ORIGINAL ARTICLE

Transdermal Permeation of Acyclovir by Iontophoresis Technique

M. Gopi[1], R. Kumaravelrajan[1], P. Killivalavan[1], V. Suba[2]

[1]*Department of Pharmaceutics, C.L. Baid Metha College of Pharmacy, Thorapakkam, Chennai, Tamil Nadu, India,*
[2]*Department of Pharmacology, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India*

Abstract

Context: The approach for improving transdermal drug delivery is a technique called iontophoresis, a promising technology that has already received regulatory approval. **Aims:** The aim of the present investigation was to develop acyclovir (ACV) gel, which deliver drug through iontophoresis. Formulation variable was drug loading, current density, and hydrogen-ion concentration (pH). Factorial design was used to study the above variable with drug release at T95% as the response variable. **Settings and Design:** 2^3 factorial design was used to study the effect of pH, drug loading, and current density as independent variables, to T 95 % of drug release as dependent variables. **Materials and Methods:** The current settings were maintained at 0.5 mA. The silver wire was connected as anode at one side and silver chloride as cathode at other side. The resistor was fixed to control the flow of current from the equipment. ACV gel was prepared using Carbopol 934P. *Ex vivo* permeation study was conducted for 6 h in Franz diffusion cell using rat skin. Optimized formulation was analyzed for viscosity, flux, and *in vivo* animal study. **Statistical Analysis Used:** Numerical optimization carried out to find out the constrain variables. Contour plot and response surface were also studied for optimized one. **Results:** Two factorial interaction of independent variable with respect to dependent variable was analyzed by the kinetics of drug release. It was found that the drug release was controlled up to 6 h when variables used as pH 7.4, current input 0.5 mA, and drug loading 5% in the formulation. The Cmax and Tmax of *in vivo* study revealed 190 ng/ml at 6 h. **Conclusions:** It was found that the effectiveness of iontophoretic delivery from gel was largely affected by polarity and current density than drug loading. The optimized formulation gave desired release profile up to 6 h. Drug release kinetics seemed to be Korsmeyer-Peppas model (n = 0.875). Further *in vivo* study in animal model revealed the Cmax and Tmax reached the predicted time of delivery of drug without fluctuation in the plasma level.

Key words: Acyclovir, factorial design, iontophoresis, optimization technique

INTRODUCTION

Conventional delivery systems suffer from the limitations of minimal synchronization between the required time for therapeutically effective drug plasma concentrations and the actual drug release profile exhibited by the dosage form.[1] In some drugs, transdermal delivery offers a number of advantages with respect to oral or parenteral administration. However, only a small minority of drug molecules are able to passively penetrate the skin. Ionic, neutral, and/or polar molecules typically show limited skin penetration ability.[2] One approach for improving transdermal drug delivery is a technique called iontophoresis, a promising technology that has already received regulatory approval. In iontophoretic delivery, a small electric current is applied to the delivery system to drive the drug molecules across the skin barrier. Drugs delivered through iontophoresis are typically hydrophilic and ionic. Positively charged drugs are placed at the anode; negatively charged drugs are placed at the cathode. An electronic controller (i.e., battery/microcomputer) is used as a current and/or voltage source that drives the drug out of the donor reservoir and through the skin into the systemic circulation. To complete the electric circuit, a buffer solution is placed in a return reservoir.[3] This technique is capable of expanding the range of compounds

Address for correspondence:
R. Kumaravelrajan, Department of Pharmaceutics,
C.L. Baid Metha College of Pharmacy, Thorapakkam,
Chennai, Tamil Nadu, India. Tel.: 044-24960151.
E-mail: rkumaravelrajan@gmail.com

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that can be delivered transdermally along with the benefits of bypassing hepatic first-pass effect and higher patient compliance.[4-6]

A mathematical model of iontophoretic transdermal drug delivery was applied to study the effects of physical parameters on the cumulative amount of amitriptyline HCl.[7] Hydrogels are used as a vehicle to study the iontophoretic delivery of methotrexate.[8] The type of electrodes used also affects the iontophoretic delivery. Electrodes Ag/AgCl are the most preferred as they resist the changes in pH which are generally seen during the use of platinum or zinc/zinc chloride electrodes.[9] Other important factors affecting iontophoretic delivery include concentration of co-ions (buffers), current strength, type of current used, type of skin used, concentration of solute in the donor, temperature of acceptor phase, the charge on the drug, and the type of vehicle used.[10] Acyclovir (ACV), a synthetic analog of 2′-deoxiguanosine, approved for the treatment of herpes simplex infection over ten years ago, remains a potent and reliable antiviral agent.[11] The aim of the present investigation was to develop Acyclovir hydrogel to deliver through iontophoresis technique. Formulation variable was drug loading, current density, and hydrogen ion concentration (pH). Factorial design was used to study the above variable with drug release at 95% as response variable.

