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Research Article

DESIGN AND EVALUATION OF MATRIX TYPE OF TRANSDERMAL PATCHES OF METHOTREXATE

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ABSTRACT

The present investigation highlights the design and evaluation of matrix type transdermal patches of Methotrexate (MTX) with various polymer concentration of Hydroxypropyl methyl cellulose (HPMC) and Ethyl cellulose (EC). Methotrexate which is an anticancer drug which have the half life of about 3 hours and bioavailability of about 60% used for the treatment of cancer. F_1 and F_2 formulation with HPMC in the ratio 1:1 and 1:2, F_3 and F_4 formulation with EC in the ratio 1:1 and 1:2 were prepared. The drug polymer interaction studies were carried out and found the drug is compatible with polymer. The prepared patches were evaluated for their physicochemical characterization followed by the *in vitro* evaluation test. The various parameters like thickness weight variation, Percentage moisture absorption, Percentage moisture loss, microbial count, drug content and *in vitro* diffusion was studied. It was observed that viscosity had a major influence on drug release from hydrophilic matrixes. F_1 formulation drug release is 96% at end of 8 hours effectively when compared to EC patches which have drug release of only 86%. The F_1 formulation showed a good dissolution profile due to decrease in viscosity of polymer. The accelerated short term stability study indicated that the optimized formulation F_1 has very good stability at 40 ^{o} / 75% / RH for a period of 3 months .Hence we conclude that Methotrexate transdermal patches suits the ideal requirements of transdermal drug delivery system and also improves the patient compliance.

Keywords: Ethyl cellulose, hydroxy propyl methyl cellulose, Transdermal drug delivery system.

INTRODUCTION

Transdermal drug delivery system (TDDS) is a well accepted means of delivering many drugs to the systemic circulation and currently transdermal patch devices are used o treat motion sickness, hypertension, angina, female menopause, severe pain states[1]. Transdermal medication offers many advantages such as it delivers a steady infusion of a drug over an extended period of time[2]. It can increase the therapeutic value of many drugs by avoiding specific problem associated with drug such as GIT, low absorption, decomposition due to first pass effect. The simplified medication regimen leads to improved patient compliance and reduce inter and intrapatient variability[3, 4].

Methotrexate is a folic acid antagonist, is classified as a cytotoxic antimetabolite. Methotrexate was selected because of its shorter half-life of 3hrs and bioavailability of about 60%[5]. It has been used for many years in the treatment of various types of cancer and is also used to treat autoimmune

disorders such as rheumatoid arthritis, psoriasis, inflammatory bowel diseases[6], Wegener's granulomatosis[7], myasthenia gravis[8], bullous pemphigoid[9], polymyalgia rheumatic [10], inflammatory muscle diseases[11] and sarcoidosis[12,13]. Recently MTX has been found to be effective against chloroquine resistant and chloroquine sensitive plasmodium falciparum malaria *in vitro*[14]. It has also be found to be useful as a steroid sparing agent in granulomatous conditions such as idiopathic orbital inflammatory syndrome[15], xanthogranulomas[16] and Erdheim-Chester disease[17]. In the treatment of autoimmune conditions, methotrexate is typically given once weekly, but numerous errors were reported[18, 19]. Because of the immunosuppressive properties that make this drug so valuable in the treatment of autoimmune disorders, daily dosing can lead to serious patient harm[20]. The systemic use of this drug causes numerous side effects, the most important being the hepatic toxicity. To reduce such effects, it would be preferable to deliver methotrexate by the transdermal route. A major problem is that the drug is water soluble and is mostly in ionised form at physiological pH, so its capacity for passive diffusion is limited[21]. The objective of the present study was to design and evaluate transdermal polymeric matrix films of HPMC and EC containing Methotrexate to avoid the hepatic first pass metabolism and improve the therapeutic efficacy of the drug.

MATERIALS AND METHODS

Methotrexate was a generously gifted by M/S Dabour India Ltd., Ghaziabad. The polymers Ethyl Cellulose (EC) and Hydroxy Propyl Methyl Cellulose (HPMC) were procured from Loba Chemicals Ltd., Mumbai. All other chemicals and solvents used were of analytical grade.

Fabrication of films[22]

The solvent casting technique was used to formulate the methotrexate transdermal patch with various proportions.F1 and F2 formulation with drug: HPMC ratio- 1:1, 1:2, F3 and F4 formulation with drug: EC- 1:1, 1:2 were prepared. The drug polymer solutions were dispersed in a casting solvent (acetone: distilled water-9:1 ratio). The polymeric dispersion was poured into a glass mould (5cm x 5cm). To control the rate of evaporation of solvent, the mould was covered with a funnel of suitable size and the casting solvent was allowed to evaporate overnight to obtain the dried films .The films were cut into small patches containing equivalent of 2mg of the drug per patch and stored between sheets of wax paper in desiccators for further evaluations.

Solubility study

The solubility studies were performed in various solvents by adding excess amount of drug in each case and keeping excess drug containing solvent flask on a wrist action shakes (REMI equipment) Mumbai for 24 hour at 25°C, after 24 hour, solutions were analysed spectrophotometric using UV1700 shimadzu spectrophotometer at 303nm.

