International Journal of Pharmacy Research and Technology
2012, Volume 2, Issue 2, 47-53
ISSN 2250 – 0944 (Online)
ISSN 2250 – 1150 (Print)

Research Article

Formulation, Evaluation and Optimization of Transdermal Drug Delivery System of Methotrexate Using Different Ratio of Eudragit RLPO / RSPO

Nirav A. Singhal[1*], Girish K. Jani[2], Anil Bhandari[1], S. M. Vijayendraswamy[3], Nishant Upadhyay[3]
[1]Department of Pharmaceutical sciences, Jodhpur National University, Jodhpur, Rajasthan.
[2]S.S.R College of Pharmacy, Silvasa-Sayli road, Silvassa-Sayli Road, Sayli, UT of Dadra & Nagar Haveli-396230.
[3]Dept. of Pharmaceutics, Bhagwan Mahavir College of Pharmacy, Surat, Gujarat, India
***Corresponding author:** Tel: +91(0)9427676829; ayushya_4u@yahoo.co.in
Received: 16/09/2012, Revised: 19/11/2012 Accepted: 09/12/2012

ABSTRACT
The present investigation was aimed to development, evaluation and optimization of transdermal preparations capable of administering Methotrexate (MTX), which is at present considered to be most effective for rheumatoid arthritis, more safely than conventional oral preparations while permitting high compliance. Transdermal patches of Methotrexate were prepared by solvent casting method using Eudragit RLPO: Eudragit RSPO in different ratios along with Di butyl -n- phthalate as plasticizer. MTX containing transdermal patches were formulated by using central composite design having 2 independent variables at 3 levels. Independent variables were total amount of polymers (X_1) and % of Eudragit RSPO (X_2). The prepared formulations were evaluated for various physicochemical properties like flexibility, thickness, smoothness, weight variation, Tensile Strength, Folding Endurance and Drug Content and was found to be flexible, smooth, uniform thickness and weight, suitable drug content (98 to 99.9 %) and good Folding endurance (> 100). The prepared formulations were also evaluated for *in-vitro* drug release and *Ex-Vivo* drug diffusion characteristics. Statistical Optimization carried out for various responses like 'K' of zero order, $T_{50\%}$ and $T_{80\%}$. Optimized Formulation was found to provide more controlled diffusion of drug. Release kinetics of Optimized Formulation followed zero order drug diffusion. Hence Optimized Transdermal Patch could be a promising delivery system for Methotrexate with sustained release action and improved drug availability.

Keywords: Central composite design, Transdermal Patch, Methotrexate, Eudragit RLPO/RSPO.

INTRODUCTION

Transdermal patches are innovative drug delivery systems intended for skin application to achieve a systemic effect. A transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a time-released dose of medication through the skin to treat systemic conditions. Transdermal drug delivery system offers a variety of significant clinical benefits over other systems, such as tablets and injections. For example, it provides controlled release of the drug, and produces a steady blood-level profile, leading to reduced systemic side effects and, sometimes, improved efficacy over other dosage forms by avoiding hepatic first pass metabolism [1,2]. The success of Transdermal Therapeutic System has created much interest in the pharmaceutical industry and has activated research activities related to it. Worldwide market revenues for transdermal drug delivery systems are at US$4 billion with the growth rate expected to increase 12% annually through 2015 [3].

Rheumatic disease is among the autoimmune disorders and characterized by symmetrical, erosive synovitis, and in some cases, extra articular involvement [4]. Rheumatoid Arthritis (RA) affects about 1% of world population. The disease strikes women three times more often than men. The estimated cost of RA to the National Health Service is £240–600m per year and may be as high as £1.3bn. Social cost of RA is also considerable, with significant numbers of patients being unable to work, requiring residential home care and having reduced life expectancy [5]. Diseases Modifying Antirheumatic Drugs (DMARDs) like Methotrexate, sulfasalazine, hydroxychloroquine and cyclosporine, either alone, or in combination, have been the principal therapies for RA in the last decade. It is now well established that early therapy with DMARDs is critical for better long term outcome in RA [6]. Methotrexate (MTX) is currently being used as one of the most widely prescribed drugs for the treatment of RA due to its efficacy and safety. However, even at low and intermittent doses, oral administration of MTX exhibits high interindividual variability, gastrointestinal and hepatic toxicity [7]. However, the systemic use of this drug causes numerous side effects like hepatic toxicity, bone marrow depression, leucopenia, thrombocytopenia, anaemia, ulcerative stomatitis, nausea, abdominal distress, etc [8]. So, it is desirable to deliver MTX by the transdermal route.

