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Influence of ethylcellulose on release of lamivudine from HPMC K4M transdermal patches

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ABSTRACT

The purpose of the present research was to formulate HPMC K4M based lamivudine transdermal patches and to study the influence of ethyl cellulose on its characteristics. TDDS formulations were prepared and characterized for the tensile strength, percentage elongation, water vapour transmission studies (WVT), water vapour absorption studies (WVA), FTIR, SEM, and *in vitro* drug release kinetics. The prepared patches showed good elasticity. The IR spectral studies showed that the drug remained in its normal form without undergoing any interaction with the polymers. The optimized formula based on drug release (F5) showed drug dissolution of 98.72% after 14 h.

Key words: Transdermal patches, Lamivudine, hydrophobic polymer, *in vitro* dissolution, water vapour transmission studies,water vapour absorption studies.

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 h) and requires frequent administration for a prolonged period of time (life-long in AIDS and for one year in hepatitis patients)[1,2]. Transdermal route is, therefore, is one of the promising alternatives to achieve constant plasma levels for prolonged periods of time, which additionally could be advantageous because of less frequent dosing regimens.

Earlier research showed that cellulose acetate and ethylcellulose were employed to prepare transdermal films using diclofenac sodium and salicylic acid as model drugs[3]. Further, keterolac tromethamine from cellulose matrices containing HPMC, methylcellulose and sodium carboxy methylcellulose were also studied earlier[4]. Lamivudine ethosomes were prepared and characterized for transdermal delivery[5]. As per best of our knowledge, transdermal patches for the delivery of lamivudine are not found in the literature. Hence the present work was aimed at formulation and evaluation

of lamivudine transdermal patches using HPMC matrix polymer. The influence of hydrophobic polymer such as ethylcellulose in different proportions was studied. Further the effect of plasticizers/penetrating enhancer like, glycerol was also investigated.

MATERIALS AND METHODS

Materials: The materials used were obtained as follows: Lamivudine from Nishka Scientific and Reference Laboratories, Hyderabad; HPMC K4M from Central Drug House Pvt Ltd., Delhi; Ethylcellulose from S.D. Fine-Chemicals Ltd., Boisar, Mumbai; Glycerol and from Qualigens Fine Chemicals, Mumbai. All other reagents used in the study were of analytical grade.

Preparation of lamivudine patches: Suitable glass rings of 5.5 cm diameter were taken and placed over a petri dish containing mercury substrate[6]. Initially the volume required was calculated, and exactly the same volume (5 mL) was transferred with a pipette into the rings, for all formulations. In the entire patches the drug:polymer ratio was kept at 1:3 (180 mg drug and 150 mg HPMC)[6]. HPMC and Lamivudine were dissolved in a minimum quantity of water and to this ethanol, as a solubiliser and evaporating agent, was included and mixed thoroughly for 10 min. An inverted funnel was placed over the petri dishes, for constant drying. The patches were dried at room temperature for 48 h and all the formulations were stored in a tightly closed desiccator. The formulations F2 and F5 contained HPMC polymer matrix and drug (3:1), the ethylcellulose (EC) as a hydrophobic polymer, which was solubilised in dichloromethane. HPMC and drug were dissolved in alcohol. About 1 mL of dichloromethane was added to obtain clear solution and glycerin as plasticizer (0.023 g the weight of HPMC) was added. Table 1 shows the formulation of lamivudine transdermal patches.

Table 1: Formulation of lamivudine (LAM) transdermal patches

Formulation	LAM	HPMC	E.C	Alcohol	D CM	Glycerol	Water	Chloroform
F1	150	540	-	4	-	-	1	1
F2	150	540	67.5	4	1	0.023	-	-
F3	150	540	135	4	1	0.023	-	-
F4	150	540	202.5	4	1	0.023	-	-
F5	150	540	540	4	1	0.023	-	-

All values are in g.

