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Research Article

Enhanced Percutaneous Permeability of Acyclovir by DMSO from Topical Gel Formulation

Sanjay Dey[1], Bhaskar Mazumder[1], J. R. Patel[2*]

[1]*Department of Pharmaceutical Sciences, Dibrugarh Universitry, Dibrugarh, Assam 786004, India*
[2]*Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi District Bhind (M.P.) 477660, India*

ABSTRACT
The aim of this study was to investigate the effect of DMSO on the permeation of acyclovir in the form of topical gel formulations. Different formulations were prepared containing carbopol 934P, acyclovir (1 % w/w) and selected concentration of DMSO (0 to 10% w/w) to evaluate drug content, spreadibility, pH, viscosity, and in-vitro permeation through mouse epidermis and porcine skin. FTIR spectrometry was used to investigate physical state of drug in the gel formulations. The mechanisms of drug permeation were evaluated by FTIR spectrophotometer and histopathological studies. The carbopol 934P gel was found to contain 95.62 to 98.89 % of acyclovir and spreadibility was found in the range of 10.75 to 11.75 g.cm/sec. The pH of all formulations was found near to the skin pH value. The viscosity of the formulations was found inversely proportional with drug permeation. A maximum permeation flux of acyclovir (463.42±36.41μg/cm^2.h) through porcine skin was observed with an enhancement ratio of 1.55, when DMSO was incorporated at a concentration of 10%w/w in gel system. The FTIR spectra revealed the absence of drug-polymer interaction. From FTIR spectroscopy and histopathological studies it was evident that the permeation of acyclovir, across mouse and porcine skin, were increased in presence of DMSO which can be attributed to the partial extraction of lipids in the stratum corneum. The results suggest that DMSO may be useful for enhancing the skin permeability of acyclovir from transdermal therapeutic system containing carbopol 934P gel as reservoir.

Keywords: Topical gel, acyclovir, dimethylsulphoxide, carbopol 934P, porcine skin, mouse epidermis.

INTRODUCTION

Acyclovir, cyclic purine nucleotide 2´-deoxyguanosine analogue derived from guanosine, chemically {9-[(2-hydroxyethoxy) methyl] guanine}, is clinically useful in the treatment of herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus, and Epstein Barr virus infections. [1-3] The unique feature of acyclovir is its selectivity as a substrate for virus specified thymidine kinase (TK) encoded by HSV and VZV. [4-5] Intravenous, oral and topical routes are common to administer different antiviral agent. In the case of skin infection, topical applications have several advantages, including convenience and reduction of side effects. Absorption of orally administered acyclovir is slow, variable and incomplete, with bioavailability of ~15-30 %. [6] An *in-vitro* study using porcine buccal tissue indicated that buccal transport of acyclovir occurs predominantly by a passive diffusion mechanism, probably through paracellular route. [7] The physicochemical properties of acyclovir are: molecular weight (225.21 daltons), partition coefficient (log Ko/w-1.56), melting point (256.5^{o}C), and dissociation constant (2.94 and 9.23). [8-10] Therefore, this compound may serve as a good model drug for the transdermal drug delivery.

***Corresponding author: Mr. J. R. Patel**, Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi District Bhind (M.P.) 477660, India **Phone No:** +91-9893612009
Email: jrpatel_rns@yahoo.co.in

The greatest obstacle in the transdermal drug delivery is stratum corneum, because it provides a rate-limiting step for drug absorption. [11] Many studies showed that lipid domain, the integral component of the transport barrier, must be breached if the drug is to be delivered transdermally at an appropriate rate. [12] Several enhancement techniques have been developed to overcome the impervious nature of the stratum corneum. A popular technique is the use of chemical permeation enhancers, which alters reversibly the permeability barrier of the stratum corneum. DMSO has been extensively used as permeation enhancers in the permeation of hydrophilic and lipophilic drugs. [13-15] It was also reported that DMSO blocks HSV-1 productive infection at different stages with positive cooperativity. [16] This property of DMSO makes it an interesting excipient to work with especially in case of transdermal formulations as it can serve a dual purpose of enhancing drug permeability and blocking HSV-1 productivity.
The carbopol resins are very high molecular weight polymers of acrylic acid cross-linked with polyalkenyl ethers, and they have been used for development of bioadhesive controlled release drug delivery systems owing to their bioadhesive properties.
In the present study, the enhancing effect of DMSO on the *in-vitro* permeation of acyclovir from gel formulation was investigated.

