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Research article

Optimization and characterization of transdermal film of curcumin containing natural oils as permeation enhancer by response surface methodology

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Key words: Curcumin, herbal drugs, osteoarthritis, optimization, natural oils.

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Abstract

The purpose of present study was to develop optimized transdermal drug delivery system of curcumin by utilizing response surface methodology to study the combined effect of independent variables like concentration of hydroxyl propyl methyl cellulose, ethyl cellulose and natural oils as permeation enhancers which significantly influenced characteristics like elongation and in vitro drug permeation. Linear model fitted the best for all the formulations. According to Derringer's desirability prediction tool, the composition of optimized film containing peppermint oil was found to contain 200mg of HPMC, 150mg of EC, and 0.35ml of peppermint oil and incase of films containing jojoba oil, the composition of optimized film was, 300mg of HPMC, 100.2mg of EC, and 0.39ml of jojoba oil. Under these conditions, the optimized patch exhibited a predicted value of % elongation and in vitro drug permeation of 66.44%, 85.50%, respectively (peppermint oil) and 79.7, 97.1%, respectively (jojoba oil). Jojoba oil showed better characteristics than peppermint oil and can be better substitute for synthetic permeation enhancers. FTIR was also performed which revealed no interaction between drug and excipients. It can be concluded that if proper optimization is carried out for herbal formulations, they can be the first choice for people compare to synthetic drugs.

Introduction

Osteoarthritis (OA) is a chronic inflammatory disease that affects people of age ≥60.It is reported to affect 14-47% of Indian population. [1-2]. Hormonal, genetic, aging, metabolic and mechanical factors regulate the biology of the articular cartilage by complex molecular mechanisms [3]. Major causes behind OA are obesity, joint location, genetic predisposition etc. Clinical implications associated with OA are: Joint pain and stiffness occurs which is related to use, pain occurs on movement with restricted range and cracking of joints may occur. [4]. The treatments available for OA are acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) cyclooxygenase COX-2 selective inhibitors , opoid analgesics and opoids are taken in case of severe pain. There are many problems associated with the above mentioned drugs like (a) NSAIDs do not slow down the advancement of ailment as they do not impede any immune-inflammatory reactions but only interfere in the production of prostaglandin by cyclooxygenase. [5-6]. (b) It has been reported in literature that a huge segment of patients neither show any response to the current treatments available nor reprieve the treatments available in the market. (c) Prolonged use of these drugs lead to adverse reactions such as Liver infection, gastrointestinal damage and may lead to heart failure [7-8]. Therefore there is a need of both effective and safe new drug substances to overcome the situation. Since ages the medicines were derived from plants [9]. The demand of herbal drugs is therefore increasing day by day to treat various ailments with less toxic effects and better therapeutic effects. Another key reason to proceed from synthetic to herbal drugs is the liberation of human and veterinary medicines in the environment by various routes is becoming the significant issue of human and environmental health concern. For example diclofenac is a widely used non-steroidal anti-inflammatory drug (NSAID) is known to inhibit the cyclooxygenase activity, responsible for the synthesis of prostanoids which can increase the synthesis of other eicosanoids and chronic exposure to environmental diclofenac may result in side effects such as kidney failure and intestinal pathology which may be detrimental to the health and survival of aquatic vertebrates [10]. Various herbal preparations are available in the market but they are either not stable or are having solubility problems leading to less or zero therapeutic effect. Therefore, Novel drug delivery system has come into the picture. [11]. Inclusion of plant actives in novel drug delivery system overcomes the problems

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associated with crude plant extracts [12-13]. From time immemorial, *curcumin* (Zingiberaceae), the principal plant active of renowned Indian spice turmeric (*Curcuma longa)* has been used as inflammatory agent in ayurvedic medicine system. *Curcumin* interplays with multiple molecular targets i.e. TNF-α, IL-1β, IL-6, IL-8, NF-κB, and COX-2 [14]. However its low aqueous solubility leads to its low oral bioavailability i.e. only 40-45% of its oral dose reaches systemic circulation owing to its metabolism in intestinal mucosa and by liver which in turn restricts its oral use [15-16]. Innumerable problems are associated with the oral ingestion of drugs such as many patients may experience gastrointestinal irritation and intolerance from single dose and many may develop severe gastrointestinal irritations (ulceration) on prolonged treatment. Pain medications if administered orally may have undesirable effects on cognition and level of consciousness. The topical preparations allow for pain relief without undue risk of undesired sedation and altered cognition [17]. Therefore there is a need to develop transdermal drug delivery system of anti-inflammatory herbs to overcome these difficulties and low oral bioavailability of curcumin also motivates us to study topical preparation. Moreover presently the herbal drugs present in the market are not properly optimized in respect to excipients utilized which restricts their utilization as compare to synthetic drugs [18-19]. So the aim of the present study is to prepare optimized transdermal film of curcumin containing natural oils i.e jojoba oil and peppermint oil as permeation enhancers by utilizing factorial design. EC is nontoxic, nonallergic polymer having good film forming properties for the preparation of tough films. Hydrophilic polymer, HPMC was added to EC to increase the release rate. This can be attributed to the leaching of soluble polymer on contact with water leading to formation of pores thus boosting the release of drug [20-21]. In the first step designing of experimental trials using multiple factorial design technique was carried out, while in the second step optimization of best composition of variables for the transdermal patch was performed employing Derringer's desirability functional method. Design-Expert software 11 was used to design trials and to obtain optimized formulation by analyzing response surface plots.

Experimental

Materials and methods

Chemicals

Curcumin and hydroxy propyl methyl cellulose (HPMC) was obtained as gift sample from Sanat products limited, Delhi (vkumar@sanat.com), ethyl cellulose (EC), dibutyl phthalate (DBT), polyethylene glycol 400 (PEG 400), jojoba oil and peppermint oil were purchased from VK chemicals, Ambala Cantt.

Experimental design

Preliminary study indicated that the factors such as proportion of HPMC and EC and percentage of natural oils were majorly influencing the mechanical property of film and dissolution behavior. Therefore, in the present study multiple factorial design was adopted to optimize the above formulation factors and to evaluate the main effect on responses such as % elongation of film and permeation behavior. All the independent and dependent variables were mentioned in Table 1 and Table 2. Statistical analysis for the present study was performed employing 45 day trial version of Design-Expert software (Version DX11.0, Stat-Ease Inc., Minneapolis, MN, USA). Experimental design of different batches of matrix film and the obtained responses are presented in Table 2 [22-23].

Preparation of transdermal patches

The transdermal patches were prepared by solvent casting technique by using bangles. The casting solutions for transdermal patches were prepared as per composition stated in Table 3 and Table 4. Weighed quantities of HPMC and EC were dissolved in measured quantities of ethanol and chloroform (1:1). The drug *curcumin* 20mg is dissolved in the solvent mixture along with natural oils as permeation enhancer which was mixed thoroughly to form homogeneous mixture. This mixture was then poured onto the mercury surface placed in the petridish in between bangle and then covered using glass funnels and allowed to dry at room temperature for 24 hr by solvent evaporation. The patches were removed and cut into required dimension. The prepared patches are kept in dessicator for 2 days for further drying and wrapped in aluminium foil and then packed in self sealing covers [24].

Table 1. Variables and their levels in 3^3 level factorial experimental design for patches containing peppermint oil as permeation enhancer.

Variable	Levels		
	High (+1)	Medium (0)	Low (-1)
A:HPMC (mg)	300	200	100
B:EC (mg)	200	150	100
C: Peppermint oil (ml)	0.3	0.4	-
Dependent variable (response)			
R1	% Elongation	Maximizing	
R2	% Drug release	Maximizing	

Table 2. Variables and their levels in 3^3 level factorial experimental design for patches containing jojoba oil as permeation enhancer.

