Formulation and Evaluation of Transdermal Patch from Lamivudine and Stavudine-Loaded Ethosomes

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ABSTRACT

The purpose of the work was to formulate and evaluate the Lamivudine and Stavudine-loaded ethosome transdermal patches for the controlled delivery of the drug in the body. Patches were formulated using various ratios of polymer $HPMCK_{15}M$, ($PVPK_{30}$) Polyvinyl Pyrrolidone and Ethyl Cellulose. Transdermal patches were formulated by solvent evaporation method. The drug polymer interaction was investigated by FTIR and the results indicated no incompatibility. Lamivudine and Stavudine patches were evaluated for various parameters like thickness, folding endurance, percentage moisture loss, percentage moisture absorption, drug content uniformity, stability studies, *in vitro* skin permeation, skin irritation test. All formulations possess excellent physicochemical properties and exhibited negligible skin irritation with good physical stability. Permeation study was performed by using modified Franz diffusion cells. On the basis of drug release and physicochemical values, formulation TF5 was considered as an optimized formulation which shows higher percentage of drug release at 12 hours. Percentage drug release of Lamivudine and Stavudine-loaded ethosome transdermal patches was observed to be 95.24% Lamivudine and 94.24 % Stavudine drug at 12 hrs. Release kinetics studies revealed that the drug release from formulations TF5 followed Higuchi kinetics.

Keywords: Lamivudine, Stavudine, ethosome,Transdermal patches, $HPMCK_{15}M$, *In vitro*.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus that causes irreversible destruction of the immune system. During the last decade, even though attempts were being made to eradicate HIV but it was found that eradication of HIV is highly unlikely, and effective antiretroviral therapy is required on a long-term basis to maintain viral suppression and reduce disease progression. Lamivudine is a commonly used hydrophilic antiviral drug for treatment of acquired immunodeficiency syndrome (AIDS and hepatitis. Lamivudine has a short biological half-life (4-6 hour) and requires frequent administration for a prolonged period of time (lifelong in AIDS and for one year in hepatitis patients). Transdermal route is, therefore, a better alternative to achieve constant plasma levels for prolonged periods of time, which additionally could be advantageous because of less frequent dosing regimens.

Stavudine is used in the treatment of HIV-1 infection, but is not a cure. It is not normally recommended as initial treatment.Stavudine can also reduce the risk of developing HIV-1 infection after coming into contact with the virus either at work (e.g., needlestick) or through exposure to infected blood or other bodily fluids. It is always used in combination with other HIV medications for the better control of the infection and a reduction in HIV complications.

The major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a non-invasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration, gastric irritation and discomfort associated with parenteral drug administration can be avoided. Flexible liposomes are common vectors in transdermal drug-delivery systems, with relatively good liquidity and deformability. In recent years, ethosomes have become new liposome carriers with high deformability; high entrapment efficiency and a good transdermal permeation rate in the drug delivery system, and are suitable for transdermal administration. Compared with other liposomes, the physical and chemical properties of ethosomes make these more effective for drug delivery through the stratum corneum into the blood circulation, which is very important in the design of a transdermal drugdelivery system.[1,2]Transdermal drug delivery is the non-invasive delivery of medications from the surface of skin-the largest and most accessible organ of human body- through its layers, to the circulatory system. TDDS offers many advantages over conventional injection and oral methods. It reduces the load that the oral route commonly places on the digestive tract and liver. It enhances patient compliance and minimizes harmful side effects of a drug caused from temporary overdose. [3-7]

Methodology

Preparation of Standard Curve [7]

Stock solution: Accurately weighted 100 mg of Lamivudine and Stavudine was dissolved separately in 10 ml of methanol in 100 ml of volumetric flasks and volume was made up to 100ml with pH 7.4 phosphate buffer to get a solution 1000µg/ml concentration.

Standard solution: From primary stock solution of 10 ml was pipette out in a 100 ml of volumetric flask and volume was made up to the mark with pH 7.4 buffer to get a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 1ml to 8ml were transferred in to 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 10-60 µg/ml. Absorbance of each solution was measured at 270 nm and 263 nm against pH 7.4 phosphate buffer as a blank. A plot of concentrations of drug versus absorbance was plotted.

FT–IR spectral analysis

The development of a successful formulation depends only on a suitable selection of excipients. Hence the physical state of the drug Lamivudine, Stavudine and the polymers, EC, HPMCK_{15}M , PVPK_{30}, PEG-400,

Tween 80 and Methanol individually and the combination of drug and polymers used for ethosomes preparation are studied by FTIR (Fourier transform infrared spectroscopy) to know the drug–polymer compatibility. The physicochemical compatibility of the drugs and the polymer was obtained by FTIR studies. The interpretation values of the FTIR are mentioned in the Table

Preparations of transdermal patches[8]

The transdermal patches of composition listed in Table no.2 were prepared by solution casting technique employing a glass substrate (Bangles wrapped with aluminium foil). Membrane type transdermal systems with ethosomes containing 150 mg Lamivudine and 15 mg Stavudine prepared using HPMC alone and by employing various proportions of HPMCK_{15}M, PVP$K_{30,}$ and Ethyl Cellulose. The polymers was accurately weight and dissolved in a suitable solvent mixed until clear solution formed with magnetic stirrer then added drugs to the uniform polymeric solution and mixed completely to form uniform solution. PEG400 added as a plasticizer and tween-80 was used as a penetration enhancer. The polymer solution was poured into bangles placed in a suitable level, hard rigid surface and patches were dried at a room temperature in a dust free environment for 24 hrs. an inverted funnel was covered over the bangles to avoid fast evaporation of the solvent. Patches of 3.14 cm^2 were prepared by cutting and packed in an aluminum foil and kept in a desiccator.

Evaluation of Transdermal Patches

Thickness of patches [9]

The thickness of Patches was measured by digital vernier calipers with least count 0.001mm at three different sites average of three reading was taken with standard deviation.

Weight variation [9]

The three disks of 3.14 cm^2 was cut and weighed on electronic balance for weight variation test. The test was done to check the uniformity of weight and thus check the batch- to- batch variation.

Drug content

Accurately weighed patches were individually dissolved in minimum quantity of methanol and made volume up to 100 ml with PBS pH 7.4 solutions; 10 ml was transferred to flask and made volume 100 ml. The absorbance was recorded. The blank solution was made in the same manner except the patches without drug were used.[9]

Table: 1 Composition of transdermal patches

Formulation		TF1	TF2	TF3	TF4	TF5	TF6	TF7
Drug from ethosomal formulation (mg)	Lamivudine	150	150	150	150	150	150	150
	Stavudine	15	15	15	15	15	15	15
HPMCK_{15}M (mg)		400	350	300	250	-	-	-
PVPK_{30} (mg)		-	50	100	150	300	250	200
EC (mg)		-	-	-	-	100	150	200
PEG-400*(ml)		0.2	0.2	0.2	0.2	0.2	0.2	0.2
Tween 80 (*ml)		0.15	0.15	0.15	0.15	0.15	0.15	0.15
DCM/Methanol (ml)		10	10	10	10	10	10	10

Key-*based on polymer weight

Percentage Moisture content [10]

The films were weighed & placed in desiccators containing calcium chloride at 40^0c in a dryer for at least 24 hrs or more until it gives a constant weight. The % of moisture content was the difference between constant weight taken and the initial weight and as reported with percentage by weight moisture content.

$$\text{Percentage moisture lost} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Percentage Moisture absorption/uptake [10]

The films of which the size 3.14cm^2 were put in a desiccators with silica gel for 24 hrs and weighed the patches were transferred to another desiccators containing saturated solution of KCL(84% RH) after equilibrium was attained. Patches were taken out and weighed. Moisture uptake was calculated with following formula

$$\text{Percentage moisture absorption} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Swelling index[11]

The patches of 3.14 cm² were weighed and added into Petri dish which contains 10 ml double distilled water and were permeated to absorb moisture at a fix time interval check the increase weight of the patches. Continue this process till same weight observed until weight remaining the same over a period of time. Swelling index (% S) was determined by applying the formula.

