—Regular Articles—

Design and Development of Hydroxypropyl Methycellulose (HPMC) Based Polymeric Films of Methotrexate: Physicochemical and Pharmacokinetic Evaluations

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The present investigation was aimed to evaluate the possibility of using different concentrations and polymeric grades of hydroxypropyl methylcellulose (K4M, K15M and K100M) for transdermal delivery of methotrexate, an immunosuppressant drug for rheumatoid arthritis. The matrix films were evaluated for their physicochemical characterization followed by *in vitro* and *in vivo* evaluation. Selected formulations were subjected for their *in vivo* studies on healthy rabbits following balanced incomplete block design. The relevance of difference in the *in vitro* dissolution rate profile and pharmacokinetic parameters (C_{max}, t_{max}, $AUC_{(s)}$, $t_{1/2}$, K_{el}, and MRT) were evaluated statistically. The thickness and weight of the patch increased with the increase in polymeric grade and content. Fourier transform infrared spectroscopy and differential scanning calorimetry results confirm that there is no interaction between drug and polymer used. X-ray diffraction study reveals an amorphous state of drug in the matrix films. The *in vitro* drug release followed Higuchi kinetics ($r=0.972$–997; $p<0.001$) as its coefficient of correlation values predominates over zero order and first order release kinetics. *In vitro* dissolution profiles and pharmacokinetic parameters showed a significant difference between test products ($p<0.01$), but not within test products. A quantitatively good correlation was found between per cent of drug absorbed from the transdermal patches and $AUC_{(s)}$. A significant *in vitro*/*in vivo* correlation was observed when per cent drug released was correlated with serum drug concentration. Out of the various formulations made, the selected formulations are better in their *in vitro* dissolution and pharmacokinetic characteristics and thus hold potential for transdermal delivery.

Key words——transdermal drug delivery; methotrexate film; cygnus' sandwich patch holder; interaction study; *in vitro* and *in vivo* evaluation

INTRODUCTION

The success of Transdermal Therapeutic System has created much interest in the pharmaceutical industry and has activated research activities related to it. In the present decade, a good number of drugs have been reported for their transdermal applications: scopolamine, nitroglycerin, nicotine, estrogen, testosterone, fentanyl, buprenorphine, lignocaine, clonidine, oxybutynin and diclofenac.[1] These drugs are being investigated and/or developed for transdermal therapeutic system either for academic research or for commercial purpose.[1,2] Transdermal administration of drugs avoids many of the problems that arise with conventional oral route and with the more invasive methods of drug delivery. Transdermal delivery is best suited for drugs, which display high toxicity and/or narrow therapeutic windows.[1,3]

The present work is aimed at the development of matrix-type transdermal drug delivery system of methotrexate,[4] an antifolate class of antineoplastic agent, which acts by inhibiting the enzyme dihydrofolate reductase. Methotrexate is currently being used as one of the most widely prescribed drugs for the treatment of rheumatoid arthritis (RA) due to its efficacy and safety.[5] However, even at low and intermittent doses, oral administration of methotrexate exhibits high inter-individual variability, gastrointestinal and hepatic toxicity. Whereas intravenous route results in systemic toxicity, intraarticular route is painful, and iontophoretic administration causes irreversible damage of skin.[6,7] Weekly pulse therapy with low dose methotrexate as an immunosuppressant is now the first-line therapy for the treatment of RA not responsive to non-steroidal anti-inflammatory drugs alone.[5,6] Along with weekly pulse therapy, patients are being supplemented with folic acid without compromising the methotrexate efficacy. Folic acid/folinic acid is known to inhibit the effect of methotrexate. At present its main targets are largely unknown.[8] The

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low molecular weight (454.5) would theoretically allow the transdermal delivery of the 5–15 mg once a week, to treat RA. Methotrexate in hydrophilic gels with or without penetration enhancer showed promising clinical results. There is insufficient information available on factors that might contribute to the skin permeation and systemic delivery of methotrexate suitable for a specific therapeutic need.[8] Treatment of RA required a longer duration, hence the use of chemical penetration enhancers should be restricted in topical formulation of methotrexate, as it may produce some undesired effects on skin. The skin enzymes get deactivated in the presence of some penetration enhancers.[9] Moreover, high potency of methotrexate, chronic nature of disease, and inter and intra-patient absorption variability, strongly supports a rationale to develop non-invasive topical delivery system of methotrexate for the treatment of rheumatoid arthritis.

An appropriate selection of polymer matrix is necessary in order to develop successful transdermal drug delivery system. The primary objective of the present study was to design and develop a transdermal patch of methotrexate employing varying concentrations of HPMC K4M, K15M and K100M with plasticizer glycerin.

MATERIALS AND METHODS

Materials Hydroxypropyl methycellulose (HPMC) K4M, K15M and K100M were gifts from Colorcon Ltd, UK. Methotrexate (MTX) was a gift from Dabur Research Foundation, Ghaziabad, India. ScotchPak™ 1022 release liner and ScotchPak™ 1109 backing membrane were obtained from 3M Drug Delivery Systems, MN, USA. Other solvents and reagents used were of AnalaR grade.

