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Original Article

Formulation optimization and characterization of transdermal film of curcumin by response surface methodology

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ABSTRACT

Objective: India is referred as goldmine of herbal drugs but still lack of optimization of herbal drugs, which has kept us on the back foot. The rationale of the study is to prepare optimized transdermal drug delivery system of curcumin employing response surface methodology to study the collective effect of independent variables like concentration of ethyl cellulose, hydroxyl propyl methyl cellulose and dibutyl phthalate which significantly influenced characteristics like percentage elongation and *in vitro* drug release.

Method: Twenty formulations containing varying concentrations of polymers and permeation enhancer were prepared using solvent casting technique.

Result: The study revealed that the effect of dibutyl phthalate (DBP) concentration was the highest on percentage elongation ($P < 0.0001$), while hydroxy propyl methyl cellulose (HPMC) concentration exhibited pronounced effect on drug release ($P < 0.0001$) through dialysis membrane. Linear model fitted the best for curcumin release and elongation for all formulations. According to Derringer's desirability prediction tool, the composition of optimized film was found to be 242.14% of HPMC, 109.59% of ethyl cellulose (EC), and 1.03% of DBP. Under these conditions, the optimized patch exhibited a predicted value of %elongation and *in vitro* drug release of 94.35% and 80.0306%, respectively, which was comparable to the actual values of percent elongation and *in vitro* drug release i.e. 95.02% and 81.03% respectively. FTIR and thermal studies were also performed which revealed no interaction or complexation between drug and excipients. The *ex vivo* study performed using rat skin showed that the cumulative drug release from the optimized patch showed flux of (30.68 ± 18) μg/cm^2/h.

Conclusion: It can be concluded that in future if proper optimization of herbal formulations is carried out, they can become the first choice for patients as compare to synthetic drugs.

1. Introduction

Osteoarthritis (OA) has emerged as one of the main causes of disability in patients affecting 9.6% men and 18% women having age ≥ 60 worldwide, while in India it is affecting 14%–47% of population. Every year, in India 15 million people are grabbed by this leading cause of disability (Bhasker et al., 2016; Mahajan, Verma, & Tandon, 2005). The severity of disease depends on various factors such as joint dislocation, obesity, genetic predisposition, trauma, gender, joint malignment, muscle weakness, etc (Herrero-Beaumont et al., 2017). Nowadays young athletes, juveniles, middle age people are also diagnosed with this disorder. Common clinical symptoms related to OA are pain on movement, joint pain depending on use, cracking of the joints and stiffness of joints of hands, knees, feet, spine and hips (Rahmati, Mobasheri, & Mozafari, 2016). First line treatment used in case of mild to moderate pain of osteoarthritis are non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen and COX-2 cyclooxygenase selective inhibitors. In case of moderately severe pain, tramadol and other opoid analgesics are recommended and for severe pain opoids are prescribed. Further treatment is supplemented with various food supplements (glucosamine & chondroitin) and hyaluronic acid injections are also injected to improve the functioning of joints. There are many problems associated with the above mentioned drugs like (a) NSAIDs don't interfere with any immune-inflammatory reactions but affect only small area of inflammatory

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cycle i.e. prostaglandin production by cyclooxygenase, therefore they can't retard the progression of disease (Burch, Codding, Patel, & Sheldon, 2004; Kivitz, 2008). (b) Large part of the population either doesn't show any response to the recent treatments available in the market or show partial negligence towards them. (c) Moreover, extensive use of these drugs for long time may lead to multiple side effects like gastrointestinal damage, liver infection and heart failure in some cases (Park et al., 2013; Rathore, Mahid, Paul, Saxena, & Das, 2007). Therefore, safe and effective therapeutic strategies and options are needed to overcome the situation. Since ages the medicines were derived from plants (Pan et al., 2014). Natural drugs are gaining attention in the modern world to treat ailments with better therapeutic effects and minimal side effects. Another key factor to move from synthetic to herbal drugs is the release of veterinary and human drugs into the environment by numerous routes which has become a major issue of human and environmental health concern (Mehinto, 2009). The drug delivery system used for administering these herbal medicines to the patient is traditional and out-of-date, resulting in the reduced efficacy of the drug due to poor solubility, poor stability in highly acidic pH, first pass hepatic metabolism, chances of toxicity and poor stability. This leads to level of drug below therapeutic concentration in blood resulting in minimal or zero therapeutic effect. Novel herbal drug delivery system has gained attention in the recent years for delivery of herbal plant actives (Dhiman, Nanda, & Ahmad, 2012). Inclusion of plant actives in novel drug delivery system prunes the problems associated with crude plant extracts (Goyal, Kumar, Nagpal, Singh, & Arora, 2011; Lubick, 2010).