MATERIALS AND METHODS

The following materials were obtained from the authenticated manufacturers and used as received: acyclovir (matrix Laboratories, India); carbopol 934P and glycerin (Loba Chemie Pvt Ltd, India); methylparaben and propylparaben (Himedia Laboratories Pvt Ltd, India); Triethanolamine (SD Fine Chemical, India), dimethylsulphoxamide (Allied Chemical Corporation, India)

Preparation of acyclovir gel

Different formulations were prepared, containing the above materials, as mentioned in Table-1. Carbopol 934P (1.5 % w/w) and purified water were taken in a mortor and allowed to soak for 24 h. To this required amount of drug (1 % w/w) dispersed in water was added. Carbopol 934P was then neutralized with sufficient quantity of triethanolamine. Glycerin (10 % w/w), mehylparaben (0.1 % w/w) and propylparaben (0.05 % w/w) were added slowly with continuous gentle trituration until the homogenous gel formed. A similar procedure was followed for the formulations, containing DMSO and placebo formulations. It was kept for 24 h at ambient conditions after which evaluation was carried out. To access the reproducibility of the procedure, three batches were prepared for each formulation.

Physicochemical evaluation of gels

Drug content studies

1.0 g of each gel formulations were taken in 100 ml volumetric flask containing 20 ml of saline phosphate buffer (pH 7.4) and stirred for 30 min. The volume was made up to 100mL and 1mL of the above solution was further diluted to 50mL with saline phosphate buffer (pH 7.4). The resultant solution was filtered through membrane filter (0.45µm). The absorbance of the solution was measured spectrophotometrically at 251.5 nm using placebo gel as reference.

Spreadibility

The spreadibility of the gel formulation was determined, by measuring diameter of 1g gel between horizontal plates ($20\times20cm^2$) after 1min. The standardized weight tied on the upper plate was 125 g. The spreadibility was calculated by using the following formula. [17]

$$S = \frac{m.l}{t} \text{ - - - - - - - - - - - - - } (1)$$

Value S is spredibility, m is the weight tied to the upper slides, l is the length of glass slide, and t is the time taken

Determination of pH

2.5 g gel was accurately weighed and dispersed in 25 ml of purified water. The pH of the dispersion was measured using pH meter (Toshniwal, model CL54), which was calibrated before each use with buffered solution at 4.0, 7.0 and 9.0.

Viscosity measurement

Viscosity of different formulations was determined using Brookfield viscometer (Brookfield Engineering Laboratories, USA) with spindle no. 6 at 10 rpm at temperature 37±0.5°C.

Preparation of mouse epidermis

The abdominal hair of Albino male mice, weighing 20-25 g of 8-10 weeks old was shaved using hand razor 24 h. before use in the experiment. After anaesthetizing the mouse with chloroform the abdominal skin was surgically removed from the animal and adhering subcutaneous fat was carefully cleaned with hot water cotton swab and kept in freeze. Finally, the epidermis was taken and examined microscopically to ensure the integrity of the skin.

Preparation of porcine skin

Pig weighing 20.0 Kg, 8 weeks old, sacrificed by termination of the resuscitation, and was used. Pig ears were obtained within 30 min after termination of the resuscitation and cleaned under running water before whole skin membrane were removed from the underlying cartilage. Hairs were cut and the whole membranes were frozen in deepfreeze. Permeation experiments were performed by soaking the whole membranes in water for 120 sec at 60°C, followed by blunt dissection. The frozen whole membranes were thawed before epidermal membranes were prepared.

***In-vitro* permeation studies**

Modified Keshary-Chein diffusion cells [18] were used in the *in-vitro* transdermal permeation studies. The skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The effective diffusion area was 3.14 cm^2, and the volume of the receptor compartment was 60 ml. 1 g of carbopol 934P gel, without or with DMSO (5 % w/w and 10 % w/w) containing 10 g of acyclovir, were placed in the donor cell. In this study, 60 ml of saline phosphate buffer (pH 7.4) solution was used as receptor medium. The receptor medium was maintained at 37±0.5°C and stirred magnetically at 500 rpm. 1 ml of sample were withdrawn from the receptor compartment at predetermined time interval for 6 h period, and replaced by same volume of fresh prewarmed saline phosphate buffer (pH 7.4) solution to maintain constant volume. The amounts of acyclovir in the samples were assayed spectrophotometrically at 251.5 nm against appropriate blank.