Preparation of Transdermal Films Matrix type transdermal patches containing methotrexate were prepared using varying concentrations of HPMC K4M, K15M, and K100M individually, keeping drug concentration constant (5 mg/2.25 cm^2 patch). The drug : polymer ratios used were 1 : 2, 1 : 3, 1 : 4, 1 : 5, 1 : 6, and 1 : 7. The required amount of drug and polymer were dispersed separately in casting solvent (acetone : distilled water in 9 : 1 ratio) and the polymeric dispersion was sonicated for 2 min, to remove entrapped air bubbles. These two were then mixed and glycerin (150% w/w of polymer weight) was incorporated as plasticizer. The polymeric dispersion of drug was poured into a glass mould (6 cm×6 cm) fabricated in the laboratory. To control the rate of evaporation of solvent, the mould was covered with a funnel of suitable size. The casting solvent was then allowed to evaporate overnight to obtain the dried films. The films were cut into small patches containing equivalent of 5 mg of drug per patch. Backing membrane was then glued and the films were stored between sheets of wax paper in desiccators until further evaluations.

Physicochemical Characterization

Thickness The thickness of the films was assessed using digital instrument (FT–1000 Spectralab, India). The polymeric film was placed on iron surface and the probe was placed on film surface. The apparatus was calibrated with calibration film of 103±3 μm (Spectralab, India). The thickness was measured at randomly chosen 5 different points of each patch in order to ensure uniform thickness.

Weight Variation The patches were subjected to weight variation by individually weighing ten randomly selected patches. Such determinations were carried out for each formulation.

Film Folding Endurance This was determined by repeatedly folding of the patches at the same place until it shows any crack or break. The number of times the film could be folded without breaking/cracking gave the value of folding endurance. Five randomly selected patches of each formulation were tested.

Flatness Longitudinal stripes from the 5 randomly selected medicated films of each formulation were cut out. The length of each strip was measured, and variations in the length due to non-uniformity of flatness were measured. Flatness was calculated by measuring constriction of strips using the formula

$$\% \text{ Constriction} = \frac{l_1 - l_2}{l_2} \times 100$$

Where,

l_1=initial length; l_2=cutted film length

0% constriction was considered to be 100% flatness.

Content Uniformity Assay of each of the ten randomly selected medicated patches was carried out to determine the drug content. The patch was dissolved in 2 ml of the casting solvent and the volume was adjusted to 100 ml with 0.1*N* NaOH. The solution was filtered, suitably diluted, and content per film was estimated spectrophotometrically (UV-1700 PharmaSpec, Shimadzu, Japan) at 303.0 nm using

standard curve with OD=0.0522×conc.+0.0162 (r =0.9998; $p<0.001$).

Surface pH The films were kept in contact with 0.5 ml of distilled water for 1 h. The surface pH was measured by means of pH paper placed on the surface of the swollen patch. A mean of two readings was recorded.

Fourier Transform Infrared Spectroscopic Studies A IR Prestige-21 FTIR (Shimadzu, Japan) spectrometer equipped with attenuated total reflectance (ATR) accessory was used to obtain the infrared spectra of drug matrix as well as placebo films. Analysis of pure drug, polymers and their physical mixture (in 1 : 1 ratio) was carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr. All the powder samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For each spectrum, 32 scans were obtained at a resolution of 4 cm^{-1} from 4000–600 cm^{-1}.

Differential Scanning Calorimetric Studies Thermal analysis was carried out using differential scanning colorimeter (Q10, TA Instruments, Waters Inc., USA) with a liquid nitrogen cooling accessory. The analysis was performed under purge of dry nitrogen gas (50 cc min^{-1}). High purity indium was used to calibrate the heat flow and heat capacity of the instrument. Sample (2.5–5 mg) placed in aluminum crucible cell was firmly crimped with the lid to provide an adequate seal. The sample was heated at from ambient temperature to 250℃ at pre-programmed heating rate of 10℃ min^{-1}.

All the samples were analyzed in the same manner. In case of two component systems, physical mixtures of individual component (150 μm) in equal weight ratios were prepared in glass mortar and pestle.

X-ray Diffraction Studies The X-ray diffractometer (Regaku-Miniflex, Japan) used consisted of 30 kV, 15 mA generator with Cu-Kα radiation anode tube. Diffraction pattern of pure drug, placebo films and drug loaded matrix films (1 : 2 ratio) were scanned over 2θ range of 2° and 60° at a rate of 2° per min in 0.02° 2θ step size.

***In Vitro* Dissolution Studies** The *in vitro* dissolution study of each selected transdermal patch was determined on USP dissolution apparatus equipped with a fractional collector (TDT-08L, Electrolab, India). A Cygnus' sandwich patch holder, a slightly modified form of FDA's sandwich patch holder was used to ensure patch-to-patch reproducibility of transdermal film.[10,11] The dissolution vessels contained 500 ml of phosphate buffer pH 7.4 maintained at 32±0.5℃ (the skin surface temperature) and paddle speed set at 50 rpm. Patch assembly was carefully placed at the bottom of the vessel and was centered using a glass rod. Five ml sample was withdrawn at one hour time intervals until the completion of drug release. The withdrawn sample was replenished with 5 ml of fresh media.

The withdrawn samples were analyzed for drug content by measuring the absorbance at 303.0 nm using UV-Visible spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan). Three such determinations were carried out for each formulation. The content of methotrexate was calculated from the standard curve [O.D.=0.0525×conc.+0.0136 (r= 0.9997; $p<0.001$)]. The *in vitro* dissolution profiles, namely, cumulative drug release, dissolution rate constant, and dissolution half life ($t_{50\%}$) were calculated.

