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FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES OF METHOTREXATE

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ABSTRACT

The present study was to develop transdermal matrix patch of Methotrexate and assess its feasibility for transdermal application. The low permeability of skin is the rate-limiting step for delivery of most of the drugs. Studies were carried out to investigate the effect of permeation enhancers like DMSO, tween-80, eucalyptus oil and olive oil on the permeation of Methotrexate from matrix patch through skin. The formulations were subjected to *in vitro* drug release, *in vitro* permeation studies, and stability studies. The results of Methotrexate transdermal matrix patch showed that the most promising formulation was FT1 (formulation containing EC: PVP, 3:2; Methotrexate 20%; dibutylpthalate 30% and 2% tween-80 all in %w/w). This formulation was able to deliver drug up to 24 h at a flux of 43.91µg/cm^2/h across rat skin. Thus optimized transdermal matrix patch of Methotrexate using polymers such as EC and PVP with tween-80 as permeation enhancers demonstrated their ability to give sustained release, because of excellent release and permeation of drug and its influence on antidepressant efficacy. The developed formulation of Methotrexate is expected to improve the patient compliance, form better dosage regimen and provide maintenance therapy to patients suffering from cancer.

KEYWORDS: Transdermal, Matrix Patch, Methotrexate, Bioavailability.

INTRODUCTION

At present, the most common form of delivery of drugs is the oral route because it has advantage of easy administration.[1] But it also has significant drawbacks namely poor bioavailability due to first pass metabolism and the tendency to produce fluctuation in plasma dug concentration due to the frequency in dosing which can be both cost prohibitive and inconvenient.[2,3] Transdermal drug delivery systems those can deliver medicines via the skin

portal to the systemic circulation at a predetermined rate and maintain clinically effective concentrations for prolonged period of time.[4] Avoidance of first pass hepatic metabolism, thus increasing bioavailability and efficacy of drugs.[5] Extended therapy avoiding frequent dose administration.[6] Reduce side effects due to optimization of the blood concentration time profile.[7] Erythema, itching and local edema can be caused by the drug, adhesive or by the excipients in the patch formulation.[8,9]

This investigation focused on development of low dose maintenance therapy for anticancer drug, to reduce the risk of potential side effects, improve the patient compliance in depressant patients. The present study is focused on the development of suitable transdermal drug delivery system (Patches) for sustained delivery of methotrexate.

MATERIALS AND METHODS

Methotrexate was obtain from Yarrow Chem Product, Mumbai, India as gift. Ethylcellulose, Polyvinylpyrrolidone (PVP K-30) and Tween-80 were purchased from SD Fine Chem Ltd., Mumbai, India. All chemicals used in this experiment are laboratory grade.

Preformulation Studies

Physical appearance: The drug sample was purchased from Yarrow Chem Products, Mumbai, India. The supplied powder of drug sample was a crystalline, white to off white in colour powder of odourless and bitter in taste.

Methods of Determination of λmax: The Methotrexate exhibits peak maximum absorbance at 303 nm in 0.1N Hydrochloric acid. Preparation of standard solution: 100mg of Methotrexate was exactly weighed in volumetric flask of 100 ml & solubilised in small volume of PH 7.4 phosphate buffer. The volume was made up with the 0.1N Hydrochloric acid to get a concentration of 1000µg/ml (SS-1). From this SS-2 was prepared containing 100µg/ml.

Methods of Preparation of standard calibration curve of methotrexate: The concentration of 5 µg/ml, 10 µg/m, 15 µg/ml and 40 µg/ml, respectively were prepard using pH 7.4 phosphate buffer. Different aliquots were analysed at 303 nm by using UV-visible spectrophotometer (Model-1700, Shimadzu, Japan) against pH 7.4 phosphate bufferas a blank. Absorbance was recorded and standard curve was plotted absorbance on y axis and concentration on x axis for linear relationship.

Methods of Drug-Excipients interaction study: The Fourier transform infra-red (FTIR) profile of drug alone and physical mixtures of Methotrexate with ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) were recorded in order to determine the physicochemical compatibility between drug and polymers used in the formulation. A pellet of pure drug and physical mixture of drug and polymers (1:1) were prepared by compressing with IR grade potassium bromide in a 100:1 ratio by applying 5.5 metric ton of pressure in hydraulic press. The pellet was mounted in IR compartment and scanned between wave number 4000-450 cm-1 using FTIR spectrophotometer (Model-8400 S, Shimadzu, Japan). The obtained FTIR spectra of drug, polymers and physical mixture of Methotrexate with polymers (EC and PVP) were compared with the references for obtained peak of functional groups.

Methods of formulation of methotrexate transdermal patches

The matrix type transdermal patches of Methotrexate were prepared by solvent evaporation technique by using different ratio of ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) polymers. The polymers EC and PVP were weighed and mixed in different ratios by keeping the total polymers weight at 1.6 g added in a chloroform solvent using magnetic stirrer. The dibutyl phthalate 30% w/w of polymer was incorporated as plasticizer. Drug 20 % w/w of polymer weight was added slowly to the polymers solution and mixed thoroughly by continuous stirring for 30 minutes to obtain a homogenous solution. The five formulations were prepared by using same drug and different polymers ratio without permeation enhancer in order to determine the optimum combination of drug and polymers. On the basis of preliminary studies, the optimized polymers ratio 3:2 (EC:PVP) were mixed with the different permeation enhancers like DMSO, Tween-80, eucalyptus oil and olive oil. The permeation enhancers were added in three different concentrations i.e. 2%, 5% and 10% w/w of total polymers weight for each. The resulting drug-polymers solution was poured in petridish of 64 cm2. The aluminum foil was uniformly spread on petridish on which drug-polymers solution was poured. The rate of evaporation was controlled by inverting a funnel over the petridish and the solvent was allowed to evaporate for 24 h at room temperature. After 24 h, the films were collected and a wax paper was applied on other side of the films as a release liner to complete the formulation.

Physicochemical evaluation of Methotrexate Patches

Physical appearance: All formulated transdermal patches were visually inspected for colour, clarity, entrapment of any air bubble, flexibility and smoothness, which on a large part determines patient acceptability of the patch and also therapeutic efficacy.

Thickness: Thickness of transdermal patch was measured by using digital thickness gauge (Muttato Japan). Thickness of rectangular patch (2x2 cm) was determined with a four different points and average thickness was taken. Same was performed for other patches also.

Weight variation: Weight variation study of transdermal patches was performed by individually weighing 10 randomly selected patches of sizes 4.52 cm2 on digital weighing balance and average weight was calculated. The individual weight of patches should not deviate significantly from the average weight.

