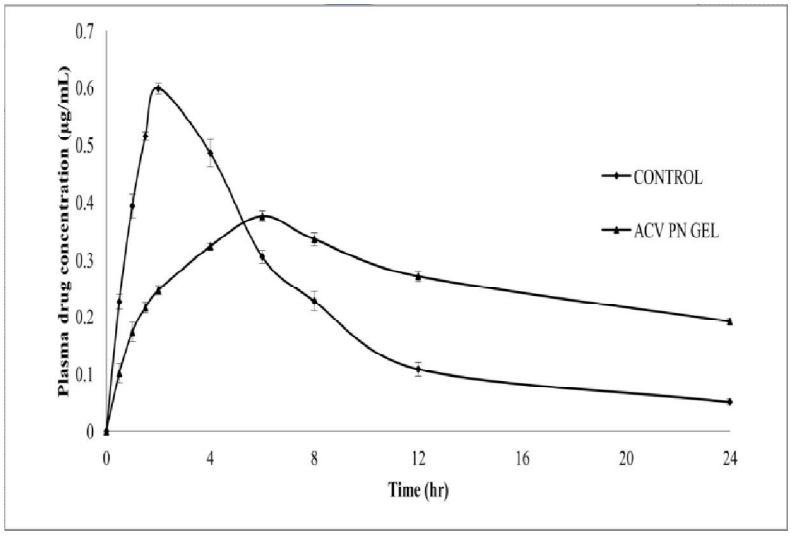


Fig. 9: Mean plasma concentration Vs Time profile of ACV followed by the treatment with optimized ACVPN gel (ACVPN2) and Control (mean±SD; n=6).

Stability studies

The change in morphological behaviour was monitored periodically. The change in physical appearance, vesicle size and entrapment efficiency were evaluated for 180 days (Fig. 10&11). From the microscopical examinations we could not find significant ($p<0.05$) morphological changes. However, we noticed slight increase in micro viscosity and integrity of proniosomal gels. This may be due to during storage water molecules intercalate with polar head groups of lecithin results in slight increase in gel consistency. Concomitant evaporation of little portion of ethanol also improves in microviscosity. A significant increase of particle size observed up on storing at room temperature indicates the effect of temperature. Competence of cholesterol with ACV in hydrophobic bilayer resulted in slight reduction of entrapment efficiency of all the formulations except ACVPN1 (without cholesterol) ($p<0.05$) during storage at room temperature. No congealing observed on storage at 4°C. The formulations more stable at 4°C compare to room temperature.

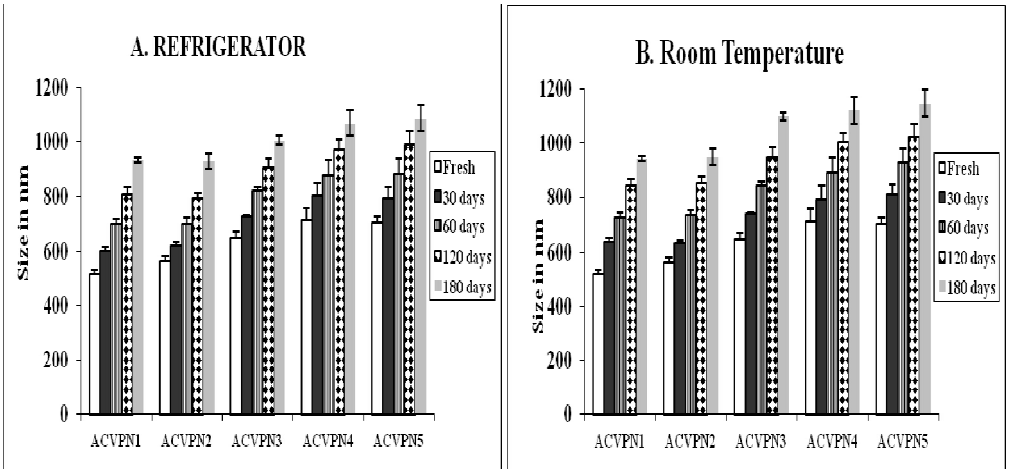


Fig. 10: Change in Particle size ACV containing proniosomal gel formulations during storage at **(A)** Refrigerator **(B)** Room Temperature.