Skin Irritation Studies Skin irritation studies were carried out in the healthy rabbits weighing 1.934 ±0.166 kg. The dorsal surface of the rabbits was cleared and hair was removed by shaving. The patches were placed over the skin with the help of surgical adhesive tape. They were removed after 24 h and the skin was examined for any untoward reaction.

***In Vivo* Studies** The test formulations were tested for their bioavailability on nine healthy rabbits weighing 1.934±0.166 kg following balanced incomplete block design. The protocol of the study in rabbits was approved by the institutional animal ethical committee (Reg. No. 621/02/ac/CPCSEA), Birla Institute of Technology, Mesra, Ranchi, India. The hair of a skin area of around 50 cm^2 was shaved covering both sides of the vertebral column of each rabbit and care was taken to avoid the damage of skin during shaving. The formulation was applied on the shaved surface 24 hours after hair removal. Blood samples were collected from ear vein prior to application of films and then at 1, 2, 4, 6, 8 and 24 h post application of films. Due to the small size of the animal and damage to the ear vein, it was not advisable to withdraw the blood for more than the above mentioned intervals. The withdrawn blood sample was set aside for 15 min and then centrifuged for 10 min at 5000 rpm. The supernatant serum samples were stored in well closed tubes under refrigeration (−20℃) until further analysis.

A previously reported spectrophotofluorometric

procedure with some modifications were used to analyze methotrexate in the serum samples.[12] Acquisition of kinetic data and fluorescent measurement were made on a spectrofluorophotometer (RF 5301 PC, Shimadzu, Japan) equipped with RF-530XPC computer software at $\lambda_{exc}=375$ nm and $\lambda_{em}=456$ nm. Excitation and emission spectra were recorded in a 10 mm quartz cell at ambient temperature conditions. The pharmacokinetic parameters were calculated using non-compartmental pharmacokinetics data analysis software, WinNonlin™ version 5.0.1.

Statistical Evaluations The relevance of difference in the *in vitro* dissolution rate profile and pharmacokinetic parameters was evaluated statistically. The data were tested by two-way analysis of variance.

RESULTS AND DISCUSSION

Matrix dispersion type transdermal films of methotrexate were prepared using varying ratio of drug : polymer (HPMC K4M, K15M and K100M) to get the desired drug release profile. The thickness, weight variation, and drug content values of the formulations made are shown in Table 1. Irrespective of the grade and concentration of HPMC used, the drug content per patch was found within 4.896 to 4.972 mg per patch, but the thickness and weight of the patch increased with the increase in polymer content. The results of flatness study showed that none of the formulations had the difference in the strip lengths before and after longitudinal cut, indicating 100% flatness, and thus they could maintain a smooth surface when applied onto the skin. Folding endurance values of matrix films found more than 250 indicating good strength and elasticity, which is explained by the linear nature of the cellulose structure. The surface pH of all formulations was in the range of 5.5–6.0, the pH range of skin and hence no skin irritation was expected. Amongst the various formulations made, using different concentrations and grades of HPMC, six formulations (A–F) were selected on the basis of drug content and release pattern for their *in vivo* studies.

Table 1. Physical Characteristics of Methotrexate Transdermal Patches

Sr. No.	Composition	Thickness (μm)	Weight (mg)	Content (mg)
	MTX : K4M			
1.	1 : 2	6.22 (0.833)	36.661 (0.199)	4.921 (0.011)
2.	1 : 3	6.45 (1.035)	45.436 (0.239)	4.975 (0.013)
3.	1 : 4	110.13 (0.853)	55.871 (0.212)	4.966 (0.016)
4.	1 : 5	169.60 (1.174)	78.373 (0.167)	4.942 (0.010)
5.	1 : 6	259.30 (0.943)	84.507 (0.169)	4.972 (0.011)
6.	1 : 7	324.91 (0.865)	101.932 (0.309)	4.957 (0.012)
	MTX : K15M			
7.	1 : 2	68.70 (0.823)	39.666 (0.199)	4.904 (0.015)
8.	1 : 3	113.60 (1.173)	47.514 (0.155)	4.924 (0.014)
9.	1 : 4	157.81 (0.919)	59.173 (0.205)	4.933 (0.011)
10.	1 : 5	223.88 (0.782)	83.86 (0.251)	4.896 (0.012)
11.	1 : 6	309.90 (0.876)	102.462 (0.187)	4.946 (0.012)
12.	1 : 7	367.60 (0.576)	115.671 (0.242)	4.931 (0.024)
	MTX : K100M			
13.	1 : 2	78.91 (0.876)	40.836 (0.189)	4.898 (0.010)
14.	1 : 3	138.80 (0.789)	48.255 (0.121)	4.948 (0.018)
15.	1 : 4	181.93 (0.875)	63.328 (0.181)	4.962 (0.021)
16.	1 : 5	250.38 (1.059)	86.574 (0.116)	4.924 (0.011)
17.	1 : 6	343.72 (0.823)	110.732 (0.211)	4.931 (0.010)
18.	1 : 7	399.00 (1.054)	121.363 (0.190)	4.963 (0.017)

Values in parentheses indicate standard deviation; Mean of 10 readings.

Drug Polymer Compatibility Study Apart from physical characteristics, compatibility between a drug and polymer is a factor in determining the effectiveness of polymeric delivery systems. Herein to consider compatibility between polymer and drug we refer to solubility and/or interaction with no alteration in the chemical structure of the polymer or the drug.[13] Because each drug has its own characteristic chemical and physical properties, no delivery vehicle prepared from a particular polymer will serve as universal carrier for all drugs. The possible drug-polymer interaction was studied by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) analysis of pure substances, placebo films and drug loaded matrix films.

