

## A novel transdermal patch of Lamivudine: *Invitro-Invivo* characterization

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### ABSTRACT

The work was carried out to formulate and evaluate the matrix transdermal patches of Lamivudine for the controlled delivery of drug in the body. The transdermal patches were prepared by the solvent casting method using Span-80 as a permeation enhancer and were prepared in different drug: polymer (Lamivudine: HPMC and Lamivudine: EC) ratios of 1:2.5, 1:5, without permeation enhancers and with permeation enhancers. The prepared transdermal patch were found to be good physicochemical properties, shows no skin irritation on the rat skin and subjected to *in vitro* drug permeation study by using rat skin in phosphate buffer pH 7.4 for 24 hours. The comparative statistical analytical data (ANOVA) showed 'p' value < 0.0001 which suggest that the prepared formulation are extremely significant for transdermal delivery. The formulation (FPE<sub>2</sub>) ratio 1:5 of (Lamivudine: HPMC) patch with permeation enhancer showed best result among all the formulation. This formulation (FPE<sub>2</sub>) was subjected to *in-vivo* studies by using rat. The *in-vivo* studies of the patches were administrated transdermally to rat skin and while a standard solution of 10µg/ml was used as a control, and collected the plasma drug sample at different time interval and analyzed by HPLC. The *in-vitro in-vivo* correlation studies shows the regression value (R) 0.863 and correlated and to be linear.

**Keywords** *Invitro-invivo* correlation, Lamivudine, Transdermal patches, antiretroviral agent, Span80

### INTRODUCTION

Transdermal drug delivery is the non-invasive delivery of medications from the surface of the skin through its layers, to the circulatory system. The current transdermal delivery system has evolved as a successful alternative to systemic drug delivery. Most transdermal patches are designed to release the active ingredient at a zero-order rate for a period of several hours to days following application to the skin.<sup>1</sup>

Acquired immune deficiency syndrome (AIDS), which threatens to cause a great plague in the present generation, was first identified in California in 1981. AIDS is considered to be an epidemic disease. The annual number of AIDS deaths can be expected to increase for many years to come, unless more effective and patient compliant anti-retroviral medications are available at affordable prices. The major drawbacks of anti-retroviral drugs for the treatment of AIDS are their adverse side effects during long-term therapy, poor patient compliance and huge cost of the therapy.<sup>2</sup>

Lamivudine (3TC), an anti-retro viral is commonly used in the treatment of HIV infected patients. Most of the anti-retro viral including Lamivudine are virustatic in nature and they must be administered for the life span of the patient. Despite being quite effective on oral administration, 3TC exhibits dose dependent toxic side effects. Side effects often require dosage reduction or even cessation of treatment, since conditions like lactic acidosis may even be fatal. The problems associated with oral administration of 3TC led us to explore the possibilities of designing novel drug delivery system for Lamivudine with an alternative route of administration. Novel drug delivery carriers such as transdermal patches are very versatile to suit the delivery of various drug molecules. Thus it was proposed that a non invasive zero-order delivery such as the transdermal route is desirable.<sup>3</sup>

### MATERIALS AND METHODS

#### MATERIALS

Lamivudine were procured from Aurobindo Pharma Pvt Ltd., Hyderabad. Hydroxy propyl methyl cellulose E15LV, Ethyl cellulose, Span80, Chloroform was purchased from Loba Chemie Pvt Ltd., Mumbai. Glycerol was purchased from Spectrum reagents and chemicals Pvt Ltd., Cochin. Equipments used were Digital weighing balance, Dial calliper, pH meter, Orbitek shaker, Franz diffusion cell (60ml), UV/Visible Spectrophotometer, FTIR and HPLC Spectrophotometer, Stability chamber, REMI CPR-24 centrifuge, Magnetic stirrer.

## METHODS

### COMPATIBILITY STUDY OF DRUG AND POLYMER

FTIR absorption spectra 2 mg of the substance being examined was dried in hot air oven at 50°C for one hour to remove moisture and triturated with 300 mg to 400mg of finely powdered and dried potassium bromide. The mixture was grinded carefully, spread uniformly in a suitable disc of 13 mm diameter and put in vacuum to a pressure of about 800 Mpa(8t.cm-2). The same procedure was repeated for the polymers and the physical mixture of drug and the polymers.