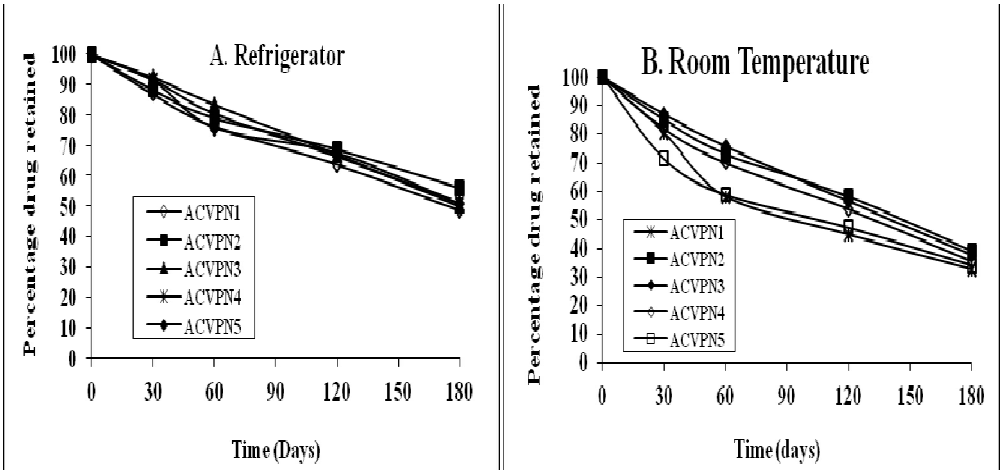


Fig. 11: Percentage retention of ACV in proniosomal gel formulations upon storage at **(A)** Refrigerator **(B)** Room temperature.

CONCLUSION

Acyclovir containing proniosomal gels formulated by co acervation phase separation method and successfully developed. All the prepared ACVPN gels evaluated for its morphological characters. The optimized formulation (ACVPN2) showed highest entrapment efficiency (86.32±1.84). Effect of cholesterol was also evaluated. Significant higher increment in enhancement ratio assessed from all the ACVPN gels compared to control. 3.07 fold increment in bioavailability of ACVPN2 than control reveals the potential of proniosomes in releasing of drug applied through transdermal route. Stability studies were performed to evaluate the stability of proniosomes. All the proniosomal gel formulations were more stable at refrigerator temperature.

CONFLICT OF INTEREST

The authors confirm that this research article has no conflict of interest.

ACKNOWLEDGEMENTS

The authors are very much thankful to Mr. Dasari Prashanth Reddy, Chairman, Trinity College of Pharmaceutical Sciences for providing the necessary facilities.