Variable	Levels		
	High (+1)	Medium (0)	Low (-1)
A:HPMC (mg)	300	200	100
B:EC (mg)	200	150	100
C: Jojoba oil (ml)	0.3	0.4	-
Dependent variable(response)			
R1	% Elongation	Maximizing	
R2	% Drug release	Maximizing	

Table 3. Multiple Factorial experimental design for the preparation of different batches and their obtained responses for patches containing peppermint oil as permeation enhancer.

Std	Run	Factor 1 A:HPMC	Factor 2 B.EC	Factor 3 C:peppermint oil	Actual R1 Elongation	Actual R2 Drug permeation	Predicted R1 Elongation	Predicted R2 Drug permeation
12	1	200	200	0.4	75.8	78	70.84	78.05
13	2	100	200	0.4	55.6	74.4	56.26	76.33
3	3	100	200	0.3	53.5	72.9	52.56	75.08
10	4	100	100	0.3	45	94.2	47.46	91.23
18	5	200	100	0.4	68.6	90.4	65.74	94.21
14	6	300	150	0.4	77.5	89.6	82.87	87.85
9	7	100	100	0.3	45.04	94.5	47.46	91.23
11	8	300	200	0.4	80.5	83.4	85.43	79.78
8	9	200	100	0.3	60.3	89.4	62.05	92.96
7	10	300	100	0.3	80.2	94.3	76.63	94.68
5	11	200	150	0.3	68.1	79.4	64.60	84.88
2	12	200	200	0.3	72.4	76.3	67.15	76.80
4	13	300	150	0.3	75.5	87.2	79.18	86.60
16	14	100	150	0.4	52.3	86.2	53.70	84.41
15	15	200	150	0.4	70.2	81.2	68.29	86.13
19	16	100	100	0.4	48.6	95.6	51.15	92.49
17	17	300	100	0.4	85.5	96.4	80.32	95.93
1	18	300	200	0.3	78.6	81.62	81.73	78.52
6	19	100	150	0.3	50.2	85.3	50.01	83.16

Table 4. Multiple factorial experimental design for the preparation of different batches and their obtained responses for patches containing jojoba oil as permeation enhancer.

Std	Run	Factor 1 A:HPMC	Factor 2 B:EC	Factor 3 C:Jojoba oil	Actual R1 Elongation	Actual R2 Drug permeation	Predicted R1 Elongation	Predicted R2 Drug permeation
15	1	100	150	0.3	55.4	87.4	53.11	83.68
14	2	200	150	0.3	70.4	80.6	66.39	85.67
17	3	200	100	0.3	62.5	91.5	64.27	93.44
4	4	300	150	0.4	74.6	91.6	81.85	89.36
9	5	100	100	0.4	50.3	96.5	53.19	93.15
3	6	100	200	0.4	56.5	75.5	57.42	77.60
16	7	300	100	0.3	81.4	94.9	77.55	95.43
5	8	200	150	0.4	71.3	82.6	68.58	87.37
1	9	300	200	0.4	82	84.4	83.97	81.58
7	10	300	100	0.4	85.9	97.1	79.74	97.13
12	11	100	200	0.3	55.4	73.7	55.23	75.90
6	12	100	150	0.4	53.2	87.5	55.30	85.38
18	13	100	100	0.3	46	94.6	51.00	91.45
13	14	300	150	0.3	76.2	88.5	79.66	87.66
2	15	200	200	0.4	75.4	79.5	70.69	79.59
11	16	200	200	0.3	74.2	77.2	68.51	77.89
8	17	200	100	0.4	68	91.6	66.46	95.14
10	18	300	200	0.3	76	82.6	81.78	79.88

Preformulation study of the drug: *Curcumin*

Preformulation study is defined a study of physical and chemical properties of a drug substance alone and in combination with excipients. Organoleptic properties, melting point, solubility studies and calibration of *curcumin* was done. The objective of the study is to generate information useful in developing stable and bioavailable dosage form.

FTIR study

The FTIR of *curcumin*, polymers and the patches containing *curcumin* was done at a resolution of $4cm^{-1}$ over 400-$4000cm^{-1}$ wavelength region. The FTIR spectrophotometer (model: Shimadzu Analytical India Pvt. Ltd (SAIP).) employed Attenuated Total Reflectance –FTIR technique to determine IR transmission spectra and record the characteristic peaks [23].

Folding endurance

The folding endurance was determined manually by taking a strip of patch (4X2cm) and repeatedly folding it at the same place till it breaks. Folding endurance is considered as the number of times the film is folded at the same place without breaking/cracking [24].

Thickness

Thickness of the patches was determined by using digital vernier calliper (Mityutoyo, Japan) at different locations. Average values and standard deviations were determined (25).

Weight variation

Weight variation was done by weighing $1.76cm^2$ area of film accurately on digital balance (Sartorious, Germany BT224S, d=0.1mg) and the average of three determinations was calculated [26].

Percentage elongation

The elongation percentage was calculated using custom elongation testing apparatus; the percentage elongation was determined by using equation 1.

$$\% \text{ elongation} = [L2-L1]/L1*100$$

Where, L1 is the initial length and L2 is the final length of the film [23].

Percentage moisture content [25]

The prepared films were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 24 h. After 24 h, the films were are weighed and determined the percentage moisture content from the below mentioned

$$\text{Percentage moisture content} = (\text{Initial weight} - \text{Final weight})/\text{Finalweight} \times 100.$$

Moisture uptake, swelling ratio and erosion studies [27]

1cmX1cm patch specimens were taken for moisture uptake, swelling ratio and erosion studies. For moisture uptake study, the patch specimens were initially weighed (W_0) and then placed in stability chamber (model: Climate Chamber ICH/ICH L, Memmert GmbH þ Co. KG, Germany) set at 25±2°C temperature and 75% relative humidity. The specimens were removed after constant weight is achieved (W_u).Moisture uptake was calculated by the following equation:

$$\text{Moisture uptake} = W_u\text{-}W_0/W_0$$

For swelling ration and erosion studies patches were dried overnight at 60±2°C. Then the patches were weighed (W_0) and dipped in 5ml of distilled water. After that the patches were placed in stability chamber set at 25±2°C temperature and 75% relative humidity for 48hours. These hydrated patches were weighed(W_s) and then dried again at 60±2°C overnight, and weighed again(W_d).the percentage swelling ratio and percentage erosion were then calculated by following formulas given below;

$$\% \text{ Swelling ratio} = W_s - W_0 / W_0 X\ 100$$

$$\% \text{ Erosion} = W_0 - W_d / W_0 X\ 100$$

Percent drug content

Patches of 2cmX2cm are cut from different locations. Each patch sample was soaked in phosphate buffer (pH 5.5) in 10ml volumetric flask and then sonicated at 25°C for 30min. The solutions were then centrifuged for 15min at 5200rpm. Finally the solutions were filtered through 0.45mm filters and analyzed spectrophotometrically [28].

In vitro drug release studies

The formulated patches are adhered to the cellophane paper and then attached to the franz diffusion cell such as that the drug releasing surface was towards the receptor compartment with diffusion area of $0.785cm^2$. The receptor compartment is filled with phosphate buffer of pH 7.4 at 37°C.The buffer is stirred magnetically. At predetermined intervals, samples (5ml) are withdrawn and replaced with the same volume of phosphate buffer. The collected samples were diluted with equal volumes of ethanol and the absorbance was recorded at 416.0 nm [29-31].

Results and discussion

Preformulation studies

Organoleptic properties of *curcumin* were determined by various tests. *Curcumin* was yellowish orange powder with acrid taste without any odor. Its melting point was found to be 181°C±1°C.