The main objective of this study was to development of a matrix dispersion type transdermal drug delivery system (TDDS) of methotrexate. The low molecular weight of MTX (454.5 g/mol) would theoretically allow the delivery of the 5–15 mg dose once a week, to treat RA [9]. Treatment of RA required longer duration of therapy; hence the use of chemical penetration enhancers should be restricted in topical formulations of MTX, as they may produce some undesired effects on skin. The skin enzymes get deactivated in the presence of some penetration enhancers [10]. Moreover, high potency of MTX, chronic nature of disease, and inter and intra-patient absorption variability strongly provide a rationale for developing a noninvasive topical delivery system of MTX for the treatment of recalcitrant RA [11].

The primary objective of the present study was to design and develop transdermal patches of MTX using mixed grades and ratios of polymers viz. Eudragit RLPO and RSPO with plasticizer like di-n-butyl phthalate using Central composite design. The Independent variables for the present study were: Total amount of polymer (X_1) and

% of Eudragit RSPO (X_2). The dependent variables studied were $T_{50\%}$(Y_1), 'K' of zero order equation (Y_2) and $T_{80\%}$(Y_3).All these polymers are water insoluble and films prepared from Eudragit RLPO are freely permeable to water and Eudragit RSPO films are only slightly permeable to water. These properties make the polymers in mixed ratios suitable for the preparation of matrix TD films [12].

Response surface methodology (RSM) is one of the popular methods in the development and optimization of drug delivery systems. Based on the principles of design of experiments (DOE), the methodology involves the use of various types of experimental designs, generation of polynomial mathematical relationships and mapping of the response over the experimental domain to select the optimum formulation [13-15]. Central composite design (CCD) having 2- independent variables at 3-level is one of the RSM designs available for statistical optimization of the formulations [16].

MATERIALS AND METHODS

Materials

Methotrexate was obtained as the gift sample from the Aan Pharmaceuticals Ltd, Ahmedabad. Eudragit RLPO & Eudragit RSPO was obtained as the gift sample from Glenmark pharmaceutical Ltd, Mumbai. Dibutyl-n-phlthalate purchased from the local dealer. Apart from these, all other chemicals used in study were analytical grade reagents.

Methods

Preparation of Methotrexate (MTX) Transdermal Films

Transdermal patches containing MTX were prepared by the solvent evaporation technique. Transdermal films of MTX were made by using mixed grades of Eudragits RLPO: RSPO in the ratios of 100:00, 50:50, and 00:100. Ten percent w/v polymer solution was made by dissolving the respective amount of polymer in a mixture of methanol and dichloromethane (in the ratio 40:60) as casting solvent. An appropriate amount of methotrexate was dissolved separately in 0.1 ml dilute methanolic hydrochloric acid. Next, 2 ml methanol was added and the MTX dispersion sonicated for 1 min. These solutions were mixed with plasticizer di-n-butyl phthalate (15% w/w based on polymer weight) with stirring. The films were cast on glass mould [17, 18]. To control the rate of evaporation of solvent, the mould was covered with funnel of suitable size. The casting solvent was allowed to evaporate overnight to obtain dried films. The films were cut into 9 cm^2 (3 cm × 3 cm) patches containing the equivalent of 5 mg of the drug per patch. Backing membrane was glued and the patches were stored between sheets of wax paper in desiccators. Table 1 represents the composition of Methotrexate along with its polymers where as **Table 1** represents the variable levels of Eudragit RLPO and Eudragit RSPO used in the study.

Table 1: The composition of Methotrexate along with the variable levels of Eudragit RLPO and Eudragit RSPO

Batch	Amount of Drug (mg)	Total amount of Polymer (mg) (X_1)	Amount of Eudragit RLPO		Amount of Eudragit RSPO (X_2)		Amount of Plasticizer (DBP) (%)	Amount of Solvents (% w/w)	
			%	mg	%	mg		Methanol	Dichloro-methane
F1	5	25	100	25	0	0	15%	40	60
F2	5	50	100	50	0	0	15%	40	60
F3	5	75	100	75	0	0	15%	40	60
F4	5	25	50	12.5	50	12.5	15%	40	60
F5	5	50	50	25	50	25	15%	40	60
F6	5	75	50	37.5	50	37.5	15%	40	60
F7	5	25	0	0	100	25	15%	40	60
F8	5	50	0	0	100	50	15%	40	60
F9	5	75	0	0	100	75	15%	40	60

Experimental design

Central composite statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the *in-vitro* release of formulations. A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 8.0.6., Stat-Ease Inc., Minneapolis, MN).