### PREPARATION OF STANDARD

#### CURVE

**Stock solution** was prepared by dissolving 100 mg of lamivudine in 100 ml of phosphate buffer ph 7.4.

**Standard solution** 10 ml of stock solution was made to 100 ml with ph 7.4. From this solution 1-8 ml were transferred into 10 ml standard flask and make up with buffer. Thus the final concentration ranges from 10-60 µg/ml and measure the absorbance at 270 nm.

#### DOSE DESIGNING

Based on the Pharmacokinetic parameters of Lamivudine, the amount of drug required to achieve the effective plasma concentration was calculated using the equation<sup>4</sup>,

$$Kp = (C_{\text{plasma}})_{\text{ss}} \times K_{\text{el}} \times V_d$$

Where,  $(C_{\text{plasma}})_{\text{ss}}$  - The drug level at steady state,  
 $K_{\text{el}}$  - Elimination rate constant  
 $V_d$  - Volume of distribution of drug.

### FABRICATION OF TRANSDERMAL PATCHES

**Technique:** Solvent casting method.

Four types of formulations were prepared by using two different polymers in different ratios. First two formulations were prepared by using HPMC alone having Drug & Polymer ratio 1:2.5, 1:5 using water as a solvent and the next formulations were formulated using EC having Drug & Polymer ratio 1:2.5, 1:5 using chloroform as a solvent with Span 80 (1%) as permeation enhancer, Glycerol (3%) was used as a plasticizer. The films were cast on to suitable designed and fabricated glass mould and then dried in hot air oven at 40°C for 6 hrs. The films were removed by using sharp blade by inserting along the edges of the film. The dried films were wrapped in aluminium foils and stored in a closed container in cool place away from light.

### FABRICATION OF LAMIVUDINE TRANSDERMAL PATCHES OF 3.14 SQ CM

S.No	Ingredients	F <sub>1</sub> W	F <sub>2</sub> W	F <sub>3</sub> W	F <sub>4</sub> W	FPE <sub>1</sub>	FPE <sub>2</sub>	FPE <sub>3</sub>	FPE <sub>4</sub>
1.	Lamivudine	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2.	Hydroxy Propyl Methyl Cellulose (mg)	12.5	25	—	—	12.5	25	—	—
3.	Ethyl cellulose (mg)	—	—	12.5	25	—	—	12.5	25
4.	Span 80	—	—	—	—	1%	1%	1%	1%
5.	Glycerol	3%	3%	3%	3%	3%	3%	3%	3%

### PHYSICO-CHEMICAL EVALUATIONS

**Thickness of the patch** Thickness of the patch was measured by using dial gauge in mm.

**Folding endurance** The folding endurance was measured manually for the prepared film .A strip of film was cut evenly and folded at the same place till it broke .The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance<sup>5,6,7,8,9</sup>.

**Percentage of moisture absorption**<sup>10</sup> To check the physical stability of the film in high humidity condition, accurately weighed film were placed in a desiccators containing saturated solution of aluminum chloride (79.5% relative humidity) for 3 days The film were reweighed and percentage moisture absorption were calculated using the formula.

Percentage of moisture absorption =  $(\text{Final weight} - \text{Initial weight} \times 100) / \text{Initial weight}$

**Percentage of moisture loss**<sup>10</sup> To check the extent of moisture loss from freshly prepared film. Accurately weighed film were placed in a desiccator containing fused anhydrous calcium chloride for 72 hrs. After 72 hrs the film were reweighed and percentage moisture loss was calculated using the formula.