MATERIALS AND METHODS

Materials

ACV received as a gift sample from Twenty First Century Pharmaceuticals Pvt. Ltd. (Chennai), Carbopol 934, triethanolamine, methylparaben, propylparaben, glycerin, and citric acid were purchased from Loba chemie Pvt. Ltd. (Mumbai). Dimethyl sulfoxide (DMSO) was obtained from Merck Specialities Pvt. Ltd. (Mumbai). Acetic acid, disodium hydrogen phosphate, sodium chloride, and potassium hydrogen phosphate were purchased from SD Fine-Chemicals Ltd. (Mumbai).

Methods

Fourier transform infrared (FTIR) spectroscopy

The l compatibility between ACV and the excipients used in research was tested by IR spectroscopy using ABB Bomem IR spectroscopy. The FTIR spectra of samples were obtained, using an FTIR spectrophotometer. About 2 mg of the samples were mixed with dried potassium bromide of equal weight and compressed to form a KBr disc. The samples were scanned from 500 to 4000/cm.

Differential scanning calorimetry (DSC)

Assessment of possible incompatibilities between an active pharmaceutical ingredient and different excipients forms an important part of the preformulation stage during the development of a solid dosage form. DSC allows the fast evaluation of possible incompatibilities because it shows changes in the appearance, shift or disappearance of melting endotherms and exotherms, and variations in the corresponding enthalpies of reaction. The DSC thermograms of pure drug and drug with polymer were recorded. The samples were separately sealed in aluminum cells and set in NETZSCH DSC. The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 50-300°C.

Experimental design

A three-factor and two-level factorial design (2^3) was used as the experimental design. The independent variables studied were the pH (X1), current input (X2), and drug loading (X3). The total number of experiments was 8 [Tables 1 and 2]. Design expert 10.0 Stat-ease employed to optimize the process parameters. Time to release 95% of drug ($T_{95\%}$) was used as dependent variable. The following polynomial equation generated to study the interaction.

$$Y = B0 + B1 (X1) + B2 (X2) + B3 (X3) + B12 (X1X2) + B13 (X1X3) + B23 (X2X3) + B123 (X1X2X3)$$

Where, Y is dependent variable, B0 arithmetic mean response of nine batches, and B1 estimated coefficient for factor X1. The main effects (X1, X2, and X3) represent the average result of changing one factor at a time from its low-to-high

Table 1: Matrix table

Formulation no.	X1	X2	X3
1	1	1	−1
2	−1	1	−1
3	−1	1	1
4	1	−1	−1
5	1	−1	1
6	−1	−1	1
7	1	1	1
8	−1	−1	−1

Table 2: Matrix design for formulations

Formulation no.	pH	Voltage (mA)	Drug loading (%)
F1	7.4	0.7	3
F2	3	0.7	3
F3	3	0.7	5
F4	7.4	0.5	3
F5	7.4	0.5	5
F6	3	0.5	5
F7	7.4	0.7	5
F8	3	0.5	3

value. The interaction term (X1, X2, and X3) shows how the response changes when three factors are simultaneously changed. The data demonstrate that both X1 (pH) and X2 (current in mA) and X3 (drug loading) affect the time required for drug release ($T_{95\%}$). From the results, it can be concluded that an increase in the amount of the polymer leads to decrease in the release rate of the drug and drug release pattern may be changed by appropriate selection of the X1, X2, and X3 levels.

Preparation of ACV gel

Carbopol 934P (1.25% w/w) and purified water were taken in a mortar and allowed to soak for 24 h. To this, required amount of drug (5% 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 trituration until the homogenous gel formed, then added DMSO (5% w/w) to this formulation.[12]

Preparation of iontophoresis

Iontophoretic transport can be regulated by varying the applied current density and area of application. The current settings were maintained at 0.5 mA. The silver wire was connected as anode at one side and silver chloride as cathode at other side. The resistor was fixed to control the flow of current from the equipment.