This design is characterized by set of points lying at 4-edges and 4-midpoint of each edge of a square and 1-center point replicates (n = 4). For central composite designs having two factors (where, α=1), Suitable models include linear, second order and quadratic models. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2), and the predicted residual sum of square (PRESS), analyzed by Design-Expert® software. Linear model:

$$Y = \beta_0 + \beta_1 A + \beta_2 B$$

Second order (2 factors interaction) model:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB$$

Quadratic model:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2$$

Where Y is the measured response associated with each factor level combination; β_0 is an intercept; β_1 to β_{22} are regression coefficients computed from the observed experimental values of Y from experimental runs; and A, B and C are the coded levels of independent variables. The

terms AB, A^2 and B^2 represent the interaction and quadratic terms, respectively [19].

Fourier Transform Infrared Spectroscopic Studies

For designing a transdermal drug delivery system, it is imperative to investigate drug-excipients interaction and in consequence, their compatibility. To investigate the possible interaction between MTX and polymeric materials of the patches FT-IR analysis was carried out on pure substances and physical mixtures [12] at Shree Dhanvantary pharmaceutical and analytical centre, Kim.

Evaluation of Transdermal patches of Methotrexate

Thickness

It was assessed at different points of the patch using micrometer screw. Ten randomly selected patches of each formulation were tested for their thickness. The thickness was measured at 5 separate points of each patch in order to ensure uniform thickness [12].

Weight Variation

The patches were subjected to weight variation by individually weighing 10 randomly selected patches. Such determinations were carried out for each formulation [12].

Flatness

Longitudinal strips from the five 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 = cut film length; 0% constriction was considered to be 100% flatness [12].

Table 2: Analysis of kinetic models of ex-vivo drug diffusion mechanism of various Formulations of MTX Patches

Formu. Code	Y_1: $T_{50\%}$ (h)	Y_3: $T_{80\%}$ (h)	Higuchi Eq.		Peppas Eq.			First Order Eq.		Zero Order Eq.	
			N	R^2	N	Log K	R^2	N	R^2	Y_2: K	R^2
Std. Diffusion in 24 h	***12***	***19.2***	**16.959**	**0.880**	**0.899**	**0.738**	**0.993**	**-0.050**	**0.685**	**4.166**	**1.000**
F1	11.83	17.64	16.863	0.817	1.224	0.376	0.999	-0.052	0.686	4.477	0.988
F2	13.74	20.17	15.536	0.808	1.291	0.219	0.999	-0.042	0.705	3.877	0.985
F3	16.41	23.35	12.672	0.785	1.321	0.090	0.999	-0.024	0.848	3.176	0.975
F4	12.89	18.94	15.337	0.802	1.274	0.274	0.998	-0.036	0.820	4.084	0.982
F5-T1	14.86	21.71	14.200	0.808	1.267	0.210	0.999	-0.030	0.802	3.544	0.984
F5-T2	15.45	22.14	13.740	0.794	1.329	0.118	0.999	-0.028	0.810	3.438	0.979
F5-T3	15.56	22.34	13.530	0.789	1.362	0.070	0.999	-0.027	0.815	3.390	0.977
F5-T4	15.40	21.85	13.950	0.795	1.293	0.167	0.998	-0.029	0.798	3.489	0.979
F6	18.26	26.14	11.030	0.791	1.352	0.006	0.999	-0.018	0.902	2.762	0.978
F7	14.15	21.01	14.873	0.805	1.298	0.192	1.000	-0.034	0.779	3.714	0.984
F8	17.02	24.37	12.076	0.795	1.296	0.102	0.999	-0.021	0.878	3.021	0.979
F9	20.80	29.88	9.277	0.785	1.360	0.092	1.000	-0.014	0.921	2.326	0.975

Folding Endurance

Film folding endurance was determined by repeatedly folding the patches at the same place until they show a crack or break. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance. Five randomly selected patches of each formulation were tested [17].

Tensile strength

In order to determine the elongation as a tensile strength, the polymeric patch was pulled by means of a pulley system; weights were gradually added to the pan to increase the pulling force till the patch was broken. The elongation i.e. the distance traveled by the pointer before break of the patch was noted with the help of magnifying glass on the graph paper [18]. The tensile strength was calculated as kg cm^{-2}.

Surface pH

The surface pH of the patches was determined to investigate the possibility of any irritation side, *in-vivo*, because an acidic or alkaline pH may cause irritation to the Skin. Therefore, the idea behind the test is to keep the surface pH of skin. For the determination of surface pH, three patches (3×3 cm^2) from each formulation were kept in contact with 1 mL of distilled water for 2 h, in test tubes. Excess water from the tubes was drained and the pH was noted by means of pH paper placed on the surface of the swollen patch. A mean of two readings was recorded [18].

Content Uniformity

Assay of each of the 10 randomly selected medicated patches of specified area (9 cm^2) were dissolved in 5 mL of casting solvent and the volume was made up to 10 mL with phosphate buffer pH 7.4; dichloromethane was evaporated using a vacuum evaporator at 45 °C. A blank was prepared using a drug-free patch treated similarly. The solutions were filtered through a 0.45 µm membrane, diluted suitably and absorbance was read at 303 nm in a double beam UV-Visible spectrophotometer [20].