Determination of absorption maxima (λ max)

The wave length of light in the ultra violet range at which the compound shows the maximum absorbance is called as λmax. Calibration Curve of *Curcumin* at 416.0 nm is taken and curve show in Figure 1.

Correlation Co-efficient (r^2) = 0.999 Equation for regressed line y =0.2043x-0.0044, Slope of regressed line =0.2043

Where x = Concentration (µg/ml), y = Absorbance (nm)

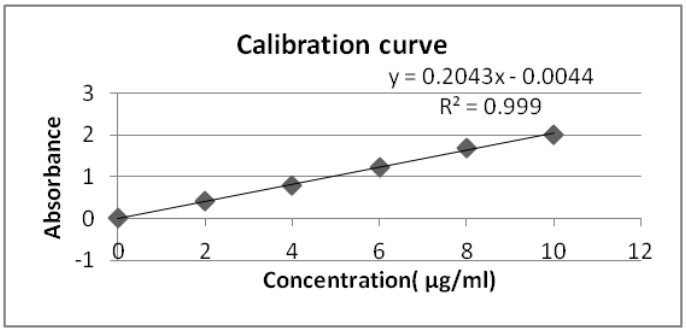


Figure 1. Calibration curve of Curcumin

FTIR study

The FT-IR spectra of pure curcumin showed absorption bands at 3629 cm^{-1} which indicate the presence of OH group. Peak at 2933cm^{-1} indicate the presence of C-H stretching. Peak at 1716cm^{-1} shows ketone C-O stretching and peak at 1312cm^{-1} is attributed to enol C-O peak. Peak at 1653 cm^{-1} show the presence of C=C stretching. Peak at 1196 cm^{-1} indicates the presence of O-H bend (phenolic). CH vibration of aromatic ring occurs at 814cm^{-1}. Mixing curcumin with polymers in the form of physical mixture and development of optimized formulation did not show interference with the characteristic drug peaks. This finding confirms compatibility of the drug with the studied additives (Figure 2).

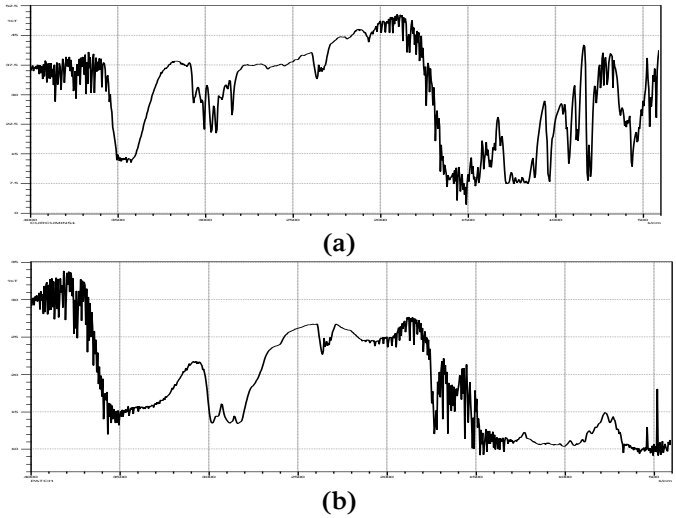


Figure 2. IR spectras of (a) Curcumin (b)Transdermal Patch.

Physicochemical evaluation of transdermal patches

Physicochemical evaluation of the prepared formulations (Figure 3) was carried out in terms of weight variation, thickness, folding endurance, moisture content, moisture uptake, swelling ratio, erosion and drug content. Variables like Moisture content, Moisture uptake, swelling ratio and erosion have a large impact on release behavior of drug from transdermal patches [24].

Table 5. Physicochemical and mechanical properties of curcumin patches containing peppermint oil as permeation enhancer.

Run	A:HPMC (mg)	B:EC (mg)	C: Peppermint oil (ml)	Wt. variation ±SD(mg)	Thickness ±SD (mm)	% Moisture content ±SD	Folding endurance ±SD	% Drug content ±SD	Moisture uptake ±SD (mg)	% Swelling ratio ±SD	% Erosion ±SD
1	200	200	0.4	206±0.6	0.18±1.4	3.5±1.5	11.0±0.8	94.3±0.4	20.04±0.5	22.7±0.3	1.54±0.9
2	100	200	0.4	198±2.1	0.17±0.5	3.14±1.8	11.8±0.3	97.5±1.3	20.03±1.3	22.4±0.4	1.93±1.0
3	100	200	0.3	198±3.3	0.17±0.3	3.1±0.4	10.9±2.2	97.1±0.2	20.15±0.1	22±0.2	1.9±0.3
4	100	100	0.3	195±0.1	0.15±1.4	2.0±0.3	9.0±0.3	98.5±1.3	16.4±1.4	16.03±1.2	1.55±0.5
5	200	100	0.4	205±0.4	0.16±0.4	3.4±0.1	12±1.1	97.6±1.1	20.4±0.3	22.2±0.5	1.94±0.2
6	300	150	0.4	209±0.4	0.19±0.4	3.93±1.6	11.6±1.1	96.5±0.4	23.2±0.2	24.1±0.2	1.92±0.2
7	100	100	0.3	195±0.1	0.15±1.4	2.0±0.3	9.0±0.3	98.5±1.3	16.4±1.4	16.03±1.2	1.55±0.5
8	300	200	0.4	210±0.4	0.19±0.3	3.9±0.4	11.2±0.4	95.6±0.2	26.12±0.3	24.2±0.1	1.8±0.2
9	200	100	0.3	205±0.2	0.16±0.3	3.3±0.2	11.9±1.0	98±1.2	20.2±0.2	22.1±0.4	1.90±0.3
10	300	100	0.3	208± 1.1	0.18±0.3	4.25±0.4	11.9± 1.2	98± 0.1	25.9± 1.1	24.2±1.1	2.0 ±0.2
11	200	150	0.3	209± 0.6	0.18±0.1	3.52±0.1	12.9±0.5	98±0.1	20.2±0.1	22.1±1.2	1.9±0.1
12	200	200	0.3	205±0.4	0.18±1.4	345±1.6	10.0±0.5	94.2±0.6	19.04±0.5	21.9±0.3	1.53±0.2
13	300	150	0.3	209±0.3	0.19±0.3	3.92±1.6	11.2±0.2	96.2±0.2	23.1±0.3	24.0±0.3	1.91±0.3
14	100	150	0.4	200±0.3	0.18±0.2	2.4±0.4	12.3±0.3	96.2±0.2	17.5±0.3	19±0.2	1.91±1.1
15	200	150	0.4	209± 1.6	0.18±0.2	3.53±0.4	13±2.2	98±0.2	20.3±0.15	22.3±1.8	1.93±0.2
16	100	100	0.4	196±0.2	0.15±1.3	2.1±0.4	9.5±0.3	99.0±1.2	16.5±1.1	16.03±1.2	1.61±0.4
17	300	100	0.4	208± 2.1	0.18±0.3	4.35±0.5	12± 1.5	99± 0.2	26± 2.1	24.20±1.2	2.1 ±0.1
18	300	200	0.3	209±0.3	0.19±0.4	3.8±0.5	11.0±0.3	95.5±0.3	26±0.2	24.1±0.2	1.8±0.3

The moisture uptake, Moisture content, Moisture uptake, swelling ratio and erosion of all the formulations was determined (Table 5 and Table 6). It can be concluded that as the concentration of hydrophilic polymer (HPMC) increases in the patches, the Moisture content, Moisture uptake, swelling ratio and erosion of patches increases as the hydrophilic part of the polymer blend dissolves and erodes easily on contact with aqueous media.