S (percentage) = Wt – Wo / Wo x 100

Where, S percent swelling, Wt patch weight at time t.

Wo patch weight at time zero.

Folding endurance[12]

This was obtained by constantly folding one patch at the same place without breaking gave the value of folding endurance. This test performed to check folding ability of transdermal patches also indicate brittleness of patches, more brittle patch when folding endurance value le

Percentage Elongation [12]

A film strip (4 x 1cm) was cut on a glass plate with a sharp blade. The % elongation break is to be determined by observing the length just before the breaking point with formula by pointer on the graph paper.

% Elongation = [Final length – Initial length] * 100/Initial length

Tensile Strength [13]

The tensile strength of the patches was found by the apparatus and the design of instrument such that, it had one wooden frame that horizontally placed having fixed scale. On the top of frame two clips were attached to hold patches that under study. From two clips one clips fixed & other moved. Instrument also has pulley to hold weight a patch, weight applied to one end of pulley and other end attached to the fixed clip. During the test wooden platform not dislocate from the original place so platform was fixed carefully to avoid dislocation. Three patches were cut for study having 3.14 cm^2 sizes. Thickness and breadth of patches were noted at three sizes and calculated average value. Rate of stress changes was maintained constant with the addition of 0.5g per 2 minutes. The elongation was observed and the total weights taken were used for

calculation. Formula for tensile strength :

$$\text{Tensile strength} = F/a.b\ (1+L/l)$$

Where,

F is the force required to break; 'a' is width of film; 'b' thickness of film; L is length of the film; l is an elongation of film at break point

***In-vitro* permeation studies** [13-18]

Franz diffusion cell (fabricated in our Lab.) with a diameter 3.7 cm was used in in-vitro release studies. A glass tube with both end open, 10 cm height and 3.7 cm outer diameter was used as a permeation cell. A transdermal patch sample was accurately placed on a semipermeable cellophane membrane to occupy a circle of 3.7 cm diameter. The loaded membrane was stretched over the lower open end of a glass tube of 3.7 cm diameter and made water tight by rubber band. The tube (donor compartment) was immersed in a beaker containing 100 ml of phosphate buffer pH 6.8 (receptor compartment) .The cell was immersed to a depth of 1 cm below the surface of buffer. The system temperature was maintained at 37°±1° and speed was maintained at 30 rpm throughout the experiment by magnetic stirrer. The samples 3 ml were withdrawn at different time intervals and analyzed without dilution or filtration for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.

Drug release kinetics

Drug release mechanism of drug through film investigated and analyzed with the following mathematical release models:

Kinetics Model		**Equation**
Zero order	:	$Q = Q_o - K_o t$
First order	:	$Q = Q_o (1 - e^{-K1t})$
Higuchi model	:	$Q_t = K_H t^{1/2}$
Hixson-Crowell's cube root model	:	$\sqrt[3]{Q_o} - \sqrt[3]{Q_t} = K_{HC} t$
Korsmeyer-peppas model	:	$\frac{Q_t}{Q_\infty} = K_k\ t^n$

Where, Q_t – drug release at time t

Q_o – initial amount of drug

K_O, K_1, K_H, K_{HC} and K_K are the equations coefficients. The selection of suitable model based on observed data matches the value expected. The zero order models represent an ideal release profile to achieve the prolonged pharmacological action applicable to transdermal systems. In this kinetics release rate of drugs is not depends on its concentration. The first order models used to describes absorption and elimination of drugs and shows concentration dependant release rate of drug. Higuchi model applicable to water soluble and low soluble drugs in solid matrices and release of hydrophilic drug describes the release of water-soluble drug from insoluble matrix by diffusion process and depends on square root time. The Hixson- Crowell model discuss the release from system where surface area and particle diameter changes and doesn't involve diffusion mechanism. Korsmeyer-Peppas model describes the amount of drug release relates exponentially to the elapsed time, used to study the release of polymeric dosage forms and this model applicable in the case of unknown release mechanism or more than one type of release mechanism.

Stability studies[14-18]

Stability studies were performed at the different storage condition $25^{O}C\pm2^{0}c$ temp., 60%±5% RH and $40^{O}C\pm2^{0}c$ temperature, 75%±5% RH, for 90 days on optimized formulation batches (FT5).The parameters studied for stability studies are thickness, drug content, assay, moisture content and uptake and in vitro drug permeation.

Skin irritancy study [16-19]

Skin irritation study was performed on healthy rats weighing between 200-250 g. the hairs of albino rats were withdraw from dorsal side by clipping skin portion 1 day prior of the experiment. The experimental rats were distributed into 4 groups (n=2), group I acts as control, group II with patch TF5 formulation, group III received a blank transdermal patch and group IV received a 0.8% (v/v) formalin solution as a irritant. Rats back side skin area was removed 24 hours before experimental study. Optimize patch was used on the clean area of rat with the help of adhesive tape. After 24 hour, removed patch by using alcohol swab and observed visually for signs of edema or erythema. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of R.K.D.F college of Pharmacy,Bhopal, M.P. and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

RESULT AND DISCUSSION:

Standard curve of Lamivudine

Table no.2 and Fig-1 shows the standard curve for Lamivudine in phosphate buffer pH 7.4. The method obeyed Beer's law limit in the concentration range of 2-12 mcg/ml at 270 nm with a regression value of 0.996

Table no2: Standard curve of Lamivudine.

S.No	Concentration (Mcg/ml)	Absorbance at 270 nm
0	0	0
1	2	0.055
2	4	0.096
3	6	0.145
4	8	0.182
5	10	0.218
6	12	0.263

Standard curve of Stavudine

Table no.3 and Fig-2 shows the standard curve for Stavudine in phosphate buffer pH 7.4. The method obeyed Beer's law limit in the concentration range of 2-12 mcg/ml at 263 nm with a regression value of 0.997

Table no3: Standard curve of Stavudine.