Fourier Transform Infrared Spectroscopy The DRS-FTIR spectrum of physical mixture of drug : polymer (1 : 1) compared with individual spectrum

of HPMC K15M and pure MTX (Fig. 1). The DRS-FTIR spectral analysis of methotrexate showed the principal peaks at about 1693 cm^{-1} (-COOH), 1643 cm^{-1} (-CO-NH), 1539, 1500 cm^{-1} (aryl system), and 831 cm^{-1} (aromatic ring system) confirming the purity of the drug as per established standards.[14,15] The DRS-FTIR spectra of physical mixture seemed to be only a summation of drug and HPMC K15M spectra. The DRS-FTIR spectrum of physical mixture, showed the major peaks which correspond to drug and polymer respectively.

Further, the ATR-FTIR spectra of drug loaded matrix films prepared from varying grade of HPMC polymers were compared with individual spectrum of placebo polymeric films and pure MTX (Fig. 2). ATR is quick and non-destructive sampling technique for obtaining the IR spectrum of materials surface.[16] This technique required minimum, or no, sample preparation, but an intimate optimal contact between the sample and the ATR crystal is crucial. ATR-FTIR spectra of placebo HPMC films gave the characteristic peaks for HPMC at about 1643, 1109 and 1033 cm^{-1} vibration region. In the drug loaded matrix films the characteristic peaks for HPMC and MTX were preserved. In the films the intensity of peaks corresponding to drug were reduced, may be due to the inhibition of the methotrexate crystal growth. The reduction in peak intensity and broadening may occur due to change of crystalline nature of drug to amorphous state, which is in agreement with those reported by Kotiyan and Vavia.[17] Formation of amorphous state of drug in matrix film was further verified by X-ray diffraction analysis. As the characteristic peaks of drug and polymer were preserved in the physical mixture and matrix films, particularly in the fingerprint region, and also no new peaks were found, no interaction is inferred. These results are in agreement with our earlier work reported in literature.[18]

In this study, it has been observed that there is no chemical interaction between methotrexate and polymers used. Some changes in the peaks are observed, which indicate that there may be some physical interaction related to the formation of weak to medium intensity hydrogen bonding between polymers and drug, but *in vitro* release studies showed that this type of interaction did not interfere with the release of drug from different grades of HPMC polymer matrix.

Differential Scanning Calorimetry The DSC was used in order to detect formulation incompatibilities resulting from drug: polymer interactions. The thermograms of the pure drug, pure polymer (HPMC K4M, K15M, and K100M) and their physical mixtures at 1 : 1 ratio are shown in Fig. 3. In pure methotrexate, a broad endothermic effect was observed over a temperature range of 40–145℃ may be due to hydrate form of MTX.[14] A broad endothermic peak, due to the dehydration process was also observed over a temperature range of 60–120℃ for all grades of HPMC polymers. Such dehydration endotherm was also observed in case of binary physical

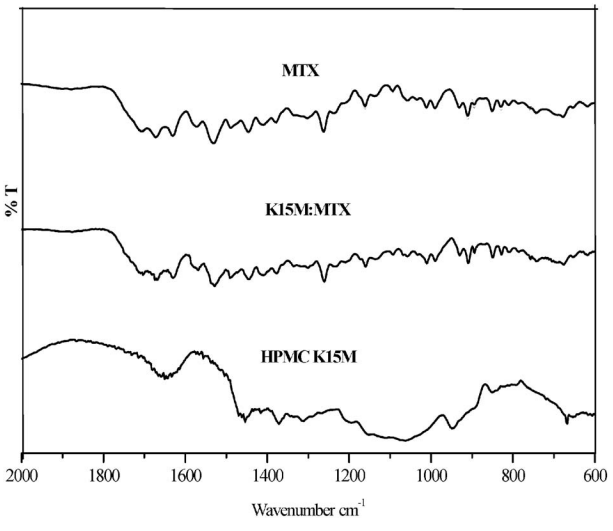

Fig. 1. DRS-FTIR Spectra of Pure Methotrexate, Pure HPMC K15M and their Physical Mixture (1 : 1)

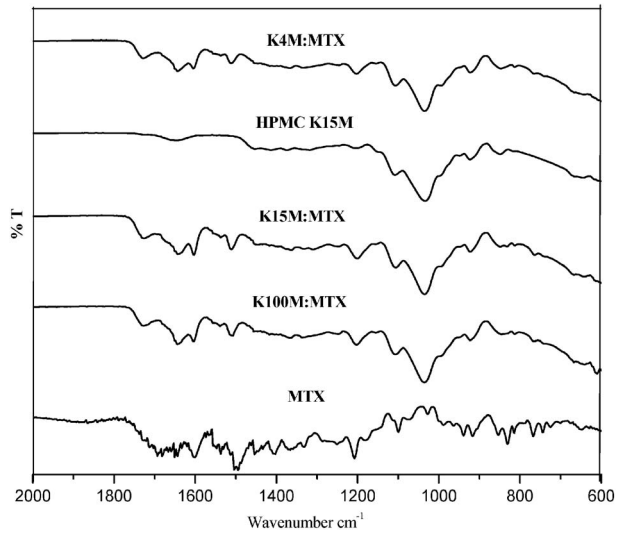

Fig. 2. ATR-FTIR Spectra of Pure Methotrexate, Placebo Film of HPMC K15M and Matrix Films of Methotrexate: HPMC (K4M, K15M and K100M)

mixture. Further, in the binary mixtures, the drug melting signal was clearly distinguishable (Fig. 3). On the other hand pure HPMC polymers did not show any endothermic peak in this region. Since no new endothermic events were observed in physical mixture, one can state that no incompatibility was found between drug and polymer used. This study indicates that, these polymeric grades are well established in formulating matrix type patches because of their compatibility.[19]