Analysis of suitability of model

The essential components of this statistical design comprise of input variables and their response, experiment design, running experiment, statistical analysis and optimization of formulation. Multiple factorial designs was used to study the combined effect of process variables like HPMC concentration, EC concentration and natural oils concentration at different variables on dependant variables i.e. percent elongation and drug permeation. To analyze the adequacy of models, experimental data was fitted to various polynomial models including linear, interactive, cubic and quadratic and statistical tests such as sequential model sum of squares and model summary was prepared (Table 7 and Table 8).

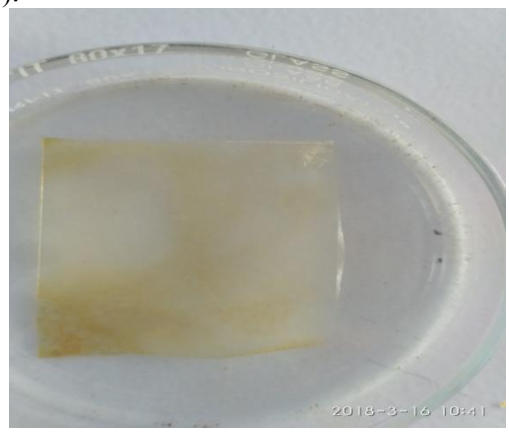


Figure 3. The prepared transdermal patch of curcumin.

Table 6. Physicochemical and mechanical properties of curcumin patches containing jojoba oil as permeation enhancer

Run	A:HPMC (mg)	B:EC (mg)	C:Jojoba oil (ml)	Wt. variation ±SD (mg)	Thickness ±SD (mm)	% Moisture content ±SD	Folding endurance ±SD	% Drug content ±SD	Moisture uptake ±SD (mg)	% Swelling ratio ±SD	% Erosion ±SD
1	100	150	0.3	200±0.2	0.18±0.1	2.41±0.3	12.4±0.2	96.4±0.1	17.4±0.2	19.2±0.1	1.9±1.2
2	200	150	0.3	209± 0.5	0.18±0.2	3.53±0.2	12.9±0.4	98.2±0.2	20.3±0.2	23±1.1	1.92±0.2
3	200	100	0.3	205±0.1	0.16±0.4	3.4±1.2	11.9±1.0	98.3±0.1	20.3±0.3	22±0.2	1.91±0.2
4	300	150	0.4	209±0.2	0.19±0.5	3.9±2.2	11.7±1.2	96.6±0.2	23.3±0.3	24.2±0.1	1.93±0.3
5	100	100	0.4	196±0.3	0.15±1.2	2.2±0.4	9.6±0.2	99.2±1.1	16.7±0.2	16.3±0.2	1.7±0.3
6	100	200	0.4	198±2.2	0.17±0.4	3.1±0.5	11.9±0.4	97.7±0.2	20.2±1.2	22.5±0.3	1.9±1.3
7	300	100	0.3	208± 0.6	0.18±0.2	4.2±0.5	11.9± 1.1	98.5± 0.2	25.8± 1.2	24.3±0.2	2.1 ±0.4
8	200	150	0.4	209± 0.5	0.18±0.3	3.6±1.5	13.1±2.1	98.4±0.2	20.4±0.1	22.4±1.4	1.9±0.4
9	300	200	0.4	210±0.2	0.19±0.2	3.9±1.3	11.2±0.2	95.8±0.1	26.3±0.2	24.3±0.2	1.6±0.4
10	300	100	0.4	208± 1.0	0.18±0.2	4.4±0.4	12.2± 1.2	99.2± 0.1	26.1± 0.1	24.3±1.1	2.2 ±0.2
11	100	200	0.3	198±2.0	0.17±0.2	3.2±1.3	10.9±2.3	97.3±0.1	20±0.2	22.2±0.3	1.8±0.4
12	100	150	0.4	200±0.2	0.18±1.3	2.6±1.3	12.4±0.4	96.3±0.3	17.3±0.2	19.2±0.3	1.92±1.2
13	100	100	0.3	195±0.2	0.15±0.2	2.1±0.3	9.1±0.2	98.7±1.1	16±1.2	16.4±1.3	1.56±0.3
14	300	150	0.3	209±0.4	0.19±0.3	3.9±1.2	11.4±0.3	96.3±0.3	23±0.2	24.1±0.4	1.92±0.2
15	200	200	0.4	206±0.5	0.18±0.3	3.6±1.2	11.2±0.5	94.5±0.2	21±0.4	22.4±0.4	1.53±0.4
16	200	200	0.3	205±0.3	0.18±0.3	3.5±2.2	10.2±0.4	94.4±0.5	19.2±0.3	21±0.4	1.54±0.4
17	200	100	0.4	205±0.3	0.16±0.3	3.5±0.4	12.1±1.2	97.8±0.2	20.5±0.2	22±0.4	1.95±1.4
18	300	200	0.3	209±0.2	0.19±0.3	3.9±0.2	11.2±0.4	95.7±0.5	26.3±0.1	24.3±0.3	1.9±0.2

Table 7. Fit summary model for the measured responses R1 and R2 for patches containing peppermint oil as permeation enhancer.

Response 1: Elongaton

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9198**	**0.8906**	**Suggested**
2FI	0.1432	0.9352	0.8970	
Quadratic	0.0304	0.9613	0.9170	Aliased

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	81375.95	1	81375.95			
Linear vs Mean	**3033.51**	**3**	**1011.17**	**69.85**	**< 0.0001**	**Suggested**
2FI vs Linear	76.62	3	25.54	2.18	0.1432	
Quadratic vs 2FI	70.66	2	35.33	5.06	0.0304	Aliased
Residual	69.87	10	6.99			
Total	84626.60	19	4454.03			

Response 2: Drug permeated

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.0502**	**0.8175**	**0.7693**	**Suggested**
2FI	0.2036	0.0532	0.8423	0.7813	
Quadratic	0.0012	0.0939	0.9509	0.8993	Aliased

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	1.399E+05	1	1.399E+05			
Linear vs Mean	**856.97**	**3**	**285.66**	**27.88**	**< 0.0001**	**Suggested**
2FI vs Linear	47.41	3	15.80	1.78	0.2036	
Quadratic vs 2FI	78.72	2	39.36	14.29	0.0012	Aliased
Residual	27.54	10	2.75			
Total	1.409E+05	19	7415.92			

Table 8. Fit summary model for the measured responses R1 and R2 for patches containing jojoba oil as permeation enhancer.

Response 1: Elongation

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.8614**	**0.8078**	**Suggested**
2FI	0.2607	0.8756	0.7899	
Quadratic	0.1188	0.9053	0.7867	Aliased

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	81972.01	1	81972.01			
Linear vs Mean	**2190.03**	**3**	**730.01**	**36.22**	**< 0.0001**	**Suggested**
2FI vs Linear	83.21	3	27.74	1.53	0.2607	
Quadratic vs 2FI	75.01	2	37.51	2.72	0.1188	Aliased
Residual	123.91	9	13.77			
Total	84444.17	18	4691.34			

Response 2: Drug permeated

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**		**0.8217**	**0.7694**	**Suggested**
2FI	0.3023		0.8348	0.7877	
Quadratic	0.0078		0.9314	0.8691	Aliased

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	1.347E+05	1	1.347E+05			
Linear vs Mean	**786.01**	**3**	**262.00**	**27.11**	**< 0.0001**	**Suggested**
2FI vs Linear	36.85	3	12.28	1.37	0.3023	
Quadratic vs 2FI	65.01	2	32.50	8.74	0.0078	Aliased
Residual	33.46	9	3.72			
Total	1.357E+05	18	7536.32			

