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Development and evaluation of transdermal gel of acyclovir using Nerolidol as penetration enhancer

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Abstract
The aim of this study was to investigate the effect of Nerolidol on the penetration of Acyclovir in the form of transdermal gel formulation. Different formulations were prepared containing different concentrations of Nerolidol (0-5%) using Aloe vera gel as a base. Formulations evaluated for pH, Viscosity, Percent drug content, Spreadability, Diffusion study and In-vitro permeation study. Rheological study showed that viscosity is inversely proportional to the rate of shear and thus the system shows pseudoplastic behaviour. pH of all the formulations was found to be around 6 which is skin compatible. The percent drug content was found to be within the range of 98 to 101% for all the formulations. Spreadability of formulations is found to be in the range of 11.25 to 12.66 gm.cm/sec. Stability studies showed that the formulation is stable at 40^{o}C at 75% RH for a period of one month. It was evident that the permeation of Acyclovir was enhanced, across freshly excised hairless rat abdominal skin, in presence of Nerolidol which can be attributed toincreased lipid disruption in the stratum corneum.
Comparison of F5with marketed formulation (Acivir, Cipla) for permeation of Acyclovir across hairless rat abdominal skin, showed greater flux 12.89 mg/cm$^{2.}$ as compared to flux 4.74 mg/cm^{2}.hr of marketed formulation. F0 (without Nerolidol) showed better flux of 6.59 mg/cm^{2}.hr than the marketed product, this can be attributed to the use of Aloe vera gel as an absorption base which consists of lignins, that intensify penetrative properties.

Keywords: Acyclovir, Nerolidol, Aloe vera gel, Transdermal gel

Introduction
Herpes simplex virus-1 (HSV-1), an alpha herpes virus, is a large DNA virus that causes primary herpetic gingivostomatitis, mucocutaneous orofacial disease, and ocular disease; recurrent lesions are common on the face and on the lips and less common intraorally. HSV infections are among the most common diseases of humans, with an estimated 60–95% of the adult population being infected by at least one of them.Available therapies include topical acyclovir, oral acyclovir, penciclovir, docosanol, and valacyclovir.Acyclovir is a guanosine analogue antiviral drug. It is one of the most commonly used antiviral drugs for the treatment of herpes simplex virus infections, as well as in the treatment of varicella zoster (chickenpox) and herpes zoster (shingles).Oral bioavailability of Acyclovir is only 20%. It is commonly marketed as tablets (200 mg, 400 mg, 800 mg and 1 gram), topical cream (5%), intravenous injection (25 mg/mL) and ophthalmic ointment (3%). Cream preparations are used primarily for labial herpes simplex. The intravenous injection is used when high concentrations of acyclovir are required. The ophthalmic ointment preparation is only used in herpes simplex keratitis. Acyclovir belongs to BCS Class III i.e it has low permeability, hence it also faces limitations while administering topically. This problem can be overcome by using chemical penetration enhancers in the formulation. Nerolidol (terpene), a naturally occurring volatile oil, appear to be promising candidate for clinically acceptable enhancer. Aloe vera gel possess soothing, moisturizing and healing properties. Anthraquinones in Aloe vera breakup residue, pus and lifeless cells, bring blood to the region and flush out material from the

wounds and ulcers. The lignins, a major structural material of cellulose content allows for penetrative properties. Aloe vera can soak into the skin upto seven layers deep. Lignins penetrate the toughened areas of skin being beneficial for skin problems.The above properties of Aloe vera gel makes it a suitable candidate for use as a base for topical formulations.

Materials and Methods

Acyclovir was obtained as a gift sample from Cipla Ltd. Mumbai, India. Nerolidol (98%, Mixture of cis & trans) was purchased from Sigma Aldrich. Another excipients Methyl Paraben andPropyl Paraben were of AR Grade and were purchased from Modern Scientifics, Nashik. All the other chemicals used were of high standard and purity obtained from commercial sources.