Scanning electron microscopic analysis[7]: Morphological characteristics of TDDS patches were studied by SEM analysis and optical microscopy. The SEM analysis was performed using a scanning electron microscope (Exacta Optec, Model B3, Optec Optical Technology, Germany). Prior to examination, samples were mounted on an aluminium stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (200 Å) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 0.5 kV and resolution of 4000.

Fourier transform infrared spectroscopic (FTIR) spectral analysis[7]: The FTIR spectra (400 to 4000 cm^{-1} and resolution of 4 cm^{-1}) of the lamivudine, polymers and optimized formulation (F5) were measured by preparing dispersion in dry KBr using Shimadzu FTIR 8400S (Shimadzu Analytical India Pvt. Ltd., Mumbai, India). The transmission minima (absorption maxima) in the spectra obtained with these polymers were compared. The presence of additional peaks corresponding to the functional groups was noted.

Water vapor transmission studies (WVT): About 1 g of calcium chloride was accurately weighed and placed in dried empty vials having an equal diameter. The polymer patches were pasted over the brim with the help of an adhesive, and then the vials were weighed and placed over a mesh in desiccators containing 200 mL of saturated potassium chloride solution. The desiccators was tightly closed and the humidity inside was measured by using a hygrometer and was found to be 56% RH and 84% RH respectively[3]. Then the vials were weighed after 1, 2, 3, 4, 5, 6 and 7^{th} day. The results were tabulated and a graph of cumulative amount water vapor transmitted vs time was plotted.

Water vapor absorption studies (WVA[3]): Accurately weighed patches were placed onto a dry glass slide, which was kept in a desiccator containing 200 mL of saturated potassium chloride solution. The desiccators were tightly closed and the humidity inside the

desiccator was measured by using a hygrometer and was found to be 56% RH and 84% RH respectively before and after saturation. The patches were weighed after 1, 2, 3, 4, 5, 6 and 7th day. The results were tabulated and a graph of cumulative percent water vapor absorbed vs time was plotted

Tensile strength and percentage elongation[8]: To determine the tensile strength of transdermal patches, the polymeric film was sandwiched separated by corked linear iron plates. One end of the patches was kept fixed with the help of an iron screen and the other end was connected to a freely movable thread over a pulley. The weights were added gradually to the pan attached to the hanging end of the thread. A pointer to the thread were used to measure the elongation of the patch. The weight just sufficient to break the patch was noted. The tensile strength was calculated using the following equation.

$$\text{Tensile strength} = \frac{F}{ab(1 + L/1)}$$

F is the force required to break; 'a' is width of patch; 'b' is thickness of patch; L is length of the patch; l is an elongation of patch at break point. In another study, tensile strength of the patch was determined with the help of texture analyzer. The force and elongation were measured when the patches broke. The patches were casted on mercury and taken in rectangular containers using a proportionate quantity of the solution calculated on the basis of the area. The patches were cut into strips of 1 cm width and 15 cm length. The patches were fixed onto the tensile strength apparatus in such a way that the length of the patch between the jaws was initially 10 cm. The trials where the breakage occurred at the jaw were invalid and the result was repeated on another strip. The Tensile strength was calculated by the following formula.

$$\text{Tensile strength} = \frac{\text{Break force}[1 + \text{change in length}]}{\text{width}[\text{initial length of the film}]}$$

The percent elongation was determined by noting the length just before the break point and substituting the formula

$$\% \text{ Elongation} = \frac{[\text{Final length - Initial length}]}{\text{Initial length}} \times 100$$

Drug content uniformity[9]: A film of area about 0.7539 cm^2 was triturated with a 5 mL of pH 7.4 phosphate buffer using mortar and pestle; transferred to a volumetric flask and about 50 mL of pH 7.4 phosphate buffer was added to it. It was kept aside for 1 h to allow the drug to dissolve thel drug present in the patch and the volume was made up to 100 ml with the same buffer. Then the absorbance of this solution was measured after suitable dilution at 268 nm against phosphate buffer of pH 7.4 as blank using a UV-visible spectrophotometer (Double Beam, Jasco V-530, Japan). The content of lamivudine was calculated using standard graph.