Curcuma longa L. popularly known as Turmeric or golden spice is a well-known plant known for its medicinal value. From bygone time, curcumin is utilized in diverse applications belong to Zingiberaceae family as inflammatory agent in ayurveda. It acts via multiple molecular targets i.e. IL-1β, 6, 8, TNF-α, COX-2, and NF-κB (Liu et al., 2015). It has been investigated in literature that it possesses low aqueous solubility which leads to poor bioavailability and optimum therapeutic concentration is difficult to achieve. Only 40%−85% of curcumin dose passes unchanged through gastrointestinal tract and most of the absorbed dose is metabolized in liver and intestinal mucosa which restricts its oral dose (Chen et al., 2012; Gadekar, Saurabh, Thakur, & Saurabh, 2012). Numerous problems are associated with oral intake of drugs/pharmaceutical agents such as various patients experience intolerance from a single dose and gastrointestinal irritation owing to sensitivities. As a consequence of protracted treatment, many others develop such intolerance or may develop ulcerations like stern gastrointestinal irritations. Erratic rates of absorption are observed in patients having largely differing metabolism. Undesirable effects on level of consciousness and cognition are observed in patients taking muscle relaxants and pain medication. The topical preparations give relief from pain without any risk of sedation and cognition (Archer & Pettit, 1997). Thus in order to triumph over these difficulties novel transdermal drug delivery systems are required to be developed. Moreover presently the herbal drugs present in the market are not properly optimized/standardized in respect to excipients utilized which restricts their utilization as compare to synthetic drugs (Agarwal & Singh, 2002; Marwick, 1995). The novelty and objective behind the present study is that though many articles are published on curcumin and though it's a common drug molecule available in various formulations but still its preparations are not used to that extend as that of synthetic drugs to treat osteoarthritis. So the aim of the present study is to prepare optimized transdermal film of curcumin with respect to excipients utilized by incorporating the combination of EC and HPMC by utilizing RSM. EC is a nonallergic, nontoxic polymer having possessing good properties to form tough films. HPMC, hydrophilic polymer is added to increase the release rate of the drug which leads to formation of pores on contact with aqueous media leading to release of drug (Kandavilli, Nair, & Panchaganula, 2001; Rao, Ramakrishna, & Diwan, 2000). Firstly, Box-Behnken 3-level, 3-factorial design is utilized to design experimental designs. Secondly, Derringer's desirability tool is employed to optimize the formulation with respect to variables. Design-Expert software was used to design trials and to obtain optimized formulation by analyzing response surface plots.

2. Materials and methods

2.1. 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 (DBP), polyethylene glycol 400 (PEG 400) were purchased from VK chemicals, Ambala Cantt.

2.2. Animals

For *in vivo* studies healthy albino rats weighing between (180–220) g of either sex with no prior treatment were chosen. The animals were kept in the laboratory for 10 d to adjust in that environment and housed in metal cages at (23 ± 2) °C and (50 ± 10)% RH. They are given food and water ad libitium. Animal study was done with due permission from Institutional Ethical Committee (Regn. No.873/PO/Re/S/2005/CPCSEA) in pharmacology division, Guru Gobind Singh College of Pharmacy, Yamunanagar (Haryana).

2.3. Experimental design

Drug permeation and mechanical properties of the film are affected by the concentration of HPMC, EC and permeation enhancer. Thus in order to study the consequence of responses i.e. percent elongation of film and drug release and optimization of formulation factors, Box–Behnken statistical design was chosen. In Table 1, all the dependent and independent variables were stated. For the present work, statistical analysis was conducted utilizing Design-Expert software, trial version of 45 d (DX10.0 version, Stat-Ease Inc., Minneapolis, MN, USA). The designed combinations of excipients by the software were mentioned in Table 2 (Ahmed & El-Say, 2016; Parhi & Suresh, 2016).