S.No	Concentration (Mcg/ml)	Absorbance at 263 nm
0	0	0
1	2	0.018
2	4	0.035
3	6	0.049
4	8	0.062
5	10	0.079
6	12	0.093

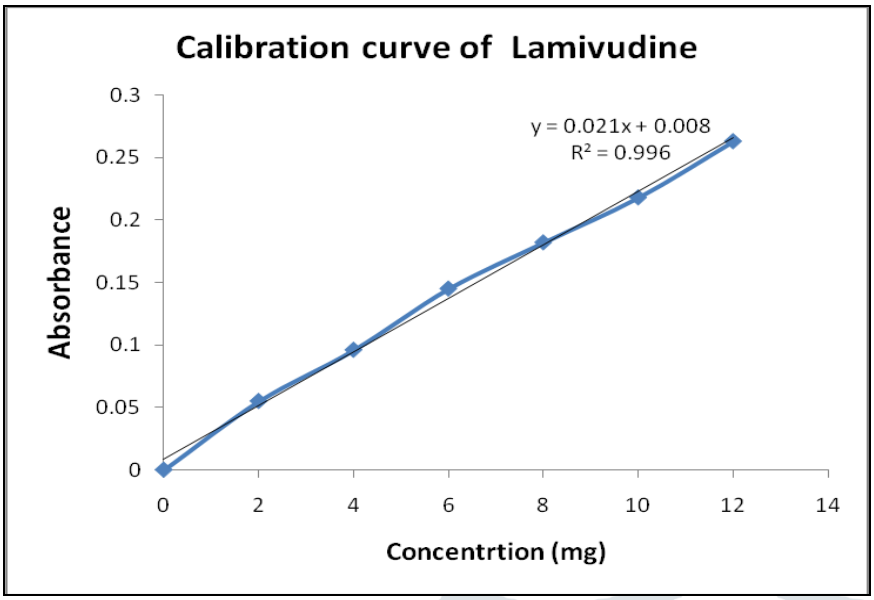

Fig: 1 Standard curve of Lamivudine using Phosphate buffer 7.4 pH at 270 nm

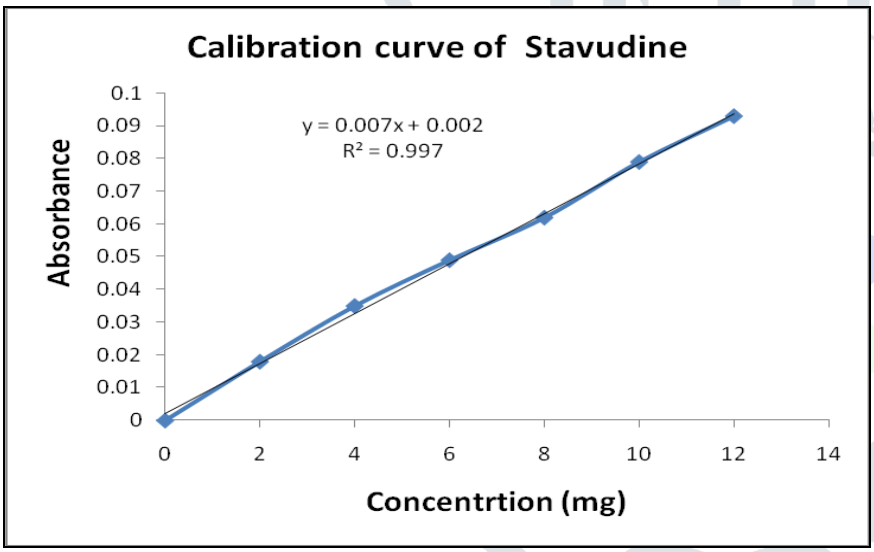

Fig: 2 Standard curve of Stavudien using Phosphate buffer 7.4 pH at 263 nm.

FTIR Studies for Transdermal Patches

The FTIR spectra are recorded over the wave number range of 4000–400 cm^{-1}. The Lamivudine drug showed different peaks e.g. C–H stretching at 2960, aromatic C=C at 1496, O–H stretching at 3587, C-H stretching at 2960, N-C streching at 2837 & 2731, C=N at 1650 and aromatic C-N at 1087 & 1317 cm^{-1} which confirms the purity of the Lamivudine drug. The same peaks are also found in the FT-IR spectra of the formulations, showing that no drug– polymer interaction occurred. In FT–IR studies the characteristic peak due to pure lamivudine has appeared in the spectra of Transdermal patches also.

The FTIR spectra of pure Stavudine drug also showed a sharp peak of aromatic C=O stretching structure at 1694.3 cm^{-1}, N–H stretching at 3169.5 cm^{-1} and at 2882.5 cm^{-1}.

The identical peaks are also present in drug loaded HPMC, Ethyl Cellulose,PEG and PVP mixed polymeric Transdermal patches.

Therefore, there was no alteration and no interaction was observed between polymer and drug in combination. All the characteristic peaks of Lamivudine and Stavudine are present in combination, thus indicating compatibility between drug and polymers and finally confirm that there was no chemical modification of the drug has been taken place.(fig 3-7)