***In vitro* evaluation[9]:** *In vitro* diffusion studies were performed on Keshary-chein diffusion cell for all the patches. The patches of 0.75 cm^2 area were used from each patch formulation. The sigma dialysis membrane was previously hydrated by soaking it in distilled water for 15 min, after which it was fixed to the donor compartment**.** The patch was placed over the dialysis membrane in the donor compartment. The receptor compartment was filled with phosphate buffer of pH 7.4[10]. A Teflon® coated magnetic bead was placed in the receptor compartment and the whole assembly was placed on a magnetic stirrer and temperature maintained at 37 ±5 ^oC. The buffer was stirred at 50 rpm for all formulations. Samples of 2 mL were withdrawn at time intervals of 5, 10, 15, 30, 45, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720, 780 and 840 min and were suitably diluted and the absorbance was measured at 268 nm using a UV-visible spectrophotometer (Double Beam, Jasco V-530, Japan). The volume of the receptor compartment was maintained constant by replacing the equal volume of buffer after collection of the samples.

RESULTS AND DISCUSSION

Transdermal patches of lamivudine were successfully prepared using solvent casting method. The patches were smooth, flexible and uniform in thickness.

FTIR studies for transdermal patches: The FTIR spectra were recorded over the wave number range of 3500–400 cm^{-1}. The Lamivudine drug showed different peaks at C–H =2958, C=C = 1605, 1499, 1450.2, O–H = 3264.5, N=N = 1499 and Cl = 1200–1400 cm^{-1} of benzene which confirms the purity of the Lamivudine drug. The FTIR studies also indicated four bands present in the lamivudine spectrum at 3445.91, 2958.0, 1801.6 and 1651.1 cm^{-1}, due to the formation of N-H, O-H, C=O, C=N linkage respectively**.** The same bands were also found in the spectra of the formulations, showing that no drug-polymer interaction occurred. In FTIR studies the characteristic peak due to pure lamivudine has appeared in the spectra of transdermal patches. The identical peaks were also present in drug loaded HP MC, ethylcellulose mixed polymeric transdermal

patches. Therefore, all the characteristic peaks of lamivudine were present in combination, thus indicating compatibility between drug and polymers and finally confirm that there was no chemical modification of the drug has taken place[8]. The results are shown in Fig. 1.

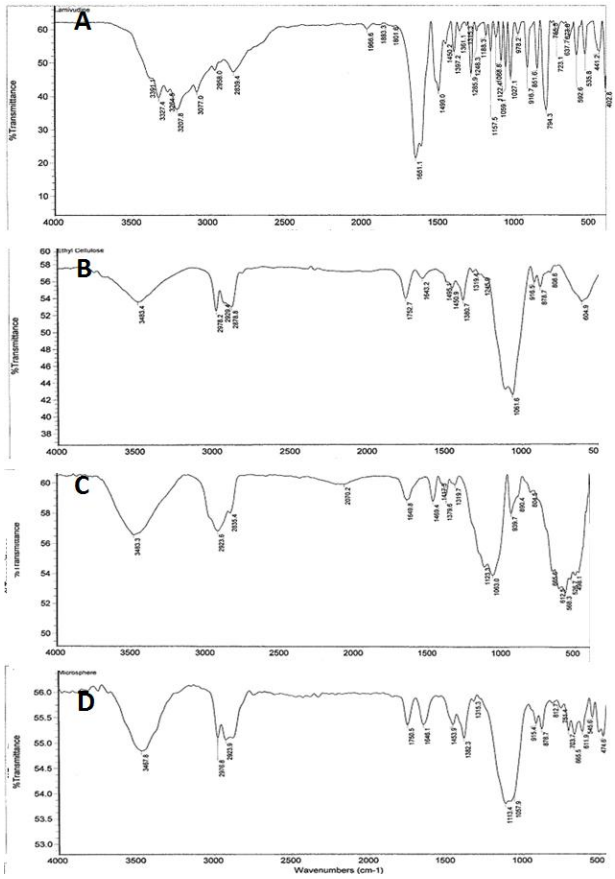

Fig.1: FTIR spectra of (A) lamivudine, (B) ethylcellulose, (C) HPMC and (D) formulation of patch

Water vapor transmission (WVT) at 56% RH: The percent WVT studies reveal that all the patches transmitted water vapor when exposed to 56% RH as depicted in Fig. 2. The optimized formulation F5 showed maximum WVT was 0.4 g after seven days at 56% of RH. The results also indicated that, WVT through all the patch formulations followed zero order kinetics.