Determination of acyclovir retained in the skin

At the end of the *in-vitro* permeation experiment, the skin sample was removed from the diffusion cell and washed with distilled water to remove the adhering gel and blotted dry with tissue paper. The treated skin area weighed and cut into small pieces and extracted with 10 ml of saline phosphate buffer (pH 7.4) solution for 24 h and filtered through membrane filter (0.22 µm). Then the sample was diluted and the amount of acyclovir retained in the skin sample was estimated spectrophotometrically at 251.5 nm.

Permeation data analysis and statistics

The permeation profiles were constructed by plotting the cumulative amount of acyclovir permeated per unit skin surface area (Q, $\mu g/cm^2$) versus time (h). The steady state flux (Jss, $\mu g/cm^2.hr$) of acyclovir was calculated from the slope of the plot using linear regression analysis. [19] The lag time (t_L) was determined from the x-intercept of the slope at the steady state. The steady state diffusion coefficient (cm^2/hr), permeability coefficient (Kp, cm/hr) and partition coefficient (k) of the drug through skin was calculated using the following equation. [20]

$$t_L = \frac{h^2}{6D} \text{------------------------} (2)$$

$$K_p = \frac{J_{SS}}{C} \text{------------------------} (3)$$

$$K_P = \frac{K.D}{h} \text{------------------------} (4)$$

Where ‘C’ and h’ are the initial concentration of acyclovir in the gel and thickness of the membrane respectively. The

Table 1: Drug content, spreadibility, pH and viscosity of gel formulations prepared at DMSO concentration of 0 %, 5 % and 10 % w/w. (Mean±S.D.; n=3)

DMSO (%w/w)	Formulation Code	%Drug content ±S.D.	Spreadibility (g.cm/sec)±S.D.	pH±S.D.	Viscosity (P) ±S.D.
0	S_1	98.89±0.01	11.75±0.98	6.82±0.03	204.15±24.56
5	S_2	95.62±0.12	11.08±1.23	6.89±0.02	181.40±17.38
10	S_3	97.45±0.20	10.75±0.91	6.40±0.02	165.91±13.74

Table 2: The permeability data of acyclovir delivered from gel formulation containing different concentration of DMSO through mouse epidermis and porcine skin. (Mean±S.D.; n=3)

Skin	Form. Code	Q_6[1] (μg/cm^2)	Jss[2] (μg/cm^{2}hr)	t_L[3] (hr)	D[4] (cm^2/hr×10^{-6})	Kp[5]	ER[6]
Mouse skin	S_1	1529.93±105.45	251.81±24.46	0.39±0.12	15.38±2.16	3.19±0.40	1.0
	S_2	1859.32±98.67	302.25±32.89	0.32±0.05	18.75±1.18	3.97±0.33	1.21
	S_3	2819.42±123.42	463.42±36.41	0.23±0.07	26.08±1.75	5.97±0.48	1.84
Porcine skin	S_1	889.83±113.67	251.81±24.46	0.32±0.06	18.75±1.32	1.45±0.08	1.0
	S_2	1048.18±89.31	302.25±32.89	0.26±0.14	23.07±1.71	2.25±0.14	1.18
	S_{13}	1378.43±126.42	463.42±36.41	0.19±0.02	31.58±2.02	2.94±0.04	1.55

1. Cummulative amount permeated at 6 h. 2. Steady state flux 3. Lag time
4. Diffusion coefficient 5. Permeability coefficient 6. Enhancement ratio.