Formulation design

Preparation of transdermal gel of Acyclovir

Required amount of drug (5%) and preservatives were weighed and mixed properly in mortar pestle. Methyl Paraben (0.15%) and Propyl Paraben (0.08%) were used as preservative. Then small amount of Aloe vera gel was added to it in a successive manner and mixed until a homogenous formulation is formed. Appropriate Nerolidol concentration was added and mixed with the formulation. Finally, remaining amount of Aloe veragel to make up the final volume was added with mixing.

The formulations are varied for the concentration of the penetration enhancer (Nerolidol) from 1-5% (i.e. Formulation F1 to F5) as it was found safe within this range. Above 5% Nerolidol can be irritating to the skin.

Fourier Transform Infrared (FTIR) analysis

The FT-IR spectrum of Acyclovir was recorded using FTIR spectrophotometer (Shimadzu 84005) using Potassium bromide through diffuse reflectance attachment cell.

Drug excipient compatibility testing

Sample preparation

Samples as mentioned below were prepared manually by mixing them so as to form a uniform mixture.

Drug: Aloe vera gel (1:1) w/w

Drug: Nerolidol (1:0.25) w/w

Drug: Methyl paraben: Propyl paraben (1:0.1:0.15) w/w

Mixture of above (1:1:0.25:0.25) w/w

Storage conditions

The above prepared samples were kept in sealed vials for 7 days at 40ºC and 75% RH.

Observations for change in organoleptic properties

Organoleptic properties of the above samples were inspected manually with respect to reference samples. Organoleptic properties are important parameter to be evaluated as change in organoleptic properties is an indication of instability of formulations.

Spectroscopic analysis

Each sample was analyzed using UV spectrophotometer and the shift in λmax (if any) of the drug in presence of other formulation ingredients was observed.

Determination of pH

pH of each formulation was determined by using pH meter (pH meter CL54+) which was calibrated before with buffer solutions of pH 4, 7 and 9.

Determination of Viscosity

Viscosity of each formulation was determined using Brookfield viscometer (Brookfield viscometer; type DV-II PRO) with spindle LV 4, at room temperature and at 5, 10, 20, 50 and 100 rpm.

Drug content

Gel formulationequivalent to 10 mg of drug was taken in 100 ml volumetric flask containing 20 ml of phosphate buffer pH 7.4 and sonicated for 15 minutes. Volume was made up to 100 ml.1ml of above solution was further diluted to 10 ml with phosphate buffer of pH 7.4.The resultant solution was subjected to UV spectrophotometric analysis at 252 nm and the absorbance was noted down.

Spreadability

To determine spreadability of the gel formulations, two glass slides of standard dimensions were selected. Formulation whose spreadability was to be determined was placed over one slide and the other slide was placed over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present and the adhering gel was wiped off. The two slides were placed onto a stand such that only the lower slide is held firm by the opposite fangs of the clamp allowing the upper slide to slip off freely by the force of weight tied to it. 20 gm weight was tied to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using the following formula.

$$s = m \, . \, l/t$$

Value “s” is spreadability, “m” is the weight tied to the upper slides, “l” is the length of glass slide, and “t” is the time taken.

Diffusion study

The diffusion of Acyclovir from gel formulations was studied through egg membrane and cellophane membrane using the Franz diffusion apparatus. The donor cell was filled with gel formulation equivalent to 15 mg of drug. The receptor compartment was filled with phosphate buffer of pH 7.4. The temperature of the receptor compartment was maintained at 37 ± 0.5ºC by circulating hot water through the jacket of Franz diffusion cell. The samples were removed at predetermined intervals at 0.5,1,2,4,6 hours and replaced immediately with equal volume of receptor solution to maintain sink conditions. The aliquots were analyzed at 251.4 nm by UV spectrophotometer.