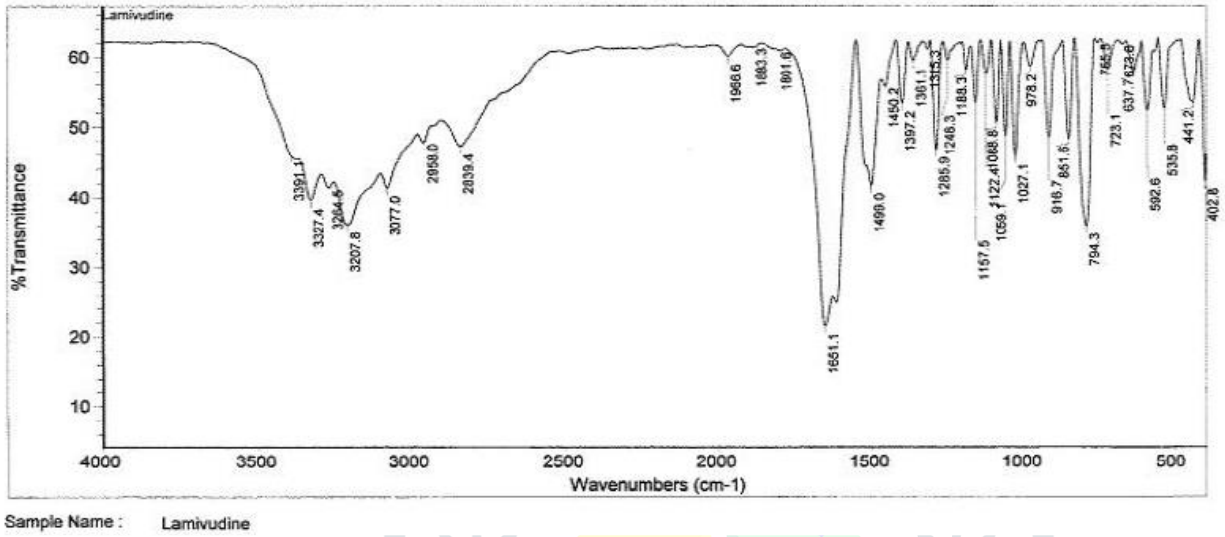

Fig 3: FTIR of Lamivudine

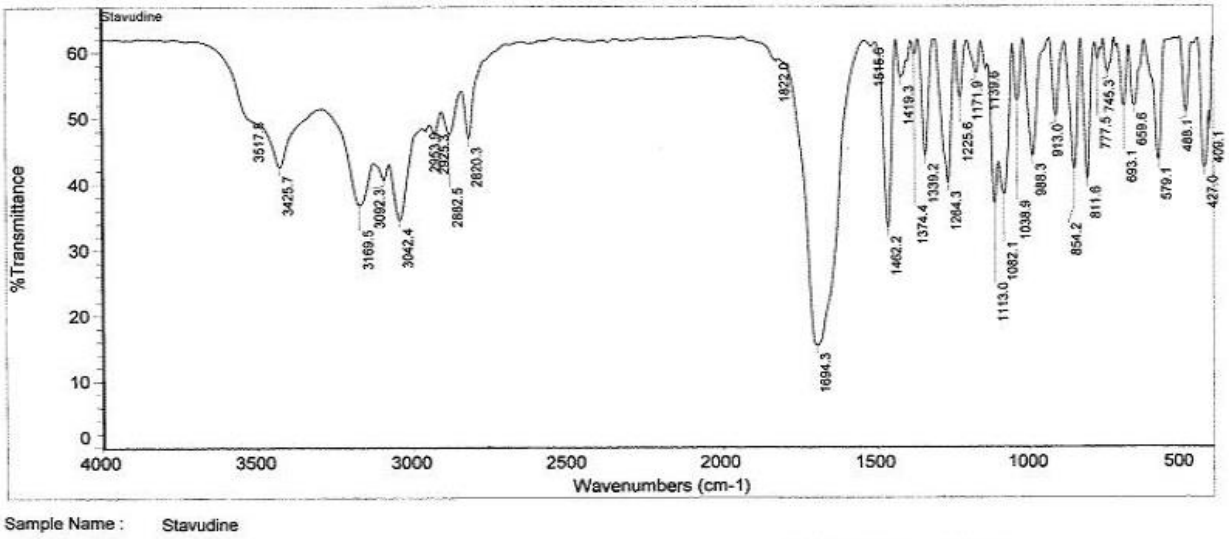

Fig4 : FTIR of Stavudine

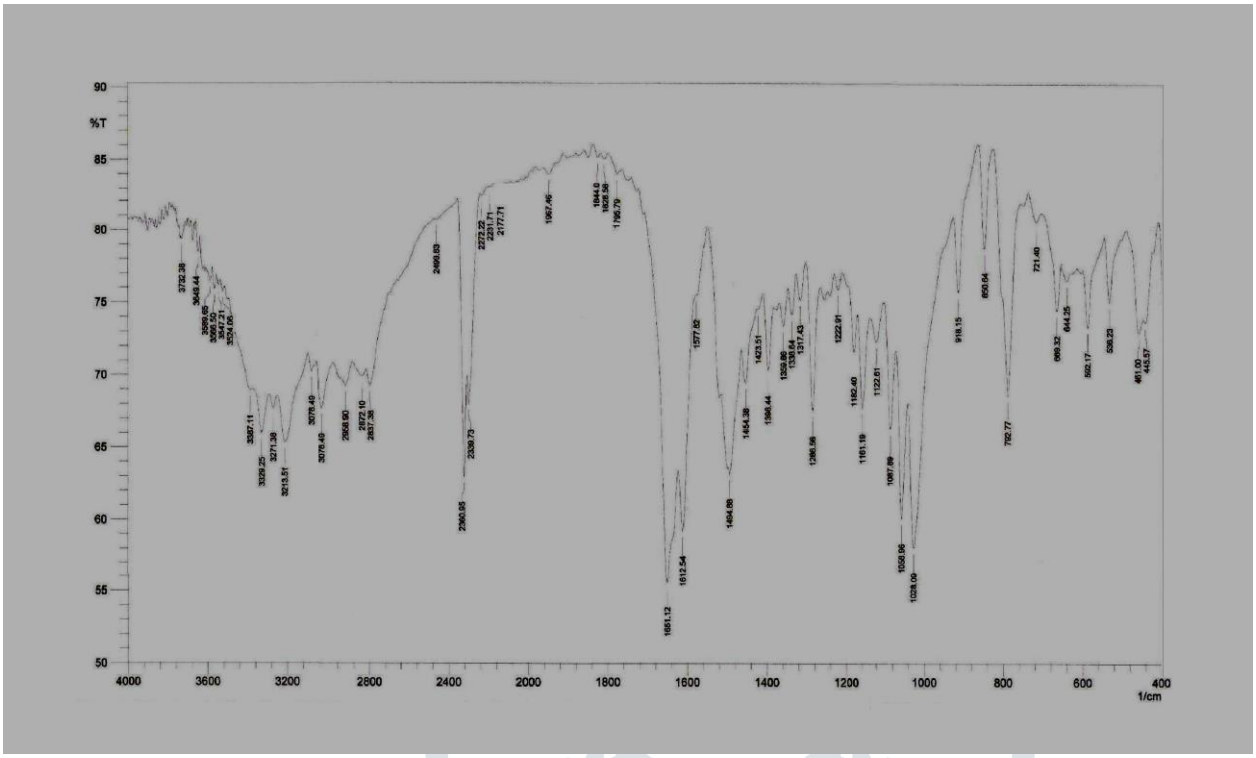

Fig 5: FTIR of Lamivudine and Stavudine Loaded ethosome

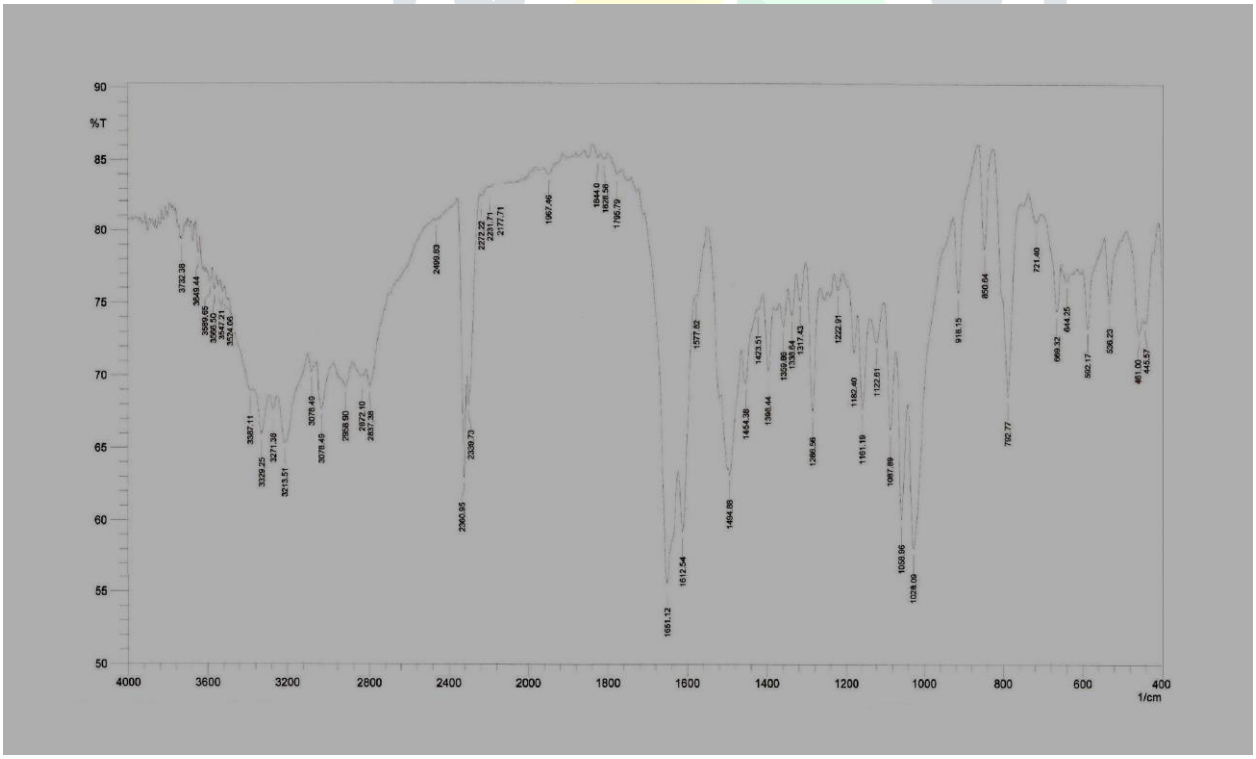

Fig 6: FTIR of Transdermal Patch Formulation

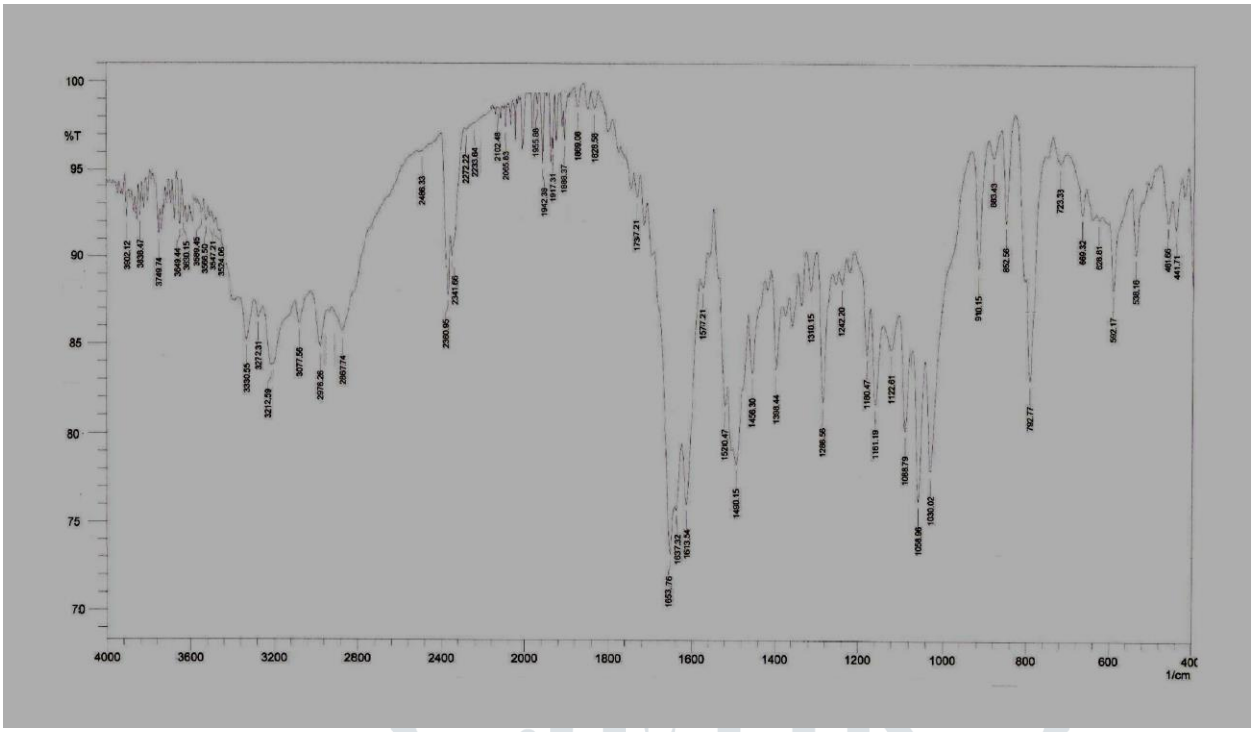

Fig 7: FTIR of Transdermal Path containing Lamivudine and Stavudine Loaded ethosome

Formulations of Transdermal Patches :

Seven formulations of Lamivudine and Stavudine loaded ethosome tansdermal patches compose with different polymers HPMCK_{15}M, PVPK_{30}, Ethyl cellulose. Methanol and Dichloromethane were used as a casting solvent. PEG400 used to give plasticity to patches while Tween 80 is used to enhance penetration of drug through transdermal systems. The polymeric solution was poured into bangles placed in a suitable level, hard rigid surface and patches were dried at a room temperature in a dust free environment for 24 hrs. an inverted funnel was covered over the bangles to avoid fast evaporation of the solvent. Patches of 3.14 cm2 were prepared by cutting and packed in an systems were smooth, thin and flexible. The preparation method of patches was found satisfactory.

Evaluation of Transdermal patches

Table 4 and 5 shows the physicochemical evaluation like the Thickness, Folding endurance, Percentage moisture absorbed, Percentage moisture lost, Drug content uniformity.

Permeation studies and Permeation Kinetics

