Formulation and Evaluation of Transdermal Patch of Stavudine

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ABSTRACT: Stavudine has the half life of 1 to 1.5 hour and bioavailability in the body is 86% due to first-pass metabolism. The dose of stavudine is 40 mg two to three times daily depending on weight and gender; hence, it requires frequent dosing. Transdermal patch of stavudine was prepared to sustain the release and improve bioavailability of drug and patient compliance. Different formulations were prepared by using different concentrations of Eudragit RS 100 and Eudragit RL 100. The prepared formulations were evaluated for various parameters like weight, thickness, drug content, percentage moisture content, percentage moisture uptake, tensile strength, folding endurance, *In vitro* drug release and *in vitro* permeation studies. Also these patches were characterized by Field Emission Scanning Electron Microscopy and Fourier transforms Infrared Spectrosphotometry (FTIR). All formulations have shown 0 % constriction of the patches indicating 100% flatness of the transdermal patches. Thus, these formulations can maintain a smooth and uniform surface when they are functional onto skin. The folding endurance values of patches showed optimum flexibility of the patches. The moisture content and moisture uptake in the formulations of transdermal patch was found to be increased by increase in the concentration of Eudragit RS 100 and decreasing the concentration of Eudragit RL 100. FTIR study has shown absence of any interaction of the drug with the excipients. As concentration of Eudragit RL100 increased and subsequently the concentration of Eudragit RS100 decreased, the drug release was enhanced.

Key words: Stavudine, transdermal patch, Eudragit RS 100, Eudragit RL 100

INTRODUCTION

The drug undergoes extensive hepatic first-pass metabolism and thus lead to reduction in administered dose achieving systemic circulation. This suggests the need of an alternative route of administration, which can bypass the hepatic first-pass metabolism.[1,2] Transdermal drug delivery system (TDDS) has been a better interest in the drug administration by the skin for both local therapeutic effects and for systemic delivery of drugs.[3] Compared to conventional routes of drug administration skin as a site of drug delivery has many of important advantages together with the ability to avoid problems of gastric irritation, pH and emptying rate effects, avoid hepatic first-pass metabolism and thus increases the bioavailability of drug, decreases the risk of systemic side effects by reducing plasma concentrations compared to oral therapy, provides a sustained release of drug at the site of application, rapid extinction of therapy by removal of the device or formulation, the reduction of fluctuations in plasma levels of drugs, and evade pain associated with injections.[4] Transdermal patches offer added advantages such as maintenance of constant and prolonged drug level, reduced frequency of dosing, minimization of intra- and intr-apatient variability, self administration, and easy termination of medication, leading to patient compliance.[1] Stavudine is thymide analogue reverse transcriptase inhibitor that is active *in vitro* against HIV-1 and

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HIV-2.[5] It is absorbed rapidly following oral administration producing peak plasma concentration within 1 hour with 86 % bioavailability. Elimination half-life is 1 to 1.5 hours following single or multiple doses. In addition to pharmacokinetic properties, stavudine has low dose, low molecular weight (224.2) and extensive first pass effect.[6] All the above properties are reflecting on its suitability and necessity as a good candidate for transdermal delivery. The purpose of the present work was to develop transdermal formulation of stavudine which increases the patient compliance. Also an attempt was made to establish the best possible combination of polymeric ratio of formulated transdermal patches with maximum controlled and sustained drug release capability as well as physical stability.

MATERIALS AND METHODS

Materials

Stavudine and Eudragit RS100 & RL100 were obtained as gift samples from Hetero Labs., Hyderabad, India. Dimethyl sulphoxide (DMSO), Di-n-butyl phthalate (DBT), sodium hydroxide, chloroform, potassium dihydrogen phosphate, ammonium chloride, calcium chloride, polyethylene glycol 400 (PEG) and mercury metal were procured form FISCHER Chemic Ltd, Chennai, India. All chemical used were of analytical grade.

Methods

Development of the patch. Matrix-type transdermal patches containing stavudine were prepared by solvent evaporation technique using composition as given in Table 1. The polymers were weighed in requisite ratio and they were then dissolved in chloroform and DMSO. The *n*-butylphthalate was used as a plasticizer. The drug (40 mg) was added in the homogeneous dispersion, by slow stirring with a mechanical stirrer. The obtained uniform dispersion was casted on to petriplate of surface area about 70 sq.cm, allowed for air drying over night followed by vacuum drying for 8-10 h. The placebo film was prepared using same method excluding drug. The entire sheet was cut into small patches with an area of 4.9 cm^2, with a diameter of 2.6 cm. About 10 patches were obtained from each sheet. They were kept in desiccators until used.