In vitro permeation study

In vitro release of Acyclovir from gel formulation and the amount of drug that is permeated through freshly excised hairless rat abdominal skin was studied using the Franz diffusion apparatus. The donor cell was filled with gel formulation. The receptor compartment was filled with phosphate buffer pH 7.4. The temperature of the receptor compartment was maintained at 37 ± 0.5ºC by circulating hot water through the jacket of Franz diffusion cell. The samples were removed at predetermined intervals at 0.5,1,2,4,6 hours and replaced immediately with equal volume of receptor solution to maintain sink conditions. The removed samples were analyzed at 251.4nm on UV spectrophotometer.

Comparison with marketed formulation

The in vitro release of Acyclovir from marketed formulation (Acivir cream by Cipla) was studied through freshly excised hairless rat abdominal skin using the Franz diffusion apparatus.The marketed formulation and the prepared formulation were then compared for their drug release.

Stability study

Stability study is performed for F5 as the formulation shows greatest drug release and hence can be termed as 'best formulation' from within those that are developed.

Stability study was carried out for 1 month, the formulation was kept in stability chamber at 40ºC and at 75% relative humidity. After one month the formulation was checked for following parameters.

Physical examination

The formulation was manually examined to check any variations in the colour, odour and texture.

Result and Discussion:

Fourier Transform Infrared (FTIR) analysis

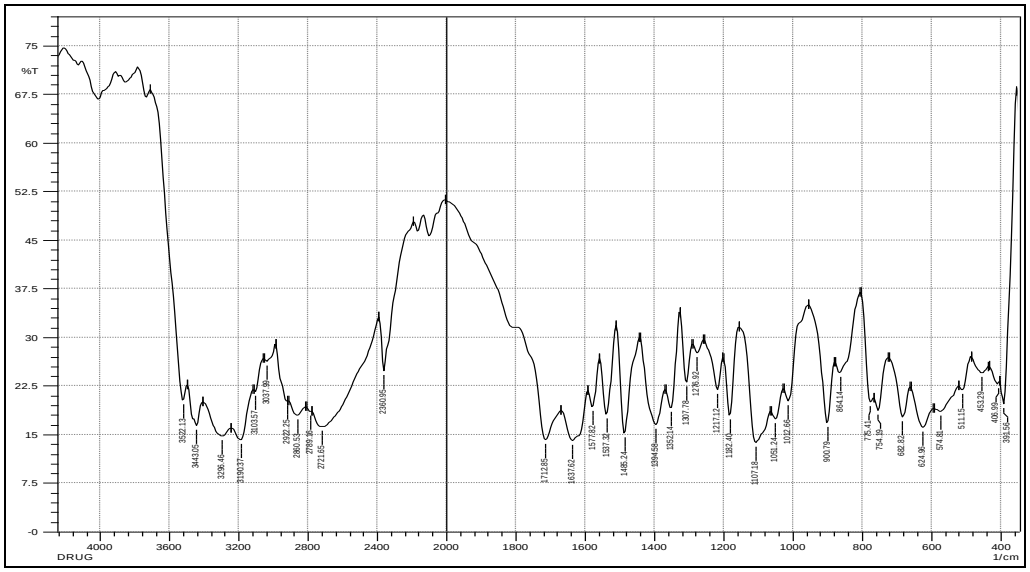

Fig: IR spectra of Acyclovir

Table: Interpretation of IR

Sr. no.	Functional Groups	Groups	Type of Vibration	STD Range (cm^{-1})	Intensity	Peak Observed (cm-1)
1.	Aromatic Ring	(- C - H)	Stretch	3150 - 3050	(m)	3103.57
		(- C = C -)	Bending	1600	(m - w)	1577.82
		(= C - H)	bending	900 -690	(s)	864.14
2.	Alkane	(-C-H-)	Stretch	3000 - 2850	(s)	2860.53
		(-CH2-)	Bending	1465	(m)	1485.24
3.	Amine	(- NH2)	Stretch	3500 - 3100	(m)	3443.05
			Bending	1640 - 1550	(m - s)	1637.63
4.	Ether	(- C - O-)	Stretch	1300 - 1000	(s)	1107.18
5.	Alcohol	(O-H)	Stretch	3500 - 3650	(m)	3522.13

Principal peaks for IR of Acyclovir are 1717, 1632, 1485, 1104 cm^{-1}. The above spectrum matches with the standard spectrum for Acyclovir.