Drug Content: To determine the drug content of transdermal patch, known amounts of Methotrexate patch was cut from casted film and dissolve in chloroform in 100 ml volumetric flask and placed in shaking incubator for 4 h. The solution was filtered through membrane filter (0.45 µm) and 1 ml solution was taken and diluted with chloroform to 10 ml. The absorbance of solution was measured at 303 nm by using UV/visible spectrophotometer (Model-1700, Shimadzu, Japan). The chloroform was used as a blank. The average reading of three patches was taken as the content of drug in one patch.

Moisture content: To determine moisture contents of transdermal patches, they were weighed individually and kept in a desiccator containing calcium chloride at room temperature for 24 h. The transdermal patches were weighed repeatedly until they showed a constant weight. The moisture content was calculated by given below formula.

$$\%\textbf{Moisture Content} = \frac{final\ weight - initial\ weight}{Initial\ weight} X100$$

Moisture uptake: Transdermal patches were kept in desiccators at room temperature for 24 h with silica gel and weighed (ws) and transfer to other desiccators to expose of 75% RH using a saturated solution of sodium chloride at 250C and patches were reweighed again and again, until a constant weight (wm) was obtained. The moisture uptake capacity was calculated according to the given formula.

$$\%\textbf{Moisture Content} = \frac{Wm - Ws}{Ws} X100$$

Flatness: Longitudinal strips from the 5 randomly selected transdermal films of each formulation were cut out. One from the center and one from the other side of patch. The length of each strip was measured and the variation in length because of the non-uniformity of flatness was measured. 0 % constriction was considered to be 100 % flatness. Flatness was calculated by measuring constriction of strip using given formula

$$\%\textbf{Construction} = \frac{I1 - I2}{I2} X100$$

Where, I1 = Initial length of each strip, I2 = Cutted film length

Folding Endurance: The folding endurance of patch was expressed as the number of folds (number of times the patch folded at the same place), either to break the preparation or to develop visible cracks. This test was performed to determine the stability of sample to withstand folding and brittleness. Folding endurance of patches was determined by repeatedly by folding a small strip of patches (approximately 2×2 cm) at the same place till it broke. The number of times patches could be folded at the same place, without breaking gave the value of folding endurance and it was recorded.

Tensile strength: The formulated patches were evaluated for its tensile strength to measure their mechanical properties. The tensile strength of the patches was determined by using a self designed assembly (Department of Pharmacy). Assembly consists of a pan hanged by using a strong thread and the other end of the thread was attached with the centre of the patch. The whole assembly was held like a beam balance and weights were kept on the pan. Weights required to break the patch was noted. Tensile strength was calculated using following formula.

Tensile Strength= Break Force/ a. b (1+ ΔL/L)

Where, a = Width of the patch, b = Thickness of the patch, L = Length of the patch, ΔL = Elongation of patch at break point, Break Force = Weight required to break the patch (Kg)

Method of pH measurement: The pH of the film-forming solutions was determined using a pH meter which was calibrated before use with buffered solutions at pH 4, 7 and 10.

***In vitro* drug release studies of methotrexate patches:** The dissolution studies were performed by using dissolution rate test apparatus (USP-II) for the assessment of the release of the drug from the transdermal patches (3.14 cm^2). The commercially available water impermeable adhesive backing membrane was placed over the patch and it was further fixed

on glass slide (2.3x2.3cm) using cyanoacrylate adhesive. Then the transdermal patch was covered with a dialysis membrane and placed at the bottom of dissolution vessels with the release surface facing upward. The apparatus was equilibrated to 32 ± 0.50C and the dissolution medium was 20% methanol in PBS pH 7.4. The paddle speed was kept constant at 50 rpm. The samples were withdrawn at appropriate time intervals upto 24 h and analyzed by UV spectrophotometer at 303 nm using 20% methanol in PBS pH 7.4 solution as a blank. After each sampling, an equal volume of fresh dissolution fluid was added to dissolution vessel to maintain a sink condition.

The drug release data of all formulations were fitted to various mathematical models such as zero order as cumulative % of drug released vs. time, first order as log cumulative % of drug remaining vs. time and Higuchi's model as cumulative % drug released vs. square root of time. To determine the mechanism of drug release from formulations, the data were fitted into Korsmeyer Peppas equation as log cumulative % of drug released vs. log time.

Amounts of drug permeated per sq. cm of patch was calculated and plotted against time. Flux was calculated as the amount of drug permeated per sq. cm per hour. The lag time (Tlag) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa.

***In-Vitro* Drug Permeation Studies:** In-vitro drug release studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 22 ml. Cellulose acetate, acetate ester of cellulose 28, has been fabricated as semi-permeable membranes for biomedical application 29. The cellophane membrane (cellulose acetate membrane) was used for the determination of drug from the prepared transdermal matrix type patches. The cellulose acetate membrane having a pore size 0.45µ was mounted between the donor and receptor compartment of the diffusion cell 30. The prepared transdermal film was placed on the cellulose acetate membrane and covered with aluminum foil. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a hot plate magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads and the temperature was maintained at 32 ± 0.5 °C, because the normal skin temperature of human is 32°C 31, 32, 33. The samples were withdrawn at different time intervals and analyzed for drug content in U.V. Spectrophotometer at 303 nm. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal in vitro drug release rate of selected TDDS.

5.5 Stability Studies of Methotrexate Patches

Stability studies of formulation FT1 was conducted according to ICH guidelines by storing at 40 °C and 75 % RH for 3 months. The samples were withdrawn at 30, 60 and 90 days and evaluated for physical appearance and drug contents. The in vitro permeation study was performed after 90 days and compared with fresh batch.

RESULT AND DISCUSSION

Preformulation Studies

The drug sample (Methotrexate) was was a crystalline, white or almost white in color powder of odorless and bitter in taste, melting point 192-193 ^{O}C and UV Absorption Maxima 303nm.