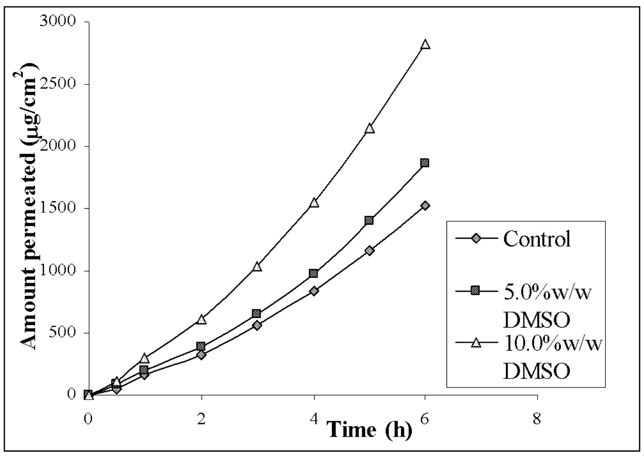


Fig. 1: Permeation profiles of acyclovir through mouse epidermis from control gel (without enhancer), and gel containing 5%w/w and 10%w/w DMSO (n=3).

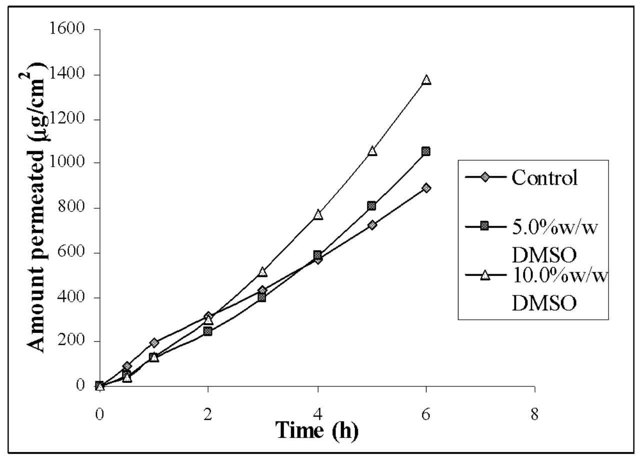


Fig. 2: Permeation profiles of acyclovir through porcine skin from control gel (without enhancer), and gel containing 5%w/w and 10%w/w DMSO (n=3).

Fig. 3: FTIR spectra of (a) acyclovir, (b) carbopol 934P and (c) physical mixture of acyclovir and carbopol 934P in 1:1 ratio