X-ray Diffractometry X-ray diffraction of pure drug, placebo films and drug loaded matrix films are shown in Fig. 4. In the matrix films the drug and polymer was used in the ratios of 1 : 2. The X-ray diffractogram illustrates the crystalline nature of methotrexate. Numerous distinctive sharp peaks occurred for methotrexate at approximately 2θ angles of 28, 29, 39 and 45 degrees. Placebo films made up of different grades of HPMC exhibited just one broad peak with low intensity at 2θ 10–35° (Fig. 4), and drug loaded matrix films also illustrate a similar broader peak, with lessened intensity. This indicates an almost amorphous state of these polymers and drug. The nature of the drug and change of its physical state from crystalline to amorphous also indicates that the drug was well dispersed in the polymeric matrix films.

***In Vitro* Dissolution** From the *in vitro* dissolution profile data, kinetics of drug release was found

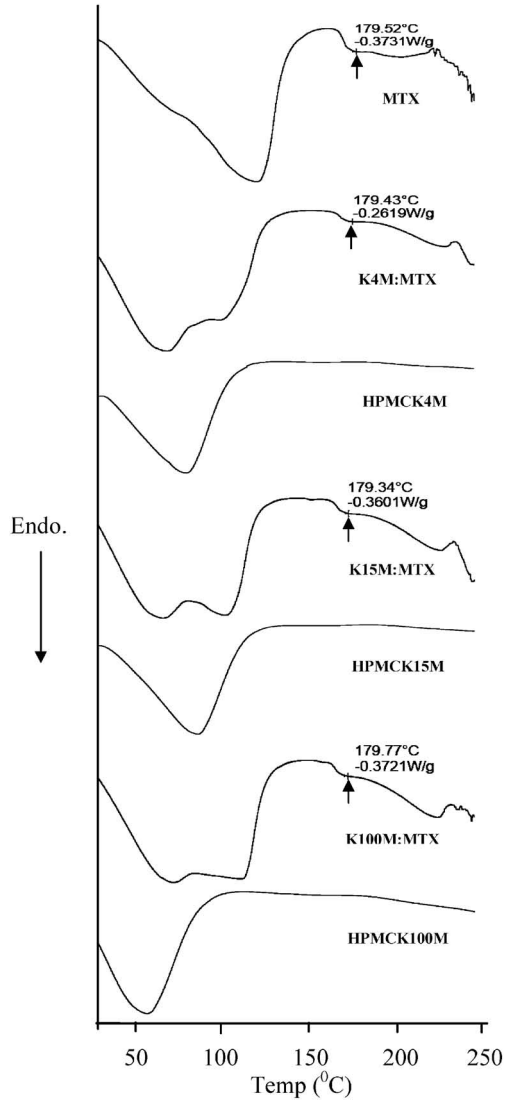

Fig. 3. DSC Thermograms of Methotrexate, Physical Mixtures of Methotrexate-HPMC (MTX-K4M, MTX-K15M and MTX-K100M) at 1 : 1 ratio and Pure HPMC K4M, K15M, and K100M

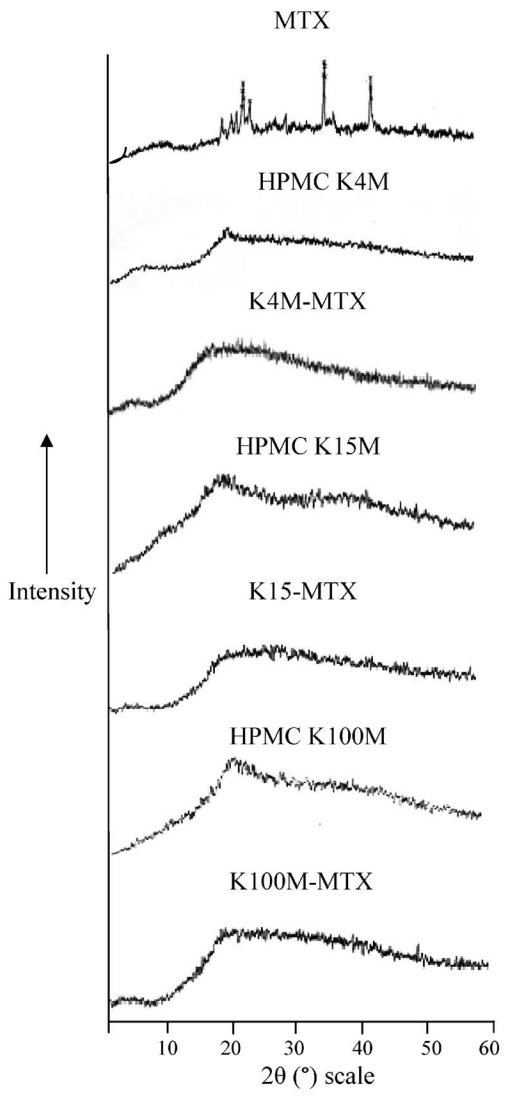

Fig. 4. X-RD Spectra of Pure Methotrexate, Placebo HPMC Films (K4M, K15M, and K100M) and Matrix Films of Methotrexate with HPMC

for zero-order (K_0), first-order (K_1) and Higuchi-type (K_h) release kinetics. Korsmeyer-Peppas semi-empirical model were also employed, in order to better characterize the drug release behavior.[20]

$$M_t/M_\infty = k\ t^n$$

Where, M_t/M_∞ is the fractional release of drug in time t, k is a constant incorporating structural and geometric characteristics of the controlled-release device, and n (the release exponent) is a parameter indicative of mechanism of drug release.