2.4. Preparation of transdermal patches of curcumin

Solvent casting technique was utilized to prepare transdermal patches. Varying concentrations of polymers and permeation enhancers were utilized to prepare casting solutions. The polymers i.e. EC and HPMC were weighed and then incorporated in equal quantities of chloroform: ethanol (1:1). A total of 20 mg curcumin was dissolved in the casting solutions along with PEG as plasticizer and DBP as permeation enhancer. All the ingredients are thoroughly mixed. The mixture was then poured in between the bangle placed

Table 1
Variables and their levels in 3^3 level factorial experimental design.

Variables	Levels		
	High (+1)	Medium (0)	Low (−1)
A: HPMC/mg	300	200	100
B: EC/mg	200	150	100
C: DBP/mL	1.5	1	0.5
Dependent variables (response)			
R1 (Elongation/%)	Maximizing		
R2 (Drug release/%)	Maximizing		

Table 2
Box-Behnken experimental design for preparation of different batches and their obtained responses.

Standard	Run	Factor A	Factor B	Factor C	Actual R2	Actual R1	Predicted R2	Predicted R1
		HPMC/mg	EC/mg	DBP/mL	Drug release/%	Elongation/%	Drug release/%	Elongation/%
10	1	300	200	1.0	70.7	75.00	69.78	76.51
7	2	200	100	0.5	79.8	74.00	78.69	78.43
20	3	100	100	1.5	75.37	60.00	72.68	62.26
16	4	100	200	1.5	60.5	56.00	57.38	52.53
2	5	100	200	0.5	59.4	31.00	57.01	36.15
5	6	100	150	0.5	61	39.00	64.69	40.10
3	7	300	150	0.5	81.6	76.00	77.32	73.50
18	8	200	150	1.5	69.4	87.00	71.30	89.20
15	9	200	100	1.0	80.2	90.00	78.84	89.31
19	10	300	100	1.5	83.3	110.00	85.30	106.81
11	11	100	150	1.0	61.8	50.00	64.84	49.53
12	12	100	150	1.0	61.8	50.00	64.84	49.53
13	13	200	100	1.0	80.2	90.00	78.84	89.31
9	14	300	200	1.0	70.7	75.00	69.78	76.51
1	15	200	200	0.5	61.8	68.00	63.32	63.31
6	16	300	100	0.5	82.2	82.00	85.00	84.68
8	17	100	100	0.5	75.2	50.00	72.38	45.90
17	18	200	150	1.5	69.4	87.00	71.30	89.20
4	19	200	150	0.5	68.5	72.00	71.01	69.94
14	20	200	100	1.0	80.2	90.00	78.84	89.31

on mercury surface in a petridish and covered with a inverted glass funnel for 24 h at room temperature to dry by evaporating the solvent. After 24 h, the prepared films were sliced into required dimensions. The patches were wrapped in aluminum foil and stored for 2 d in dessicator for further drying (Suksaeree et al., 2015).

2.5. Preformulation study of drug: curcumin

Analysis of chemical and physical characteristics of active ingredient along with its additives is called preformulation study. It serves as the base for the preparation of safe and efficacious dosage form. After procurement of drug, its preformulation study was conducted. Organoleptic properties, melting point and calibration curve of curcumin was prepared. The purpose of the study was to generate information valuable for the preparation of stable and bioavailable dosage form.

2.6. FTIR study

The FTIR studies of curcumin were performed over the 4000–400 cm^{-1} wavelength region at a resolution of 4 cm^{-1}. Attenuated total reflectance is employed to analyze the IR transmission spectra and trace the distinguishing peaks, sample in the ratio 1:100 is mixed with KBr for analysis by FTIR spectrophotometer (model: Shimadzu Analytical India Pvt. Ltd. (SAIP) (Ahmed & El-Say, 2016).

2.7. DSC study

The DSC instrument (model: NETZSCH, NETZSCH Technologies India Pvt. Ltd., Chennai) was used to determine the endothermic peak of the drug. For this study, 8 mg sample was placed in hermetically sealed DSC pan. The sample was run in the DSC instrument under liquid nitrogen atmosphere (20–250) °C at 35 °C/10.0 (K/min) heating rate (Ahmed & El-Say, 2016).