Water vapor absorption (WVA) 56% RH: WVA studies indicated that all the patches absorbed water vapor when exposed to 56% RH as shown in Fig.3. From the curves it was clearly observed in general, that all the patches absorbed water vapor till a critical value was reached and then attained equilibrium. The optimized formulation F5 showed maximum WVA was 14.82% after seven days at 56% of RH. A further exposure to the same humidity conditions, the moisture content did not increase in the patch.

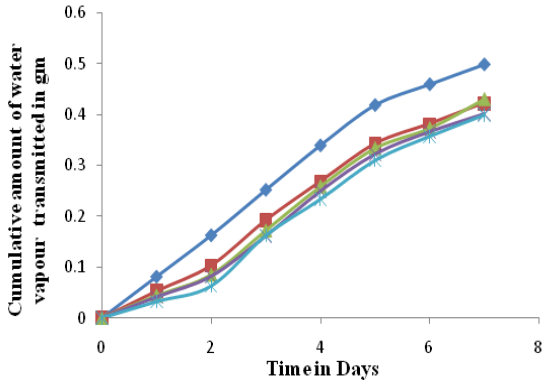

Fig. 2: Cumulative Amount of WVT at 56% RH through formulations, F1 (–◊–), F2 (–□–), F3 (–Δ–), F4 (–×–) and F5 (–*–)

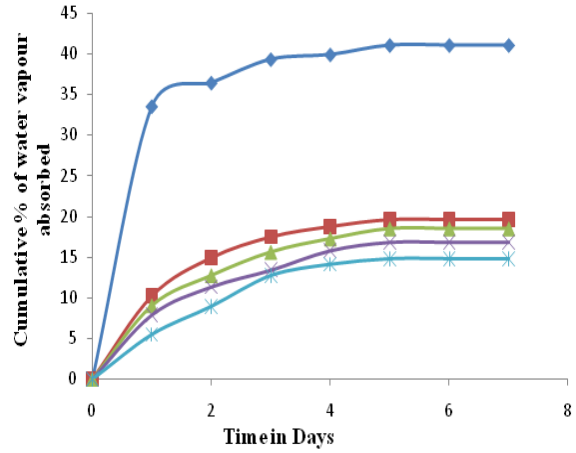

Fig.3: Cumulative % of WVA at 56% RH through formulations, F1 (–◊–), F2 (–□–), F3 (–Δ–), F4 (–×–) and F5 (–*–)

Tensile strength and percentage elongation[5]: The results of tensile strength and percentage elongation for the optimized formulation (F5) were found to be 12.25 kg/cm^2 and 2.2% respectively.

SEM Analysis: Scanning electron microscopic analysis was performed on the prepared lamivudine loaded transdermal patches to access their surface morphological characteristics and the results are shown in Figs. 4 and 5. The surface of the patches prior to *in vitro* permeation studies showed uniform, smooth surface without any pores.

Drug content uniformity: The drug content obtained from the patches was 87.96±3.45%, 93.58±5.32%, 90.77±2.77%, 89.74±3.18% and 91.69±2.68% in formulations F1, F2, F3, F4 and F5 respectively. This

indicates that the lamivudine distribution in the prepared patches was uniform and above 87.96%.