The coefficient of correlation of each of these release kinetics were calculated and compared (Table 2). The data revealed that the release pattern of formulations are best fitted for Higuchi kinetics equation as the formulation coefficient of correlation values predominates over zero-order and first order release kinetics. A significant linear correlation (0.972–0.997, $p<0.01$), was found for Higuchi-type release

Table 2. Dissolution Characteristics of the Methotrexate from Different TD Films

Sr. No.	Formulation	Zero-Order		First-order		Higuchi-Matrix		K-P	$t_{50\%}$ (h)
		K_0 (h^{-1})	r	K_1 (h^{-1})	r	K_H ($h^{-1/2}$)	r	n	
	MTX : K4M								
1.	1 : 2	11.186 (0.671)	0.755 (0.015)	0.886 (0.007)	0.931 (0.015)	22.238 (0.911)	0.972 (0.018)	0.324 (0.042)	0.52 (0.122)
2.	1 : 3	10.385 (0.738)	0.782 (0.014)	0.742 (0.011)	0.946 (0.017)	20.224 (0.866)	0.976 (0.015)	0.334 (0.012)	0.81 (0.136)
3.	1 : 4	9.214 (0.561)	0.838 (0.017)	0.581 (0.005)	0.948 (0.012)	19.712 (0.765)	0.981 (0.022)	0.344 (0.033)	1.12 (0.131)
4.	1 : 5	7.993 (0.588)	0.841 (0.013)	0.432 (0.010)	0.953 (0.025)	17.778 (1.166)	0.986 (0.013)	0.379 (0.051)	1.18 (0.168)
5.	1 : 6	7.131 (0.658)	0.852 (0.008)	0.383 (0.009)	0.967 (0.016)	17.448 (1.038)	0.989 (0.014)	0.409 (0.028)	1.55 (0.184)
6.	1 : 7	6.357 (0.515)	0.857 (0.009)	0.322 (0.006)	0.967 (0.018)	16.439 (0.885)	0.990 (0.011)	0.441 (0.044)	1.82 (0.184)
two-way ANOVA 'p'		—	—	—	—	<0.01	—	—	<0.01
	MTX : K15M								
7.	1 : 2	9.134 (0.766)	0.810 (0.022)	0.767 (0.006)	0.952 (0.019)	19.597 (1.033)	0.987 (0.018)	0.406 (0.023)	0.91 (0.116)
8.	1 : 3	7.888 (0.555)	0.829 (0.014)	0.654 (0.007)	0.959 (0.018)	18.175 (0.995)	0.989 (0.016)	0.417 (0.045)	1.23 (0.128)
9.	1 : 4	7.184 (0.608)	0.851 (0.015)	0.505 (0.009)	0.963 (0.018)	17.757 (1.122)	0.989 (0.012)	0.423 (0.038)	1.36 (0.148)
10.	1 : 5	6.422 (0.561)	0.891 (0.016)	0.405 (0.006)	0.966 (0.022)	16.754 (1.014)	0.993 (0.023)	0.427 (0.055)	1.61 (0.166)
11.	1 : 6	6.231 (0.432)	0.904 (0.012)	0.318 (0.007)	0.978 (0.016)	16.121 (1.165)	0.993 (0.014)	0.449 (0.052)	1.92 (0.158)
12.	1 : 7	5.545 (0.514)	0.926 (0.008)	0.182 (0.011)	0.989 (0.015)	14.993 (0.894)	0.996 (0.010)	0.462 (0.026)	2.22 (0.172)
two-way ANOVA 'p'		—	—	—	—	<0.01	—	—	<0.01
	MTX : K100M								
13.	1 : 2	7.831 (0.515)	0.818 (0.015)	0.714 (0.008)	0.961 (0.017)	18.011 (1.261)	0.991 (0.016)	0.430 (0.033)	1.32 (0.132)
14.	1 : 3	6.938 (0.462)	0.844 (0.017)	0.607 (0.005)	0.968 (0.013)	17.007 (1.112)	0.991 (0.013)	0.441 (0.045)	1.48 (0.128)
15.	1 : 4	6.166 (0.356)	0.861 (0.021)	0.458 (0.007)	0.968 (0.018)	15.940 (1.025)	0.994 (0.014)	0.450 (0.028)	1.86 (0.138)
16.	1 : 5	5.995 (0.488)	0.898 (0.013)	0.332 (0.005)	0.977 (0.016)	15.395 (0.821)	0.997 (0.014)	0.479 (0.031)	2.14 (0.164)
17.	1 : 6	4.657 (0.394)	0.933 (0.018)	0.240 (0.006)	0.984 (0.015)	13.758 (0.908)	0.996 (0.015)	0.490 (0.048)	2.57 (0.188)
18.	1 : 7	4.399 (0.336)	0.961 (0.011)	0.126 (0.008)	0.990 (0.017)	12.885 (1.005)	0.997 (0.007)	0.513 (0.022)	3.00 (0.184)
two-way ANOVA 'p'		—	—	—	—	<0.01	—	—	<0.01