2.8. Folding endurance

The patches were sliced into required dimension (4 cm × 2 cm). Then they were folded at the same location repeatedly until it breaks. The number of times the film is folded without cracking or breaking is calculated (Suksaeree et al., 2015).

2.9. Thickness

At different locations, digital vernier caliper (Swastik Scientific Instruments Private Limited, Maharashtra) was employed to determine the thickness of patches. Average values and standard deviations were determined (Mamatha, Rao, Mukkanti, & Ramesh, 2010).

2.10. Weight variation

The films were cut into required dimensions i.e. 1.76 cm^2. These were weighed in triplicate on digital balance (Sartorious, Germany) and average value was calculated for each combination (Ali & Hanafy, 2017).

2.11. Percent elongation

Elongation testing apparatus was employed to determine percent elongation by using equation mentioned below:

$$\text{Percent elongation} = (L2 - L1)/L1 \times 100$$

where L1 and L2 are the original and final length of the film respectively (Ahmed & El-Say, 2016).

2.12. Percent moisture content

The preweighed films were kept in dessicator containing fused calcium chloride at room temperature for 24 h. The percent moisture content was determined by reweighing the films after 24 h by the formula mentioned below (Mamatha et al., 2010):

$$\text{Percent}(\%)\text{moisture content} = (\text{Initial weight} - \text{final weight})/\text{final weight} \times 100$$

2.13. Moisture uptake, swelling ratio and erosion studies

1 cm × 1 cm dimension film was employed for erosion, swelling ratio and moisture uptake studies. The required patches were weighed initially (W_0) for moisture uptake and then placed in the stability chamber (model: SC-10 Plus, REMI Electrotechnik Limited, Vasai, India) set at 75% RH and (25 ± 2) °C. After constant weight was achieved, the specimens were removed (W_u). The

equation mentioned below was used to determine moisture uptake:

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

For erosion and swelling ratio studies, the patches were dried at (60 ± 2) °C overnight. Initial weight of patches was determined as (W_0) and then they were soaked in distilled water (5 mL). The patches are then placed in stability chamber set at 75% RH and (25 ± 2) °C for 48 h. The patches were then weighed (W_s) and dried overnight at (60 ± 2) °C and again reweighed (W_d). Following formulas were employed to determine percent swelling ratio and percent erosion are given below (Rajesh, Siddaramaiah, & Gowda, 2010):

$$\text{Percent swelling ratio} = (W_s - W_0)/W_0 \times 100$$

$$\text{Percent Erosion} = (W_0 - W_d)/W_0 \times 100$$

2.14. Percent drug content

In volumetric flask of 10 mL, required dimension of patch was drenched in phosphate buffer (pH 5.5) and sonicated at 25 °C for 30 min followed by centrifugation for 15 min at 5200 rpm. The 0.45 mm filters were employed to filter the above solutions and spectrophotometrically analyzed (Munoz, Castan, Ruiz, & Morales, 2017).

2.15. In vitro drug release studies using dialysis membrane

The prepared films were attached onto the dialysis membrane and further adhered to the franz diffusion cell. Consequently, the surface from where the drug permeates having 0.785 cm^2 area was facing towards receptor compartment. The receptor compartment contains phosphate buffer of 7.4 pH maintained at 37 °C which is magnetically swirled. At programmed timings, 5 mL samples were taken out and equal volume of fresh buffer was replaced. The samples withdrawn were diluted with ethanol and then analyzed spectrophotometrically at 416 nm (Prajapati, Patel, & Patel, 2011; Wahid, Sridhar, & Shivakumar, 2008; Young-Chang, Choi, Choi, Ki, & Bae, 2010).

2.16. Optimization of patches

Optimization of patches with respect to polymers and permeation enhancer by utilizing response surface methodology employing Design-Expert software, trial version of 45 d (DX10.0 versions, Stat-Ease Inc., Minneapolis, MN, USA) was done.