The drug permeation from the Patches is depends on the polymer type as well used concentration. In-Vitro (permeation) studies were performed with Franz cell in Phosphate Buffer Saline pH 7.4. In drug Permeation study the formulation TF5 shows maximum drug permeation 95.24 % Lamivudine drug and 94.24 % Stavudine drug at 12 hrs. The drug permeation data of TF5 was plotted for Zero order, First

order, Higuchi model and Korsmeyer-Peppas model to evaluate the permeation pattern of the dosage form. From these plots, kinetic values of the drug permeation were determined. Drug released from the matrix devices by diffusion studied with Higuchi's Model and result suggested that the drug permeation follow Higuchi model. Table 6-21)

Table 4: Physicochemical Evaluation data of Transdermal Patches of Lamivudine ans stavudine ethosomes

Formulation Code	Thickness (mm)	Weight variation (mg)	% Drug Content		Folding endurance	Tensile strength Kg/mm^2
			Lamivudine	stavudine		
TF1	0.13±0.01	0.149±0.01	96.92±3.32	95.92±3.32	56±12.04	2.46±0.81
TF2	0.18±0.02	0.150±0.005	95.59±3.14	96.59±3.14	37.4±21.0	2.79±0.80
TF3	0.13±0.004	0.153±0.021	97.41±2.17	97.61±2.18	39±18.20	2.39±0.70
TF5	0.16±0.008	0.163±0.011	99.55±2.42	98.71±1.43	59±24.33	3.84±1.80
TF5	0.34±0.09	0.150±0.017	98.12±2.02	99.65±2.42	57±22.03	3.95 ±1.84
TF6	0.35±0.003	0.154±0.014	96.91±1.42	97.51±2.17	56±10.41	2.84±1.84
TF7	0.36±0.003	0.152±0.015	97.61±1.42	98.36±2.02	58±10.41	2.94 ±1.78

Table 5 .Physicochemical Evaluation data of Transdermal Patches of Lamivudine and Stavudine ethosomes

Formulation Code	% Elongation	% Moisture Content	% Moisture uptake	Swelling index
TF1	23.23±2.51	1.92±0.35	4.77±3.13	24.21±1.38
TF2	24.10±2.12	2.3±0.77	3.5±3.7	25.81±0.72
TF3	25.65±2.61	2.9±1.29	5.4±1.22	25.49±2.12
TF5	26.71±4.12	3.1±1.82	4.6±0.85	23.38±0.74
TF5	27.94±4.71	3.35±2.78	5.5±1.45	23.12±1.25