Drug excipient compatibility testing:

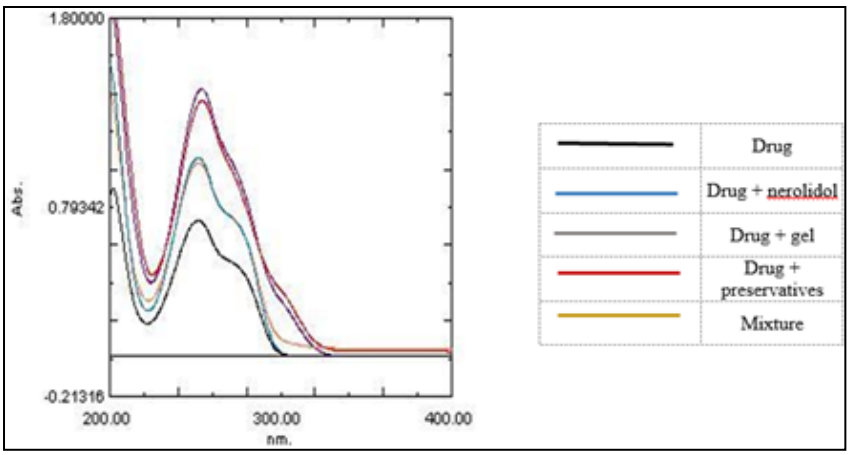

Fig: Overlay of UV spectrums

From figure it was observed that the major peak of the drug (λ_{max} 251.4nm) is not shifted in presence of other excipients. Hence it can be said that no incompatibility exists between drug and the excipients.

Determination of pH

Table: pH of formulations (Mean±S.D.; n=3)

Sr. no.	Formulation code	pH
1.	F1	6.62±0.004
2.	F2	6.63±0.04
3.	F3	6.63±0.00
4.	F4	6.62±0.004
5.	F5	6.54±0.008

pH of all formulations is found to be around 6 which is compatible with skin pH.

Determination of viscosity

Table: Viscosity of formulations.

rpm	Viscosity (cp)				
	F1	F2	F3	F4	F5
5	54708	57228	58667	54588	47870
10	30294	32573	33713	30713	27174
20	17966	18866	19646	17696	15987
50	8794	8350	9754	8686	7810
100	5165	5351	5723	5213	4739

Viscosity is an expression of the resistance of the fluid to flow; higher the viscosity, greater is the resistance.

It is observed that the viscosity of the formulations goes on decreasing as the rpm increases i.e. an inverse relationship exists between the viscosity and the shear rate. From the figures it can be said that the formulations follow pseudoplastic behavior.

Drug content

Table: Percent drug content of formulations (Mean±S.D.; n=3)

Sr. no.	Formulationcode	Drug content (%)
1.	F1	98.93±0.03
2.	F2	100.33±0.15
3.	F3	99.50±0.23
4.	F4	98.66±0.15
5.	F5	100.33±0.31

The percent drug content was found to be within the range of 98 to 101 % which complies with the USP standard.

Spreadability

Table: Spreadability of formulations (Mean±S.D.; n=3)

Sr. no.	Formulation code	Spreadability (gm.cm/sec)
1.	F1	12.16±0.15
2.	F2	11.33±0.15
3.	F3	11.25±0.35
4.	F4	12.33±0.15
5.	F5	12.66±0.07

Spreadability of formulations is found to be in the range of 11.25 to 12.66 gm.cm/sec.