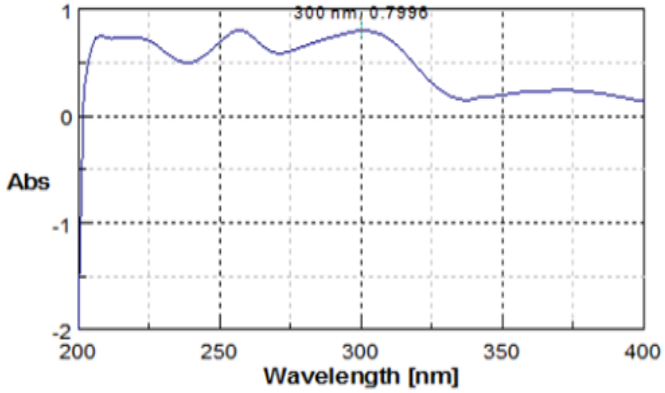

Figure 1: UV Spectra of Methotrexate

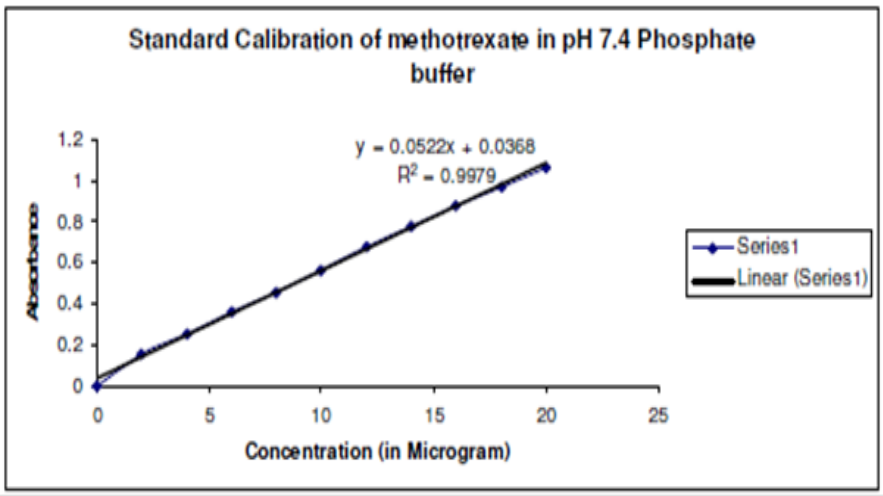

Figure 2: Calibration curve of Methotrexate.

A straight-line equation (Y= mx + c) was generated to facilitate the calculation of amount of drug. The equation is as follows.

Absorbance = 0.0522 × Concentration + 0.0368

Y = 0.0522x + 0.0368

The slope = 0.0522, The intercept = 0.0368, The correlation coefficient = 0.9979

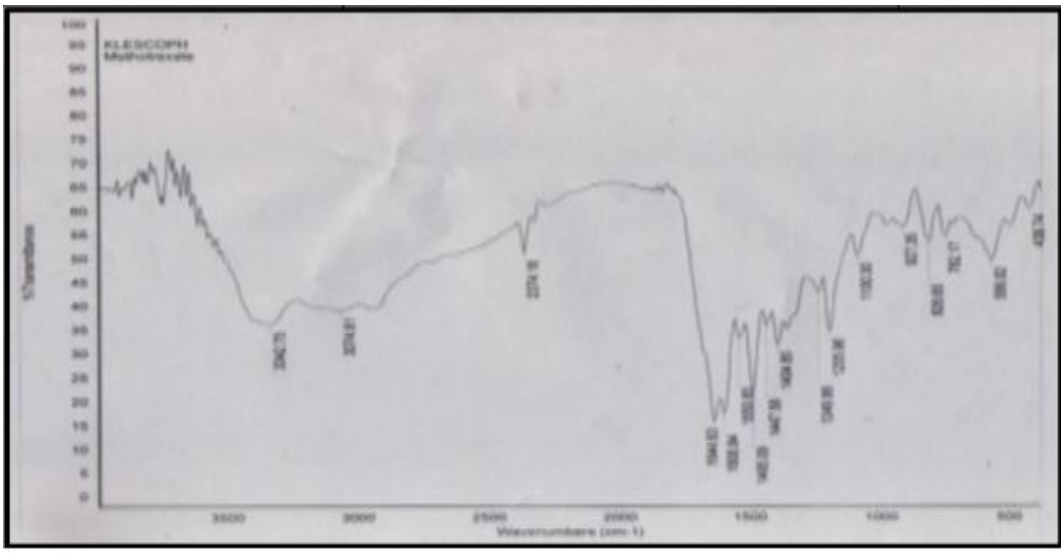

Figure 3: FTIR Spectrum of Methotrexate sample.

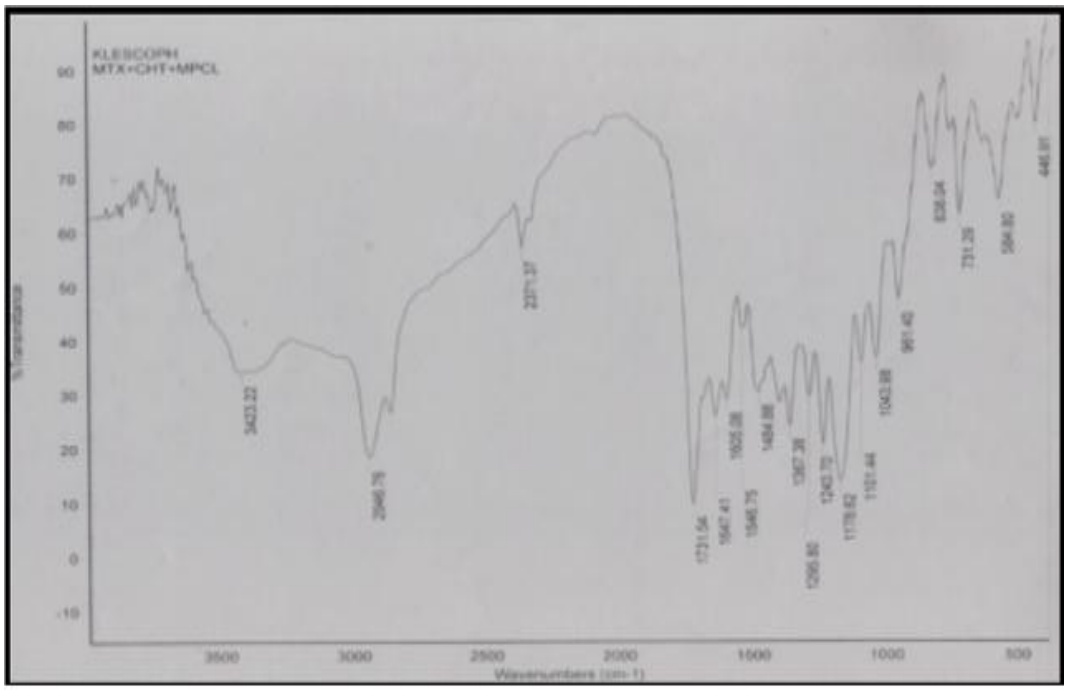

Figure 4: Reference I.R. spectrum of Methotrexate- exciepient sample.