REFERENCES

1. Nadia M V, Sara N, Carlo L, Paolo C, Patrizia S. (1998). In vitro acyclovir distribution in human skin layers after transdermal iontophoresis. Journal of controlled release. 50 (1–3): 291-296.
2. Haniza H, Siti KA, Fauziah O, Ahmad FS, Rusliza B. (2016). Antiviral nanodelivery Systems: Current trends in acyclovir administration. Journal of Nanomaterials. 1-8.
3. Agarwal S, Vsudha B, Vitta P, Raghuram AP, Pandey S, Udupa N. (2004). Effect of cholesterol content and surfactant HLB on vesicle properties of niosomes. Indian Journal of Pharmaceutical sciences. 1(1): 121-123.
4. Ahmed A A , Doaa AES, Aliaa NE. (2010). Comparative study on the effects of some polyoxyethylene alkyl ether and sorbitan fatty acid ester surfactants on the performance of transdermal carvedilol proniosomal gel using experimental design. AAPS Pharm Sci Tech. 11(4):1591-1602.
5. Arthur HK. (2000). Handbook of pharmaceutical excipients. 3rd ed., American Pharmaceutical Association, Washington, D.C. : 511-514.
6. Rizwana K, Raghuveer I. (2016). Niosomes: a potential tool for novel drug delivery. Journal of Pharmaceutical Investigation. ; 46 (2):1-10.
7. Toshimitsu Y, Brigitte S, Alexander TF. (1994). Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20,40,60 and 80) and a sorbitan triester (Span 85). International Journal of Pharmaceutics; 105: 1-6.
8. Mahmoud M, Mohammed AH, Nagia AM. (2008). Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. International Journal of Pharmaceutics ; 361(1-2): 104-111.
9. Yongmei H, Fenglin Z, Na LY, Yang KL. (2002). Studies on a high encapsulation of colchicine by a niosome system. International Journal of Pharmaceutics ; 254(1-2): 73-80.
10. Jaleh V, Seied M, Hossaini B. (2005). Sorbitan Monopalmitate-Based Proniosomes for Transdermal Delivery of Chlorpheniramine Maleate. Drug Delivery; 12 (2): 75-82.

11. Jyotsana RM, Nitesh PG, Kamal D. (2016). Formulation and evaluation of proniosomes containing lornoxicam. Drug Delivery and Translational Research. ; 6(5): 511–518.
12. Gopinath D, Ravi D, Rao BR, Apte SS, Renuka D, Rambhau D, (2004). Ascorbyl palmitate vesicles (Aspasomes): Formation, characterization and applications. International Journal of Pharmaceutics, 271(1-2): 95-113.
13. Mandawgade S, Patravale V. (2008). Development of SLNs from natural lipids: application to topical delivery of tretinoin. International Journal of Pharmaceutics. 363(1-2):132-8.
14. Vaibhav D, Dinesh M, Jain NK. (2007). Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. European Journal of Pharmaceutics and Biopharmaceutics. ; 67(2): 398–405.
15. Sunil TKN , Ramesh P. (2004). Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. Journal of Controlled Release. 95(3): 367- 379.
16. Sanjay KJ, Yashwant G, Anekant J, Kavita R. (2008). Enhanced Transdermal Delivery of Acyclovir Sodium via Elastic Liposomes. Drug Delivery. 15 (3): 141-147.
17. Vandana G, Piyush T. (2016). Dermal delivery for cutaneous malignancies: Literature at a glance. Journal of Pharmaceutical Innovation. ; 11(1):1-33.
18. Hu C, Rhodes DG.(1999). Proniosomes: a novel drug carrier preparation. International Journal of Pharmaceutics. ; 185 (1): 23- 35.
19. Raju J, Sruthi S, Suresh B, Prabhakar RV. (2011). Enhanced bioavailability of exemestane via proliposomes based transdermal delivery. Journal of Pharmaceutical Sciences, 100(8): 3208-3222.
20. Bandar EAD, Anroop BN, Rachna K , Mahesh A, Sree H. (2015). Formulation and evaluation of nano based drug delivery system for the buccal delivery of acyclovir. Colloids Surf B Biointerfaces. 136: 878-84.
21. Anroop N, Mahesh A, Al-Dhubiab BE, Jyoti W, Sree H.; Mueen, A. (2014). Enhanced oral bioavailability of acyclovir by inclusion complex using hydroxypropyl-β-cyclodextrin. Drug Delivery. 21(7): 540-547.

CITATION OF THIS ARTICLE

A Mateti, M Habibuddin, R Jukanti. Provesicular Based Colloidal Carriers For Transdermal Drug Delivery. Formulation Aspects: Bioavailability Enhancement of Acyclovir through Proniosomal Gel Formulations.. Bull. Env.Pharmacol. Life Sci., Vol10[5] April 2021 :34-46