Values in parentheses indicate±SD ($n=3$). K_0=zero-order; K_1=first-order; and K_H=Higuchi-type dissolution rate constant; K-P Korsmeyer-Peppas exponential (n) values.

kinetics. This complies with Higuchi's equation for drug release from a matrix; a slow and controlled release was observed, indicating that the drug-release mechanism was by diffusion, as proposed by Higuchi. Based on Korsmeyer-Peppas semi-empirical model, the best fitting was obtained with $n \leq 0.5$, indicating a Fickian release mechanism. In swellable systems, factors affecting the release kinetics are liquid diffusion rate and polymeric chain relaxation rate. When the liquid diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian; whereas when the relaxation process is very slow as compared to the diffusion, the case II transport occurs. When liquid diffusion rate and polymer relaxation rate are of the same order of magnitude, anomalous or non-Fickian diffusion is observed.[21] On the basis of these considerations, it is clear that the drug released from our formulations is controlled by polymer chain relaxation. This observation thus supports the results obtained with Higuchi's equation which shows that the patches released the drug by diffusion-dominated mechanism.

The observed initial burst release in formulations may be accounted to the direct exposure of the matrix films to the dissolution media. A quick release of the drug present at the surface occurs and such an initial rapid release is attributed to the fact that polymeric matrix may form loose channels within the network because of its hydrophilic nature and the dissolution of hydrophilic drug during the diffusion process. The observed initial release may be helpful to achieve the therapeutic plasma concentration of the drug in a short time along with a constant release rate for a longer period of time. Initial burst release was higher in matrix films, formulated using low viscosity grade polymer (HPMC K4M), compared to higher viscosity grade polymer (HPMC K15M and HPMC K100M). Due to initial burst effects, low dissolution half life ($t_{50\%}$) was found in case of transdermal films formulated with HPMC K4M. $t_{50\%}$ values increases linearly with increase in concentration and viscosity grade of polymer.

The dissolution rate constant was calculated for all six products of each polymeric grade and compared (Table 2). The dissolution rate constant and $t_{50\%}$ data showed a significant difference between the test products ($p<0.01$), but not within the test products ($p>0.1$), indicating that the six sets of data differ significantly. Hence, it can be inferred that the products are not same in their formulations. On the basis of physico-chemical characterization and *in vitro* release profile, six formulations were selected for their comparative *in vivo* studies.

***In Vivo* Studies in Rabbits** The skin irritation study indicated that neither the polymer nor the drug caused any noticeable irritation or inflammation on or around the patch area either during the period of study or after removal of the patch.

For a comparative bioavailability study, serum data between all test products were considered. The pharmacokinetic parameters: C_{max}, t_{max}, $AUC_{(s)}$, $t_{1/2}$, K_{el}, and MRT data are shown in Table 3. The mean plasma concentration versus time profiles of all six test products (A–F) are depicted in Fig. 5. On the basis of plasma half life, AUC ($_{0-24}$ and $_{0-\infty}$) and MRT values, the test products could be ranked as follows: F $>$ E $>$ D $>$ C $>$ B $>$ A (Table 3). The calculated parameters indicate that the biological half life ($t_{1/2}$) of methotrexate is prolonged from 4–10 h[7] to 17.07 $\pm$2.37 h (transdermal) in rabbits. Hence, the drug administered through transdermal patch will remain for longer period of time in the body and thus exert a sustained the action. The significantly less elimination rate constant $0.040 \pm 0.005\ h^{-1}$ and high mean residence time values 11.97 ± 0.73 h of methotrexate, further supports the sustained action of the drug from transdermal patches. The high AUC(s) values observed with the patches also indicate increased bioavailability of the drug, this may be due to bypass of the hepatic first pass effects and avoidance from gastric degradation. These results are in accordance with the findings reported earlier.[22,23]

Upon statistical evaluation (two-way ANOVA), a significant difference ($p<0.01$) was observed between the test products but not within the test products ($p>0.1$), when $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-\infty)}$, $t_{1/2}$, K_{el}, and MRT data generated from serum were taken into consideration, except for C_{max}, where no significant difference ($p>0.1$) was observed. Spearman rank correlation, a non–parametric statistical test,[24] demonstrated a high degree of positive correlation, showing a complete agreement in the order of ranks between percentage of drug absorbed from patches and $AUC_{(0-24)}$ ($p<0.02$; two tail) and $AUC_{(0-\infty)}$ ($p<0.02$; two tail). The increase in the amount of drug absorbed was thus associated with the increase in blood level and area under the plasma concentration curve (extent of absorption). This was

Table 3. Serum Pharmacokinetic Characteristics of Methotrexate from Different Transdermal Patches