Percentage of moisture absorption =  $(\text{Initial weight} - \text{Final weight} \times 100) / \text{Initial weight}$

#### Water Vapour transmission rate

The patches were fixed over the edge of the glass vial containing 3g of fused calcium chloride as a desiccant by using an adhesive. Then the vial was placed in a desiccator containing saturated solution of potassium chloride. The vial was taken out periodically and weighed for a period of 72 hours<sup>7, 11, 12</sup>

#### Drug content uniformity

The prepared patch was cut into small piece and put into 100ml dissolution or diffusion medium used respectively and Stirred continuously by using a mechanical stirrer and sample was withdrawn at the end of three hours and the drug content was determined, spectrophotometrically at 270 nm<sup>5</sup>.

#### Stability Study

The prepared patches were subjected to stability study by storing the patches at storage conditions specified in the ICH guidelines. The patches were stored for three months at temperature  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and at a relative humidity of  $75\% \pm 5\%$ . The stability study was conducted with regard to tensile strength, moisture content and drug content. The patches, which retained their physical properties, were further subjected to *in-vitro* permeation studies<sup>5,6</sup>.

## IN VITRO SKIN PERMEATION STUDIES OF TRANSDERMAL PATCH

### IN VITRO SKIN PERMEATION STUDIES

The Ethical clearance for the handling of experimental animals was obtained from the Institutional animal ethical committee (IAEC) formed for this purpose. The protocol of the animal study was approved by the Institutional Animal Ethical

committee, Protocol Number: **SVCP/ IAEC/ Ph.d/02/2009 dated, 7<sup>th</sup> November 2009**. The experiment was conducted according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiment on animals).

These studies were carried out using a mammalian rat skin of required thickness. The permeation cell used for this study was a specially fabricated "Modified Franz diffusion cell" were used<sup>5,6,8,13</sup>.

#### Modified Franz diffusion cell

Modified Franz diffusion cell is a skin permeation system developed by Franz and commercialized by crown glass has been frequently used for the studying the kinetics of percutaneous absorption. The modified Franz diffusion cell has receptor compartment with an effective volume approximately 60ml and an effective surface area of permeation of  $3.14\text{cm}^2$ . A rod shaped magnetic stirrer driven by a 3w synchronized motor stirs the solution and the stirring magnet rotates at constant rpm in a low viscosity receptor compartment<sup>14,15,16</sup>.

#### Operation of diffusion cell

The skin was mounted and clamped between the receptor and donor compartment. The surface area exposed to the drug patch was 3.14 Sq. cm. The cell was maintained at  $32^{\circ} \pm 0.5^{\circ}\text{C}$  using thermostatically controlled water bath. Water from the water bath was circulated through the cell, which was maintained at constant flow. The diffusion cell of 60ml capacity was filled with Phosphate buffer pH 7.4 and fixed to a thermostatically controlled magnetic stirrer, which was also maintained at the temperature  $32^{\circ} \pm 0.5^{\circ}\text{C}$ . At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analysed spectrophotometrically at 270 nm.

#### KINETIC CHARACTERISTICS OF THE DRUG RELEASE

To know the mechanism of the drug release from the patches, the results obtained from the *In-vitro* dissolution process were fitted into different kinetic equations as follows:

Zero - order drug release: Cumulative % drug release Vs Time.

First Order drug release: Log cumulative % drug retained Vs Time.

Higuchi's classical diffusion equation: Cumulative % drug release Vs Square root of time.

Peppas's Korsmeyer Exponential equation: Cumulative % drug release Vs Log time.

### THE STUDY OF IN VITRO SKIN PERMEATION KINETICS

**Permeability coefficient (P)** is the velocity of drug passage through the membrane in  $\mu\text{g}/\text{cm}^2/\text{h}$ . The permeability coefficient was calculated from the slope of the graph of percentage of drug transported versus time as,

$$P = \text{Slope} \times V_d / S$$

Where,  $V_d$  is the volume of donor solution and  $S$  is the surface area of tissue.

**Flux (J)** is defined as the amount of material flowing through a unit cross-sectional barrier in unit time. It is calculated by<sup>17,18</sup>,

$$\text{Flux (J)} = P \times CD$$

Where,  $CD$  = concentration of donor solution and  $P$  = permeability.