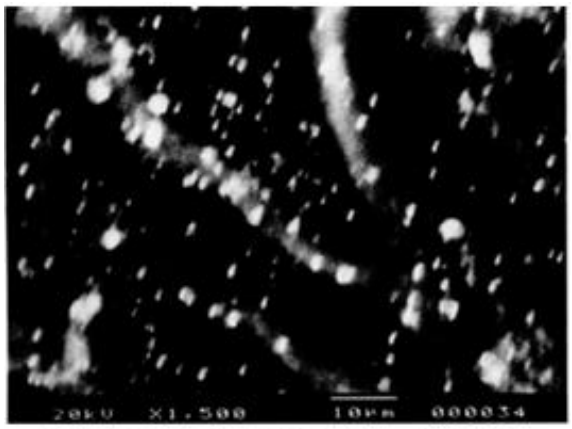

Fig.4: SEM analysis of LAM transdermal patch F2

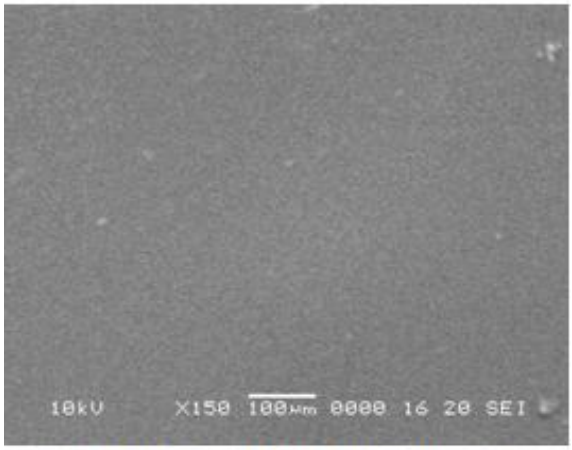

Fig. 5: SEM analysis of LAM transdermal patch F5

Drug content uniformity: The drug content obtained from the patches was 87.96±3.45%, 93.58±5.32%, 90.77±2.77%, 89.74±3.18% and 91.69±2.68% in formulations F1, F2, F3, F4 and F5 respectively. This indicates that the lamivudine distribution in the prepared patches was uniform and above 87.96%.

***In vitro* drug release studies:** During this investigation totally transdermal patch formulations containing different polymers and plasticizer/penetration enhancer were studied.The drug:polymer ratio was kept constant, at 1:3 for all patches.

The formulation F1 contains only polymer HPMC and drug (3:1) lamivudine. Glycerol was included in this formulation at 20% the dry weight of HPMC to improve plasticity of the patch. The other patches were fabricated from aqueous solution of HPMC with hydrophobic polymer such as ethylcellulose (EC); in the ratios of 8:1, 4:1, 2:1 and 1:1 dry weight of HPMC was used. Further, containing plasticizer/penetration like glycerol was included.

The total area of each patch was 26.43 sq. cm. From which the required area of 0.75 sq. cm. cut from the patch. This cut patch was used for *in vitro* studies, each of the above patches was subjected to *in vitro* diffusion studies using sigma dialysis sac, (12000 Daltons) as a support to patches, in Keshary-Chein diffusion cells. The primary *in vitro* data are tabulated in Table 2.

Table 2: Zero order data of LAM formulations F2-F5

Time (min)	Cumulative percentage released			
	F2	F3	F4	F5
5	16.67±2.84	14.45±2.81	16.19±3.12	16.53±2.92
10	35.10±1.68	34.07±2.27	42.64±3.09	22.84±2.98
15	47.88±1.78	43.64±2.77	66.70±2.45	47.94±2.60
30	61.37±3.27	50.99±2.91	78.01±3.20	64.70±2.80
45	69.90±2.38	58.78±3.00	79.19±2.77	68.18±2.98
60	75.76±1.71	61.53±2.41	80.10±2.65	71.34±2.51
120	81.17±3.05	68.81±2.50	85.37±2.94	79.71±2.08
180	86.29±2.93	76.28±3.24	88.90±1.42	86.67±2.71
240	91.12±3.17	82.00±3.23	90.38±3.01	90.79±2.78
300	92.68±2.30	88.83±2.79	91.20±3.15	91.44±3.01
360	92.79±2.08	92.67±2.15	91.91±2.58	92.19±2.42
420	93.54±2.21	94.17±1.85	92.51±2.80	92.63±2.73
480	94.38±2.52	94.90±2.22	93.00±3.32	93.85±2.83
540	95.30±2.94	95.30±2.94	93.64±2.55	94.17±2.24
600		95.46±2.85	94.19±2.48	94.26±2.44
660			95.67±2.73	95.15±3.00
720			96.33±1.40	96.02±1.58
780				97.50±2.34
840				98.71±2.24