2.17. Ex-vivo drug permeation studies using rat skin

For the *ex-vivo* study, the abdominal skin of the euthanized rats was shaved with the help of electrical clipper, and then it was washed with the phosphate buffer of pH 7.4 before excising. The extraneous tissues and subcutaneous fat was removed with the scalpel from the excised skin. The formulated optimized patches were adhered to the hairless rat skin and then adhered to franz diffusion cell so as the surface from which drug releases with 0.785 cm^2 diffusion area was towards the receptor compartment. Phosphate buffer (pH 7.4) was filled in the receptor compartment swirled magnetically and maintained at 37 °C. The samples (2 mL) were withdrawn at predetermined intervals and the equal quantity of phosphate buffer was introduced. The withdrawn samples were diluted with same quantity of ethanol and the analyzed spectrophotometrically at 416 nm (Prajapati et al., 2011; Wahid et al., 2008; Young-Chang et al., 2010; Pichayakorn et al., 2013).

3. Results and discussion

3.1. Preformulation studies

Various tests were used to analyze the organoleptic properties of curcumin. Curcumin was observed to yellowish orange powder without any odor having acrid taste. Its melting point was observed to be (181 ± 1) °C. Solubility study of curcumin was analyzed in water [(161 ± 4.5) μg/mL], ethanol [(9200 ± 0.12) μg/mL], and methanol [(9000 ± 0.23) μg/mL] respectively.

λmax is the wavelength of light in the ultraviolet region at which maximum absorbance is exhibited by the compound. At 416 nm, the calibration curve of curcumin was taken. Correlation co-efficient (R^2) = 0.999, The Regression line equation: $y = 0.2043x - 0.0044$, regressed line slope = 0.2043, where y stands for absorbance in nm and x stands for concentration in μg/mL (Fig. 1) (Priyadarshini, 2014).

3.2. Thermal analysis

Analogous to the drug melting endotherm, a distinctive peak of pure curcumin was observed at 180.5 °C by transition temperature analysis. The peak of EC was at 181.4 °C and HPMC at 79 °C, DBP at 254.1 °C and of PEG400, peak occurred at 47.2 °C. Peak of optimized transdermal film didn't indicated different phase transition temperatures showing a shift due to the presence of excipients, presenting a broad peak at 182.0 °C having area of 57.7 J/g that didn't influence the distinctive drug melting endotherm (Fig. 2).

3.3. FTIR study

FTIR spectra (Fig. 3) of pure curcumin displayed a peak at 3629 cm^{-1} indicating OH group presence. C—H stretching is affirmed by peak at 2933 cm^{-1}. C═O (keto) stretching is indicated by the peak at 1716 cm^{-1} and C—O enol peak is observed at 1312 cm^{-1}. C═C stretching is indicated by the presence of peak at 1653 cm^{-1}. O—H bend (phenolic) is indicated by peak at 1196 cm^{-1}. Peak at 814 cm^{-1} indicates CH vibration of aromatic ring. Blending of curcumin with polymers and development of optimized formulation didn't show any interference with the characteristic peaks of the drug which indicates compatibility of curcumin with incorporated polymers.

3.4. Physicochemical evaluation of transdermal film

Various tests like thickness, folding endurance, weight variation, drug content, swelling ratio, erosion, moisture content and

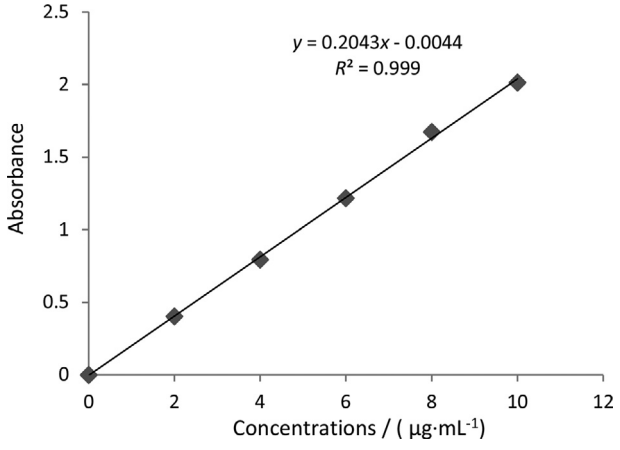

Fig. 1. Calibration curve of curcumin.