**Enhancement ratio ( $E_r$ )** was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by,

Enhancement ratio = Permeability coefficient of drug with enhancer / Permeability coefficient of drug alone

### SKIN IRRITATION TEST

The skin irritation test was done on healthy rabbit weighing between 2-3 kg. The rabbits were divided into 5 groups with three each. On the previous day of the experiment, the hair on the backside of the rabbit was removed. Drug loaded polymeric film of  $3.14 \text{ cm}^2$  was placed on the left dorsal surface of the rabbit. The skin was examined for erythema/edema<sup>6,7,8,19</sup>.

Group – I : Served as normal, without any treatment

Group – II : Control- applied with marketed official adhesive tape in SP

Group – III: Blank – applied with drugless polymeric patches

Group – IV: Test – applied with drug loaded polymeric patches

Group – V: Applied with 0.8%v/v aqueous solution of formalin as standard irritant

### STATISTICAL ANALYSIS BY ANOVA

The in-vitro permeation data were statistically compared and analysed using Graph Pad Instat – 3 software version. The Tukey – Kramer multiple comparisons test was utilized to find the 'q' and 'p' values and the significance of the formulations were studied. If the 'p' is  $<0.05$ , the formulations are said to be significant.

### INVIVO STUDIES OF TRANSDERMAL PATCHES

Six Wister rats of 180-220gm weight are to be utilized for the study. The rats are to be allowed to acclimatize for 1 week before the day of administration. The rats were fasted, but allowed access to water on the day before the study<sup>20</sup>.

#### Standard concentration solution of lamivudine

A mixture of acetonitrile and water (65:35%V/V) was used as the mobile phase and  $C_{18}$  column as the stationary phase. A stock solution of the pure drug was prepared by dissolving 50mg of Lamivudine in 50ml of the mobile phase. This stock solution was further diluted with the mobile phase to obtain the concentration of  $10\mu\text{g}/\text{ml}^3$ .

Six groups of rats were taken for the animal studies and each group containing one rat. Rats are to be anesthetized before the experiment. The hair on the abdominal site is clipped before the experiment. The skin was gently wiped with water and alcohol swab and patted dry. The patch of  $3.14\text{sq.cm}$  size patch containing 5mg lamivudine was fixed over the prepared skin. Blood samples of approximately 0.15ml were collected from the jugular vein in the dried heparinized tube at 0.5, 2, 4, 8, 16, 24 hrs after transdermal administration, and they are frozen at  $-20^\circ\text{C}$  as soon as possible and stored until the analysis. The HPLC analytical reports of the blood samples collected at different hours were compared with that of standard concentration of Lamivudine.

### IN-VITRO / IN-VIVO CORRELATION

*In vitro-in vivo* correlation is the demonstration of the direct relationship of *in vitro* dissolution rate / diffusion rate of drugs and there *in vivo* bioavailability. Correlation is used to ensure batch-

to-batch consistency in the physiologic performance of a drug product by use of such *in vitro* values and to serve as a tool in the development of a new dosage form with desired *in vivo* performance.

There are two basic approaches by which a correlation between dissolution/diffusion testing and bioavailability can be developed<sup>21</sup>.

1. By establishing a relationship, between the *in vitro* dissolution/diffusion and the *in vivo* bioavailability

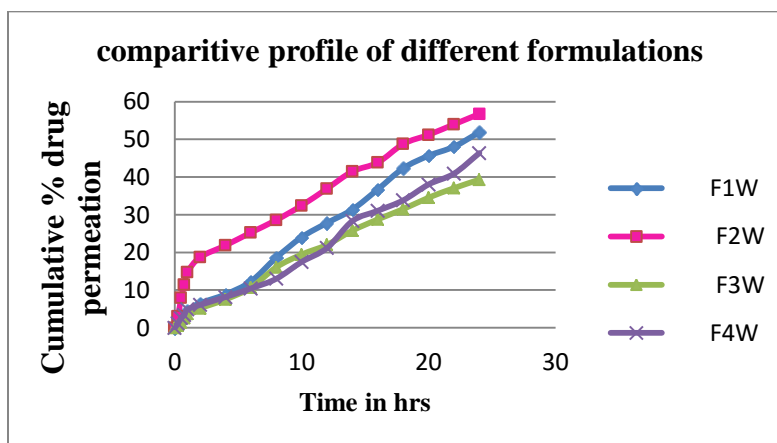
parameters. If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or level A correlation.