Characterization of Transdermal Patches

Physical appearance

All the prepared patches were visually inspected for color, clarity, flexibility and smoothness.

Thickness and Weight Variation[23]

The thickness of the patches was assessed at six different points using screw gauze and the average weight of three patches was calculated.

Percent Moisture Absorption[24]

The percent moisture absorption test was carried out to check the physical stability and integrity of the films at high humid conditions. In the present study the moisture absorption capacities of the films were determined in the following manner.

After 3 days the films were taken and weighed the percentage moisture absorption of three films was found.

$$\% \, Moisture \; Absorption = \frac{Final\ Weight - Initial\ Weight}{Initial\ Weight} \times 100$$

Percent Moisture Loss[25]

This test was also carried to check the integrity of films at dry condition. Three films of 2 cm^2 area was cut out and weighed accurately and kept in a desiccator containing fused anhydrous calcium chloride. After 72 hours the films were removed and weighed. Average percentage moisture losses of three films were found out.

$$\% \, Moisture \; Loss = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100$$

Microbial count[26]

The protective cover sheets ('release liners') of 3 patches of the transdermal preparation were removed using sterile forceps, and place them, the adhesive side upwards, on sterile glass or plastic trays. The adhesive surface with sterile gauze (or a woven filter type mono filament polymer grid) was covered, if necessary, and transferred 3 patches to a minimum volume of 500 ml of buffered sodium chloride and peptone solution pH 7.4 containing suitable in activators such as polysorbate 80 and / or lecithin. Shake the preparation vigorously for at least 30 min and transfer the suitable solution in the agar plate and place in the incubator for one day.

Water vapour transmission rate (WVTR)

Glass vials of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. About 1g of anhydrous calcium chloride was placed in the cells, and the respective polymer film was fixed over the brim. The cells were accurately weighed, and kept in a close desicator containing, saturated solution of potassium chloride to maintain a humidity of 84% .the cells were taken out and weighed after 6,12,24,36,48,72 hour of storage.

Estimation of % entrapment[27]

Three films (2×2 cm^2) from each film were taken in separate 10 ml volumetric flask. 10 ml of phosphate buffer (pH 7.4) was added and continuously stirred for 24 hours. The solutions were filtered, diluted suitably and analyzed at 303 nm in an U.V Spectrophotometer. The average values were determined. The results were analyzed for mean and standard deviation.

***In vitro* drug release 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 methotrexate, was fitted in to different kinetic equation for transdermal drug delivery system.

Stability studies[35]

The stability studies were conducted according to ICH guidelines by storing the replicates of the TDDS at 40±0.5° C and 75 ± 5 % for period of three months. The sample were withdrawn at 3 weeks and analyzed for physical appearance, drug content, *in-vitro* diffusion studies.

RESULTS AND DISCUSSION

In the present study, the transdermal patches of Methotrexate were prepared by using different polymers such as hydroxy propyl methyl cellulose and Ethyl cellulose by solvent casting method.

Physio-Chemical Parameters

All the Physio-Chemical Parameters were showed in Table 2. The weight variation of formulated films was found to be in the range of 19.91 ± 0.06 mg to 22.22 ± 0.24 mg and thickness of formulated films was found to be in the range of 272.6 mm ± 1.82 mm to 283.0 ± 1.56 mm. The Percentage drug content of formulated films was found to be in the range of 93.22 ± 0.017 to 98.17 ± 0.066 per 3.14 cm^2 strip. However, there was increased moisture content with an increase in hydrophilic polymers. The Percent moisture uptake was found to be more in films containing EC polymers because it absorbs moisture. The moisture loss in the range of 6.5 ± 0.3 to 11.3 ± 0.03 and moisture absorption in the range of 7.25 ± 0.14 to 10.24 ± 0.05 found to be satisfactory. No Microbial growth for patch F1, F2, F3 & F4 was observed. Therefore the films are expected to maintain a smooth surface when applied on to skin.

In vitro drug release studies

The release of a drug from a transdermal drug delivery system occurs by diffusion, which involves transport of a drug from the polymer matrix in to the *in vitro* study medium depending on concentration[36, 37]. As the gradient varies, the drug is released and the distance for diffusion becomes increasingly greater. This could be an explanation why the drug diffuses comparatively at a slower rate as the distance for diffusion increases [38].Initially a rapid drug release was observed, which gradually approached plateau values (Fig.1), thus confirming the controlled release behavior of matrix formulations.

A suitable proportion of HPMC and EC may be used to achieve prolonged release of the drug. Release rates were decreased when the concentration of HPMC increased in the formulations. This is because as the proportion of this polymer in the matrix increased, there was a decrease in the amount of water uptake and thus fewer drugs was released.

The EC was use it retards the release of the drug from the matrix due to the more hydrophobic nature, therefore the prolonged drug release was obtained. The formulation F1 and F2 containing HPMC showed 96.15 % & 88.46 % drug release over 8 hrs due to hydrophilic nature of the polymer. Whereas the formulation F3 and F4 containing EC Showed 86.29 % & 81.34 % drug release in 8 hrs due to hydrophobic nature of the polymer.