***In-vitro* drug release studies**

The *in vitro* dissolution study of each selected transdermal patch was determined on USP dissolution apparatus. A sandwich patch holder, a slightly modified

form of FDA´S sandwich patch holder was used to ensure patch to patch reproducibility of transdermal film the dissolution vessel contained 500 ml of phosphate buffer 7.4 pH maintained at 37 ± 0.5ºC paddle speed set at 50 rpm. Patch assembly was carefully placed at the bottom of the vessel and was centered using a glass rod. 5 ml sample was withdrawn at regular time intervals until completion of drug release. The withdrawn samples were analyzed for drug content by measuring absorbance at 303 nm using UV-visible spectrophotometer [18, 20]. The content of MTX was calculated from the standard curve. The *in vitro* dissolution profiles (cumulative drug release) were calculated.

***Ex-vivo* drug diffusion studies**

The rat abdominal skin was excised. The hairy and underlying tissue was removed. The membrane was washed thoroughly with distilled water and saline solution. It was soaked in the saline solution overnight. It was washed several times before use. The rat skin was then cut into appropriate size and mounted at the junction between donor and receptor chamber of diffusion. The matrix formulation to be tested was cut into appropriate size patches and was placed over the optimized skin. It was then covered with aluminum foil as the occlusive backing. The donor compartment was clamped over it. With the help of springs, making sure that there were no bubbles in the receptor compartment. The whole system was sandwiched between the donor and the receptor compartments and secured with a clamp, with the receptor compartment containing phosphate buffer solution of pH 7.4.The agitation speed of 50 rpm and temperature of 37±0.5ºC were maintained during the experiment. Samples of 3 ml were withdrawn at predetermined time interval for 24 hour. The samples were then analyzed for drug content using UV double beam spectrophotometer at 303 nm [20].

Optimization data analysis and model-validation

ANOVA provision available in the software was used to establish the statistical validation of the polynomial equations generated by Design Expert®. A total of 12 runs were generated by Central Composite Design. All the responses observed were simultaneously fitted to first order, second order and quadratic-models and were evaluated in terms of statistically significant coefficients and R^2 values. The optimized checkpoint formulation was prepared and evaluated for various response properties.

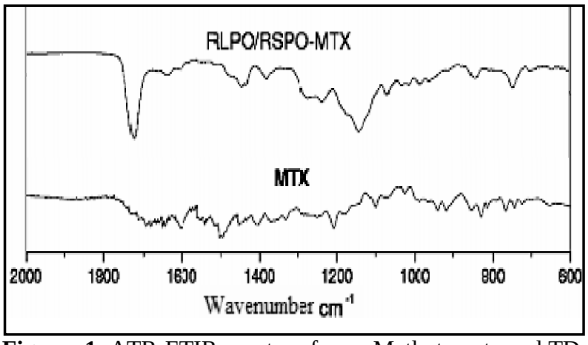

Figure: 1. ATR-FTIR spectra of pure Methotrexate and TD films of MTX:Eudragit RLPO/RSPO.

RESULTS AND DISCUSSION

Drug Interaction study: FTIR is a simple and quick technique for obtaining the IR spectrum of powder samples with KBr. The FTIR spectral analysis of MTX powder confirming the purity of the drug as per established standards. The FTIR spectrum of physical mixture of polymer and drug (1:1 ratio) showed the major peaks which correspond to drug. It can be inferred that there is no interaction between drug and polymer in physical mixture used, indicating their compatibility (**Figure 1**).

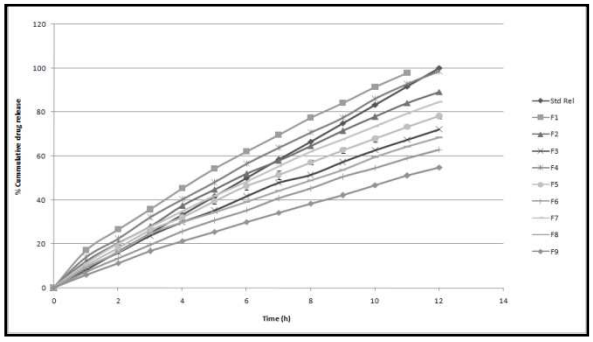

Figure 2: *In-vitro* drug release study of various formulation of Methotrexate Transdermal Patch