The basic *in vitro* data obtained were tabulated as shown in Table 1 for formlations F2-F5. The data indicated that the release of drug from F2, F3, F4 and F5 was 95.30% in 9 h 95.46% in 10 h 96.33% in 12 h and 98.71% in 14 h, respectively. The results are summarized in Table 3 and Figs. 6-9.

Table 3: Drug release kinetic data of LAM formulations F1-F5

Formulation	T_{50} (h)	Zero-order		First-order		Higuchi	Korsmeyer Peppas		Best fit
		R^2	K_0 ($mg.hr^{-1}$)	R^2	K_1 (hr^{-1})	R^2	R^2	N	
F1	0.027	0.996	10.81	0.81018	0.50836	0.988	0.995	0.685	a
F2	0.029	0.999	10.326	0.78983	0.37816	0.973	0.995	0.839	a
F3	0.028	0.998	10.410	0.73551	0.43531	0.969	0.987	0.811	a
F4	0.027	0.999	10.871	0.75967	0.46044	0.973	0.988	0.718	a
F5	0.033	0.996	20.597	0.85588	0.27861	0.965	0.986	0.942	a

R^2 = correlation coefficient, N = Koresmeyer Peppas constant, a = Peppas, b= Higuchi, c = First order

Lamivudine release from the patches of HPMC:EC in different proportions, was found to follow first order kinetics. An increase in the proportion of EC in HPMC matrix, did not significantly increase the amount of drug release, but definitely increased the duration of the release. Also, it was seen that the duration of release gradually increased with an increase in EC proportion, as compared to that of HPMC. Formulation F2 was selected from this set for further studies to determine the influence of plasticizer/penetration enhancer, since this patch showed maximum percentage release and for the longest duration of time amongst the four patches studied with HPMC.

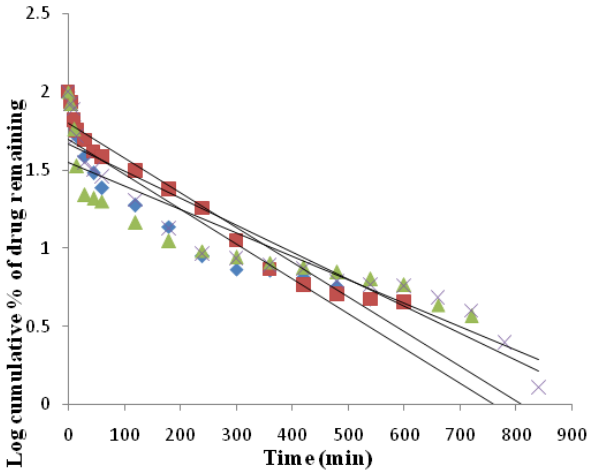

Fig.7: Comparative first order plots for formulations, F2 (–◊–), F3 (–□–), F4 (–Δ–) and F5 (–×–)

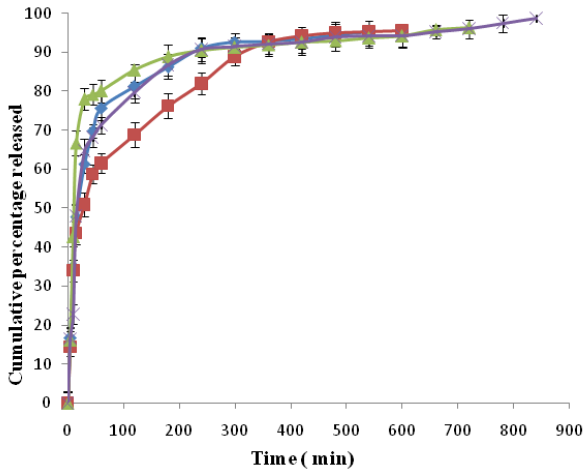

Fig.6: Comparative zero order release plots for LAM formulations, F2 (–◊–), F3 (–□–), F4 (–Δ–) and F5 (–×–)