Formulation of Methotrexate Transdermal Patches

The transdermal patches were prepared by using ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) polymers in different composition. The EC and PVP are most commonly used polymers in transdermal drug delivery system because of their

compatibility with drugs and sustained release properties. In preliminary studies, various formulations were prepared with or without plasticizer. The transdermal patches prepared without plasticizer were found to be brittle and hence di-n-butyl phthalate was used as plasticizer to reduce the brittleness of the transdermal patches. The studies indicated that addition of di-n-butyl phthalate at 30% w/w of total dry polymers weight produces smooth, uniform and flexible films. Hence, further formulations were prepared by using plasticizer at 30% w/w of polymers weight in all the patches. Hence, on the basis of preliminary formulation studies, the optimum polymer ratio was subjected with various penetration enhancers used in different concentration in order to enhance the *in vitro* permeability of drug molecule through skin. The fabricated films were evaluated for various physiochemical parameters and the composition of formulations is given in table bellow:

Table 1: Composition of Methotrexate transdermal patches.

S. No.	Formulation Code	Methotrexate (% w/w)	EC:PVP (Ratio)	Permeation Enhancer (% w/w)
1.	F1	20	4.5 : 0.5	-
2.	F2	20	4 : 1	-
3.	F3	20	2 : 1	-
4.	F4	20	3:2	-
5.	F5	20	2:3	-
6.	FD1	20	3:2	DMSO 2%
7.	FD2	20	3:2	DMSO 5%
8.	FD3	20	3:2	DMSO 10%
9.	FT1	20	3:2	Tween-80 2%
10.	FT2	20	3:2	Tween-80 5%
11.	FT3	20	3:2	Tween-80 10%
12.	FE1	20	3:2	Eucalyptus oil 2%
13.	FE2	20	3:2	Eucalyptus oil 5%
14.	FE3	20	3:2	Eucalyptus oil 10%
15.	FO1	20	3:2	Olive oil 2 %
16.	FO2	20	3:2	Olive oil 5 %
17.	FO3	20	3:2	Olive oil 10 %

All formulations containing dibutyl phthalate (30% w/w of polymers weight) as plasticizer and chloroform as solvent system.

Physiochemical Evaluation of Methotrexate Patches

The prepared transdermal patches were evaluated for their physiochemical characteristics like physical appearance, thickness, weight uniformity, drug contents, moisture contents, moisture uptake, flatness, folding endurance, tensile strength and pH. The results of physicochemical characteristics are given in Table bellow:

The formulated patches were found to be clear, smooth, uniform, flexible in their physical appearance and free from entrapment of air bubble. The weight of transdermal patched varied from 164.37 to 172.01 mg which indicated that the prepared different batches of transdermal films were similar in weight. The thickness of different batches were found in range from 0.246 to 0.276 mm. A low standard deviation value in the film thickness measurement ensures the uniformity of formulated patches. No significant difference in drug content was observed in all the formulated patches which were found in range from 94.12 to 98.23%. The obtained results indicated that the method used for the preparation of transdermal patches was capable of possessing uniform drug content due to the homogenous dispersion of the drug.

The moisture content and moisture uptake of various formulations showed that with increasing in concentration of hydrophilic polymer (PVP) both percentages of moisture content and moisture uptakes were increases. The similar results have been reported by other researchers (Gupta and Mukherjee, 2003). The percentage of moisture contents and moisture uptake were found in the range from 1.64 ± 0.31 to 6.38 ± 1.04 and 2.43 ± 0.55 to 9.41 ± 0.75 respectively. The results indicated that the hydrophilicity of the polymers is directly proportional to the percent of moisture contents and moisture uptake. The low percentage of moisture content in formulations could help them to remain stable and prevents them from being completely dried. Also, low moisture uptake protects the material from microbial contamination and bulkiness of the patch. Flatness studies were performed to determine the formulation construction. 100 % flatness of all the formulated patches indicated that there was no amount of constriction in formulated transdermal patches. Thus, formulated transdermal patches could better maintain a smooth surface when applied onto the skin.

Tensile strength of transdermal patches are important since they are expected to be sufficiently flexible and to have a mechanical strength on skin for a long period of time. Tensile strength results showed that strength of films were in a range from 0.346 to 0.438 kg/mm2. The folding endurance determines the ability of patch to withstand rupture. It was measure manually and was found to be in the range from 34 to 48. The result indicated that the patches of all batches would not break and would maintain their integrity with general skin folding when used. (Mamatha *et al.,* 2010). The pH of dermatological product has an important role to be safe and non irritant. Formulations placed on the skin should possess a pH between 4 and 7 (Hadgraft, 2001). The pH of film forming solutions was determined and

it was observed in the range from 5.8 to 6.6. The results indicated that prepared patches have desirable pH as a dermatological preparation.

Table 2: Physiochemical evaluation of Methotrexate transdermal Patches.

F. Code	Thickness (mm)	Weight Variation (mg)	Drug Content (%)	Flatness	Folding Endurance	Tensile Strength (kg/mm2)	pH	F. Code
F1	0.273 ± 0.014	164.87 ± 2.08	96.25 ± 0.42	100	42 ± 4.08	0.417 ± 0.02	5.8	F1
F2	0.254 ± 0.017	164.37 ± 1.48	97.26 ±1.42	100	48 ± 6.50	0.438 ± 0.04	5.8	F2
F3	0.266 ± 0.008	167.19 ± 1.88	94.12 ± 0.74	100	44 ± 3.43	0.393 ± 0.01	5.8	F3
F4	0.260 ± 0.012	165.20 ± 2.08	96.20 ± 1.11	100	39 ± 4.69	0.404 ± 0.03	5.7	F4
F5	0.268 ± 0.011	166.49 ± 1.11	95.03 ± 1.56	100	34 ± 3.08	0.357 ± 0.06	5.7	F5
FD1	0.265 ± 0.016	164.40 ± 1.89	96.78 ± 2.14	100	38 ± 5.37	0.370 ± 0.07	6.5	FD1
FD2	0.276 ± 0.010	166.72 ± 1.92	94.38 ± 0.92	100	36 ± 3.11	0.352 ± 0.03	6.6	FD2
FD3	0.269 ± 0.016	169.61 ± 2.33	96.20 ± 0.61	100	38 ± 4.15	0.346 ± 0.05	6.6	FD3
FT1	0.261 ± 0.022	165.20 ± 1.69	97.64 ± 1.04	100	37 ± 5.12	0.371 ± 0.02	6.3	FT1
FT2	0.256 ± 0.023	167.57 ± 2.12	95.68 ± 0.62	100	36 ± 3.91	0.397 ± 0.04	6.3	FT2
FT3	0.274 ± 0.013	168.97 ± 2.93	95.73 ± 1.80	100	40 ± 4.84	0.361 ± 0.02	6.4	FT3
FE1	0.246 ± 0.027	165.40 ± 2.18	98.23 ± 0.78	100	35 ± 4.32	0.394 ± 0.03	6.1	FE1
FE2	0.256 ± 0.014	167.60 ± 1.34	95.53 ± 1.21	100	38 ± 2.54	0.403 ± 0.04	6.5	FE2
FE3	0.267 ± 0.012	166.76± 2.76	97.19 ± 0.96	100	35 ± 3.63	0.372 ± 0.03	6.6	FE3
FO1	0.265 ± 0.016	168.56 ± 1.91	94.88 ± 1.13	100	36 ± 6.72	0.346 ± 0.02	5.7	FO1
FO2	0.273 ± 0.009	167.95 ± 4.32	94.58 ± 1.34	100	40 ± 3.91	0.363 ± 0.04	5.7	FO2
FO3	0.272 ± 0.014	172.01 ± 2.77	96.43 ± 0.69	100	43 ± 4.18	0.358 ± 0.05	5.7	FO3