Evaluation of polymeric films

Determination of weight, thickness and tensile strength. Six pieces (1 cm x 1 cm) were precisely cut from films. Pieces were weighed and their thickness was determined by using a micrometer (Mitutoyo, Japan). Circle pieces with 4 cm diameter from films were cut and their tensile strengths were determined using a microprocessor based advanced force gauze (Ultra Test, Mecmesin, UK) equipped with a 25 kg load cell. Film strip with dimensions 60 x 10 mm and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3 cm. During measurement, the top clamp at a rate of 2 mm/s pulled the strips to a distance till the film broke. The force and elongation were measured when the film broke. The mechanical properties were calculated according to the following formula.[7] Measurements were run in four replicates for each formulation.

$$\text{Tensile strength (Kg/cm sq.)} = \frac{\text{Force at break (Kg)}}{\text{Cross sectional area of the sample (cm sq)}}$$

Determination of drug content. Circle pieces with 1 cm diameter cut from films were dissolved in 10 ml pH 7.4 phosphate buffer solution (PBS) in the ultrasonic bath for 30 min. Solutions were diluted to 25 ml, filtered through Whatman filter paper no. 42 and analyzed spectrophotometrically at 266.5 nm (Shimadzu UV-1601 spectrophotometer, Japan), for drug content.

Determination of moisture content. The prepared films were marked, then weighed individually and kept in a desiccators containing activated silica at room temperature for 24 h. The films were weighed again and again individually until it showed a constant weight. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.[8]

Determination of moisture uptake. A weighed film kept in a desiccator at normal room temperature

for 24 h, was taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) in a desiccator until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.[8]

Determination of flatness. Longitudinal strips were cut out from each film, one from the center and two from either side. The length of each strip was measured and the variation in the length because of nonuniformity in flatness was measured by determining percent constriction, considering 0% constriction is equivalent to 100% flatness [8].

$$\text{Constriction (\%)} = \frac{\text{Final length of strip} - \text{Initial length of strip}}{\text{Final length of strip}} \; x \; 100$$

Determination of folding endurance. A strip of specific area (2 cm x 2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film was folded at the same place without breaking gave the value of the folding endurance.[9]

Field emission scanning electron microscopy (FESEM). The morphology of samples was examined by FE scanning electron microscopy, FESEM QUANTA 200FEG model, (FEI NETHERLAND make) with operating voltage ranging from 5.00 kV and with 10.0nm resolution and 100,300,900 & 238 x magnification. FESEM micrographs were taken after coating the surfaces of samples with a thin layer of gold by using BAL-TEC-SCD-005 Sputter Coater, BAL-TEC AG, Balzers, Liechtenstein; Germany under argon atmosphere to make the sample conducting. The FESEM micrographs are analyzed by xT Microscope Server software and for EDAX analysis EDAX Genesis software was used.

Fourier transforms infrared spectroscopy (FTIR). Fourier transforms infrared spectra of stavudine, placebo transdermal patch and stavudine loaded transdermal patch (F5) were recorded using Jasco V5300 (Jasco, Japan) FTIR system using potassium bromide (KBr) pellet method. Each spectrum was derived from single average scans collected in the region 4000 to 400 cm^{-1}.

***In vitro* dissolution studies.** The dissolution of patches was performed using USP Basket Type Dissolution Apparatus. The patches were placed in respective baskets with their drug matrix exposed to phosphate buffer, pH 6.8. All dissolution studies were performed at 37 ± 0.5 °C, at 100 rpm, with each dissolution jar carrying 900 ml of buffer. Samples were withdrawn at different time intervals and analyzed using a UV spectrophotometer at 266.5 nm against blank. Cumulative amounts of drug released were plotted against time for all formulations. Profile of all batches were fitted to various mathematical models such as Zero order, First order, Higuchi, Hixon and Crowell and Korsmeyer to ascertain the kinetic of drug release.[10]