Effect of formulation variables on % elongation of the film

ANOVA was used to evaluate experimental data and p value of regression coefficients was used to measure significance as shown in table 6. Percent Elongation is a good means to analyze the mechanical properties of film. A soft film has low percent elongation and tensile strength whereas hard and brittle films are characterized by low elongation and moderate tensile strength. A film having high tensile strength and high elongation is considered as soft and tough film [32]. So, in order to decrease the brittleness of film and augment the elasticity

of film required percentage of plasticizer is incorporated [33]. The elongation percentage varies from 45-85.5% (peppermint oil) and 46-85.9% (jojoba oil) as shown in Table 2. Linear model was observed to fit for the percent elongation response with a p value and F value of <0.0001 and 69.85 in case of patches containing peppermint oil and <0.0001 and 36.1 in case of patches formulated with jojoba oil respectively. It was found that linear coefficients (A, B, C) were significant for the chosen model. P value was used to judge interaction strength between variables and significance of individual coefficients [34]. It is clear from the response surface plot that both concentration of natural oils and HPMC percentage (C) are the significant variables affecting the percent elongation in positive manner with a p value of <0.0001 (Table 9). Permeation enhancers weaken the cohesion forces between polymer chains or interrupt polymer chains thereby increasing mobility of chains and hence improving flexibility of polymer matrix [35, 36]. The accuracy of the model to determine the percent elongation of film was affirmed by the ANOVA of observed values which yielded linear relationship with R^2 value of 0.8078% (Table 9). The linear equation generated by software is given below:

% Elongation = +13.28*HPMC+ 2.12*EC+1.09* jojoba oil (patches containing jojoba oil as permeation enhancer)

% Elongation = +14.59*HPMC-2.55*EC+1.85* peppermint oil (patches containing peppermint oil as permeation enhancer).

Table 9. Analysis of variance table for measured responses for patches containing peppermint oil as permeation enhancer.

ANOVA for Linear model

Response 1: Elongaton

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3033.51	3	1011.17	69.85	< 0.0001	significant
A-HPMC	2730.79	1	2730.79	188.64	< 0.0001	
B-EC	83.59	1	83.59	5.77	0.0297	
C-peppermint oil	64.10	1	64.10	4.43	0.0526	
Residual	217.14	15	14.48			
Lack of Fit	217.14	14	15.51	19387.80	0.0056	significant
Pure Error	0.0008	1	0.0008			
Cor Total	3250.65	18				

Fit statistics

Std. Dev.	3.80	**R^2**	0.9332
Mean	65.44	**Adjusted R^2**	0.9198
C.V. %	5.81	**Predicted R^2**	0.8906
		Adeq Precision	21.7470

Response 2: Drug permeated

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	856.97	3	285.66	27.88	< 0.0001	significant
A-HPMC	38.03	1	38.03	3.71	0.0732	
B-EC	837.85	1	837.85	81.78	< 0.0001	
C-peppermint oil	7.43	1	7.43	0.7257	0.4077	
Residual	153.67	15	10.24			
Lack of Fit	153.63	14	10.97	243.86	0.0502	not significant
Pure Error	0.0450	1	0.0450			
Cor Total	1010.64	18				

Fit Statistics

Std. Dev.	3.20	**R^2**	0.8479
Mean	85.81	**Adjusted R^2**	0.8175
C.V. %	3.73	**Predicted R^2**	0.7693
		Adeq Precision	14.2019

In response surface graphs such as contour and 3D plots (Figure 4), increase in HPMC proportion in the polymer mixture provides higher tensile strength to the film as shown in figure 4a, 4b, 4c and 4d. For a drug delivery system for skin application a hard and tough film is desired [37]. In case of jojoba oil, the %R1 (85.9) was observed highest for experimental run 7 followed by 82 for experimental run 9.Similarly for peppermint oil, the % R1 (85.5) was observed highest for experimental run 17 followed by 80.5 for experimental run 8. It is revealed from Figure 4 that with increase in concentration of EC (hydrophobic polymer), decrease in tensile strength was observed. It can be concluded that the preparation

containing high concentration of HPMC and natural oils has higher tensile strength [22-23].

Effect of formulation variables on % drug permeation of the film

ANOVA was used to evaluate experimental data and p-value of regression coefficients was used to measure significance as shown in table 6. Generally, the drug permeation is a good tool to illustrate the dissolution characteristics of the film [38]. A film that has low drug permeation has poor dissolution properties. The use of the proper percentage of permeation enhancer and the type and concentration of hydrophilic/hydrophobic polymer enhances the drug release from the film [39, 40]. The data presented in table 2 demonstrated that drug release percent ranged from 72.9 to 96.4% (peppermint oil) and 73.7 to 97.1% (Jojoba oil) respectively. Linear model was observed to fit for response % drug permeation with a p and F value of <0.0001 and 27.88 (Peppermint oil) and <0.0001 and 27.11 (jojoba oil) respectively. For this model linear coefficients A and B were found to be significant. P-value was used to determine the significance of individual coefficients and interaction strength between variables. From response surface plot it is revealed that the concentration of HPMC and natural oils are the major variables influencing the drug permeation percent in positive trend with p-value of <0.0001 (Table 10). The accuracy of the model was confirmed by the R^2 value (0.7694%) for patches containing peppermint oil and 0.8217 for patches containing jojoba oil respectively as determined by the ANOVA of the observed values which yielded linear relationships (Table 5). The linear equation generated by software is given below:

% Drug permeation = +1.99*HPMC-7.7*EC+0.8500* jojoba oil (patches containing jojoba oil as permeation enhancer)

% Drug permeation = +1.72*HPMC-8.08*EC+0.6286* peppermint oil (patches containing peppermint oil as permeation enhancer).