Diffusion study

1. Using cellophane membrane

Table: Cumulative amount of drug released (Q) /cm^2 at different time intervals across cellophane membrane. (Mean±S.D.; n=3)

Time (hr)	Q (mg/cm^2)						
	F0	F1	F2	F3	F4	F5	Marketed
0.5	170.04±3.10	288.47±2.33	174.51±6.31	129.08±3.04	254.95±6.19	505.21±5.26	141.00±1.05
1	329.43±2.57	601.29±2.80	572.98±22.74	620.65±1.81	541.70±3.39	827.71±2.33	236.33±2.10
2	591.57±6.08	931.98±2.11	781.30±10.17	628.84±3.03	777.81±11.81	1252.25±2.80	246.76±2.27
4	754.64±28.69	1220.22±2.00	870.16±3.69	800.15±11.57	932.73±1.98	1576.98±3.33	295.56±4.26
6	1066.79±4.03	1250.01±2.33	1396.74±11.93	1474.95±1.28	1523.36±1.28	1579.22±2.57	396.46±1.26

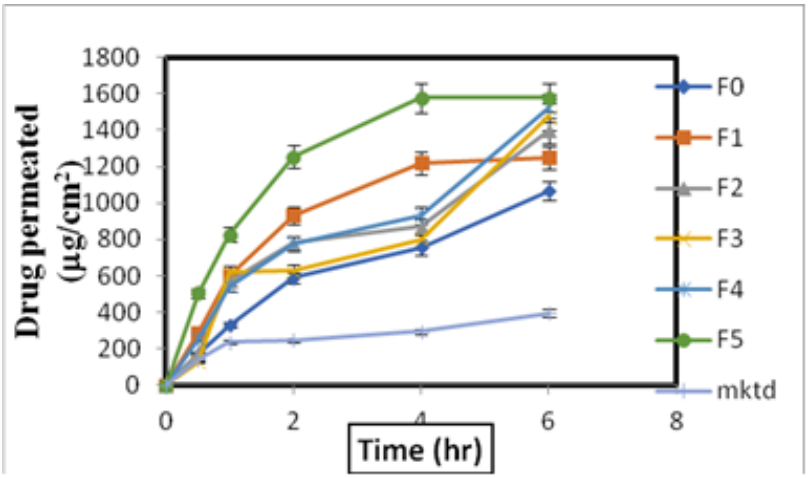

Fig: Plot of cumulative amount of drug release v/s time for different formulations of Acyclovir gel across cellophane membrane.

The release profiles were constructed by plotting the cumulative amount of Acyclovir diffused per unit membrane surface area (Q, mg/cm^2) versus time (hr). The steady state flux (Jss, mg/cm^2. hr) of Acyclovir was calculated from the slope of the plot using linear regression analysis.

Table: Results of drug diffusion parameters across cellophane membrane (Mean±S.D.; n=3)

Formulation code	Q_6 (mg/cm^2)	Jss (mg/cm^2·hr)
F0	1066.79±4.18	177.06±3.08
F1	1250.01±2.33	198.6±0.19
F2	1396.74±2.40	202.9±1.33
F3	1474.95±1.28	214.4±0.46
F4	1523.36±1.28	225.0±0.51
F5	1579.22±3.2	240.9±0.56
Marketed	396.46±1.26	166.1±0.38

It is observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

2. Using egg membrane

Table: Cumulative amount of drug released (Q) /cm^2 at different time intervals across egg membrane. (Mean±S.D. ; n=3)

Time (hr)	Q (mg/cm^2)						
	F0	F1	F2	F3	F4	F5	Marketed
0.5	232.59±3.62	712.25±1.47	485.82±4.83	785.91±14.04	524.25±3.15	685.45±8.53	369.64±1.87
1	336.11±2.45	1045.90±1.29	1167.34±3.39	887.29±1.63	1118.18±4.56	869.92±2.06	533.51±1.28
2	362.17±2.45	1165.81±2.44	1211.27±2.05	1027.32±3.03	1390.78±3.62	1513.67±6.07	570.73±0.92
4	523.01±3.17	1285.01±3.62	1289.49±2.33	1314.84±2.22	1428.02±6.20	1551.65±9.66	540.19±10.75
6	695.10±2.44	1305.10±3.51	1375.14±0.87	1424.26±1.63	1475.68±1.93	1565.45±2.33	607.21±2.10