***In vitro* permeation studies.** The permeation studies were performed in a modified Keshary-Chien cell (cell capacity 45 mL, crosssectional area 4.906 cm^2) using cellophane membrane. A section of membrane was cut, measured, and placed in the donor compartment facing the drug matrix side of the patch to the membrane and backing membrane upward. The holder containing the membrane and formulation was then placed on the receiver compartment of the modified diffusion cell, containing phosphate buffer pH 6.8. The donor and receiver compartment were kept in an intimate contact by wrapping parafilm at the junction. The temperature of the diffusion cell was maintained at 37 ± 0.5 °C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred during the whole experiment using magnetic bead. The samples were withdrawn (1 ml each time) at different time intervals and an equal amount of phosphate buffer, pH 6.8, was replaced each time. Absorbances of the samples were read spectrophotometrically at 266.5 nm taking phosphate buffer solution, pH 6.8, as blank. The amount of drug permeated per square centimeter at each time interval was calculated and plotted against time. Release-rate constants for different formulations were also determined.

RESULTS AND DISCUSSION

Preliminary evaluation of transdermal patch. The physical appearance of the transdermal patch was as shown in figure 1.

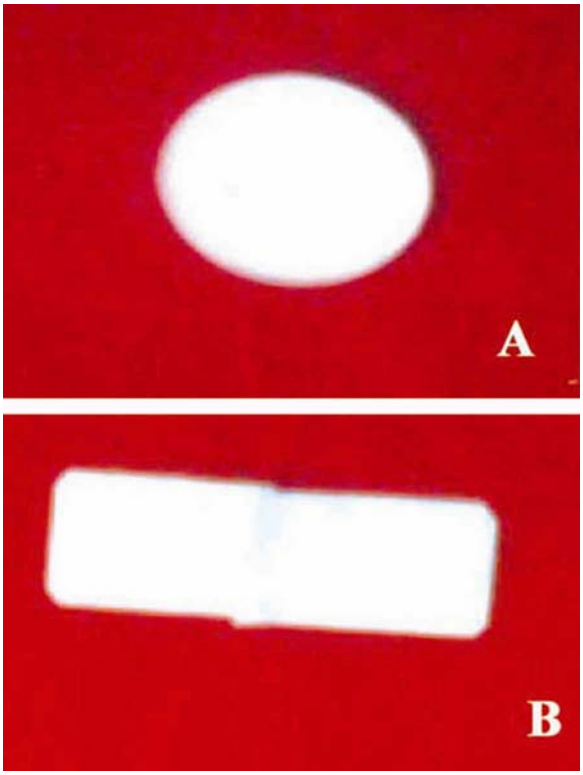

Figure 1. Stavudine transdermal patch A: Medicated Polymeric film B: Medicated Polymeric patch with adhesive.

All the formulations of transdermal patch showed thickness of 0.76 µm to 0.78 µm with average weight (for 1cm^2 patch) 0.46 mg. Additional evaluation parameters of all formulation of transdermal patch were as given in Table 2. Tensile strength of the formulations was in the range of 1.96 to 2.08 indicated that as concentration of eudragit RS 100 increases and concentration of eudragit RL 100 decreases tensile strength increases. All formulations have shown 0 % constriction of the patches. No amount of constriction in the formulated transdermal patches ensured their 100% flatness. Thus, these formulations can maintain a smooth and uniform surface when they are functional onto skin. The folding endurance values of patches were found satisfactory (Table 2) which indicates that the patches prepared were having optimum flexibility and were not brittle. The moisture content and moisture uptake in the formulations of transdermal patch was found to be increased by increase in the concentration eudragit RS 100 and decreasing the concentration of eudragit RL 100. The lower moisture content in the formulations helps them to remain stable and become a completely dried and brittle film. Again, low moisture uptake protects the material from microbial contamination and bulkiness. The drug content of the formulations were ranges from 31.40 ± 2.3 to 34.22 ± 1.7.

Fourier transforms infrared spectroscopy (FTIR). Fourier transforms infrared spectra of stavudine, placebo transdermal patch and stavudine loaded transdermal patch (F5) are shown in figure 2. Drug and formulation has exhibited identical FTIR indicting absence of any interaction of the drug with the excipients.

Drug release and *ex vivo* permeability. The drug release and permeability form the transdermal patch for all the formulations were as shown in figure 3. The formulation F5 had shown 91.88 % of cumulative percentage drug released in 24 h. It was

Table 1. Formulation codes with quantities.