Ex vivo permeation studies

Franz diffusion cells were used in the *ex vivo* permeation studies. The rat skin was mounted in between the compartments of the diffusion cell with stratum corneum facing the donor compartment; the volume of the receptor compartment was 20 ml. About 2 g of gel was applied on the donor compartment. In this study, 20 ml of saline phosphate buffer (pH 7.4) solution was used as receptor medium. The receptor medium was maintained at 37°C ± 5°C and stirred magnetically at 200 rpm. Anode was fixed in the donor compartment and cathode was fixed in the receptor compartment. About 1 ml of sample were withdrawn from receptor compartment at predetermined time interval for 6 h period, and replaced by same volume of fresh pre-warmed saline phosphate (7.4 pH) solution to maintain constant volume. The samples were assayed spectrophotometrically at 254 nm against appropriate blank.[13]

Flux rate determination

Franz diffusion cells were used. The cellophane membrane was mounted between the compartments of the diffusion cell in donor compartment. The volume of the receptor compartment was 20 ml. About 0.2 g of gel was applied on the donor compartment. In this study, 20 ml of saline phosphate buffer (pH 7.4) solution was used as a receptor medium. The receptor medium was maintained at 37°C ± 5°C and stirred magnetically at 200 rpm. Anode was fixed in the donor compartment, and cathode was fixed in the receptor compartment. About 1ml of sample was withdrawn from receptor compartment at predetermined time interval for 6 h period and replaced by same volume of fresh pre-warmed saline phosphate (7.4 pH) solution to maintain constant volume. The samples were assayed spectrophotometrically at 254 nm against appropriate blank.[14]

In vivo permeation study of optimized formulation (IAEC/L/09/CLBMCP/2017)

To investigate the *in vivo* potential of iontophoretic delivery with iontophoresis, the study was carried in hairless rats. To restrict the area of application of the developed gel formulation and to hold the gel at the applied site, the "O" ring-shaped spacer was taken and 0.2 g of gel was applied with applicator; the anode was placed in gel formulation whereas cathode was placed elsewhere on the body of the animal. The electrodes were first connected to the electroporation device to give 0.5 mA for iontophoresis.[15] Blood collected continuously at various time intervals were analyzed using HPLC method. The curve stripping of plasma concentration was done by PK solutions 2.0.68. Blood sampling: Blood samples were withdrawn at 1-6 h after transdermal application (F9). Blood samples were centrifuged at 1500 rpm for 10 min at 4°C; plasma samples were immediately analyzed by high-performance liquid chromatography (HPLC).

Analytical method

The plasma concentrations of ACV were determined by HPLC using a mobile phase of methanol:0.1% formic acid in water (5:95), pumped at a flow rate of 1.0 ml/min, and the eluate monitored at a wavelength of 254 nm. The injection volume was 10 µl, and the chromatography was carried out on a Luna C8 (5 µ, 150 mm × 4.6 mm i.d.) column. Samples were injected at ambient temperature during analysis. The chromatographic system was equipped with a pump (Spectra-physics, SP 8700, California, USA), an auto sampler (Spectra-physics, AS 1000), a UV detector (Spectra-physics, UV 100), and an integrator (Spectra-physics, SP 4270). A reverse-phase column (ZorbaxODS, 4 ± 6 mm, 25 cm 64.6 mm i.d; Dupont de Nemours, Wilmington, DE, USA) was used.[16]

RESULTS AND DISCUSSION

Drug-excipient compatibility studies: FTIR study

The FTIR spectra of ACV and carbopol 934 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. Pure ACV showed prominent peaks 3288, 1706, 1629, and 1180/cm because of O-H, C=O, C=N, and C-O-C stretching, respectively. It also exhibited peaks at 3470 and 3437 due to N-H group. The principal FT-IR absorption peaks of the drug were close to those in the spectra containing drug and polymer, indicating that ACV was unaffected by processing conditions and was compatible. The spectra are represented in Figure 1. It is clear from the results obtained that there is no positive evidence for the interaction between vehicle and drug.