In-vitro Drug Release Studies of Methotrexate Patches

The dissolution studies of transdermal patches are very crucial to ensure sustained release pattern. One need to maintain concentration of drug on the stratum corneum surface consistently and subsequently more than concentration of drug in the plasma to obtain a

constant permeation drug release rate. The modified paddle over disc assembly using 20% methanol in PBS pH 7.4 as a dissolution medium at 32 ± 0.50C was used to conduct dissolution studies. Result of *in-vitro* dissolution studies of prepared transdermal patches are presented in Table and Figure below:

The cumulative amount of drug release from control formulations (without enhancer) F1, F2, F3, F4 and F5 were found to be 40.70, 46.68, 52.38, 59.66 and 50.61% respectively in 24 h. The highest percentage of drug release (59.66%) was observed from formulation F4 (EC/PVP, 3:2) which was significantly *($p<0.05$)* greater than the lowest value 40.70% obtained from the formulation F1 (EC/PVP, 4.5:0.5). The percentage of drug release order was as follows: F4>F3>F5>F2>F1.

It was observed that increase in the concentration of hydrophilic polymer PVP, the rate of drug release increased, except for formulation F5. The addition of hydrophilic PVP to insoluble ethyl cellulose tends to enhance its release rate constant. This outcome can be attributed to the leaching of the soluble fraction which leads to formation of pores. Thus, decrease mean diffusion path length of drug molecule into the diffusion medium and increase in the external film area exposed to the dissolution medium, increase internal porosity and decrease the tortuosity. The initial burst release effect was observed in the all formulations. This may be because of the higher percentage of PVP in these formulations and PVP hydrophilic layer might need very little “time lag” to establish a concentration profile in patches resulting in a burst release in the dissolution studies. Similar finding have also been reported by others. The formulation F5 was showed increase in the concentration of hydrophilic polymer, the rate of drug release decreased. This may be attributed to the previous finding that higher concentration of PVP K-30 may decrease the crystalline drug in patch and thus decreased drug releases. The highest cummulative percentage of drug release *i.e.* 59.66% was observed from formulation F4 (EC/PVP, 3:2) in 24 h. Therefore, formulation F4 was selected for incorporation of permeation enhancers in three different concentrations *i.e.* 2%, 5%, and 10% in order to enhance permeation.

Effect of Dimethyl sulphoxide (DMSO): It was observed that the formulations containing DMSO as permeation enhancer, release rate was found to be directly proportional to the concentration of the DMSO (64.58, 70.49 and 78.29% for 2, 5 and 10% respectively in 24 hrs) in the transdermal patches. It has been reported that DMSO is relative polar in nature having small and compact structure which could lead to higher release rate.

Effect of tween-80: In case of batch FT, *i.e.* formulation containing tween-80 as permeation enhancer, the highest percentage of drug release (88.72% in 24 h) was observed with 2% of tween-80 (FT1). This may be due to the solubilisation effect of tween-80. But further increase in concentration of tween-80 from 5 (FT2) to 10% (FT3), decreased the percentage of drug release was observed from FT2 and FT3 of 77.32 and 70.38% respectively. Tween contribute to achieving critical micelle concentration (CMC). Concentration of surfactants above CMC could probably make micelles of drug, which could be difficult to diffuse out from the patch. Greater the amount of surfactant above CMC, greater the micelles of drug and thus, drug release rate could be retarded more.

Effect of eucalyptus oil: The transdermal patches containing 2% (FE1), 5% (FE2) and 10% (FE3) of eucalyptus oil showed 68.58, 76.83 and 83.52% drug release in 24 h respectively. It was observed that concentration of eucalyptus oil increased, the cummulative amount of drug release was also increased as shown in Table and Figure. This may be due to presence of terpene constituents in eucalyptus oil. Eucalyptus oil containing cineol as a principal terpene increases the drug release. This is probably due to increase in saturation solubility of drug in matrix.

Effect of olive oil: In case of formulations containing olive oil, the cummulative % of drug release was also increased with increase in concentration of olive oil from 2 to 5% (64.50% to 73.08% respectively). This may be due to presence of fatty acids in olive oil. But a further increase in concentration of olive oil to 10%, the % of drug release was found to be decreased (67.93 %) as shown in Table and Figure. This may be attributed to decrease in solubility of drug in the presence of high concentration of oil in matrix which cannot increase solubility of drug significantly in release media as previously reported for vegetables oils.

Table 3: *In-vitro* dissolution profile of Methotrexate from patches (F-1 to F-5).

Time (h)	Cummulative % drug release				
	F1	F2	F3	F4	F5
1	0.696 ± 0.21	1.81 ± 0.50	2.29 ± 0.46	0.826 ± 0.15	3.15 ± 0.21
2	3.81 ± 0.46	3.36 ± 0.69	4.32 ± 0.35	5.68 ± 1.13	6.55 ± 0.40
4	5.23 ± 0.23	7.63 ± 1.73	9.76 ± 1.02	11.52 ± 0.63	11.25 ± 1.16
6	12.50 ± 1.28	13.80 ± 1.58	17.52 ± 3.05	18.06 ± 1.65	19.32 ± 2.81
8	20.81 ± 2.13	18.49 ± 2.08	24.41 ± 4.46	28.72 ± 3.11	25.51 ± 0.64
10	23.89 ± 1.77	25.56 ± 4.19	33.87 ± 1.78	36.55 ± 1.57	31.85 ± 1.13
12	27.66 ± 0.61	32.34 ± 1.16	39.67 ± 3.10	43.37 ± 3.02	37.02 ± 2.22
18	35.15 ± 2.86	40.76 ± 3.53	47.16 ± 1.77	52.61 ± 2.28	43.96 ± 2.39
24	40.70 ± 0.49	46.68 ± 1.76	52.38 ± 0.63	59.66 ± 0.86	50.61 ± 0.96

Table 4: *In vitro* dissolution profile of Methotrexate from patches (FD-1to FD-3) & (FT-1 to FT-3).