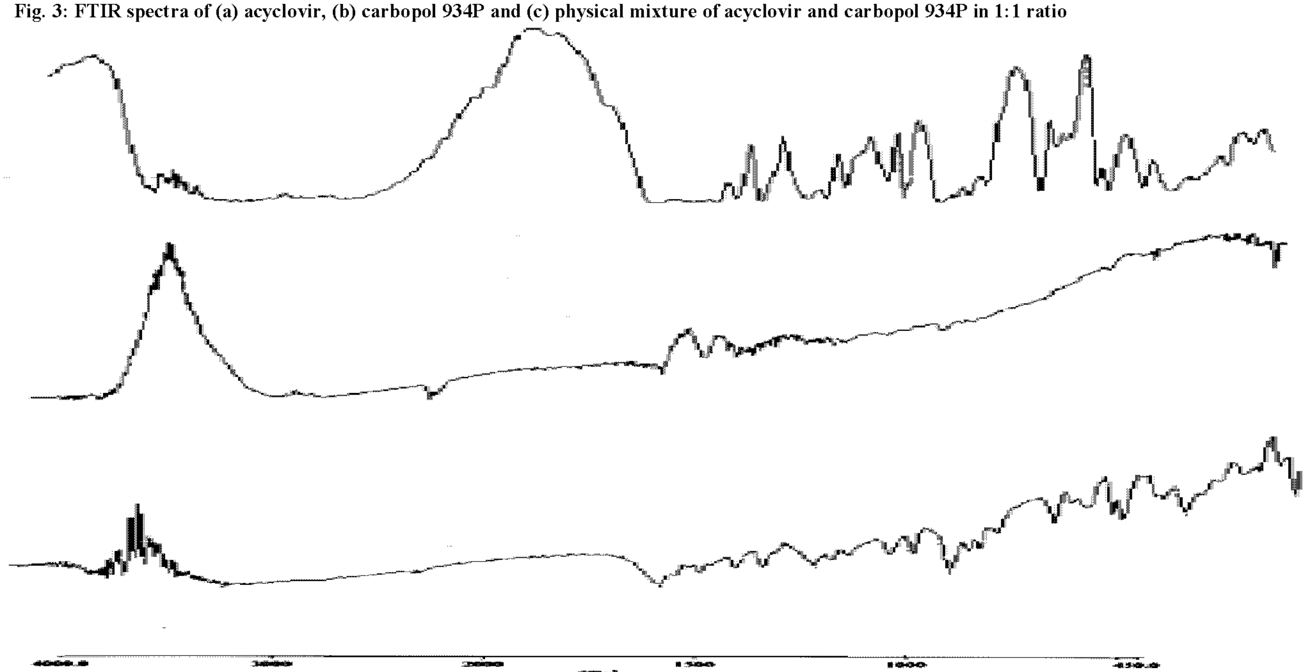
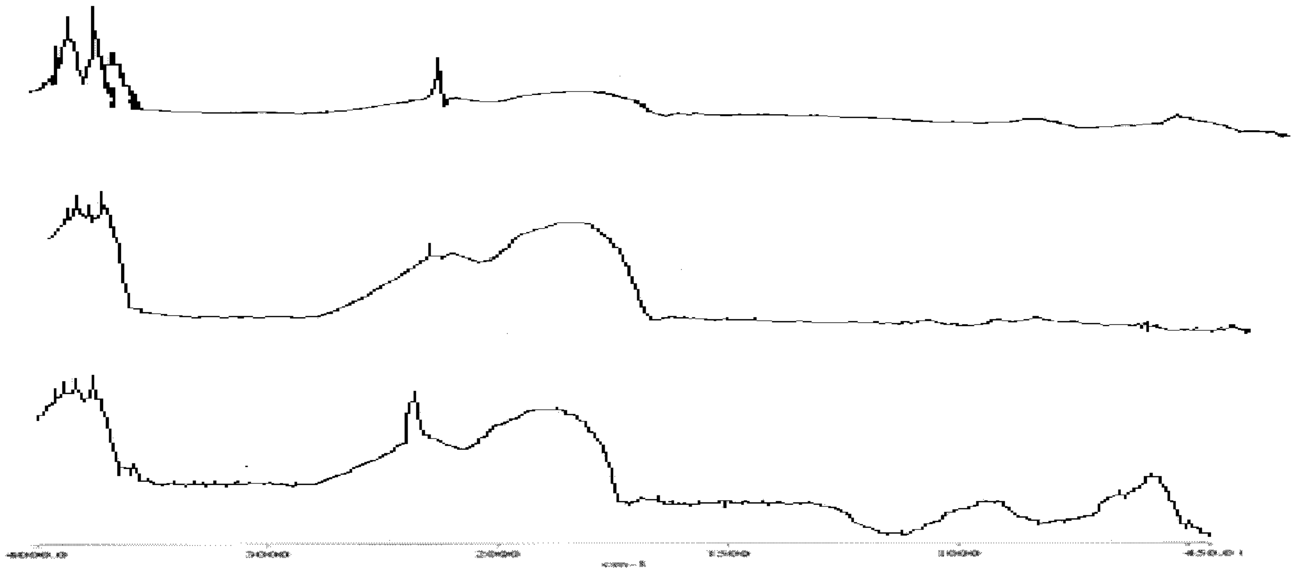


Fig. 4: FTIR spectra of (a) fresh skin, pretreated skin with (b) DMSO (5%w/w) and (c) DMSO (10%w/w)



permeation enhancing effect of DMSO was calculated in terms of enhancement ratio (ER) by using the following equation. [21]

$$ER = \frac{\text{Aciclovir flux with enhancer in gel}}{\text{Aciclovir flux without enhancer in gel (control)}} \quad ----(5)$$

The data are presented as mean± S.D. of three experiments. Statistical analysis was performed using one-way ANOVA. Correlation analyses were performed by the least squares linear regression method. A value of $p<0.05$ was consider statistically significant.

FTIR spectroscopy studies

Determination of drug-polymer interaction

FTIR spectra of pure acyclovir, carbopol 934P, and their physical mixture at a ratio 1:1 were examined using a Perkin Elmer spectrophotometer (Japan). The samples were prepared in KBr disks compressed under a pressure of 6 ton/cm^2. The wave-number selected ranged between 400 and 4000cm^{-1}.