TF6	26.02±4.19	3.13±0.98	4.45±1.06	24.20±1.37
TF7	23.98±4.18	2.7±0.97	4.59±1.05	25.13±1.36

Table6: *In-vitro* Drug Permeation of Lamivudine from TF1 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF1			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.41	0.000	25.18	74.82	1.401	1.874
2	2.00	0.301	39.75	60.25	1.599	1.780
3	2.45	0.477	52.02	47.98	1.716	1.681
4	2.83	0.602	69.20	30.8	1.840	1.489
5	3.16	0.699	79.89	20.11	1.902	1.303

Table 7: *In-vitro* Drug Permeation of Lamivudine from TF2 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF2			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	8.23	91.77	0.915	1.963
2	1.414	0.301	15.19	84.81	1.182	1.928
3	1.732	0.477	28.86	71.14	1.460	1.852
4	2.000	0.602	37.07	62.93	1.569	1.799
5	2.236	0.699	47.85	52.15	1.680	1.717
6	2.449	0.778	61.02	38.98	1.785	1.591
8	2.828	0.903	69.98	30.02	1.845	1.477
10	3.162	1.000	81.02	18.98	1.909	1.278
12	3.464	1.079	82.23	17.77	1.915	1.247

Table 8 *In-vitro* Drug Permeation of Lamivudine from TF3 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF3			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	7.92	92.08	0.899	1.964
2	1.414	0.301	13.28	86.72	1.123	1.938
3	1.732	0.477	25.81	74.19	1.412	1.870
4	2.000	0.602	31.67	68.33	1.501	1.835
5	2.236	0.699	47.82	52.18	1.680	1.718
6	2.449	0.778	56.12	43.88	1.749	1.642
8	2.828	0.903	62.21	37.79	1.794	1.577
10	3.162	1.000	74.81	25.19	1.874	1.401
12	3.464	1.079	86.62	13.38	1.938	1.126

Table 9: *In-vitro* Drug Permeation of Lamivudine from TF4 through skin membrane.

Time in (hrs)	Square root time	Log time (hrs)	TF4			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	9.01	90.99	0.955	1.959
2	1.414	0.301	15.02	84.98	1.177	1.929
3	1.732	0.477	28.97	71.03	1.462	1.851
4	2.000	0.602	34.87	65.13	1.542	1.814
5	2.236	0.699	48.01	51.99	1.681	1.716
6	2.449	0.778	59.02	40.98	1.771	1.613
8	2.828	0.903	70.17	29.83	1.846	1.475
10	3.162	1.000	81.14	18.86	1.909	1.276
12	3.464	1.079	93.21	6.79	1.969	0.832

Table 10: *In-vitro* Drug Permeation of Lamivudine from TF5 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF5			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	9.21	90.79	0.804	1.971
2	1.414	0.301	16.80	83.20	1.088	1.943
3	1.732	0.477	34.95	65.05	1.579	1.793
4	2.000	0.602	41.26	58.74	1.616	1.769
5	2.236	0.699	48.85	51.15	1.689	1.709
6	2.449	0.778	61.02	38.98	1.785	1.591
8	2.828	0.903	72.14	27.86	1.858	1.445
10	3.162	1.000	82.21	17.79	1.915	1.250
12	3.464	1.079	95.24	4.76	1.979	0.678

Table 11: *In-vitro* Drug Permeation of Lamivudine from TF6 through skin membrane

Time in (hrs)	Square root time (hrs)	Log time (hrs)	TF6			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	7.90	92.1	0.931	1.961
2	1.414	0.301	14.24	85.76	1.154	1.933
3	1.732	0.477	22.84	77.6	1.377	1.882
4	2.000	0.602	34.71	65.29	1.540	1.815
5	2.236	0.699	41.08	58.92	1.614	1.770
6	2.449	0.778	69.24	30.76	1.773	1.610
8	2.828	0.903	75.12	24.88	1.840	1.488
10	3.162	1.000	80.92	19.08	1.876	1.396
12	3.464	1.079	84.15	15.85	1.908	1.281

Table 12 *In-vitro* Drug Permeation of Lamivudine fromT F7 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF7			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	8.23	91.77	0.915	1.963
2	1.414	0.301	14.99	85.01	1.176	1.929
3	1.732	0.477	26.98	73.02	1.431	1.863
4	2.000	0.602	35.42	64.58	1.549	1.810
5	2.236	0.699	48.22	51.78	1.683	1.714
6	2.449	0.778	55.69	44.31	1.746	1.647
8	2.828	0.903	61.21	38.79	1.787	1.589
10	3.162	1.000	68.21	31.79	1.834	1.502
12	3.464	1.079	75.24	24.76	1.876	1.394

Fig 8 *In-vitro* Drug Permeation Lamivudine of TF1 to TF7

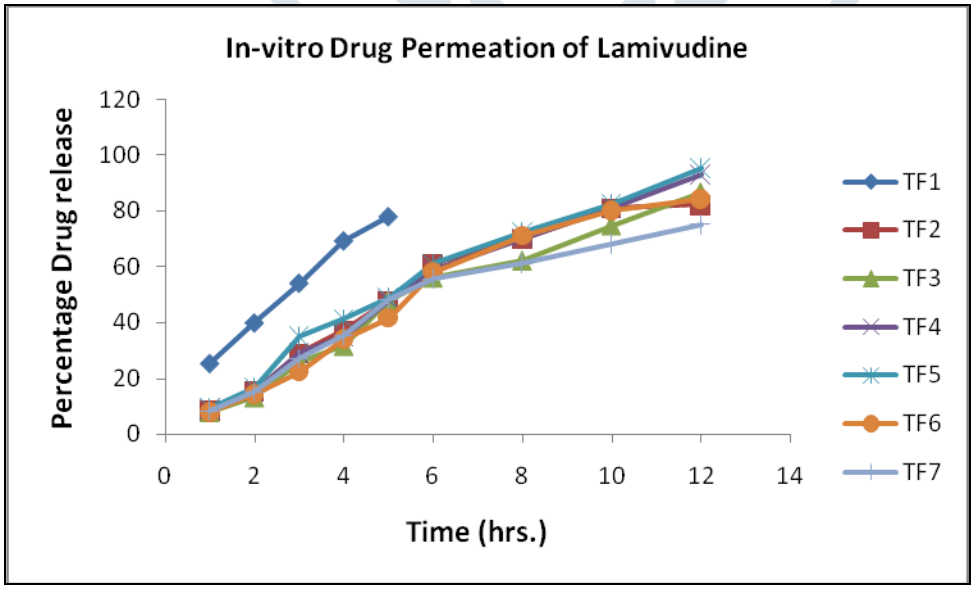

Table 13 *In-vitro* Drug Permeation of Lamivudine Kinetics

Time (hrs)	TF1	TF2	TF3	TF4	TF5	TF6	TF7
1	25.18	8.23	7.92	9.01	9.21	7.90	8.23
2	39.75	15.19	13.28	15.02	16.80	14.4	14.99
3	52.02	28.86	25.81	28.97	34.95	22.4	26.98
4	69.20	37.07	31.67	34.87	41.26	34.1	35.42
5	79.89	47.85	47.82	48.01	48.85	41.8	48.22
6		61.02	56.12	59.02	61.02	69.4	55.69
8		69.98	62.21	70.17	72.14	75.2	61.21
10		81.02	74.81	81.14	82.21	80.2	68.21
12		82.23	86.62	93.21	95.24	84.5	75.24