DSC

ACV showed [Figure 2] endothermic peak at 248.33°C which corresponds the melting point of ACV. However, drug with polymer endothermic peak shown at 246.74°C, which may be due to decreased crystalline behavior of drug to amorphous state during mixing with polymer such as Carbopol 934 was reported.[17,18]

Ex vivo permeation study: Effect of formulation variable on drug release

Permeation of ACV through animal skin was studied by *ex vivo* in all developed formulation. In formulation F1, initial drug release was found to be 28.1% and 78.4% at 6 h (formulation containing 3% of drug loading with applied current of 0.7 mA at pH 7.4) [Figure 3]. Contrarily, F2 gave better release profile of 55.6% and 98.2% due to the drug loading (3%) and pH (3.0) with increased current density (0.7 mA). In case of formulation F3, drug release found to be slightly increased to 15.1% and 39.8% at 1st h and 6th h, because of current density during experiment was 0.7 mA. To study the effect of pH on 0.5 mA and 3% of permeation enhancer formulation, F4 tested and diffusion was found to be 26.7% and 62.6% but release observed incomplete.

On the other hand, the maximum release was obtained with formulation F5 (contains 5% permeation enhancer and 7.4 pH with a consistent voltage of 0.5 mA) of 95.2 % and 63.2% at 6th h and 1st h [Figure 4], hiked the release rate of the drug. Further testing with pH 3.0 gave maximum of 77.8% of drug release for formulation F6. As expected formulation, F8 also released slowly and comparable to F2 with maximum release was 55.05% and 15% at 6th h and 1st h. Finally, the drug release was not achieved more than 32 % even with formulation F7 (contains 5% drug loading and 0.7 mA, pH of 7.4).

The permeation profiles of all formulations ACV reported in Figure 5 shows that iontophoresis largely promoted the transport of ACV through animal skin at both pH 7.4 and pH 3.0, whereas the drug was transported at a very low rate in pH 3.0. After 2 h and up to 6 h, the iontophoretic permeation profiles resulted practically linear versus time, indicating the achievement of quasi-steady state conditions through the skin. The fluxes obtained through animal skin