Times (h)	Cummulative % drug release					
	FD1	FD2	FD3	FT1	FT2	FT3
1	1.55 ± 0.27	1.99 ± 0.75	6.67 ± 1.09	2.49 ± 0.32	7.31 ± 1.10	5.76 ± 0.84
2	12.32 ± 2.11	6.16 ± 1.46	17.74 ± 1.65	6.70 ± 1.14	13.82 ± 1.35	9.43 ± 1.46
4	18.13 ± 3.34	11.34 ± 1.44	31.46 ± 3.40	14.97 ± 1.24	19.39 ± 2.08	12.34 ± 0.74
6	25.86 ± 2.32	20.82 ± 2.23	42.67 ± 3.17	27.38 ± 2.58	25.87 ± 2.63	19.47 ± 2.11
8	30.92 ± 2.13	37.65 ± 3.56	50.87 ± 2.77	38.94 ± 2.63	34.60 ± 3.19	23.83 ± 1.45
10	37.72 ± 3.28	44.51 ± 3.71	59.35 ± 3.72	53.74 ± 4.08	40.61 ± 4.44	30.51 ± 2.56
12	44.17 ± 4.30	52.17 ± 4.10	65.15 ± 3.29	59.63 ± 2.48	48.26 ± 3.59	42.16 ± 4.05
18	51.08 ± 2.82	61.88 ± 2.77	72.25 ± 2.57	72.61 ± 3.75	60.51 ± 4.47	54.76 ± 4.12
24	64.58 ± 0.63	70.49 ± 1.22	78.29 ± 0.76	88.72 ± 0.93	77.32 ± 1.27	70.38 ± 0.83

Table 5: *In vitro* dissolution profile of Methotrexate from transdermal patches (FE-1to FE3) & (FO1 to FO3).

Times (h)	Cummulative % drug release					
	FE1	FE2	FE3	FO1	FO2	FO3
1	3.65 ± 0.44	1.94 ± 0.21	3.11 ± 0.77	1.72 ± 0.30	0.756 ± 0.22	1.31 ± 0.37
2	9.23 ± 1.11	5.45 ± 1.20	10.40 ± 1.14	6.54 ± 0.93	6.51 ± 0.63	3.14 ± 0.25
4	13.29 ± 1.61	9.33 ± 0.75	24.10 ± 2.02	16.18 ± 2.25	14.43 ± 2.21	8.95 ± 1.32
6	21.41 ± 2.37	14.74 ± 1.46	31.22 ± 1.73	27.29 ± 3.09	21.71 ± 1.54	14.47 ± 2.27
8	25.18 ± 2.29	32.58 ± 3.25	40.70 ± 3.39	32.37 ± 2.42	26.22 ± 2.31	20.49 ± 2.06
10	30.58 ± 1.83	39.68 ± 4.27	47.10 ± 2.28	36.89 ± 2.74	35.15 ± 3.44	28.92 ± 3.24
12	38.40 ± 3.25	47.66 ± 3.25	53.40 ± 1.14	41.17 ± 3.17	46.90 ± 2.87	40.37 ± 3.38
18	52.28 ± 4.14	60.28 ± 3.19	64.95 ± 3.10	49.18 ± 2.75	64.47 ± 3.20	53.49 ± 3.19
24	68.58 ± 0.97	76.83 ± 0.84	83.52 ± 0.81	64.50 ± 0.52	73.08 ± 1.07	67.93 ± 0.89

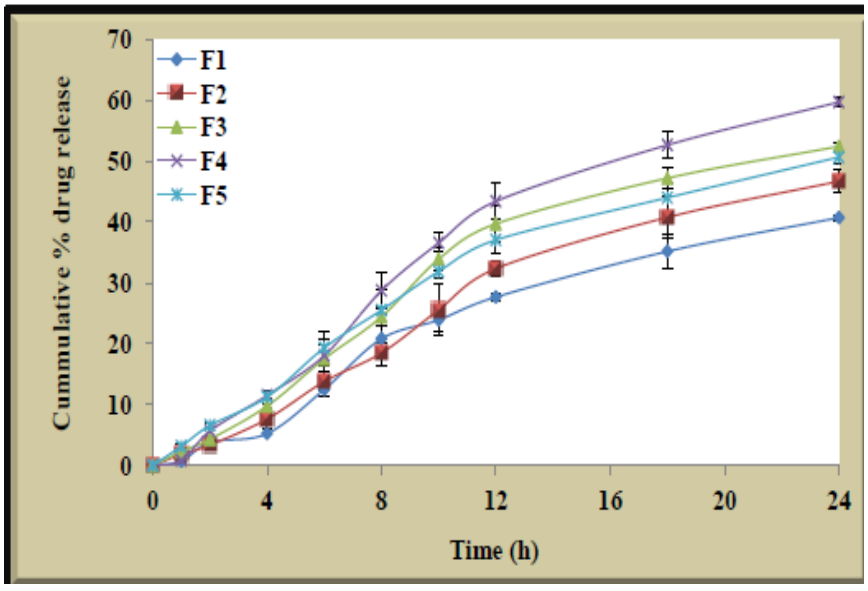

Figure 5: *In-vitro* dissolution profile of Methotrexate from transdermal patches (F-1 to F-5).

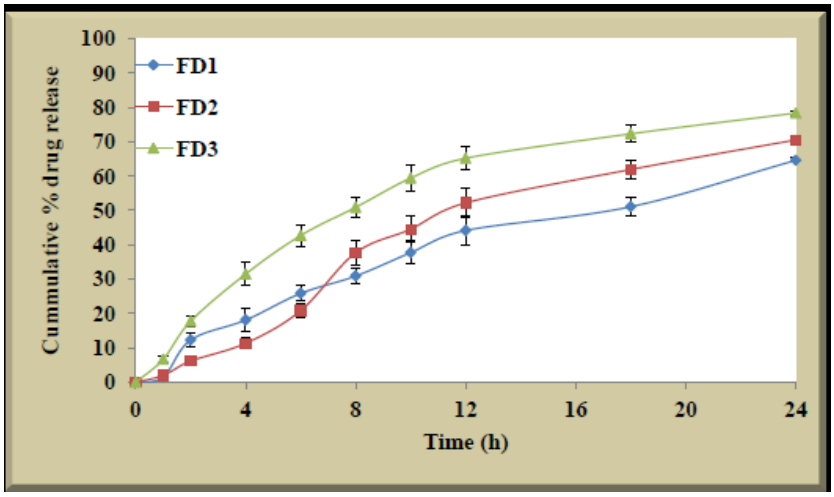

Figure 6: *In-vitro* dissolution profile of Methotrexate from transdermal patches (FD1 to FD3).