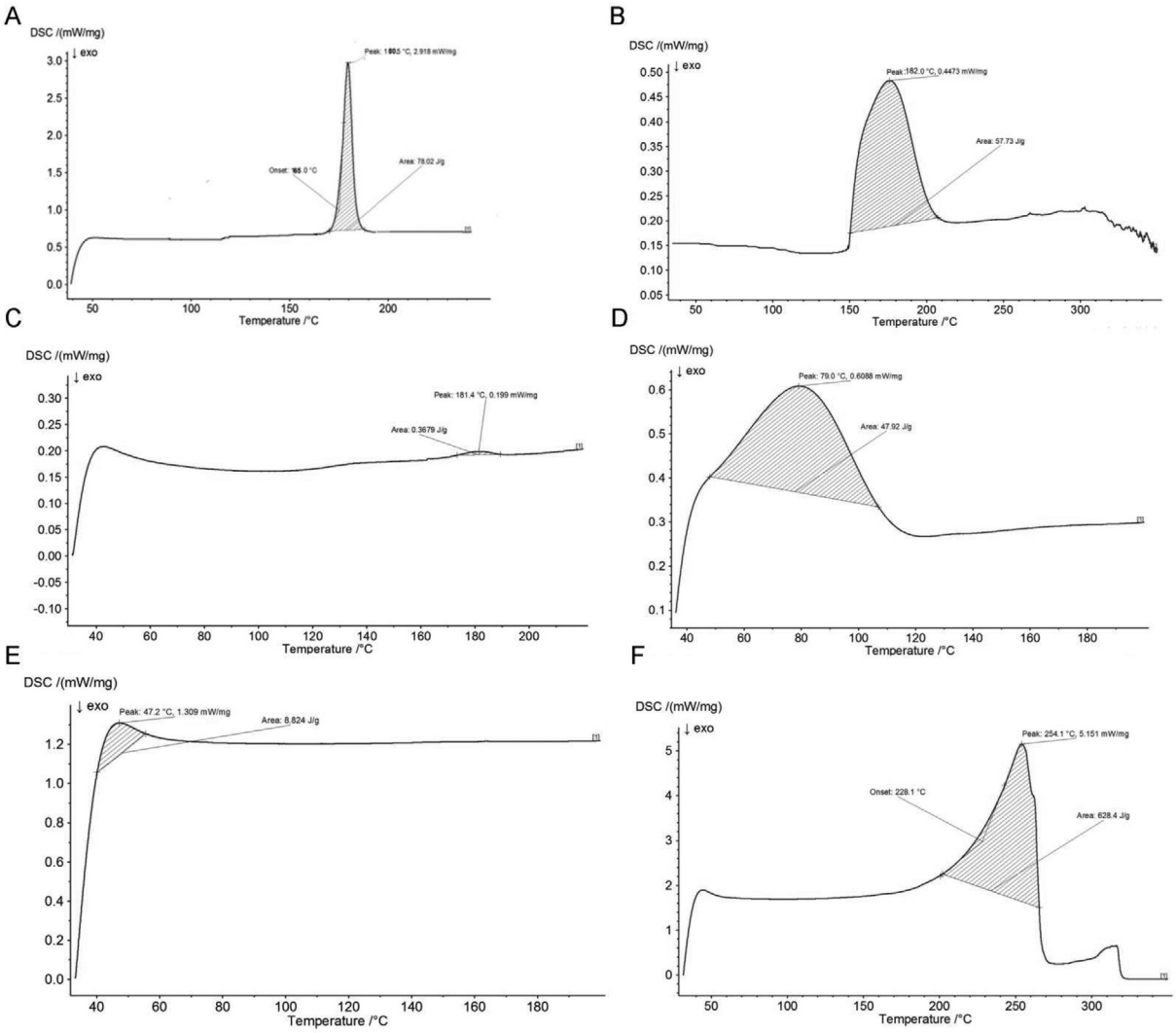

Fig. 2. DSC of curcumin (A), transdermal patch (B), ethyl cellulose (C), hydroxy propyl methyl cellulose (D), poly ethylene glycol 400 (E) and dibutyl phthalate (F).