% Drug content and physical evaluation

In the presence of the plasticizer Di-butyl phthalate, the mixtures of Eudragit RL/RS have been reported to provide films with good elasticity. To get suitable matrices for transdermal films, Eudragit RLPO/RSPO were mixed in different ratios. The thickness and weight per patch were similar for all ratios of the polymers and the drug content per patch was found to be uniform. Folding endurance values of matrix films were found to be more than 100, indicating good strength and elasticity. The endurance values decreased with an increase in the Eudragit RS content of the matrix transdermal films. The flatness study showed that none of the formulations had a difference in the strip length before and after longitudinal cut, indicating 100% flatness and thus they could maintain a smooth surface when applied to the skin. The surface pH of all formulations was in the range of 5.0–5.5, the pH range of skin, and hence no skin irritation was expected. Drug content in the prepared formulations was found to be in the range of 98-99.9%. *In-vitro* and *Ex-Vivo* drug release study of various formulation of Methotrexate Transdermal Patch were shown in **Figure 2 & 3** respectively.

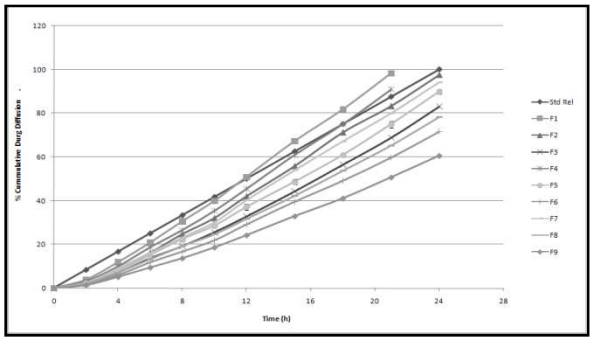

Figure 3: *Ex vivo* drug diffusion study of various formulation of MTX Transdermal Patch

Mechanism of *ex vivo* drug diffusion studies

To study the release mechanism, various kinetic models were applied to the *ex vivo* release profiles of the 12

different formulations (F1-F9 and quadruple replicates of F5). The kinetic models included in the present research work were given in Table-2. On the bases of R^2 value of 'n' of Higuchi equation and 'K' of first order equations were not considered for statistical optimization. For all the experimental design point formulations, value of 'n' of peppas equation was more than one which suggests zero order drug diffusion from all the formulations, so the further optimization of 'n' of peppas was not required to perform. Therefore 'K' of zero order equation, $T_{50\%}$ and $T_{80\%}$ were selected for further statistical optimization.

Fitting of data to the model

A two-factor, three-level central composite statistical experimental design as the response surface method requires 12 experiments. The independent variables and the responses for all 12 experimental runs are given in **Table - 2**. Twelve batches showed Y_1 (T_{50}%), were 11.83 -20.80 h, Y_2 ('K' of zero order eq.) were 2.326–4.477 and Y_3 ($T_{80\%}$), were 17.64–29.88 h respectively. All the responses observed for 12 formulations prepared were simultaneously fitted to Linear, second order and Reduced quadratic models using Design Expert® and the comparative values of R^2 and S.D. are given in (Table 3) along with the regression equation generated for each response. Responses Y_1, Y_2 and Y_3 were suggested by Design Expert® to follow Second order, linear and quadratic but by manual selection of terms A,B, AB, A^2 and B^2, they were found to follow Second order, Linear and Reduced quadratic, respectively. Only statistically significant ($p < 0.05$) coefficients are included in the equations. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response. It is evident that the total amount of polymer (A) and % of Eudragit RSPO (B) have negative effects on the response 'K' of Zero order Equation (Y_2) as well as they have positive effects on the responses $T_{50\%}$ (Y_1) and $T_{80\%}$ (Y_3)

The interaction effect of total amount of polymer (A) was seen with % of Eudragit RSPO (B) for response $T_{50\%}$ (Y_1) and $T_{80\%}$ (Y_3). Total amount of polymer (A) also showed a higher quadratic effect as compared to % of Eudragit RSPO (B) on response $T_{80\%}$ (Y_3).

Table 3: Summary of results of regression analysis for responses Y_1, Y_2, and Y_3

Model Summary Statistics							
Source	**Std. Dev.**	**p-value**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	**Model Suggestion**
Y_1: $T_{50\%}$							
Linear	0.4588	< 0.0001	0.9706	0.9641	0.9276	4.6615	
Second order	0.3225	0.0127	0.9871	0.9822	0.9486	3.3101	Suggested
Quadratic	0.2363	0.0653	0.9948	0.9905	0.9873	0.8196	
Y_2: 'K' of Zero order Equation							
Linear	0.048	< 0.0001	0.994	0.993	0.992	0.0315	Suggested
Second order	0.048	0.3923	0.995	0.993	0.990	0.0361	
Quadratic	0.049	0.4593	0.996	0.993	0.991	0.0323	
Y_3: $T_{80\%}$							
Linear	0.735	< 0.0001	0.959	0.9493	0.8959	12.178	
Second order	0.543	0.0196	0.980	0.9723	0.9021	11.448	
Quadratic	0.274	0.0069	0.996	0.9930	0.9790	2.4614	Suggested
Y_1 = 15.5313 + 2.7650 * A + 1.6657 * B + 0.5154 * AB							
Y_2 = 3.4414- 0.6685 * A – 0.4117 * B							
Y_3 = 22.10 + 3.63 * A + 2.35 * B + 0.7902 * AB + 0.73*A^2							