Code	Formulation	T_{max} (h)	C_{max} ng/ml	$t_{1/2}$ (h)	K_{el} (1/h)	AUC_{0-12} (h.ng/ml)	AUC_{0-24} (h.ng/ml)	$AUC_{0-\infty}$ (h.ng/ml)	MRT (h)
	HPMC : K4M								
A	1 : 6	6	154.28 (6.7)	13.64 (10.7)	0.047 (17.7)	1207.04 (2.7)	2334.16 (6.5)	4569.70 (9.0)	11.32 (1.6)
B	1 : 7	6	163.50 (4.7)	15.53 (7.1)	0.044 (12.2)	1224.93 (1.8)	2390.48 (3.8)	4667.09 (12.3)	11.37 (3.1)
	HPMC : K15M								
C	1 : 5	6	158.70 (5.9)	16.51 (6.5)	0.042 (17.6)	1307.74 (2.0)	2481.84 (5.9)	5479.77 (15.7)	11.67 (2.2)
D	1 : 6	8	153.52 (6.6)	17.90 (10.1)	0.040 (11.3)	1254.56 (2.1)	2560.21 (6.0)	5513.35 (9.8)	11.86 (1.5)
	HPMC : K100M								
E	1 : 4	8	154.07 (4.8)	18.47 (6.3)	0.037 (18.5)	1141.62 (3.7)	2656.78 (4.6)	5890.12 (9.2)	12.33 (1.8)
F	1 : 5	8	152.38 (5.6)	20.37 (9.6)	0.032 (8.9)	1011.79 (3.6)	2710.15 (4.2)	6874.28 (13.9)	13.24 (1.7)
two-way ANOVA 'p'			>0.1 NS	<0.01 HS	<0.05 S	<0.01 HS	<0.01 HS	<0.01 HS	<0.01 HS

Values in parentheses indicate % CV (n=3). NS: not significant; S: significant; and HS: highly significant.

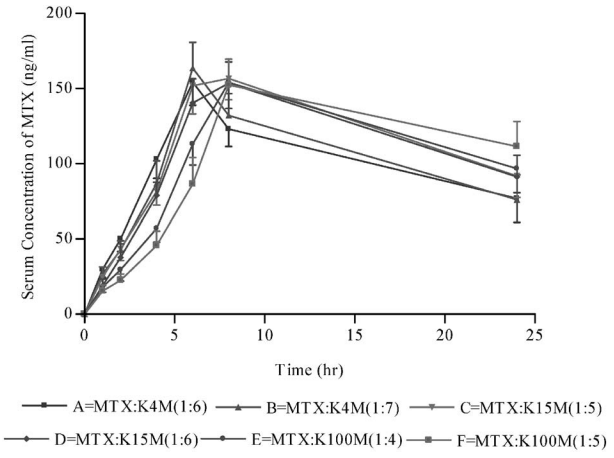

Fig. 5. Mean (±SD) Serum Concentration-Time Profile after Applying Methotrexate Transdermal Films in Rabbits (n=3)

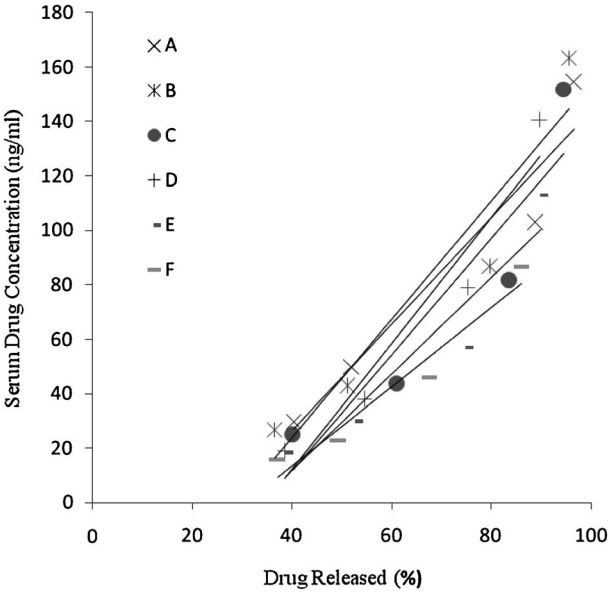

Fig. 6. A Correlation between *In Vitro* Per Cent Drug Released and Serum Drug Concentration of Selected Formulations

further quantitatively confirmed by regression of analysis showing a good correlation between percentage of drug absorbed and $AUC_{(0-24)}$ (r=0.773; $p<0.1$) and $AUC_{(0-\infty)}$ (r=0.857; $p<0.05$).

A signifiant *in vitro/in vivo* correlation was observed when per cent of drug released at a given time is correlated with plasma drug concentration. There was a point to point relationship between *in vitro* data and *in vivo* input rate of drug from the doasge form. A good correlation was observed when per cent drug released was correlated with plasma drug concentration obtained from test product A (r=0.963; $p<0.05$), B (r=0.952; $p<0.05$), C (r=0.921; $p<0.1$), D (r=0.967; $p<0.05$), E (r=0.950; $p<0.05$), and F (r=0.975; $p<0.05$) (Fig. 6).

CONCLUSION

The results of this study indicate that, polymeric grades of hydroxypropyl methylcellulose used have excellent film forming ability. Amongst the polymeric-matrix type transdermal films of methotrexate,

prepared with the different grades and ratios of HPMC and evaluated, test products A, B, C, D, E and F are better in their *in vitro* dissolution and pharmacokinetic characteristics, and thus hold potential for transdermal delivery. A slow and controlled release of drug is indicated by the fact that the per cent cumulative amount of drug release versus square root of time is found to be linear, thus supporting that the test products are suitable for transdermal films. FT-IR and DSC studies indicated no interaction between drug and polymer. The drug was distributed uniformly in the matrix and showed an amorphous nature which was confirmed using X-RD study. Further, the selected formulations, which have been duly screened, may be evaluated for their pharmacokinetic profiles in human to the best of their advantage.

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