2. By using the data from previous bioavailability studies to modify the dissolution/diffusion methodology in order to arrive at meaningful *in vitro-in vivo* correlation.

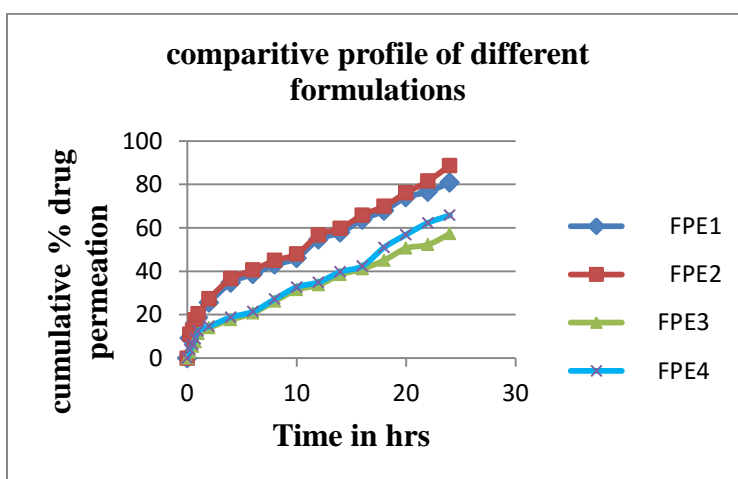
## RESULTS

### IN-VITRO SKIN PERMEATION STUDIES OF VARIOUS FORMULATIONS

Time (hrs)	Cumulative % of drug permeation							
	F <sub>1</sub> W	F <sub>2</sub> W	F <sub>3</sub> W	F <sub>4</sub> W	FPE <sub>1</sub>	FPE <sub>2</sub>	FPE <sub>3</sub>	FPE <sub>4</sub>
0	0	0	0	0	0	0	0	0
0.25	0.57	3.03	0.55	1.12	9.28	10.99	2.74	3.88
0.5	1.44	7.91	1.42	2.56	11.15	12.88	5.35	6.22
0.75	2.60	11.46	2.58	3.37	15.88	17.64	7.43	8.88
1	4.43	14.77	3.67	4.85	18.70	20.49	11.25	12.44
2	6.49	18.71	5.15	6.07	25.54	27.36	13.99	14.63
4	8.88	21.85	7.51	8.16	34.76	36.61	17.63	18.85
6	12.29	25.27	10.64	10.43	38.75	40.59	20.75	21.41
8	18.60	28.58	16.05	13.01	43.06	44.97	26.21	27.17
10	24.02	32.44	19.44	17.48	46.10	47.94	31.46	32.72
12	27.82	36.93	22.03	21.17	54.68	56.64	33.66	34.94
14	31.39	41.48	25.79	28.33	57.81	59.79	38.45	39.75
16	36.72	43.83	28.75	31.05	63.81	65.83	41.03	42.36
18	42.32	48.76	31.48	33.81	67.91	69.95	45.08	50.97
20	45.63	51.20	34.52	38.03	74.05	76.12	50.60	56.87
22	48.04	53.96	37.04	40.90	76.58	81.52	52.21	62.28
24	51.89	56.75	39.31	46.35	80.84	88.70	57.26	65.78



*In-vitro* skin permeation of various formulations (Without permeation enhancers)



In-vitro skin permeation of various formulations (With permeation enhancers)

**THE *IN VITRO* SKIN PERMEATION KINETIC DATA FOR TRANSDERMAL PATCHES (WITHOUT PERMEATION ENHANCER)**

S.No	Formulation code	Slope of zero order plot	Permeation coefficient(p)	Flux(J)
1	F <sub>1</sub> W	1.7135	2.7285	17
2	F <sub>2</sub> W	2.1916	3.4898	17.45
3	F <sub>3</sub> W	2.1792	3.4701	13.2
4	F <sub>4</sub> W	1.8520	2.9490	14.6