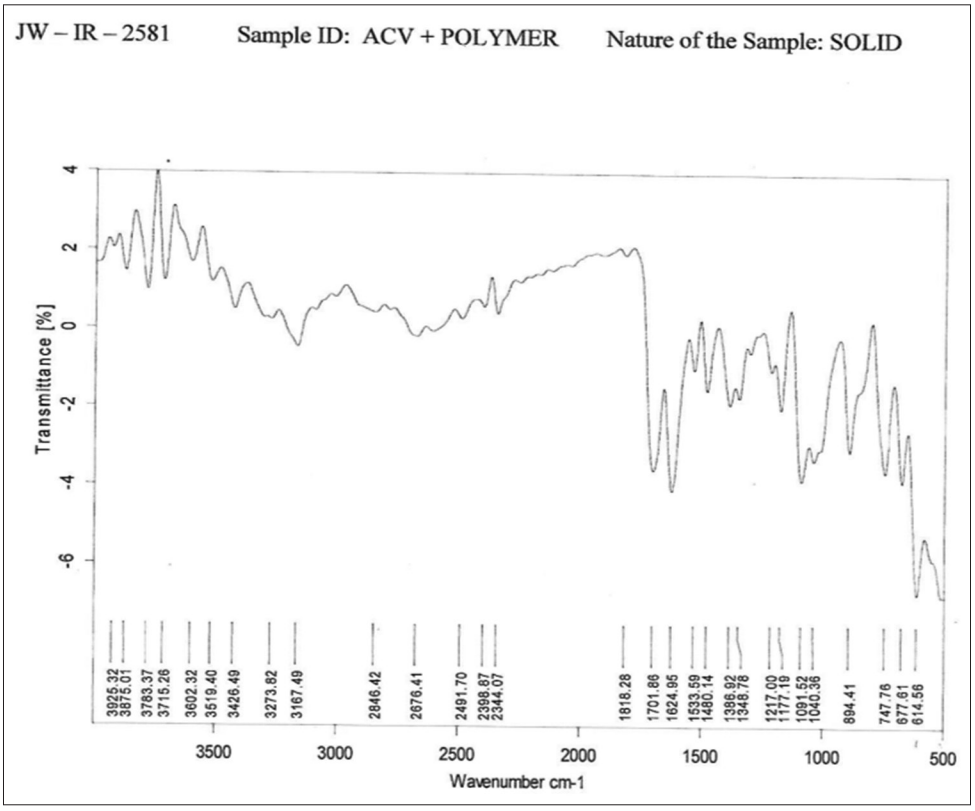

Figure 1: Fourier transformer infrared of acyclovir and polymers

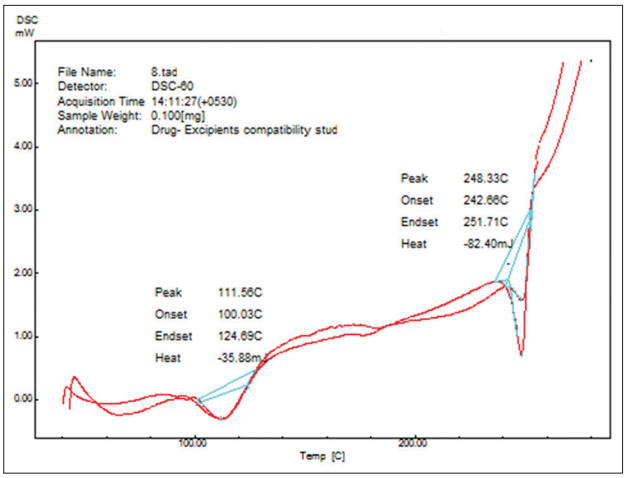

Figure 2: Differential scanning calorimetry of acyclovir pure drug and drug:polymer mixed (overlapped)

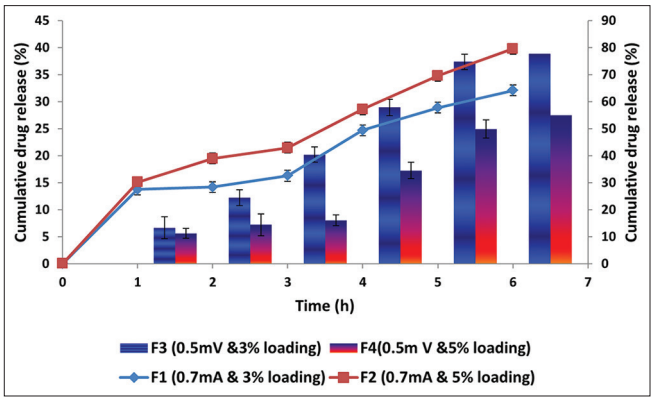

Figure 3: Effect of pH 3.0 with current density of 0.7 mA (F1, F2) and 0.5 mA (F3, F4) on acyclovir release

largely depended on current density and polarity (pH). This analogy suggests the mechanism of transport involved could be electrorepulsion at pH 3.0 and electro-osmosis at pH 7.4 and increase in permeability due to the current application. Alvarez-Figueroa *et al.*[19] reported similar behavior, but in human study.

Optimization

For optimization, effects of various independent variables on measured responses were modeled using following mathematical model equation involving independent variables and their interactions for various measured responses generated by 2^3 factorial design is as follows: Where Y is the dependent variable and results of ANOVA indicated 0.9853 [Table 3] are regression coefficients R^2, *P* value 0.064 (S). The three-dimensional response surface plots [Figure 6a and b] and corresponding contour plots relating $T_{95\%}$ indicate the controlled values of $T_{95\%}$ with the increment of all three-independent variables. The optimized (F9) formulation prepared after numerical optimization method and parameter obtained were as follows pH 7.4, voltage 0.7, and 5% drug loading. Final

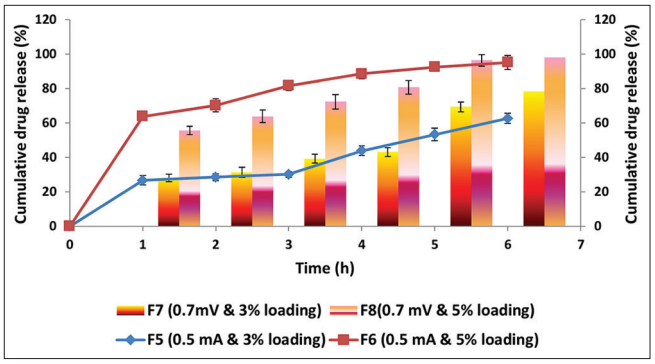

Figure 4: Effect of pH 7.4 with current density of 0.5 mA (F5, F6), and 0.7 mA (F7, F8) on Acyclovir release