FTIR spectroscopy of mouse epidermis

The mouse epidermis samples (1 cm^2) were treated with different concentration of DMSO (5%w/w and 10 % w/w) for 24 h. The trated epidermises were vacuume dried for 2 days and stored in a desiccator to remove traces of the solvent. Changes the structure of mouse epidermis were assessed by Perkin Elmer spectrophotometer (Japan) and spectra were recorded in the frequency range 400-4000cm^{-1}. Attention was focused on characterizing the occurrence of peaks near 2851 cm^{-1} and 2920 cm^{-1} which were due to symmetric and asymmetric C-H stretching absorbance, respectively, corresponding to epidermis lipids. The FTIR study was also carried out with untreated mouse epidermis (washed with only water), which served as a reference spectrum.

Histopathological study

The mouse epidermis skins applied with carbopol gel (without enhancer) or carbopol-based formulation containing DMSO (10 % w/w) for histopathological evaluation. The excised skins were 10 % formalin-fixed and paraffin embedded tissues. The sections were 4µm in thickness using microtome and then stained with Harris hematoxylin-eosin solution for the histopathological examination with 100times magnification.

RESULTS

Physicochemical properties of gel formulations as detailed in Table-1. The drug content was found in the range of 95.62±0.12 to 98.89±0.01. The spreadibility of gel formulations decreased from 11.75±0.98 g.cm/sec to 10.75±0.91 g.cm/sec with increase in DMSO concentration. The pH of all the formulations was found within the range of 6.40±0.02 to 6.89±0.02. The viscosity of gel formulations decreased with increase in DMSO concentration and it was found within the range of 204.05±24.56P to 165.91±13.74P.

The cumulative permeation of drug across the excised mouse abdominal epidermis from carbopol 934P gel containing 5 % w/w and 10 % w/w DMSO is shown in Figure 1. The cumulative amounts permeated at 6hr were 1529.93±105.45, 1859.32±98.67, and 2819.42±123.42µg/cm^2 for control gel, 5%w/w DMSO and 10%w/w DMSO. The maximum amount of acyclovir that was permeated during 6hr of study (Q_6) from carbopol 934P gel system (without enhancer) was 1529.93±105.45µg/cm^2, and corresponding flux of acyclovir was 251.81±24.46µg/cm^2.hr. A marked effect of DMSO on acyclovir permeation was observed for gel formulations containing DMSO (5%w/w and 10%w/w). The cumulative amount (Q_6) of acyclovir permeated over 6hr was found to be significantly increased, ranging from 1859.32±98.67 to 2819.42±123.42µg/cm^2 from the carbopol 934P gels containing 5 % w/w to 10 % w/w of DMSO. The corresponding flux values were ranging from 302.25±32.89 to 463.42±36.41µg/cm^2.hr. There was significant ($p<0.05$) reduction in the lag time of the prepared gel as the concentration of DMSO increased. It may be observed from the results (Table 2) that as DMSO concentration increased from 5 % w/w to 10 % w/w, the permeability coefficient of acyclovir from carbopol 934P was found to be increased significantly ($p<0.05$), when compared with control (without enhancer). There was a 1.86 fold increase in the permeability and 1.84 fold increase in the enhancement ratio of drug from carbopol 934P gel containing 10%w/w of DMSO when compared with the control. Therefore, it is interesting to note that with increase in DMSO concentration the skin permeability of acyclovir had increased significantly ($p<0.05$) through mouse abdominal skin.

Fig. 2 and Table 2 show the permeation of acyclovir from carbopol 934P gel through porcine skin. The cumulative amount permeated at 6hr was 889.83±113.67, 1048.18±89.31 and 1378.43±126.42μg/cm^2 for control gel, 5 % w/w and 10 % w/w DMSO, respectively. It was observed that the incorporation of DMSO as permeation enhancer increased the permeation of acyclovir significantly ($p<0.05$) from gel, when compared with control. There was significant ($p<0.05$) reduction in the lag time of the prepared gel, containing DMSO. There was 2.02 fold increase in the permeability and 1.55 fold increase in the permeability and 1.55 fold increase in the enhancement ratio (ER) of the drug from the carbopol 934P gel containing 10%w/w of DMSO. Thus, the permeation of acyclovir through porcine skin was increased significantly ($p<0.05$) with increase in the DMSO concentration.