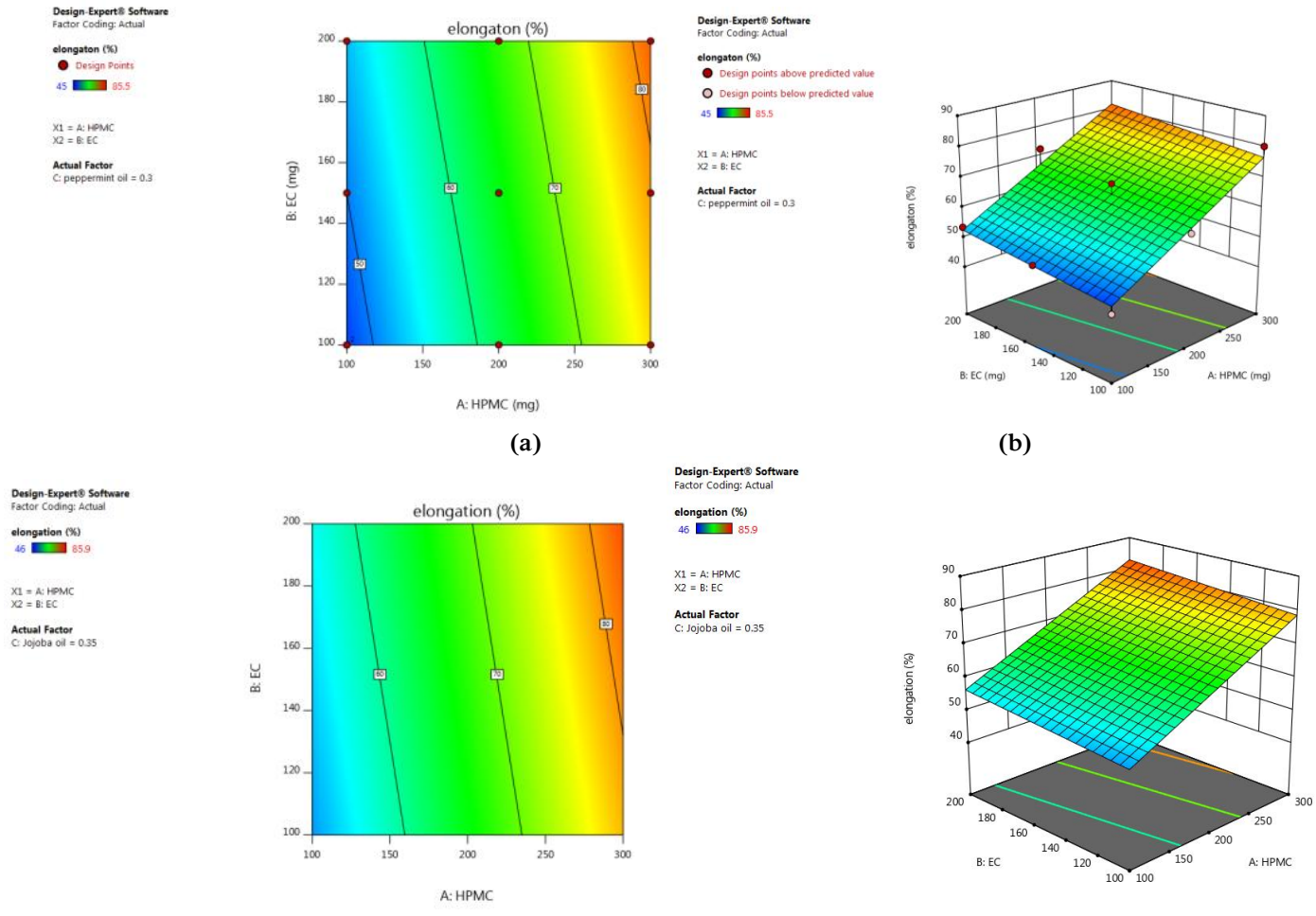


Figure 4. Contour plots and 3D response surface plots showing effect of HPMC and EC incorporating 0.35ml of peppermint oil on response R1 (%elongation) (a) and (b); Contour plots and 3D response surface plots showing effect of HPMC and EC incorporating 0.35ml of jojoba oil on response R1(%elongation) (c) and (d).

From the equation it is revealed that both factors A and C have positive influence on the %drug release of the films. It is also displayed in patches containing jojoba oil as permeation enhancer, factor A having coefficient value of 1.99 has higher impact than factor C having coefficient value of 0.8500.Similar results are observed in case of patches containing peppermint oil as permeation enhancer. Factor A has coefficient value of 1.72 while factor C has coefficient value of 0.6286. The response surface plots also show the same response as mentioned above. (Figure 5). Figure 5a, 5b, 5c and 5d reveals that as the proportion of HPMC increases in polymer mixture percent drug permeation also increases. Therefore, it was concluded that the increase in HPMC and natural oils percentage has direct influence on the film's drug permeation. With the increase in natural oil concentration (0.3- 0.4ml) and HPMC (300-100mg) concentration, drug release was found to increase. The percent drug permeation was observed to be highest (97.1%) for experimental run 7 followed by 96.5% for experimental run 9 in case of patches containing jojoba oil. Similarly, in case of patches containing peppermint oil, the drug permeation was found to be highest for experimental run 17 i.e 96.4% followed by 95.6% for experimental run 16. Linear correlation plots are illustrated between observed and predicted values for the responses (Figure 6a, 6b, 6c and 6d). The predicted R^2 value in case of patches containing jojoba oil (0.7694) was in complete agreement with adjusted R^2 value (0.8217) as the difference between the two was found to be less than 0.2 and incase of patches containing peppermint oil predicted R^2 was found to be 0.7693 and adjusted R^2 value was found to be 0.8175. [22-23] Permeation enhancers decrease the lag time which occurs due to decrease in diffusional path length of drug which is attributed to changes in stratum corneum. Jojoba oil contains fatty acids like docosenoic, eicosenoic, myristic acid and oleic acid. Myristic acid is reported to have similar composition as skin oil which leads to complete and faster absorption of drug. Oleic acid also enhances the epidermal permeability by perturbation of stratum corneum lipid bilayers and lacunae formation thereby distrupting molecular packaging and level of hydration which contributes to increased permeation [41]. Peppermint (*Mentha piperita* Linn.) itself acts as anti-inflammatory agent, can exert synergistic effect on inflammation [42]. Release of drug in patches containing peppermint oil depends on presence of terpenes i.e cineol, limonene and pinene and menthol. Terpenes loosen the existing network of hydrogen bonds between ceramides which results in higer permeation of drug across stratum corneum [41,43].

Table 10. Analysis of variance table for measured responses for patches containing jojoba oil as permeation enhancer

ANOVA for Linear model

Response 1: Elongation

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2190.03	3	730.01	36.22	< 0.0001	significant
A-HPMC	2114.71	1	2114.71	104.94	< 0.0001	
B-EC	53.76	1	53.76	2.67	0.1247	
C-Jojoba oil	21.56	1	21.56	1.07	0.3185	
Residual	282.13	14	20.15			
Cor Total	2472.17	17				

Fit Statistics

Std. Dev.	**4.49**	**R^2**	**0.8859**
Mean	67.48	Adjusted R^2	0.8614
C.V. %	6.65	Predicted R^2	0.8078
		Adeq Precision	15.5808

Response 2: Drug permeated

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	786.01	3	262.00	27.11	< 0.0001	significant
A-HPMC	47.60	1	47.60	4.93	0.0435	
B-EC	725.41	1	725.41	75.05	< 0.0001	
C-Jojoba oil	13.01	1	13.01	1.35	0.2655	
Residual	135.31	14	9.67			
Cor Total	921.32	17				

Fit Statistics

Std. Dev.	3.11	**R^2**	0.8531
Mean	86.52	**Adjusted R^2**	0.8217
C.V. %	3.59	**Predicted R^2**	0.7694
		Adeq Precision	14.4884