uptake were carried out for physicochemical evaluation of formulations. Results were shown in Table 3. Permeation behavior of drug from transdermal patches is governed by factors like moisture content uptake, swelling and erosion (Suksaeree et al., 2015). As the concentration of HPMC increases, i.e. hydrophilic part of polymer blend, the above mentioned properties of the patch were augmented. It is attributed to the fast dissolution and erosion of HPMC on contact with aqueous media. From Table 3, F10 formulation containing higher concentration of HPMC (300 mg), DBP (1.5 mL) and lower concentration of EC (100 mg) showed good results in terms of moisture uptake, moisture content, swelling ratio, erosion and drug content which may result in good permeation. With increase in the concentration of EC and decrease in the concentration of HPMC and DBP, the reverse happened. In formulation 15 where the ratio of both hydrophilic and hydrophobic polymer was same with decrease in the concentration of DBP from 1.5 to 5 mL, drug content was reduced to a great extent. EC exerted a microstructural effect on the transport of drug through films with hampers the permeability thereby release of drug from the films (Andersson, Hjärtstam, Stading, von Corswant, & Larsson, 2013). It leads to increased tensile stresses hence poor erosion and swelling of films as higher energy is required to create microvoids/cracks in the film (Hjärtstam, Borg, & Lindstedt, 1990). It is also revealed that though HPMC increases the drug release but with increase in the molecular weight of both the polymers in the film; formulation 1, drug content in film declined (Andersson et al., 2013).

3.5. Analysis of suitability of model

Input variables and responses, design of experiment, carrying out experiment along with statistical analysis and formulation optimization are the critical parameters of statistical design. Collective impact of process variables i.e. concentration of polymers; HPMC and EC along with DBP on percent elongation and drug release; dependent variables were analyzed by Box Behnken design. To investigate the adequacy of models to different polynomial models viz. interactive, cubic, quadratic and linear and statistical test i.e. sequential model sum of squares, experimental data was fitted and the summary model was prepared (Table 4).

3.6. Effect of formulation variables on percent elongation (R1) of film

Value of *P* of regression coefficients was employed to determine significance and experimental data was evaluated by ANOVA. Percent elongation is a valuable tool in order to analyze the mechanical properties of the film. Brittle and hard films exhibit low elongation with moderate tensile strength while soft and tough