Here, A= Total Amount of Polymer, B = % of Eudragit RSPO

Standardized main effects and reliability of the models

Standardized Main Effects (SME) **(Table 4)** was calculated by dividing the main effects with the standard error of the main effects [21]. Only statistically significant ($p < 0.05$) values are given. The larger SME values of A and B suggested the almost equal importance of total amount of polymer and % of Eudragit RSPO on drug release. R^2-value signifies the percentage of variability in responses that are fitted to the models. In the present study, the high R^2-value of >99% represents the reliability of the design. Additionally, the p-values of lack of fit were greater than 0.05, which further strengthened the reliability of the models.

Table 4: Standardized main effects of the factors on the responses

Factor	**Standardized main effects (SME)**		
	Y_1: $T_{50\%}$	**Y_2: 'K'[#]**	**Y_3: $T_{80\%}$**
Intercept	166.82	250.04	163.44
A-Total amount of Polymer	21.00	-34.35	26.85
B-% of Eudragit RS 100	12.65	-21.15	17.37
AB	3.20	-	4.77
A^2	-	-	3.81
B^2	-	-	-
R^2	0.9871	0.9945	0.9930
p-value of lack of fit	0.5039	0.9154	0.3604

[#] 'K' of Zero order Equation

Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are presented in **Figures 4 (I-VI)**, which are very useful to study the interaction effects of the factors on the responses. These types of plots show the effects of two factors on the response at a time.

According to **Figure 4**, it was concluded that as the Total amount of polymer (A) and/or % Eudragit RSPO in polymer mixture (B) increase/s, the value of $T_{50\%}$ (Y_1) is increased [Figure 4 (I), (II)]; As the Total amount of

polymer (A) and/or % Eudragit RSPO in polymer mixture (B) increase/s, the value of 'K' of Zero order (Y_2) is decreased [Figure 4 (III), (IV)]; and as The total amount of polymer (A) and/or % Eudragit RSPO in polymer mixture (B) increase/s, the value of $T_{80\%}$ (Y_3) is increased [Figure 4 (V), (VI)].