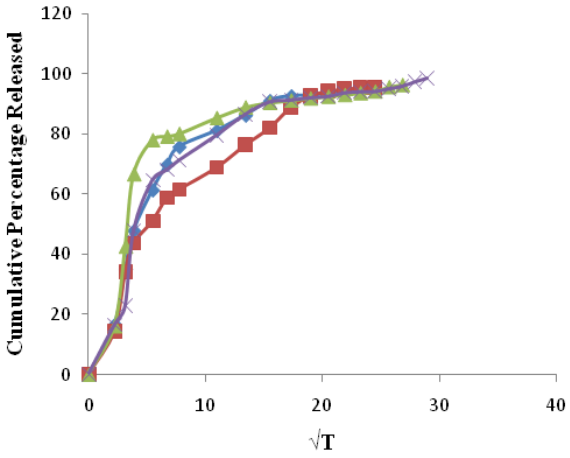

Fig. 8: Comparative Higuchi plots for LAM formulations, F2 (–◊–), F3 (–□–), F4 (–Δ–) and F5 (–×–)

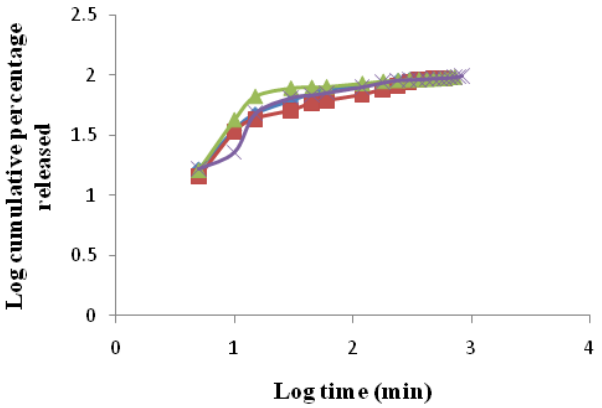

Fig. 9: Comparative Peppa's plots for LAM formulations, F2 (–◊–), F3 (–□–), F4 (–Δ–) and F5 (–×–)

CONCLUSIONS

In this work, an attempt was made to understand the influence of ethylcellulose as hydrophobic polymer included in 8:1, 4:1, 2:1 and 1:1 proportions of HPMC (w/w), on the release kinetics of drug. The FTIR characteristic peaks of lamivudine were present in combination, thus indicating compatibility between drug and polymers and finally confirm that there was no chemical modification of the drug has taken place. Further, effect of including plasticizer such as glycerol on the release rate of drug is studied. Totally, five formulations were planned, prepared and evaluated. The physical characteristics of the transdermal patches, like thickness, weight variation, surface area, density, surface pH, WVT and WVA were evaluated by standard techniques. All these patches were found to be permeable to Water vapor at 56% RH and 84% RH and the patches were found to be smooth, transparent and flexible. *In vitro* diffusion studies were carried out in Keshary-chien diffusion cells at 50 rpm and at 37±0.5^{o}C. IR spectra studies showed that the drug remains in its normal form without undergoing any interaction with the polymers. The *in vitro* drug release of optimized formulation (F5) was found 98.71% in 14 h. Results of the characterization studies indicated that good elasticity of TDDS patches.

The results of the present research gives idea about the formulation of lamivudine transdermal patches as extended release dosage form. This study will definitely useful for the researchers and scientists working in the same field. The final products developed in the research may be commercialized after the establishment of the safety and efficacy in the human volunteers.

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