**THE *IN VITRO* SKIN PERMEATION KINETIC DATA FOR TRANSDERMAL PATCHES (WITH PERMEATION ENHANCER)**

S.No	Formulation code	Slope of zero order plot	Permeation coefficient(p)	Flux(J)	Enhancement ratio(Er)
1	FPE1	3.005	4.78	23.9	1.405
2	FPE2	3.143	5.00	25	1.432
3	FPE3	2.196	3.49	17.45	1.321
4	FPE4	2.494	3.97	19.85	1.359

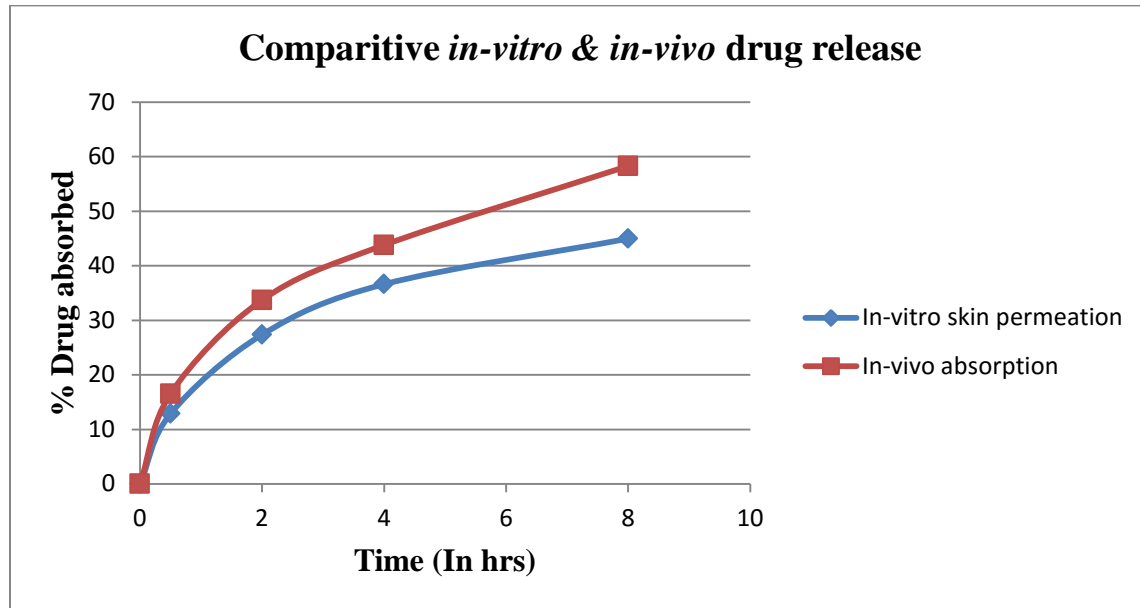
***INVIVO* DRUG ABSORPTION STUDIES**

S. No	Time (in hrs)	Mean Area Under Curve (AUC)	Concentration ( µg/ml)	% drug absorption (%)
1	Control	172950.73	10	-
2	0.5	1429283.25	8.265	16.54
3	2	2914583.07	16.85	33.71
4	4	3787204.87	21.90	43.80
5	8	5039821.69	29.14	58.52
6	16	5785842.70	33.45	66.90
7	24	6874342.48	39.75	79.50