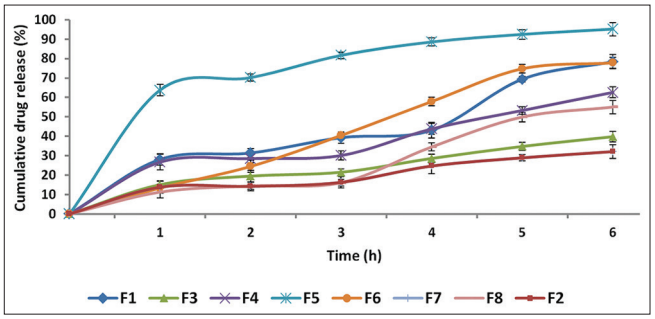

Figure 5: Comparative dissolution profile of all formulations (F1-F8)

polynomial regression equation for interaction as follows: Y=67.39A-5.27B-10.36C-9.97AB-2.74AC-3.48BC.

Evaluation of ACV hydrogel

The formulation containing carbopol 934 was slightly acidic and was neutralized with triethanolamine to optain pH of 7.4. The viscosity of the optimized formulation was 20,901.79 cps. The formulations exhibited quite low viscosity at low temperature determined by Brookfield's viscometer at room temperature. The viscosity of the formulation did not increase on increasing the temperature was observed during stability study. As a gel was formed in the room temperature, the viscosity was not affected the formulation at higher temperature. The gel formulation reflected fairly uniform drug content ensuring adequacy in the method of preparation. Drug content was found to be 99.75%. The spreadability was determined using horizontal plate method. The spreadability of the optimized formulation was 0.87 g.cm/s. The value of spreadability indicates that gels were easily spreadable with minimum application of shear even the prepared ACV gel is translucent in structure, took 120 s to spread 7 cm glass slides.

Flux determination test

The permeation profiles of acv through membranes were found to be linear. The profiles showed a straight line as a function of time [Table 4], and the ACV permeated through

membranes by a zero-order kinetic (R^2 - 0.9820 with slope value equal to 30.68 mg/cm). The permeation profiles led us to conclude that the permeation from the gel without adhesive layer was slower than that from the membrane with adhesive layer.

Comparison with market product

When the optimized formulation was compared with marketed product, the release of commercial formulation was to be 63.33% at the end of 6 h. The permeability of the drug through skin has a lot of criteria. Therefore, passive absorption of ACV is less and requires repeated use (6 times a day). Unless the suitable method available as driving forces to deliver the drug through stratum corneum, the frequency of administration cannot be reduced. Comparative profile of ACV iontophoresis with passive cream depicted as Figure 7.

Table 3: Summary of ANOVA

SD	5.52
Mean	67.39
C.V.%	8.19
R^2	0.9853
Adj R^2	0.9485
Pred R^2	0.7645
Adeq precision	13.837

SD: Standard deviation

Table 4: Determination of flux of acyclovir gel

Time (h)	Average drug release (%)	Average drug release (mg)	Rate (mg/h)
1	16.49	32.98	32.98
2	35.27	70.54	35.27
3	53.79	107.58	35.86
4	70.18	140.36	35.09
5	87.13	174.26	34.85
6	96.21	192.41	32.06

In vivo study of optimized formulation

Plasma samples were collected in different hours. After administration of Acyclovir gel, the Cmax and Tmax found to be 190.0 ng/mg/ml at 6.0 h and AUC (0-t) found to be 521 ng/ml/h. The AUMC (0-t) and cumulative AUMC (0-t) was found to be 1140.0 ng/h/ml and 2385 ng/h/m [Figure 8].

Drug release kinetics

The optimized formulation was fitted with various kinetic models to assess the drug release and it was fond to be followed zero-order profile as shown in Table 5. However, Korsmeyer-Peppas release exponent is found to be 0.875 with R^2 =0.9922. This exhibits non-Fickian Anomalous transport where rate as a function of time t^{n-1}.

