Research Article

Enhanced Percutaneous Permeability of Acyclovir by DMSO from Topical Gel Formulation

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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, spreadability, 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 spreadability was found in the range of 10.75 to 11.75 g/cm²/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±36.41 ug/cm²/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-β-(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 specific thymidine kinase (TK) encoded by HSV and VZV.¹⁻³ 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°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.

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

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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 mortar 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), methylparaben (0.1 % w/w) and propylparaben (0.05 % w/w) were added slowly with continuous gentle titration 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 100 mL and 1 mL of the above solution was further diluted to 50 mL with saline phosphate buffer (pH 7.4). The resultant solution was filtered through membrane filter (0.45 µm). The absorbance of the solution was measured spectrophotometrically at 251.5 nm using placebo gel as reference.

Spreadability

The spreadability of the gel formulation was determined, by measuring diameter of 1g gel between horizontal plates (20×20cm²) after 1 min. The standardized weight tied on the upper plate was 125 g. The spreadability was calculated by using the following formula.

\[ S = \frac{m \cdot I}{t} \]  

Value S is spreadability, m is the weight tied to the upper slides, I 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-Chen 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 cm², 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, µg/cm²) versus time (h). The steady state flux (Jss, µg/cm²/hr) of acyclovir was calculated from the slope of the plot using linear regression analysis [19]. The lag time (tL) was determined from the x-intercept of the slope at the steady state. The steady state diffusion coefficient (cm²/hr), permeability coefficient (Kp, cm/hr) and partition coefficient (k) of the drug through skin was calculated using the following equation.

\[ t_L = \frac{h^2}{6D} \]  

\[ K_p = \frac{J_{ss}}{C} \]  

\[ K_p = \frac{KD}{h} \]  

Where ‘C’ and ‘h’ are the initial concentration of acyclovir in the gel and thickness of the membrane respectively. The
Table 1: Drug content, spreadability, pH and viscosity of gel formulations prepared at DMSO concentration of 0 %, 5 % and 10 % w/w. (Mean±S.D.; n=3)

<table>
<thead>
<tr>
<th>DMSO (%w/w)</th>
<th>Formulation Code</th>
<th>%Drug content ±S.D.</th>
<th>Spreadability (g/cm²/sec)±S.D.</th>
<th>pH±S.D.</th>
<th>Viscosity (P) ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>S₁</td>
<td>98.89±0.01</td>
<td>11.75±0.98</td>
<td>6.82±0.03</td>
<td>204.15±24.56</td>
</tr>
<tr>
<td>5</td>
<td>S₂</td>
<td>95.62±0.12</td>
<td>11.08±1.23</td>
<td>6.89±0.02</td>
<td>181.40±17.38</td>
</tr>
<tr>
<td>10</td>
<td>S₃</td>
<td>97.45±0.20</td>
<td>10.75±0.91</td>
<td>6.40±0.02</td>
<td>165.91±13.74</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Skin</th>
<th>Form. Code</th>
<th>J₀ (µg/cm²)</th>
<th>ts (µg/cm²/hr)</th>
<th>Dᵢ (cm²/hr×10⁻⁶)</th>
<th>Kp</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse skin</td>
<td>S₁</td>
<td>1529.93±105.45</td>
<td>251.81±24.46</td>
<td>0.39±0.12</td>
<td>15.38±2.16</td>
<td>3.19±0.40</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>1859.32±98.67</td>
<td>302.25±32.89</td>
<td>0.32±0.05</td>
<td>18.75±1.18</td>
<td>3.97±0.33</td>
</tr>
<tr>
<td></td>
<td>S₃</td>
<td>2819.42±123.42</td>
<td>463.42±36.41</td>
<td>0.23±0.07</td>
<td>26.08±1.75</td>
<td>5.97±0.48</td>
</tr>
<tr>
<td>Porcine skin</td>
<td>S₁</td>
<td>889.83±113.67</td>
<td>251.81±24.46</td>
<td>0.32±0.06</td>
<td>18.75±1.32</td>
<td>1.45±0.08</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>1048.18±89.31</td>
<td>302.25±32.89</td>
<td>0.26±0.14</td>
<td>23.07±1.71</td>
<td>2.25±0.14</td>
</tr>
<tr>
<td></td>
<td>S₃</td>
<td>1378.43±126.42</td>
<td>463.42±36.41</td>
<td>0.19±0.02</td>
<td>31.58±2.02</td>
<td>2.94±0.04</td>
</tr>
</tbody>
</table>


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.
permeation enhancing effect of DMSO was calculated in terms of enhancement ratio (ER) by using the following equation. $^{[2]}$

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

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 considered statistically significant.

**FTIR spectroscopy studies**

**Determination of drug-polymer interaction**

FTIR spectra of pure aciclovir, 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/cm². The wave-number selected ranged between 400 and 4000cm⁻¹.

**FTIR spectroscopy of mouse epidermis**

The mouse epidermis samples (1 cm²) were treated with different concentration of DMSO (5% w/w and 10% w/w) for 24 h. The treated epidermis were vacuum dried for 2 days and stored in a desiccator to remove traces of the solvent. Changes in 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/cm2/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±24.56 to 165.91±13.74 P. 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/cm² for control gel, 5% w/w DMSO and 10% w/w DMSO. The maximum amount of aciclovir that was permeated during 6hr of study (Qₐ) from carbopol 934P gel system (without enhancer) was 1529.93±105.45μg/cm², and corresponding flux of aciclovir was 251.81±24.46μg/cm²/hr. A marked effect of DMSO on aciclovir permeation was observed for gel formulations containing DMSO (5% w/w and 10% w/w). The cumulative amount (Qₐ) of aciclovir permeated over 6hr was found to be significantly increased, ranging from 1859.32±98.67 to 2819.42±123.42μg/cm² 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/cm²/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 aciclovir from carbopol 934P was found to be increased significantly (p<0.05), when compared with control (without enhancer). There was a 1.38 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
total 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±13.67, 1048.18±89.31
and 1378.43±126.42μg/cm² 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 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 percutaneous
absorption of the exogenous material. [29] 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. [29] 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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REFERENCES

1. Bryson YJ. The in-vitro sensitivity of clinical isolates of varicella
zoster to virostatic and virocidal concentrations of acyclovir. In:
Proc Int Conf Human Herpeticviruses, Amo Atlanta, Mar 1980, pp
17-21.
2. Collins P, Bauce, PJ. The activity in-vitro against herpes virus of 9-
(2-hydroxyethoxymethyl) guanine (Acylogranosine). A new
antiviral agent. Journal of Antimicrobiology and chemotherapy
1979; 5: 431-446.
3. DeClercq E., Descamps J, Verhelst GA, Walker RT, Jones AS,
Torrence PF, Shugar D. Comparative efficacy of antinherpes drug
against different strains of herpes simplex virus, Journal of
Infectious Diseases 1980; 141: 563-574.
4. Frumbar PA, St. Clair MB, Spector T. Acyclovir triphosphate is a
suicide inactivator of the herpes simplex virus DNA polymerase.


19. Higuchi WI. Analysis of data on the medicament release from ointments. J. Pharm Sci. 1962; 51; 802-804.


23. Panigrahi L, Ghosal SK, Pattnaik S, Malarana L, Barik BB. Effect of permeation enhancers on the release and permeation kinetics of lincomycin hydrochloride gel formulations through mouse skin. Ind J Pharm Sci. 2006; 68(2); 205-211.