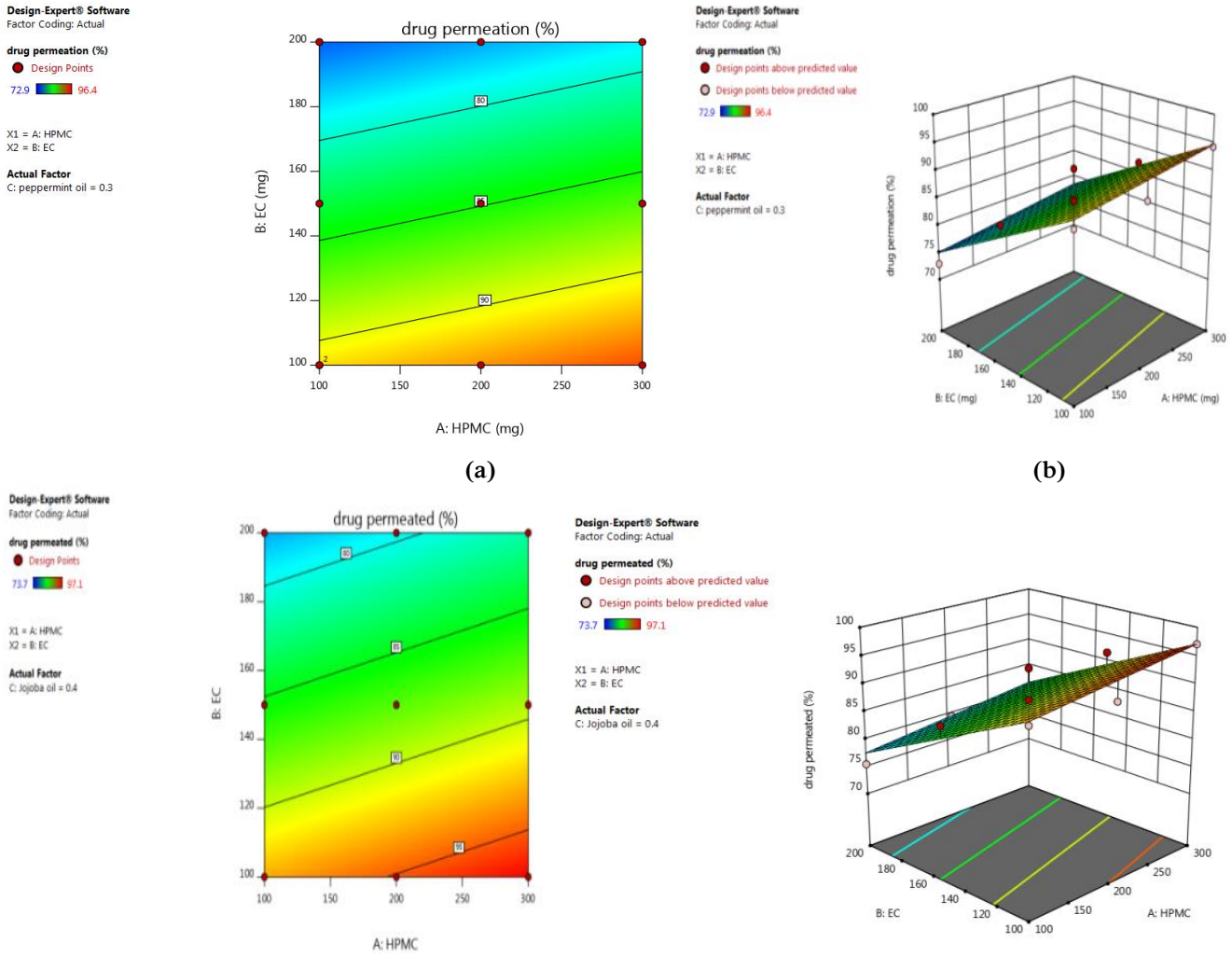
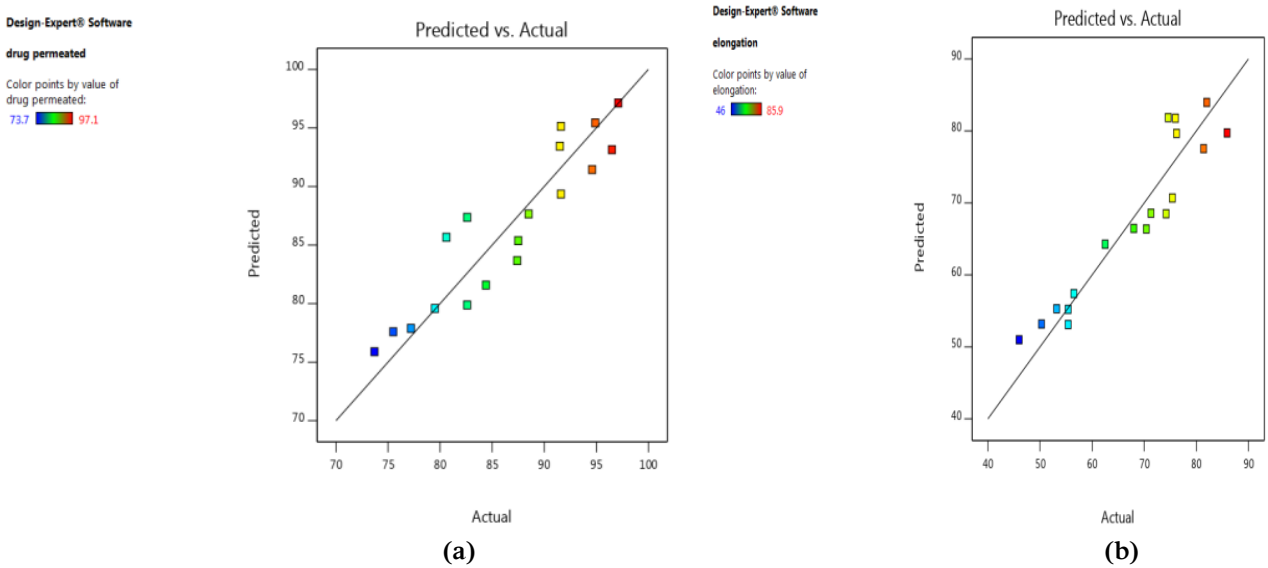
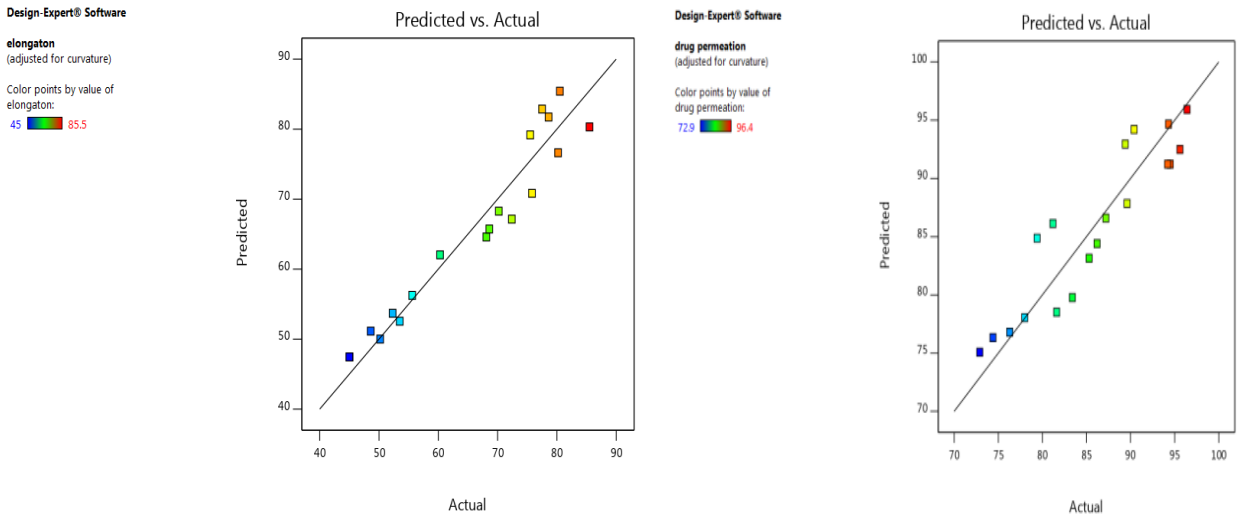


Figure 5. Contour plots and 3D response surface plots showing effect of HPMC and EC incorporating 0.35ml of peppermint oil on response R2(%drug permeation) (a) and (b); Contour plots and 3D response surface plots showing effect of HPMC and EC incorporating 0.35ml of jojoba oil on response 2 (%drug permeation) (c) and (d).





(c) **(d)**

Figure 6. Linear correlation plots (a) between actual and predicted values of % elongation(R1) and (b) between actual and predicted values of % drug release (R2) for patches containing jojoba oil (c) between actual and predicted values of % elongation(R1) and (d) between actual and predicted values of % drug release (R2) for patches containing peppermint oil.