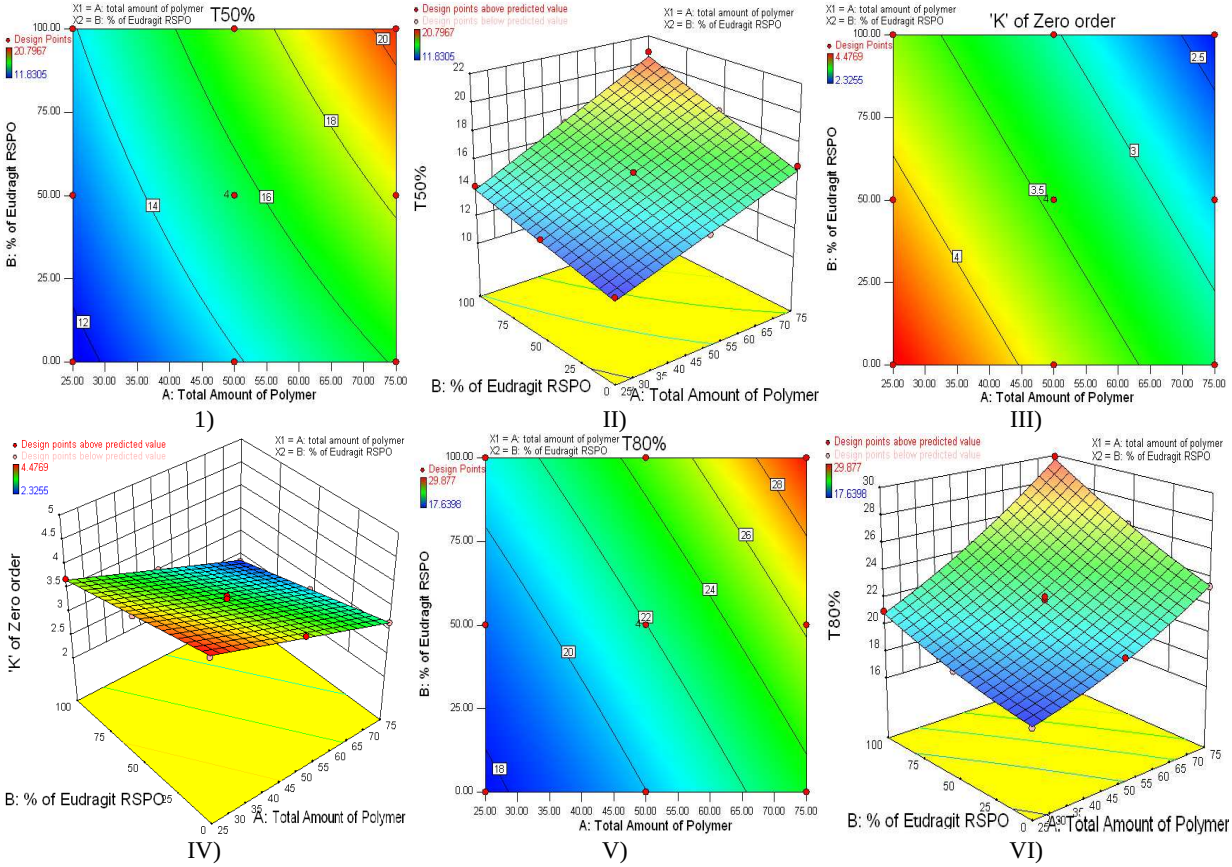

Figure 4: I) Contour plot of Predicted values of T50% I**I)** 3D surface plot of T50%, **III)** Contour plot of Predicted values of "K of Zero order" **IV)** 3D surface plot of "K of Zero order" **V)** Contour plot of Predicted values of T80% and **VI)** 3D surface plot of T80% for various formulations of Methotrexate.

Table 5: Predicted and average experimental values of response variables and % Prediction error of various optimized check point formulations of Methotrexate

Response variable	**Experimental value**	**Predicted value**	**% Prediction error**
Y_1: $T_{50\%}$	12.7458	12.7695	-0.1859
Y_2: 'K' of Zero Order Eq.	4.0895	4.1088	-0.4719
Y_3: $T_{80\%}$	19.0887	19.2000	-0.5831

Optimization

The optimized formulation was selected based on the criteria of attaining the maximum 'n' of peppas equation for patch formulations and applying constraints on T 50% (Y_1) (target to 12 h), 'K' of Zero Order Eq. (Y_2) (target to 4.166) and T80% (Y_3) (target to 19.20h). Upon 'trading off' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with polymer levels of Total amount of polymer (A), 25 mg, % Eudragit RSPO in polymer mixture (B), 50.14 %, was found to fulfill the maximum requisite of an optimum formulation because of better correlation of the theoretically obtained values of Y_1 (12.7695h), Y_2 (4.10) and Y_3 (19.2) with the standardized target values with the desirability of 0.984 (**Figure 5**). The optimized formulation was found to diffuse drug in *ex vivo* about **99.72%** drug in sustained release manner for 24 h. The release pattern of the optimized formulation was best fitted to both the zero order (K: 8.3919) and Korsmeyer-Peppas kinetics (n = 1.288). These values suggested the release to be primarily by zero order drug diffusion.

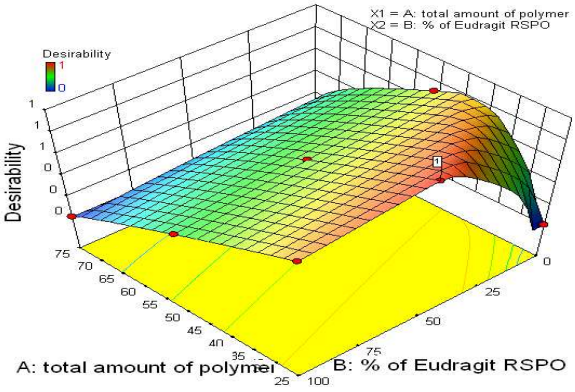

Figure 5: 3D surface response plot of desirability for obtaining optimized formulation having release profile with zero order for 24 h from various predicted formulations of RL/RSPO

Validation of RSM results

Table 5 shows predicted and experimental values of all the response variables, and the percentage error of optimum checkpoint formulations of Methotrexate.

CONCLUSION

Transdermal Patches of Methotrexate with various ratio of Eudragit RSPO/RLPO were prepared and optimized using central composite statistical design. The quantitative effect of these factors at different levels on the release rate could be predicted by using polynomial equations. Linearity observed between the actual and predicted values of the response variables suggested the prognostic ability of the RSM design. The quadratic response surface methodology studied for the release rate helped in understanding the interaction effects between the combination and ratio of the two polymers. Thus, high degree of prediction obtained using RSM is quite efficient in optimizing drug delivery systems that exhibit non-linearity in responses. *Ex-vivo* drug diffusion studies were closely met to standard zero order release and exhibited the controlled release profile within desired time duration.