Table 14: *In-vitro* Drug Permeation of Stavudine from TF1 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF1			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.41	0.000	24.08	75.92	1.382	1.880
2	2.00	0.301	38.23	61.77	1.582	1.791
3	2.45	0.477	50.31	49.69	1.702	1.696
4	2.83	0.602	67.43	32.57	1.829	1.513
5	3.16	0.699	78.264	21.736	1.894	1.337

Table 15: *In-vitro* Drug Permeation of Stavudine from TF2 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF2			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	7.03	92.97	0.847	1.968
2	1.414	0.301	11.99	88.01	1.079	1.945
3	1.732	0.477	22.81	77.19	1.358	1.888
4	2.000	0.602	30.52	69.48	1.485	1.842
5	2.236	0.699	45.24	54.76	1.656	1.738
6	2.449	0.778	54.23	45.77	1.734	1.661
8	2.828	0.903	61.21	38.79	1.787	1.589
10	3.162	1.000	70.56	29.44	1.849	1.469
12	3.464	1.079	83.61	16.39	1.922	1.215

Table 16: *In-vitro* Drug Permeation of Stavudine from TF3 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF3			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	7.62	92.38	0.882	1.966
2	1.414	0.301	12.04	87.96	1.081	1.944
3	1.732	0.477	24.81	75.19	1.395	1.876
4	2.000	0.602	30.52	69.48	1.485	1.842
5	2.236	0.699	46.24	53.76	1.665	1.730
6	2.449	0.778	55.12	44.88	1.741	1.652
8	2.828	0.903	61.21	38.79	1.787	1.589
10	3.162	1.000	72.21	27.79	1.859	1.444
12	3.464	1.079	85.612	14.388	1.933	1.158

Table 17: *In-vitro* Drug Permeation of Stavudine from TF4 through skin membrane.

Time in (hrs)	Square root time	Log time (hrs)	TF4			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	8.91	91.09	0.950	1.959
2	1.414	0.301	14.89	85.11	1.173	1.930
3	1.732	0.477	28.07	71.93	1.448	1.857
4	2.000	0.602	33.24	66.76	1.522	1.825
5	2.236	0.699	47.34	52.66	1.675	1.721
6	2.449	0.778	57.02	42.98	1.756	1.633
8	2.828	0.903	68.76	31.24	1.837	1.495
10	3.162	1.000	79.68	20.32	1.901	1.308
12	3.464	1.079	92.87	7.13	1.968	0.853

Table 18: *In-vitro* Drug Permeation of Stavudine from TF5 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)		TF5		
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	9.1	90.9	0.959	1.959
2	1.414	0.301	15.8	84.20	1.199	1.925
3	1.732	0.477	31.65	68.35	1.500	1.835
4	2.000	0.602	38.26	61.74	1.583	1.791
5	2.236	0.699	48.28	53.72	1.684	1.714
6	2.449	0.778	60.12	39.88	1.779	1.601
8	2.828	0.903	71.12	28.88	1.852	1.461
10	3.162	1.000	85.21	14.79	1.930	1.170
12	3.464	1.079	94.24	5.76	1.974	0.760

Table 19: *In-vitro* Drug Permeation of Stavudine from TF6 through skin membrane

Time in (hrs)	Square root time (hrs)	Log time (hrs)	TF6			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	8.53	91.47	0.931	1.961
2	1.414	0.301	14.24	85.76	1.154	1.933
3	1.732	0.477	23.84	76.16	1.377	1.882
4	2.000	0.602	34.71	65.29	1.540	1.815
5	2.236	0.699	41.08	58.92	1.614	1.770
6	2.449	0.778	55.23	44.77	1.742	1.651
8	2.828	0.903	66.24	33.76	1.821	1.528
10	3.162	1.000	75.12	24.88	1.876	1.396
12	3.464	1.079	80.92	19.08	1.908	1.281

Table 20 *In-vitro* Drug Permeation of Stavudine fromT F7 through skin membrane

Time in (hrs)	Square root time	Log time (hrs)	TF7			
			% Drug Permeated	% Drug Remaining	Log % Drug Permeated	Log % Drug Retained
1	1.000	0.000	7.05	92.95	0.848	1.968
2	1.414	0.301	13.99	86.01	1.146	1.935
3	1.732	0.477	21.98	78.02	1.342	1.892
4	2.000	0.602	31.42	68.58	1.497	1.836
5	2.236	0.699	39.22	60.78	1.594	1.784
6	2.449	0.778	49.69	50.31	1.696	1.702
8	2.828	0.903	61.21	38.79	1.787	1.589
10	3.162	1.000	68.21	31.79	1.834	1.502
12	3.464	1.079	75.24	24.76	1.876	1.394

Table 21 *In-vitro* Drug Permeation of Stavudine Kinetics

Time (hrs)	TF1	TF2	TF3	TF4	TF5	TF6	TF7
1	24.08	7.03	7.62	8.91	9.1	8.53	7.05
2	38.23	11.99	12.04	14.89	15.8	14.24	13.99
3	50.31	22.81	24.81	28.07	31.65	23.84	21.98
4	67.43	30.52	30.52	33.24	38.26	34.71	31.42
5	78.264	45.24	47.34	48.28	46.28	41.08	39.22
6		54.23	55.12	57.02	60.12	55.23	49.69
8		61.21	61.21	68.76	71.12	66.24	61.21
10		70.56	72.21	79.68	85.21	75.12	68.21
12		83.61	85.612	92.87	94.24	80.92	75.24

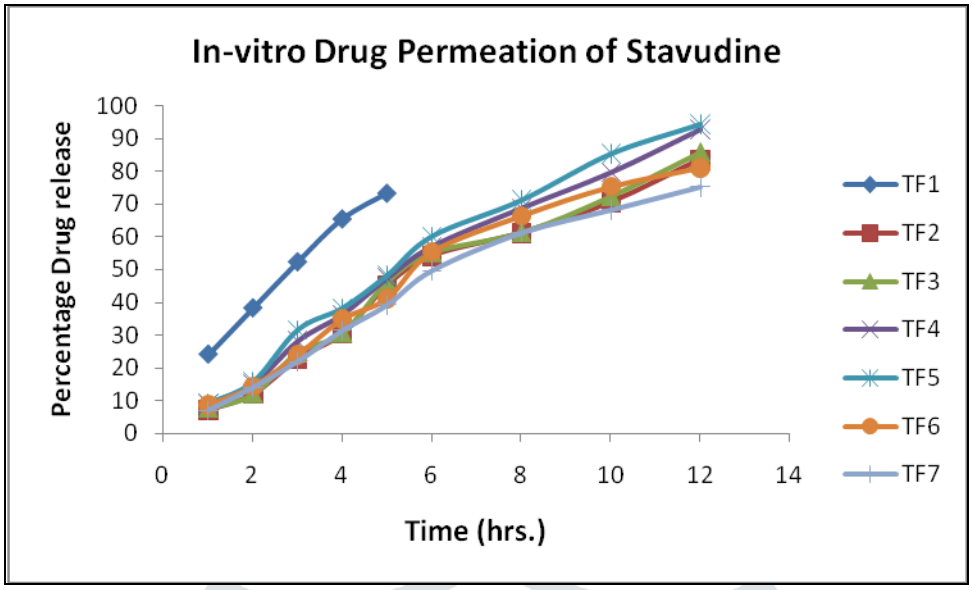

Fig 9: *In-vitro* Stavudine Drug Permeation of TF1 to TF7

Drug release kinetic modeling of optimized formula

On comparison of kinetic modeling and release profile data it was evident that Transdermal Patch containing Lamuvudine and Stavudine was found to release the drug in accordance to Higuchi kinetics, the regression coefficient was not found to be exactly near to 1, which could be due to influence of some other factors.(fig.10-11)