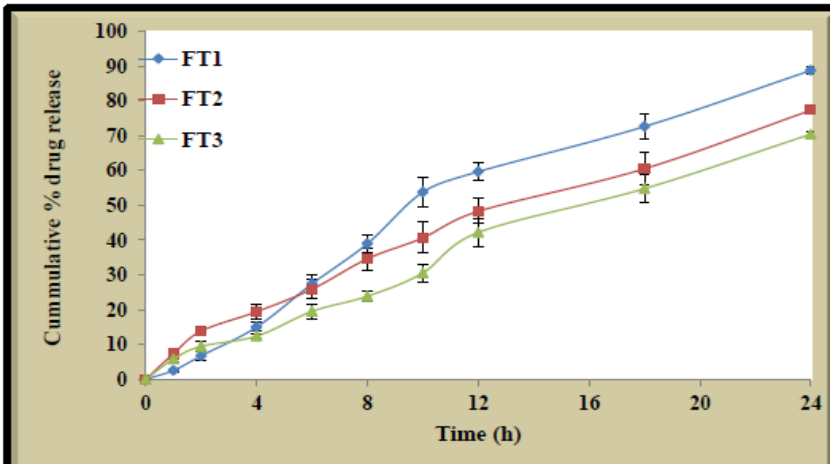

Figure 7: *In-vitro* dissolution profile of Methotrexate from transdermal patches (FT1 to FT3).

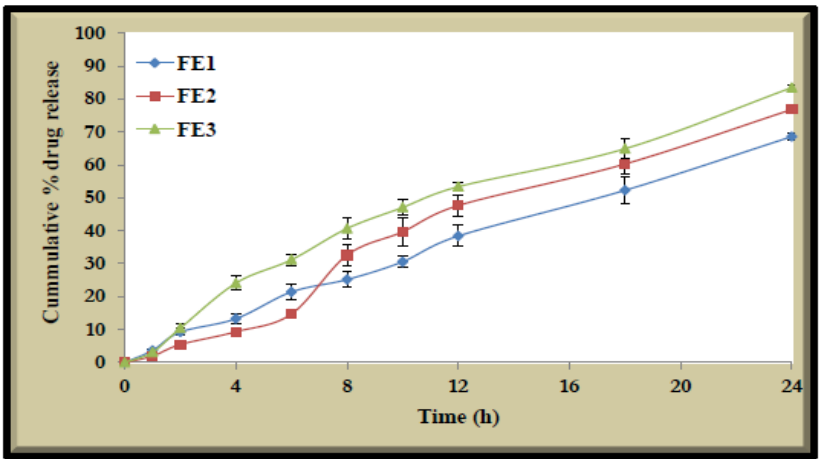

Figure 8: *In-vitro* dissolution profile of Methotrexate from transdermal patches (FE1 to FE3).

Application of Kinetic Models

In vitro drug release studies results were fitted in various kinetic models to study the release kinetics of data. Zero order as cumulative percent of drug released vs. time, first order as log cumulative percentage of drug remaining vs. time and Higuchi's model as cumulative percent drug released vs. square root of time. To determine the mechanism of drug release from formulations, the data were fitted into Korsmeyer Peppas equation as log cumulative percentage of drug released vs. log time. The value of n exponent was calculated from slope of the straight line. For matrix, if exponent n is 0.5, then diffusion mechanism is Fickian; if 0.5<n <1.0, mechanism is non- Fickian; if n is 1.0, mechanism is zero order and if n >1.0, then it is super case II transport.

The data revealed that the release pattern of formulations F1, F3, F4 and F5 was best explained by Higuchi equation (except formulation F2) according to highest linearity of r2 value. The formulations F2, FD2, FE2 and FO2 were suggested anomalous (pseudo-first order) release pattern and the formulations FT2, FT3, FE1 and FO3 showed pseudo-zero order release as reflected from higher coefficient of determination values over other models. The release pattern of formulations FD1, FD3, FT1, FE3 and FO1 was again best fitted for Higuchi's release kinetics.

Table 6: Release kinetic of Methotrexate transdermal patches.

F. Code	Zero order		First order		Higuchi Model		Korsmeyer Peppas Model
	r^2	K^0	r2	K^1	r^2	K^h	(n)
F1	0.938	1.820	0.965	0.010	0.975	11.07	1.260
F2	0.960	2.096	0.980	0.012	0.976	12.61	1.096
F3	0.923	2.349	0.958	0.014	0.970	14.37	1.065
F4	0.935	2.669	0.976	0.017	0.980	16.31	1.289
F5	0.935	2.131	0.970	0.013	0.984	13.05	0.907
FD1	0.945	2.539	0.985	0.018	0.991	15.52	1.028
FD2	0.917	3.165	0.975	0.023	0.967	19.39	1.160
FD3	0.844	3.277	0.955	0.027	0.958	18.92	0.756
FT1	0.950	3.876	0.979	0.039	0.982	23.52	1.156
FT2	0.986	2.985	0.982	0.025	0.985	17.80	0.728
FT3	0.990	2.877	0.976	0.021	0.952	16.84	0.801
FE1	0.995	2.766	0.981	0.020	0.971	16.30	0.885
FE2	0.966	3.401	0.982	0.027	0.964	20.28	1.191
FE3	0.956	3.316	0.975	0.030	0.996	20.19	0.981
FO1	0.939	2.581	0.979	0.018	0.990	15.82	1.089
FO2	0.975	3.263	0.988	0.025	0.979	19.47	1.332
FO3	0.987	3.035	0.984	0.021	0.960	17.86	1.128

In-Vitro Drug Permeation Studies

In-vitro drug release studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 22 ml. On the basis of *in vitro* dissolution studies, the best formulations F4, FD3 and FE3 was selected from every batches for *in vitro* permeation studies across excised cellophane membrane. The samples were withdrawn at different time intervals and analyzed for drug content in U.V. Spectrophotometer at 303 nm.

The permeation enhancers like DMSO, tween-80, and eucalyptus oil were evaluated and their effectiveness was determined by comparing the *in vitro* permeation and steady state flux of Methotrexate from transdermal patches with and without enhancer (control patch). The *in vitro* permeation profile is presented in Table 6.10

The DMSO demonstrated a cummulative amount of drug permeated was 637.78 ± 31.63 µg/cm2 in 24 h with flux of 27.39 ± 1.76 µg/cm2/h and Tlag was 1.65 ± 0.82 h. There was enhancement of 2.73 times. DMSO is an effective penetration enhancer that promote.

The transdermal patches containing tween-80 as permeation enhancer showed highest cummulative amount of drug permeated 1020.29 ± 40.88 µg/cm2 in 24 h with flux 43.91 ± 1.29 µg/cm2/h and enhancement of 4.36 times and Tlag was 0.72 ± 0.58 h. Tween-80 is a nonionic surfactant.