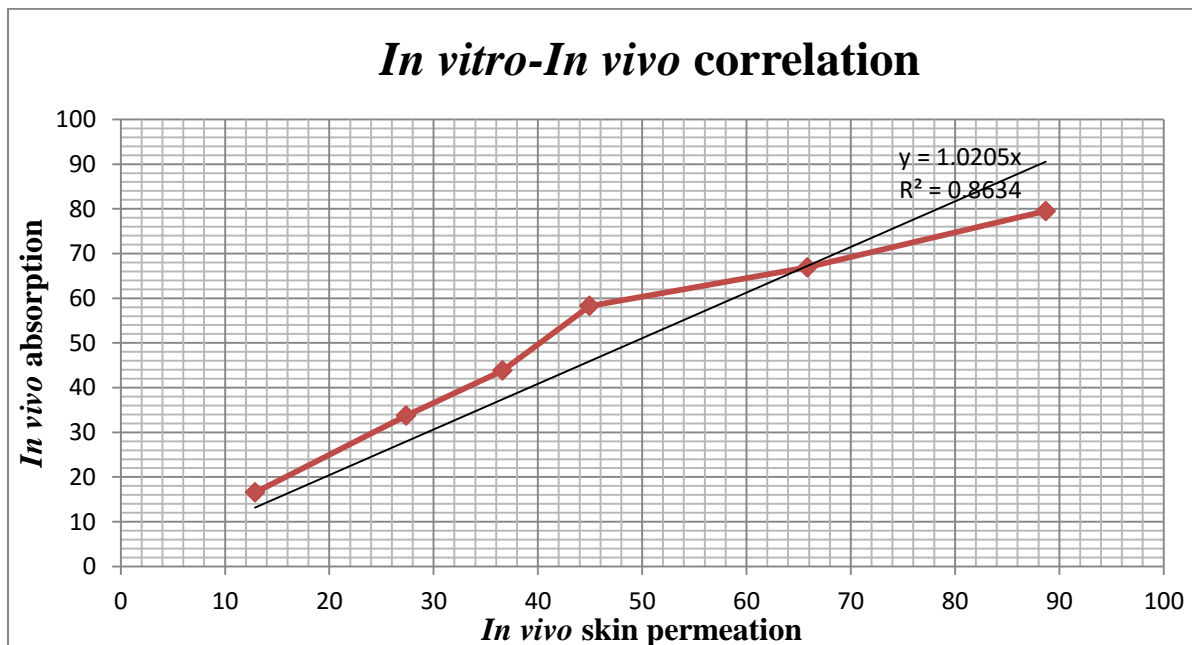
**IN VITRO- IN VIVO CORRELATION**

<i>In vitro</i> skin permeation (%)	<i>In vivo</i> absorption (%)
12.88	16.54
27.36	33.71
36.61	43.80

44.96	58.28
65.83	66.90
88.70	79.50



Comparison graph of in-vitro skin permeation and in-vivo absorption studies



## DISCUSSION

### THE *IN VITRO* SKIN PERMEATION STUDIES

The results indicated that span 80 greatly influenced the permeation of drug through the skin. Thus, suggesting the use of permeation enhancer in transdermal drug delivery system. We concluded that the mechanism of drug release as zero order and non-fickian release. The permeability parameters like  $J$ ,  $P$ ,  $E_r$  were significantly increased in formulations containing permeation enhancers.

### THE *IN VIVO* DRUG ABSORPTION STUDIES

The *in vivo* drug absorption studies reveal that there is a sustained release of drug into the rat systemic circulation of 79.5% of drug absorption was after 24 hrs.

### IN-VITRO/IN-VIVO CORRELATION

The relationship between *in-vitro* and *in-vivo* studies becomes linear with a slope of 1, then the curves are super imposable and there is a 1:1 relationship which is defined as point to point or level A correlation. In this study the slope between the *in-vitro* and *in-vivo* studies was 0.863. So, it is said to be near correlated and the curves are not super imposable and are not linear.

### CONCLUSION

It was concluded that prepared transdermal patch showed Lamivudine and HPMC in the ratio 1:5 (FPE<sub>2</sub>) formulation showed a good sustained release characteristics with the 1% Span 80 as a permeation enhancer, thus providing a better way of preventing first pass metabolism. The *in-vitro* skin permeation studies (FPE<sub>2</sub>) showed a best sustained release effect over the formulations. The comparative statically analysis done by ANOVA method proved that all the formulations is extremely significant for the transdermal route. The *in-vivo* studies (FPE<sub>2</sub>) also showed a sustained release effect of the drug into the systemic circulation. The *in-vitro/in-vivo* correlations are found to be not super imposable and said to be near correlated and linear.

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