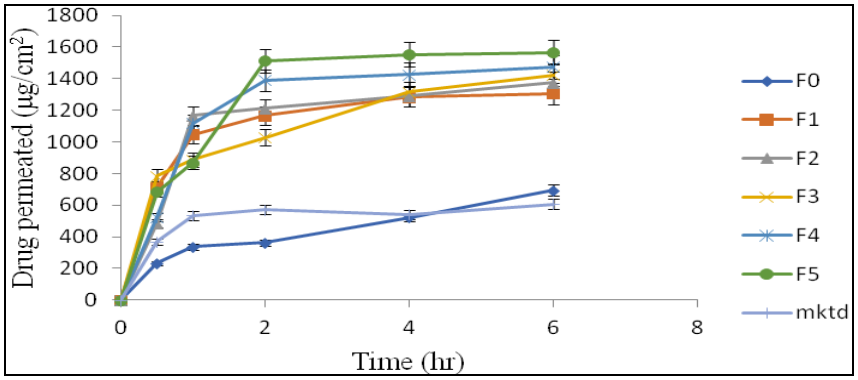

Fig 8.15: Plot of cumulative amount of drug release v/s time for different formulations of Acyclovir gel across egg membrane.

The release profiles were constructed by plotting the cumulative amount of Acyclovir diffused per unit membrane surface area (Q, mg/cm^2) versus time (hr). The steady state flux (Jss, mg/cm^2. hr) of Acyclovir was calculated from the slope of the plot using linear regression analysis. The permeability coefficient (Kp) and enhancement ratio (ER) are calculated using following equations

$$Kp = \frac{Jss}{C}$$

where, Jss is flux at steady state and C is the initial concentration of Acyclovir in the donor compartment.

$$ER = \frac{Kp\ with\ pretreatment}{Kp\ without\ pretreatment}$$

Table 8.16: Results of drug diffusion parameters across egg membrane. (Mean±S.D.; n=3)

Formulation code	Q_6 (mg/cm^2)	Jss (mg/cm^2·hr)	Kp (x10^{-3})	ER
F0	695.10±2.45	97.04±0.48	6.46±0.02	
F1	1305.10±3.51	159.57±0.31	10.63±0.02	1.64±0.002
F2	1375.14±0.87	178.30±0.33	11.88±0.01	1.83±0.004
F3	1424.26±1.63	182.37±0.53	12.15±0.03	1.87±0.009
F4	1475.68±1.93	199.10±0.37	13.27±0.02	2.05±0.004
F5	1565.45±2.33	220.06±0.92	14.47±0.15	2.27±0.009
Marketed	607.21±2.10	67.41±0.81	4.38±0.04	

Q6 = Cumulative amount permeated at 6 hr; Jss= Steady state flux;
Kp = Permeability coefficient; ER= Enhancement ratio.
It is observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

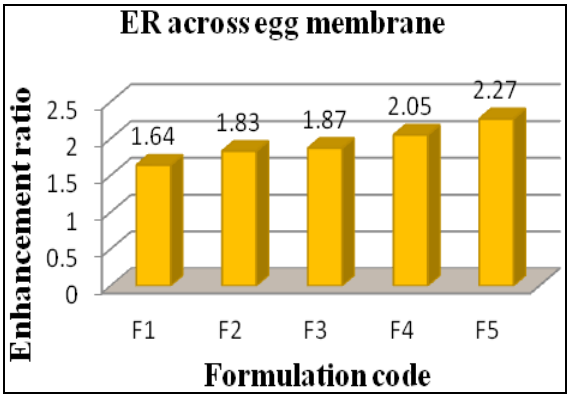

Fig 8.16: Effect of Nerolidol in terms of Enhancement Ratio across egg membrane.