Film Code	Eudragit RS 100 (mg)	Eudragit RL 100 (mg)	PEG 400 (ml)	N-butyl phthalate (ml)	Chloroform (ml)	DMSO 10% (ml)
F1	180	1620	0.08	0.04	8	0.04
F2	360	1440	0.08	0.04	8	0.04
F3	540	1260	0.08	0.04	8	0.04
F4	720	1080	0.08	0.04	8	0.04
F5	900	900	0.08	0.04	8	0.04

Table 2. Evaluation parameters of the transdermal patch.

Film code	Folding endurance	Drug content (%)	Tensile strength (g/cm^2)	Moisture content (%)	Moisture absorbed (%)
F1	93 ± 2	32.88 ± 1.2	1.98 ± 0.4	4.73 ± 0. 6	5.098 ± 0.7
F2	95 ±1	31.63 ± 1.4	1.96 ± 0.6	4.16 ± 0.7	5.896 ± 0.8
F3	97 ±1	31.40 ± 2.3	1.99 ± 0.3	3.57 ± 0.4	5.844 ± 0.5
F4	94 ±2	34.22 ± 1.7	2.04 ± 0.4	3.32 ± 0.8	8.793 ± 0.6
F5	96 ±1	32.23 ± 2.8	2.08 ± 0.5	2.24 ± 0.5	7.128 ± 0.7

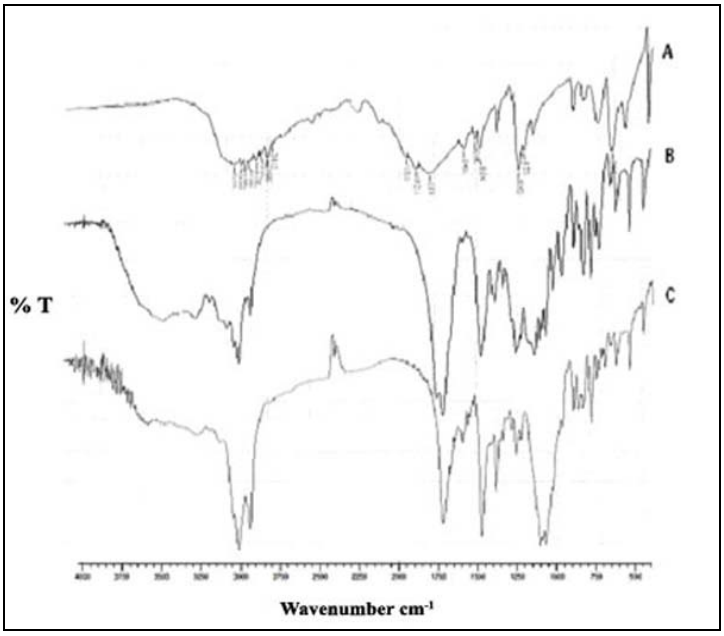

Figure 2. FTIR spectra of A: stavudine, B: Placebo patch, C: Formulation F5.

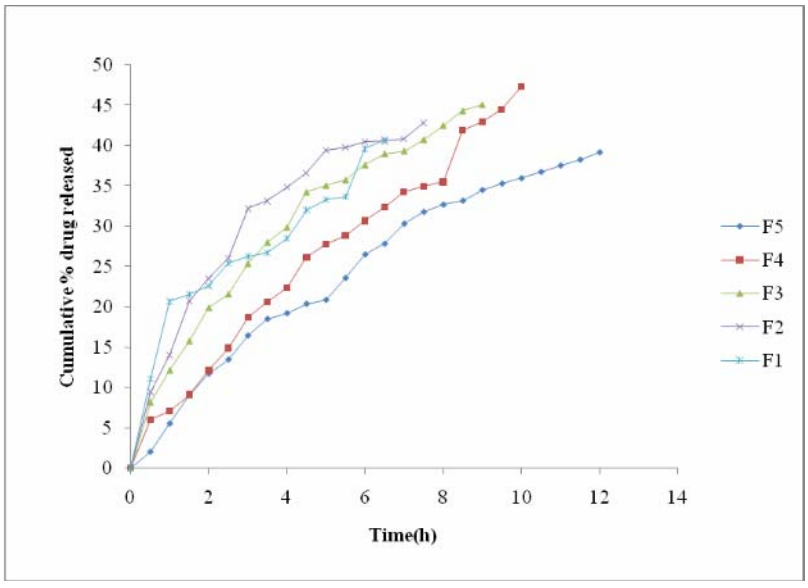

Figure 3. *Ex vivo* permeability study of all transdermal formulations.

Table 3. R^2 values of mathematical models for dissolution profiles of stavudine transdermal patch.