The skin retention of acyclovir is detailed in Table-2. The amount of acyclovir retained in the skin for both the species increased significantly ($p<0.05$) with increase in DMSO concentration. The retention of acyclovir in porcine skin was slightly higher than the retention in the mouse skin.

The FTIR spectra of acyclovir and carbopol 934P in pure form are compared with that of their physical mixture in a 1:1 ratio to obtain some information about any interaction that occurred between the drug and the vehicle. The spectra are represented in Fig. 3. It is clear from the results obtained that there is no positive evidence for the interaction between the drug and the vehicles.

The FTIR study of mouse epidermis provides an insight into the effect of DMSO on the biophysical properties of the mouse epidermis.

A typical FTIR spectrum of mouse epidermis shows characteristic peaks at 2845cm^{-1} and 2924 cm^{-1} for the symmetric and asymmetric C-H stretching for lipids and 1650-1550 cm^{-1} for two strong bond due to the amide I and amide II stretching vibrations of stratum corneum proteins. The amide I and amide II bands raised in the spectrum can be attributed to C=O stretching vibration and C-N bending vibration, respectively. There was a clear difference in the peak height and area between the FTIR spectra of untreated and treated epidermis. The rate-limiting step for dermal drug delivery is lipophilic part of stratum corneum in which lipids (ceramides) are tightly packed in bilayers due to the high degree of hydrogen bonding. The amide I groups of ceramide are bonded to amide II groups of another ceramides with hydrogen bonding and there by forms a tight network of hydrogen bonding at the head of ceramides. This hydrogen bonding makes stability and strength to lipid bilayers and thus imparts barrier properties of stratum corneum.

The photomicrogram of untreated mouse skin showed normal skin with well-defined epidermal and dermal layers. Keratin layer was well formed and lied just adjacent to the topmost layer of the epidermis. The disruption of lipid bilayers was clearly evident as distinct voids and empty spaces were visible in the epidermal region when skin treated with DMSO. On the other hand, there were no apparent sign of skin irritation (erythma and edema) observed on visual examination of the skin species treated with DMSO containing carbopol 934P.

DISCUSSION

Topical gel formulation of acyclovir using carbopol 934P as gelling agent can be prepared successfully. The uniform distribution of drug was confirmed by content uniformity studies in all formulations. The pH of all formulations was found near to the skin pH value, which showed the formulations, are compatible with skin. The viscosity of the gel formulations decreased with increasing DMSO concentration and was found inverse relationship with drug permeation.

The DMSO had shown appreciable drug permeation enhancing effect for acyclovir through mouse epidermis and porcine skin. The increased permeability and enhancement ratio (ER) might be due to extraction of lipids and protein of the stratum corneum, the main barrier to the percutanous absorption of the exogenous material. [22] From the skin retention studies it was observed that the retention of acyclovir increased with increase in the DMSO concentration in the gel formulation. It might be due to the partitioning effect of DMSO from the gel formulation to the skin. [23] But, the amount of acyclovir retained on the skin was varied from species to species, because of the variation in the skin physiology. On the other hand, the extraction of skin lipid and protein by DMSO was confirmed by FTIR spectra and histopathological studies of skin. The FTIR spectra of drug-polymer provides evidence that there was no positive interaction between the drug and vehicle other than hydrogen bonding which may have occurred between donating and accepting groups of both drug and polymer, indicating usefulness of carbopol 934P as a gelling material for topical application of acyclovir.

The enhanced permeability flux of acyclovir with 10%w/w DMSO through skin, was observed in this study, may be useful in the selection of relatively safe penetration enhancer to aid transdermal drug delivery for the treatment of HSV-1, VZV, cytomegalovirus and Epstein Barr virus infections in the skin. In the conclusion, it is also interesting to note that DMSO with acyclovir has dual affect i.e. increases the drug permeability through skin and blocks HSV-1 production. However, further studies needs to be performed to find out the influence of DMSO on the permeability of acyclovir in the human volunteers.

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