Table 7: *In vitro* permeation studies of Methotrexate from transdermal patches.

Time (h)	Cummulative amount of drug permeated (µg/cm2)				
	F4	FD3	FT1	FE3	FO2
1.	1.22 ± 0.38	3.42 ± 1.67	13.59 ± 2.58	6.41 ± 2.13	1.57 ± 0.48
2.	4.47 ± 1.17	14.22 ± 3.47	57.43 ± 6.97	32.34 ± 3.72	3.46 ± 0.83
3.	13.09 ± 2.90	40.20 ± 6.08	105.21 ± 8.77	87.85 ± 12.43	13.60 ± 2.70
4.	18.96 ± 2.35	73.53 ± 9.10	147.77 ± 16.06	110.32 ± 23.61	42.73 ± 5.26
5.	27.49 ± 4.59	109.02 ± 8.68	289.20 ± 36.40	143.87 ± 22.35	68.09 ± 9.29
6.	48.22 ± 3.78	141.14 ± 20.56	331.58 ± 22.47	180.30 ± 17.86	121.58 ± 31.12
8.	69.26 ± 7.90	167.06 ± 22.57	426.39 ± 32.22	257.31 ± 36.20	150.71 ± 27.90
10.	93.11 ± 3.53	232.83 ± 35.26	492.86 ± 46.76	314.89 ± 30.39	193.91 ± 15.04
12.	120.13 ± 6.10	295.92 ± 29.01	543.80 ± 29.69	484.24 ± 58.34	251.57 ± 44.28
18.	173.19 ± 8.56	438.55 ± 51.60	809.75 ± 59.61	666.23 ± 52.39	322.89 ± 62.36
24.	217.19 ± 4.33	637.8 ± 31.63	1020.9 ± 40.88	806.86 ± 60.25	437.84 ± 17.18

The several studies have been reported that, the essential oil like eucalyptus oil enhance the several time permeability of both lipophilic as well as hydrophilic drug. This could be probably due to binding on the stratum corneum and enhancement of the lipophilic drug

penetration by increasing the partition coefficient and hydrophilic drug penetration by increasing diffusion coefficient. It has been found to increase skin permeation by disrupting intracellular lipid in stratum corneum membrane. The result of Methotrexate permeation a lipophilic drug also support above observation. The formulation FE3 containing 10% eucalyptus oil demonstrated a cummulative amount of drug permeated 806.60 ± 60.25 µg/cm^2 in 24 h with flux 36.70 ± 2.63 µg/cm2/h and enhancement of 3.64 times and Tlag was 0.95 ± 0.36 h.

Among the penetration enhancers evaluated the following order was obtained tween-80>eucalyptus oil>DMSO>olive oil. Transdermal patches FT1 showed significantly *(P<0.05)* highest cumulative amount of drug permeated 1020.29 µg/cm2 in 24 h with flux 43.91 µg/cm2/h than control formulation F4 showed 217.19 µg/cm2 in 24 h with flux 10.06 µg/cm2/h. Although not as good as tween-80, the eucalyptus oil also exhibited good enhancing effects.

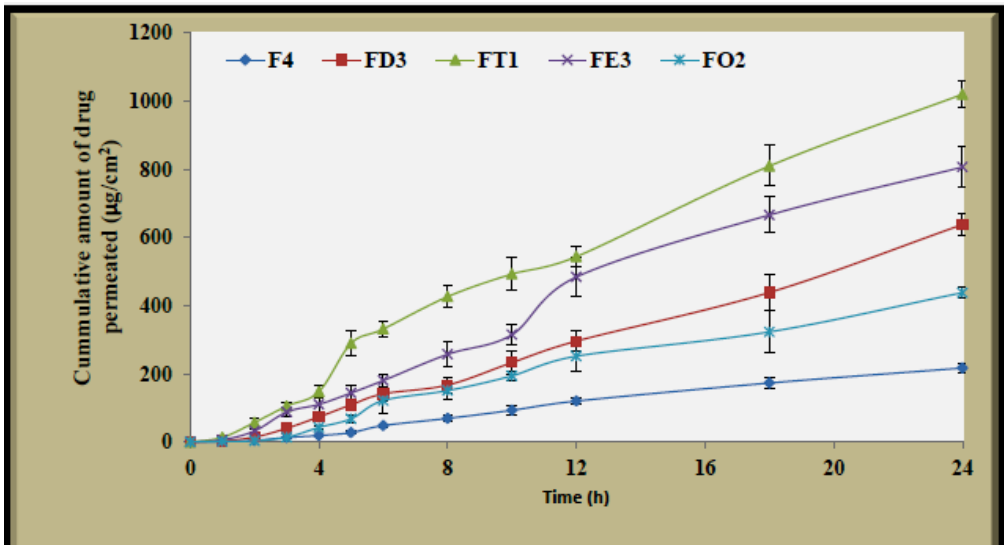

Figure 9: *In vitro* permeation studies of Methotrexate from transdermal patches.

Stability Studies of Methotrexate Patch (FT1)

The stability study of optimized formulation (FT1) was conduct according to ICH guidelines; the formulation was stored at 40 °C and 75 % relative humidity for 3 months. The result indicated that no change in physical appearance was observed after 90 days. The drug content of the patch was found 97.11, 96.91 and 96.84% after 30, 60 and 90 days respectively, indicated that no significant *(p >0.05)* change after 3 months. So on the basis of results, the optimized Methotrexate transdermal patch (FT1) was found stable enough.

CONCLUSION

The objective of the present study was to develop transdermal matrix patch of Methotrexate and assess its feasibility for transdermal application. The low permeability of skin is the rate-limiting step for delivery of most of the drugs. Studies were carried out to investigate the effect of permeation enhancers like DMSO, tween-80, eucalyptus oil and olive oil on the permeation of Methotrexate from matrix patch through skin. The formulations were subjected to *in vitro* drug release, *in vitro* permeation studies, and stability studies. The results of Methotrexate transdermal matrix patch showed that the most promising formulation was FT1 (formulation containing EC: PVP, 3:2; Methotrexate 20%; dibutylpthalate 30% and 2% tween-80 all in %w/w). This formulation was able to deliver drug up to 24 h at a flux of 43.91 µg/cm2/h across rat skin.

Thus optimized transdermal matrix patch of Methotrexate using polymers such as EC and PVP with tween-80 as permeation enhancers demonstrated their ability to give sustained release, because of excellent release and permeation of drug and its influence on antidepressant efficacy. The developed formulation of Methotrexate is expected to improve the patient compliance, form better dosage regimen and provide maintenance therapy to patients suffering from cancer. These promising results showed the feasibility of delivering Methotrexate through transdermal matrix patch.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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