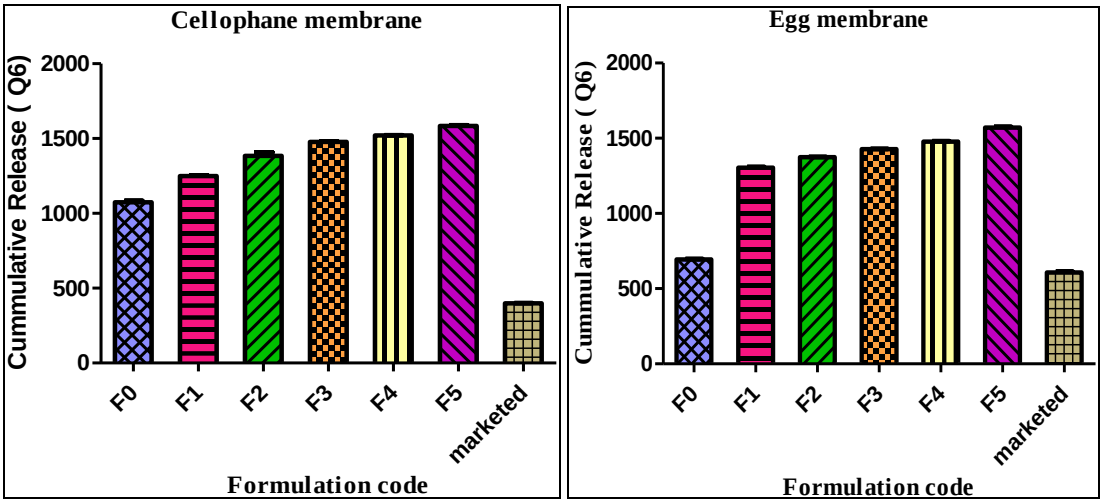

Fig 1, Fig 2

Fig: Plot of cumulative amount of drug diffused at 6 hr (Fig.1) across cellophane membrane, (Fig.2) across egg membrane

In vitro permeation studies

Permeation study of F5 formulation

From the diffusion study it was found that F5 shows maximum amount of drug release and hence can be considered as the best among all the formulations. Permeation studies are therefore carried out for this formulation.

Table: Results of skin permeation parameters for F5 across rat abdominal skin.

Time (hr)	Q (mg/cm^2)	Jss (mg/cm^2·hr)
0.5	25.55	12.89
1	59.81	
2	71.73	
4	82.90	
6	90.35	

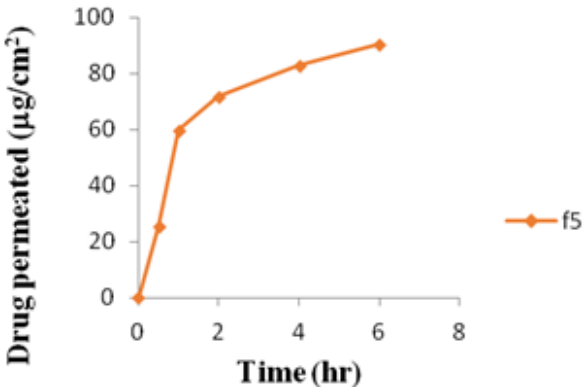

Fig: Plot of drug permeated v/s time for F5 formulation across rat abdominal skin.

The cumulative amount of drug permeated at 6 hour (Q6) and the steady state flux (Jss) for the formulation were found to be 90.35 and 12.89 respectively.

Comparison of F5 with marketed formulation and F0

Table: Cumulative amount of drug released/cm^{2}at different time intervals across rat abdominal skin

Time (hr)	Q (mg/cm^2)		
	F0	F5	Marketed formulation
0.5	15.87	25.55	11.4
1	20.34	59.81	13.63
2	21.83	71.73	16.61
4	22.57	82.90	24.06
6	52.36	90.35	33.74

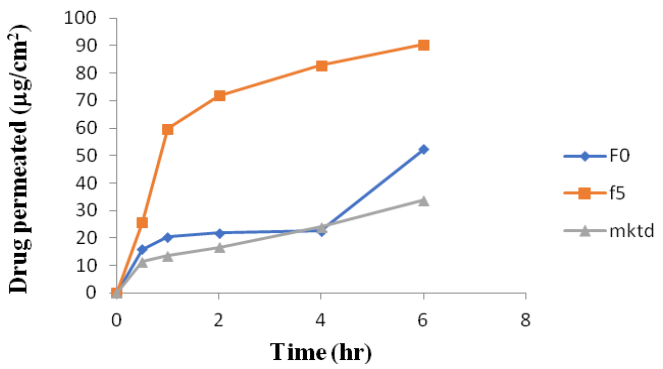

Fig: Comparative plot of drug permeated v/s time for different formulations of Acyclovir across rat abdominal skin.