REFERENCES

1. Modamio P, Lastra C F, Marino E L, "A comparative *in vitro* study of percutaneous penetration of beta-blockers in human skin" *Int. J. Pharm*, 194, 249–259, (2000).
2. Ke G M, Wang L, Xue H Y, Lu W L, Zhang X, Zhang Q, Guo H Y, "*In vitro* and *in vivo* characterization of a newly developed clonidine transdermal patch for treatment of attention deficit hyperactivity disorder in children". *Biol. Pharm. Bull*, 28, 305–310, (2005).
3. Prausnitz MR, Mitragotri S and Langer R, "Transdermal drug Delivery Technologies, Markets and Companies", *Na Rev Drug Dis*, 3(2),115-124, (2004).
4. Rang H P, Dale M M and Ritter J M, Pharmacology. Churchill Livingstone, London, 1999, pp. 293-295.
5. Scott DL, Shipley M and Dawson A, "The clinical management of Rheumatoid arthritis and Osteoarthritis: strategies for improving clinical effectiveness", *Br J Rheumato*, 37,546-54, (1998).
6. Sukhpreet V Agrawal and Pramil Tiwari, "Treatment and monitoring Cost in Rheumatoid Arthritis: Preliminary results from an Indian Setting", *Indian J. Pharm. Sci.*, 69(2): 226-231, (2007).
7. Anita T, Y Lee and Kevin Pile, "Disease modifying drugs in adult rheumatoid arthritis", *Australian Prescriber*, 26(2), 36-40, (2003).
8. VanDooren-Greebe, R J Kuijpers, A L A, Mulder J, De Boo T, van der Kerhof, "Methotrexate revisited: effects of long term treatment in psoriasis". *Br. J. Dermatol*, 130, 204–210, (1994).
9. Cronstein BN. "Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis". *Pharmacol Rev*, 57:163–172, (2005).
10. Hadgraft J, Lane ME, "Skin permeation: the years of enlightenment", *Int. J. Pharm*. 305(1-2), 2-12, (2005).
11. Hikima T, Tojo K & Maibach HI, "Skin metabolism in transdermal therapeutic systems". *Skin Pharmacol Physiol*,18:153–159, (2005).
12. Verma PRP, & Iyer SS. "Transdermal delivery of propranolol using mixed grades of eudragit: design and in vitro and in vivo evaluation". *Drug Dev Ind Pharm*, 26:471–476, (2002)..
13. Kotiyan PN, & Vavia PR. "Eudragit: role as crystallization inhibitors in drug-in-adhesive transdermal system of estradiol" *Eur J Pharm Biopharm*, 52:173–180, (1993).
14. P. Dayal, V Pillay, R J Babu, M Singh, "Box-Behnken experimental design in the development of a nasal drug delivery system of model drug hydroxyurea: characterization of viscosity, in vitro drug release,droplet size and dynamic surface tension", *AAPS Pharm. Sci.* Tech.,6E,573–E585, (2005).
15. B Singh, S K Chakkal, N Ahuja, "Formulation and optimization of controlled release mucoadhesive tablets of atenolol using response surface methodology", *AAPS Pharm. Sci. Tech.*, 7, E1–E9, (2006).
16. Shruti Chopra, Gayathri V Patil , Sanjay K Motwani, "Release modulating hydrophilic matrix systems of losartan potassium: Optimization of formulation using statistical experimental design" *European Journal of Pharmaceutics and Biopharmaceutics*, 66, 73–82, (2007).
17. Mehdizadeh A, Toliate T, Rouini M R, Abashzadeh S And Dorkoosh F, "Design and In vitro evaluation of new drug-in-adhesive formulations of Fentanyl transdermal patches", *Acta Pharm.*, 54,301-31,(2004).
18. Chandak Ashok R and Verma Priya Ranjan Prasad, "Transdermal Delivery of Methotrexate Using Mixed Grades of Eudragit: Physico-Chemical, In-Vitro, and In-Vivo Evaluations" *Clinical Res.Regu.Affairs*, 25: 2, 53-72, (2008).
19. S Nazzal, M Nutan, A Palamakula, R Shah, A A Zaghloul, M A Khan, "Optimization of a self-nanoemulsified tablet dosage form of Ubiquinone using response surface methodology: effect of formulation ingredients", *Int. J. Pharm*.,240, 103–114, (2002).
20. Bhanja Satyabrata, P Ellaiah, Rohit Choudhury., "Design and evaluation of methotrexate buccal mucoadhesive patches", *Int J Pharm Biomed Sci*, 1(2), 31-36, (2010).
21. M T H Nutan, M S Soliman, E I Taha, M A Khan, "Optimization and characterization of controlled release multi-particulate beads coated with starch acetate", *Int. J. Pharm*., 294, 89–101, (2005).