CONCLUSION

Iontophoretic transdermal delivery of ACV gel has been prepared successfully. Factorial design (2^3) with three variable and two levels was used to prepare eight formulation containing ACV 5% w/w. The pH and current density were used as a process variable, and the formulation variable was drug loading in the matrix design. From *in vitro* permeability data, it was found to be the effectiveness of iontophoretic delivery from gel was largely affected by polarity and current density than drug loading. The optimized formulation gave desired release profile up to 6 h. Drug release kinetics followed Korsmeyer-Peppas model (n = 0.875) which implies

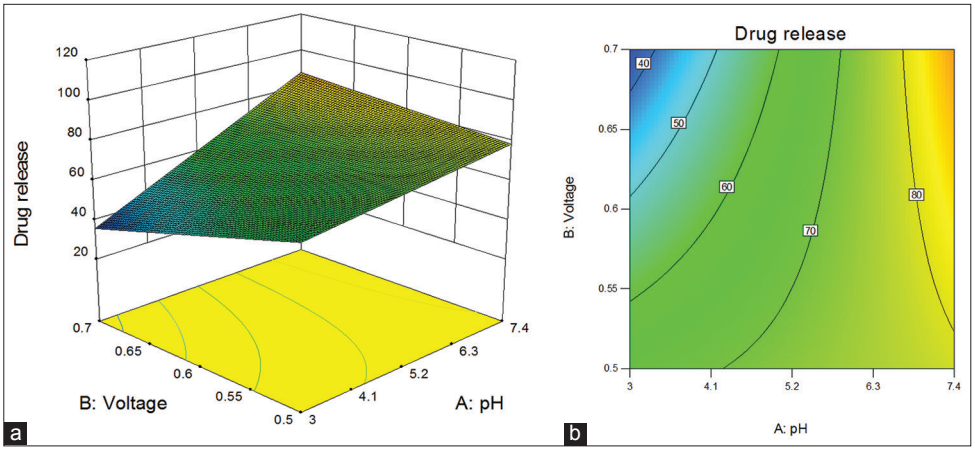

Figure 6: Effect of pH and voltage on drug release from optimized formulation. (a) Response surface, (b) contour plot

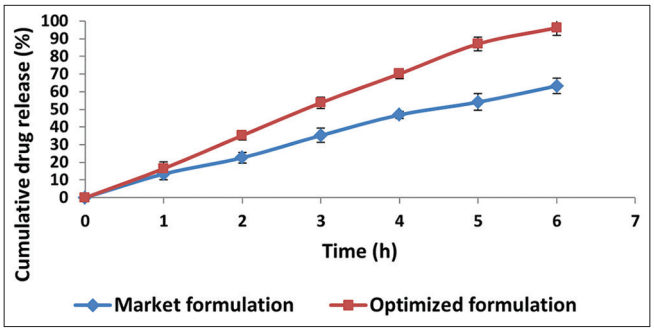

Figure 7: Comparative *in vitro* profile of optimized and marketed acyclovir gel

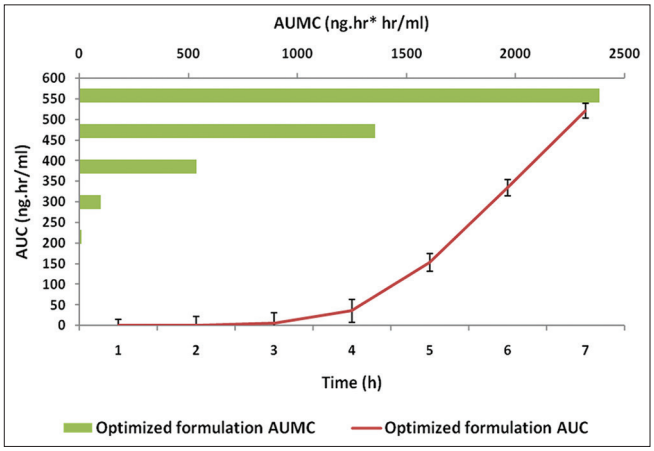

Figure 8: *In vivo* absorption profile of acyclovir gel from optimized formulation

Table 5: Fitting with various kinetics model of optimized formulation

Kinetics	Parameter	Optimized
Zero order	Slope	15.49786
	R^2	0.99138
First order	Slope	–0.23384
	R^2	0.69191
Higuchi kinetics	Slope	38.0048
	R^2	0.90252
Korsmeyer-Peppas	Slope	0.91273
	R^2	0.99227
	N	0.875

non-Fickian drug diffusion transport with zero-order drug release. Further, *in vivo* study in animal model revealed the Cmax of 190.0 ng/ml with Tmax value of 6 h without fluctuation in the plasma level. Significant pattern of the release was found when compared with the marketed preparation. However, long-term stability study and optimization of system properties might be required to improve the delivery of the drug.

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