Table: Results of drug permeation parameters across rat abdominal skin

Formulation code	Q_6 (mg/cm^2)	Jss (mg/cm^2·hr)	Kp (x10^{-3})	ER
F0	52.36	6.59	0.43	--
F5	90.35	12.89	0.85	1.97
Marketed	33.74	4.74	0.31	--

Q6= Cumulative amount permeated at 6 hr; Jss= Steady state flux; Kp= Permeability coefficient; ER= Enhancement ratio.

The maximum permeation flux of Acyclovir (12.89±0.23) through rat abdominal skin was observed with an enhancement ratio of 1.97, when Nerolidol was incorporated at a concentration of 5% in the gel system.

The rank order of the permeability coefficients of Acyclovir was found to be: F5 (5% nerolidol)>F0 (0% nerolidol)>Marketed formulation.

Stability study

Physical examination

No visible change in the formulation was observed as compared to control formulation.

Determination of drug content

Table: Drug content for stability testing

Drug content after stability	Drug content before stability
98.56	100.33

Diffusion study

Table: Results of drug diffusion parameters across egg membrane for stability testing.

Time (hr)	Q (mg/cm^2)	Jss (mg/cm^2·hr)
0.5	658.12	220.30
1	848.25	
2	1495.33	
4	1535.15	
6	1550.65	

The cumulative amount of drug permeated at 6 hour (Q6) and the steady state flux (Jss) for the formulation were found to be 1550.65 mg/cm^{2}and 220.3 mg/cm^2·hr respectively. No significant changes were observed in the formulation after its stability testing.

Summary and Conclusion

The use of chemical penetration enhancer is a promising approach to increase the permeation of drugs showing low permeability. Incorporation of Nerolidol showed improved in-vitropermeability of the drug. Maximum permeation is achieved at 5 % concentration of Nerolidol and hence F5 can be termed as the best formulation among those that are developed.

pH of all the formulations is found to be around 6 which is skin compatible. Rheological study showed that viscosity is inversely proportional to the rate of shear and thus the system shows pseudoplastic behaviour. The percent drug content was found to be within the range of 98 to 101 % for all the formulations. Spreadability of formulations is found to be in the range of 11.25 to 12.66 gm.cm/sec. Stability studies showed that the formulation is stable at 40ºC at 75% RH for a period of one month.

Statistical analysis of cumulative amount of drug permeated from various formulations at 6 hour showed marked level of significance at $p<0.05$ when compared with marketed formulation.

Comparison of F5 (containing 5% w/w Nerolidol) with marketed formulation (Acivir, Cipla) for permeation of Acyclovir across hairless rat abdominal skin, showed that greater flux 12.89 mg/cm^2·hr was obtained for F5 as compared to flux 4.74 mg/cm^2·hr for marketed formulation. This can be attributed to increased lipid disruption in the stratum corneum by Nerolidol.

F0 showed better flux of 6.59 mg/cm^2·hr than the marketed product though it did not contain Nerolidol, this increase in permeation can be attributed to the use of Aloe vera gel as an absorption base which consists of lignins, that allows for penetrative properties.

Future scope

In vivo studies: Animal studies to test general toxicity and irritancy of the formulation. These can be followed by studies in humans. Long term stability studies can be performed to determine the shelf life of the formulation. Similar approach of formulation can be used for other drug candidates having low permeability profiles. Comparative screening of different penetration enhancers can be done.

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