Optimization

As we all know that release of drug from transdermal film varies with structure and morphology of polymer matrix and physicochemical properties of drug [44]. Hydrophilic nature of HPMC(X1) exerts positive effect on release of curcumin owing to its high water absorbing property. This in turn increases the porosity and pore diameter of polymer matrix thereby allowing drug molecules to diffuse out easily [45]. Moreover increase in HPMC loading results in faster dissolution of polymer matrix resulting in formation of channels for diffusion of drug from the film [46]. On the other hand natural oils also contribute to good permeation of curcumin. It also enhances curcumin diffusion by decreasing the skin barrier resistance reversibly. It has also been indicated in literature that if the drug is soluble in polymer matrix, its thermodynamic activity increases in base resulting in its enhanced permeation [47-49]. As a final point after analysis of experimental variables an optimum formulation of curcumin film containing peppermint oil with acceptable drug release and elongation was derived to contain 200mg HPMC, 150mg EC and 0.35ml of oil (Figure 7). In case of patches containing jojoba oil the optimum formulation was found to contain 300mg of HPMC, 100.21mg of EC, and 0.39ml of oil (Figure 8). The observed, predicted values, the residuals and prediction error percentage for the optimized formulation is demonstrated in Table 11 and Table 12.

Desired mechanical and physicochemical properties were ensured by the predicted values of responses for optimized formulation. The predicted values of responses for the optimized transdermal film were very close to the observed values with no considerable prediction error percentage (below 6%) and residuals. This outcome confirms that the optimization technique used is highly reliable and reproducible for the development of curcumin transdermal film with high quality attributes [22, 23].

Table 11. Optimum combination of factors, predicted, observed values, the residuals and the predicted error percentage for the optimized formulation of curcumin loaded transdermal film incorporating peppermint oil as permeation enhancer.

Factors	Optimum			
HPMC percentage	200			
EC percentage	150			
Peppermint oil percentage	0.35			
Responses	Predicted	Observed	Residual	Prediction error (%)
% Elongation	66.44	68.3	±1.9	2.861
% Drug release	85.50	83.9	±1.6	1.871

Residual = predicted value-observed value

Prediction error = predicted value-observed value/predicted value*100

Table 12. Optimum combination of factors, predicted, observed values, the residuals and the predicted error percentage for the optimized formulation of curcumin loaded transdermal film incorporating jojoba oil as permeation enhancer.

Factors	Optimum			
HPMC percentage	300			
EC percentage	100.2			
Peppermint oil percentage	0.39			
Responses	Predicted	Observed	Residual	Prediction error (%)
% Elongation	79.7	81	±1.3	1.6311
% Drug release	97.1	96.5	±0.6	0.6179

Residual = predicted value-observed value
Prediction error = predicted value-observed value/predicted value*100.

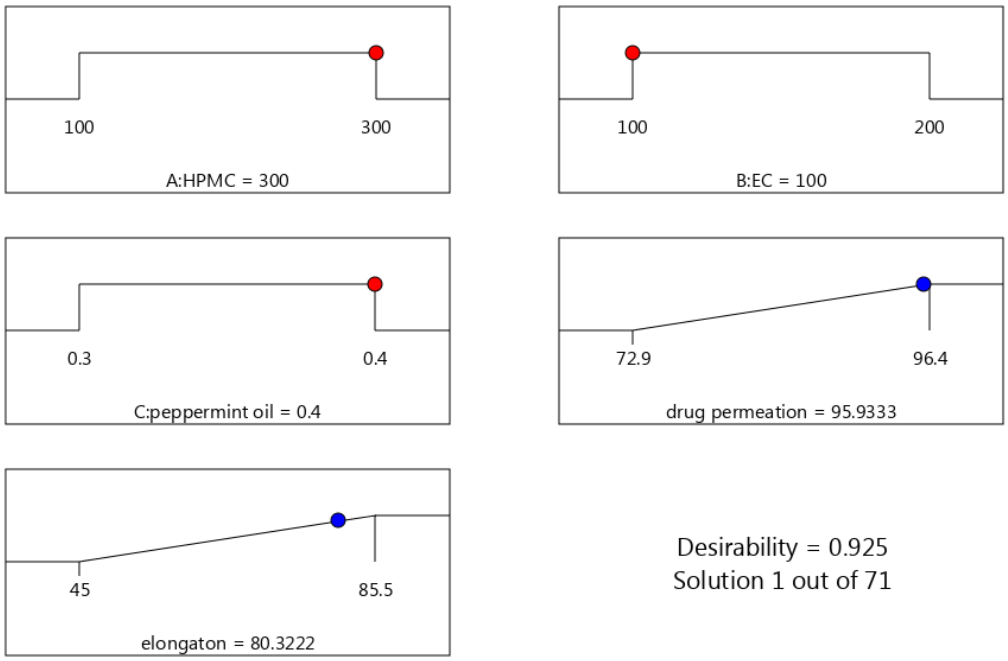


Figure 7. Desirability ramp for optimization for patches containing peppermint oil.

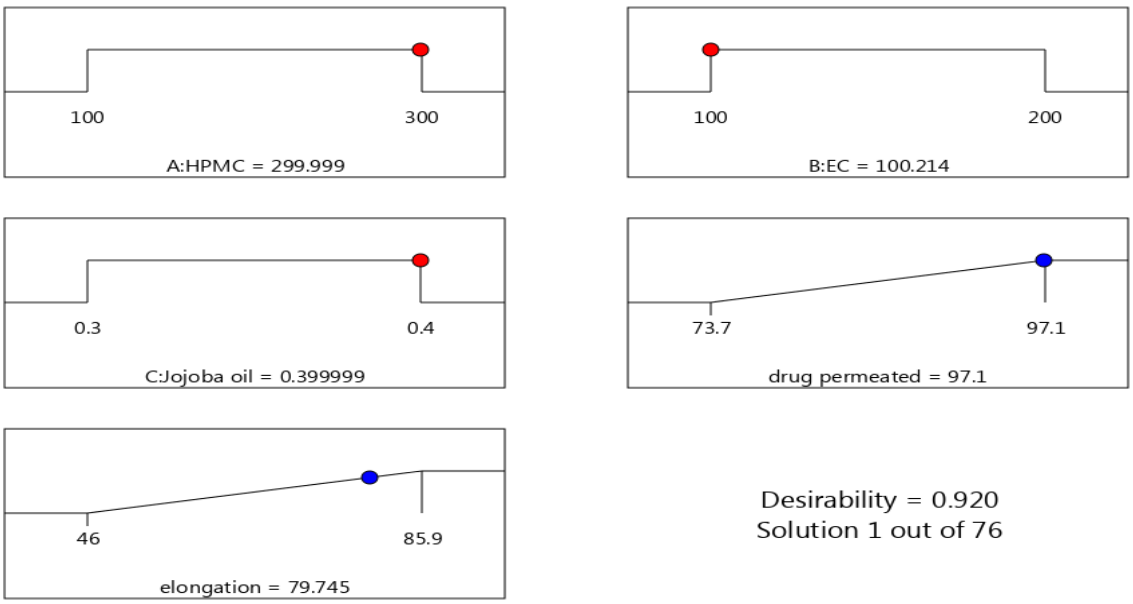


Figure 8. Desirability ramp for optimization for patches containing jojoba oil.

Conclusion

The multiple factorial designs was an efficient tool in optimizing the three variables used to prepare curcumin TDDS through studying their effect on the quality attributes and permeation behavior of the prepared curcumin-loaded transdermal films. The optimized formulations showed good physicochemical and mechanical properties as well as enhanced permeation characteristics. Patches containing jojoba oil as permeation enhancer showed better *in vitro* permeation and elongation than patches containing peppermint oil. The *in vitro* study reveals that the formulations containing natural oils as permeation enhancers and herbal drug (curcumin) has the potential to be used as effective alternative to the current drugs available in the market to treat arthritis. More research, optimization and invivo studies of herbal formulations must be done to get better products in future.

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