Formulation F1 showed best fit with higuchi equation (r^2 > 0.9892).complete and sustained release of drug methotrexate over a period of 8hours was obtained and hence F1 was selected as optimized formulation. F1 will be able to release the drug at predetermined rate in to systemic circulation for a prolonged period of time thus minimizes its dose frequency and adverse effect.

Stability study

The stability of the optimized formulation (F1) was investigated as per ICH guidelines. The formulation was stored at a temperature 40 ± 0.5 ° C and 75 ± 5% RH for 3 months. There was no significant change in release and drug content.

The solubility of drug methotrexate was determined in water, CHCL3, polyethylene glycol, propylene glycol, glycerol, methanol and octanol and it was found to be soluble in.

CONCLUSION

The Transdermal patch formulation was found to be efficacious, safe, stable and non irritant to skin. The establishment of steady state levels *in-vitro* for 8hrs shows the clear advantage of transdermal patches over current modes of administration. The moisture content in the formulations was related with ratio of HPMC and EC. It was observed that viscosity had a major influence on drug release from hydrophilic matrixes. F_1 formulation drug release is 96% at end of 8 hours effectively when compared to EC patches which have drug release of only 86%. Finally from the study it was found that Methotrexate could be given as Transdermal Patch and further *In vivo* and *In vitro* investigations are required.

Table 1: Characterization of Transdermal patches

S. No	Formulation Code	F1	F2	F3	F4
1.	Thickness (mm) ± S.D	278.2 ± 1.28	278.2 ± 1.28	278.2 ± 1.28	278.2 ± 1.28
2.	Weight variation ± S.D	21.48 ± 0.67	21.48 ± 0.67	21.48 ± 0.67	21.48 ± 0.67
3.	% Moisture absorption ± S.D	7.25 ± 0.14	7.25 ± 0.14	7.25 ± 0.14	7.25 ± 0.14
4.	% Moisture loss ± S.D	6.5 ± 0.3	6.5 ± 0.3	6.5 ± 0.3	6.5 ± 0.3
5.	Microbial count	No Growth	No Growth	No Growth	No Growth
6.	% Drug content ± S.D	98.17 ± .066	97.26 ± 0.032	95.41 ± 0.015	93.22 ± 017
7.	Water vapour transmission rate	3.65	3.82	3.98	4.30

Table 2: Results of In vitro Drug release studies

Time in hours	% Cumulative Drug release			
	F1	F2	F3	F4
0.5	30.76	26.92	23.07	26.92
1.0	44.23	42.30	30.76	30.76
2.0	48.07	46.15	44.23	42.30
3.0	52.88	52.88	48.07	50.38
4.0	69.23	65.38	50.38	52.88
5.0	72.11	69.23	65.38	69.23
6.0	76.92	73.46	76.92	72.11
7.0	84.61	80.76	80.76	76.92
8.0	96.15	88.46	86.29	81.34

Table 3: kinetic study

Formulations	Zero order	First order	Higuchi square root
F1	0.9524	0.9878	0.9892
F2	0.96710	0.8871	0.9629
F3	0.9436	0.8129	0.9867
F4	0.9724	0.7684	0.9892

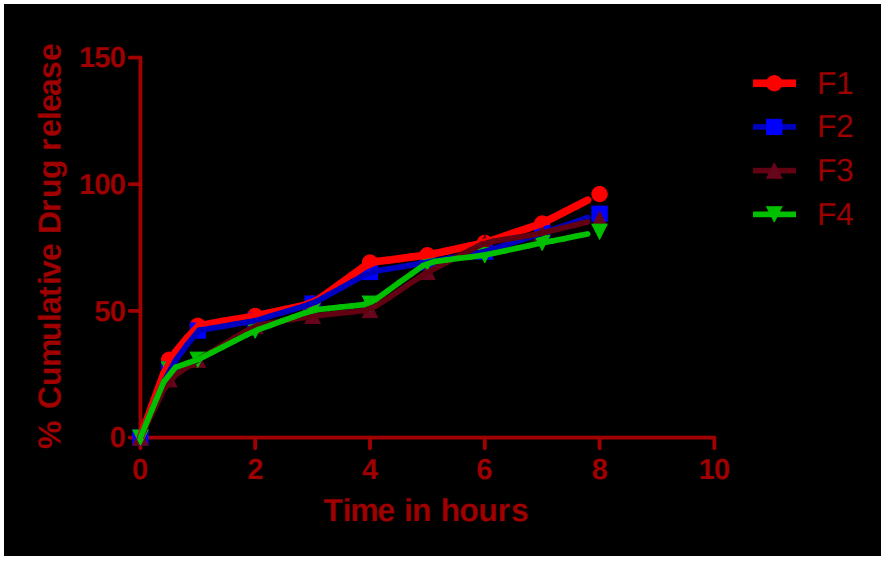

Fig. 1: *In vitro* drug release profile of Methotrexate Transdermal patches

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