Formulation Code	R^2 values of mathematical models for dissolution profiles.			
	Zero order	First order	Higuchi	Hixson-Crowell
F1	0.984	0.996	0.998	0.997
F2	0.980	0.997	0.998	0.994
F3	0.952	0.973	0.992	0.967
F4	0.973	0.994	0.998	0.988
F5	0.947	0.970	0.984	0.964

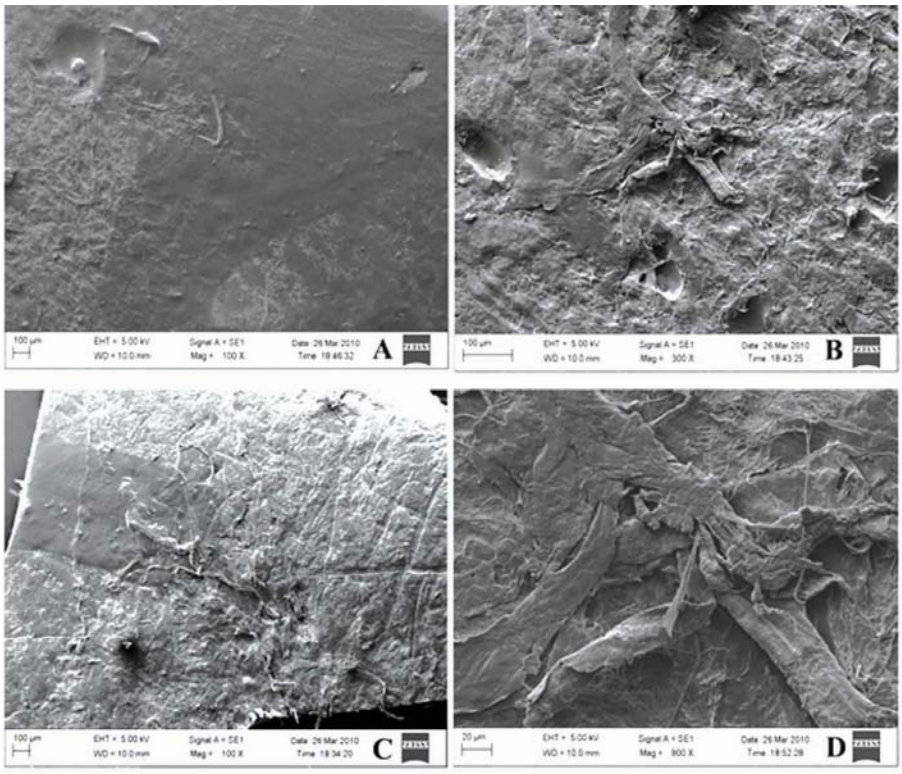

Figure 4. SEM microphotographs of drug loaded patch, A and B: Before dissolution and C and D: After dissolution.

observed that as concentration of Eudragit RL100 increased and subsequently the concentration of Eudragit RS100 decreased, the increased drug release might be attributed to higher permeability of Eudragit RL100. This was well supported by permeability study like dissolution. Formulation F5 has shown the highest permeability.

The drug release kinetics from various formulations was determined by comparing the regression co-efficient of zero-order, first order, Higuchi, Hixen crowel model as given in table 3. In case of all formulations the regression co efficient was found to be more in Higuchi model, indicating the mechanism of drug release from the prepared films to be diffusion controlled. Electron microscopic images of the patches shown in figure 4 have exposed that drug was homogeneously dispersed in the transdermal patch. It has also indicated that after the release of drug molecules, the distorted portion of the membrane has maintained elasticity in an affected small area with little effect on the other part of the membrane. This showed that very little or almost no constriction, that is, 100% flatness of the patches persists even after the patches were deprived of the drug molecules signified physical stability of the patches.

CONCLUSION

Transdermal patches of Stavudine were successfully prepared by using different concentrations of Eudragit RS 100 and Eudragit RL 100. Prepared patches were found to have smooth and uniform surface when they are functional onto skin. The moisture content and moisture uptake in the formulations of transdermal patch was found to be increased by increase in the concentration Eudragit RS 100 and decreasing the concentration of Eudragit RL 100. As concentration of Eudragit RL100 increased and subsequently the concentration of Eudragit RS100 decreased, the drug release was increased. Drug release kinetics has shown that all formulations follow in Higuchi model, indicating the mechanism of drug release from the prepared films to be diffusion controlled.

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