Table 22 : R2 value of optimized formulation TF5

Model Name	Zero order	Fist order	Huguchi model	Hixson	Kor's peppas	Best fit model
R2 value of TF5 for Lamuvudine	0.978	0.921	0.993	0.977	0.941	Huguchi model
R2 value of TF5 for Stavudine	0.977	0.948	0.992	0.988	0.983	Huguchi model

Stability Study

Stability is the essential factor for quality, safety and efficacy of product. The drug product is with insufficient stability result in altering of their physical as well as chemical characteristics. The selected formulations namely TF5 was subjected for stability studies and observed for all evaluation parameters at a temperature of 25^0C and 60% RH, 40^0C and 75% RH, at an interval of three month. There were no physical changes in flexibility and physicochemical evaluation parameter was slightly changed (Table-23)

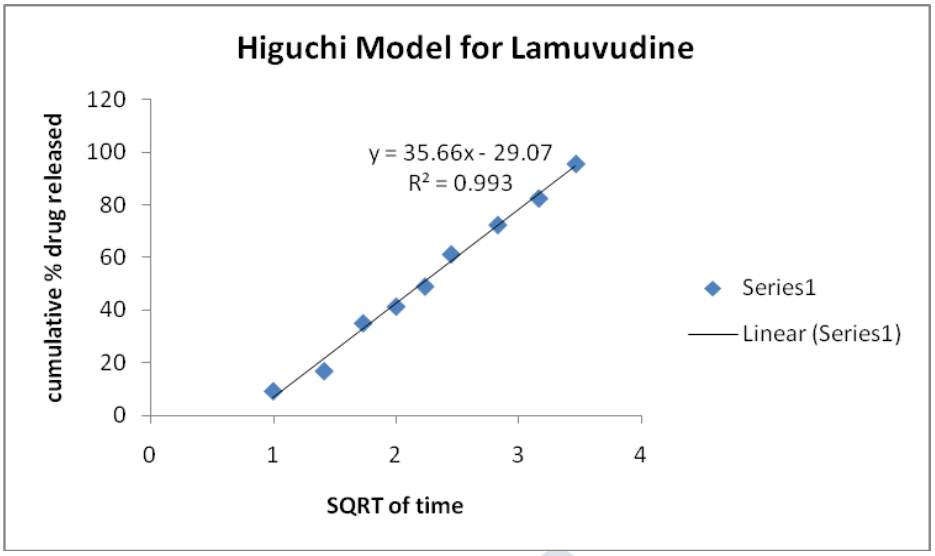

Fig 10: Kinetic Model for Lamuvudine Drug

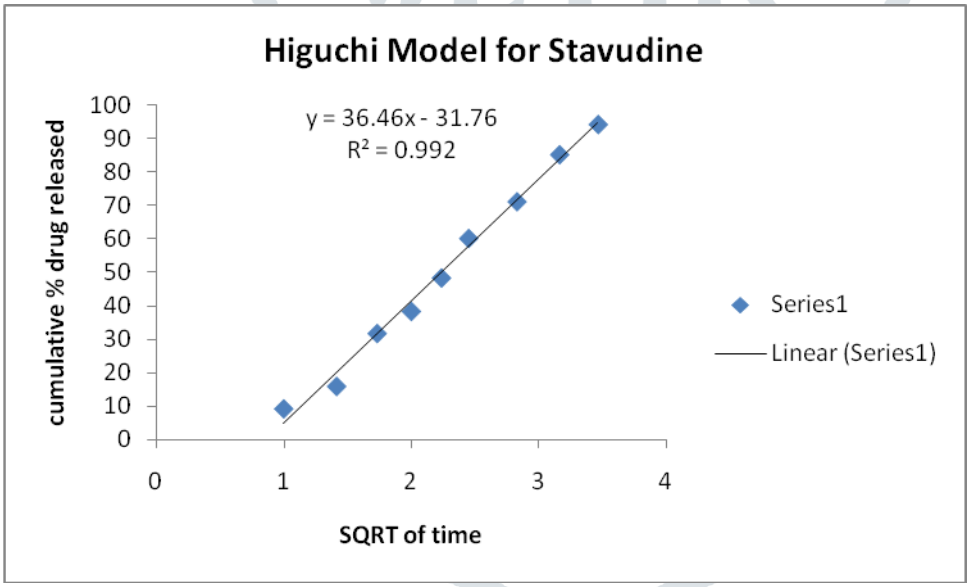

Fig 11: Kinetic Model for Stavudine Drug

Table 23 Stability Study of batch TF5

Sr. no	Evaluation Parameter	At 0 day	After 90 days
1	Thickness (mm)	0.16±0.008	0.16 ± 0.06
2	Weight variation	0.163±0.011	0.161 ± 0.011
3(a)	% Drug Content of Lamivudine	99.45±2.42	98.63 ± 1.25
(b)	% Drug Content of Stavudine	99.35±2.52	98.83 ± 1.22
4	Folding endurance	59±24.33	57 ± 24.33

5	Tensile Strength Kg/mm^2	3.85±1.80	3.55±1.80
6	% Elongation	26.71±4.12	25.99 ± 4.12
7	% Moisture content	3.1±1.82	3.1 ± 0.94
8	% Moisture uptake	4.6±0.85	4.5 ± 3.03
9	Swelling index	23.38±0.74	22.40 ± 0.71

Skin Irritation Test:

The skin irritation test carried out on albino rats for optimized formulation. The test films produced no proof of oedema and erythema this indicates suitability of these test patches for topical application.

Table 24 Data from the skin irritation study for prepared formulations.

Normal	F5:Drug	Blank film	Formalin
-	-	-	++**
-	-	-	++**

Erythema	Edema
- Nil	- Nil
+ Mild	* Mild
++ Severe	** Severe
+++ Very severe	*** Very severe

Conclusion :

TDDS are the ideal delivery system for drug that undergo hepatic first pass metabolism. Based on results of various evaluation parameters like thickness, strength, elongation, better compatibility and stability the transdermal matrix patches containing Lamivudine and Stavudune drug was successfully designed and developed by trial and error method. Formulations were prepared by employing combination of HPMCK$_{15}$M, PVPK$_{30}$, and EC in various ratios. From the research, various conclusions were drawn.

- From the evaluation results it was conclude that TF5 show highest release at 12 hrs. with suitable polymer ratio and penetration enhancer.

- From the kinetic study it was observed that Higuchi kinetics model most suitable kinetic model for drug release from all patches.
- Result of Skin irritation test on albino rats observed no oedema and erythema.
- Stability study performed on optimized formulation. No major changes showed in the parameters during study period, thus it could be concluded that formulation was stable.

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