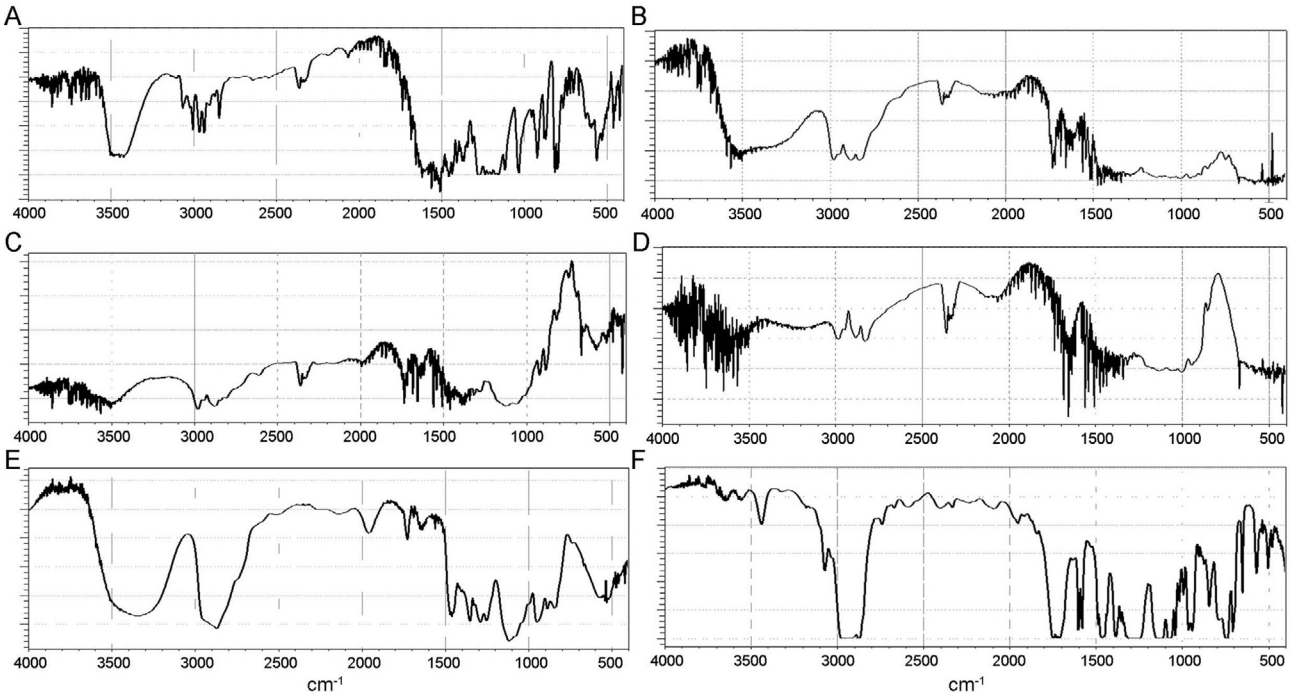

Fig. 3. IR spectras of curcumin (A), transdermal patch (B), ethyl cellulose (C), hydroxy propyl methyl cellulose (D), poly ethylene glycol 400 (E) and dibutyl phthalate (F).

Table 3
Physicochemical and mechanical properties of curcumin patches.

Run	A: HPMC/ mg	B: EC/ mg	C: DBP/ mL	Wt variation ± SD /mg	Thickness ± SD/ mm	Moisture content ± SD/ %	Folding endurance ± SD	Drug content ± SD/ %	Moistur uptake ± SD/ mg	Swelling ratio ± SD/%	Erosion ± SD/ %
1	300	200	1.0	210 ± 0.9	0.19 ± 0.2	4.0 ± 0.6	11.5 ± 0.2	95.5 ± 0.3	26.03 ± 0.4	24.0 ± 0.2	1.9 ± 0.3
2	200	100	0.5	207 ± 0.03	0.16 ± 0.5	3.55 ± 1.6	11.8 ± 1.6	97.5 ± 1.7	20.1 ± 0.3	21.5 ± 0.3	1.90 ± 1.4
3	100	100	1.5	196 ± 1.1	0.17 ± 0.4	2.6 ± 0.3	10 ± 0.4	99 ± 0.2	17.02 ± 0.4	17.2 ± 0.54	1.82 ± 0.4
4	100	200	1.5	199 ± 4.3	0.20 ± 0.3	2.6 ± 0.5	14 ± 4.2	96 ± 0.2	17.45 ± 0.21	19 ± 0.26	1.90 ± 0.4
5	100	200	0.5	199 ± 4.2	0.17 ± 0.5	3.23 ± 1.8	11.9 ± 0.4	97.4 ± 2.5	20.12 ± 1.4	22.2 ± 1.2	1.94 ± 2.0
6	100	150	0.5	200 ± 0.55	0.18 ± 0.4	2.3 ± 0.3	12.1 ± 0.2	96.1 ± 0.1	17.2 ± 0.4	18.8 ± 0.2	1.81 ± 1.6
7	300	150	0.5	209 ± 0.54	0.18 ± 0.3	4.03 ± 1.7	11.5 ± 1.3	96.6 ± 0.56	24.31 ± 0.3	24.0 ± 0.1	1.9 ± 0.21
8	200	150	1.5	209 ± 2.6	0.18 ± 0.1	3.52 ± 0.6	13 ± 3.4	98 ± 0.3	20.41 ± 0.45	22.34 ± 1.9	1.94 ± 0.3
9	200	100	1.0	208 ± 0.6	0.16 ± 0.3	3.54 ± 0.3	12 ± 1.2	97.8 ± 1.5	20.3 ± 0.4	22.1 ± 0.4	1.95 ± 0.3
10	300	100	1.5	208 ± 3.1	0.18 ± 0.2	4.25 ± 0.8	12 ± 2.5	99 ± 0.3	26.54 ± 2.5	24.20 ± 1.6	2.1 ± 0.2
11	100	150	1.0	200 ± 0.4	0.18 ± 0.43	2.5 ± 0.48	12.5 ± 0.5	96.4 ± 0.3	17.6 ± 0.31	19 ± 0.23	1.91 ± 1.3
12	100	150	1.0	200 ± 0.4	0.18 ± 0.43	2.5 ± 0.48	12.5 ± 0.5	96.4 ± 0.3	17.6 ± 0.31	19 ± 0.23	1.91 ± 1.3
13	200	100	1.0	208 ± 0.6	0.16 ± 0.3	3.54 ± 0.3	12 ± 1.2	97.8 ± 1.5	20.3 ± 0.4	22.1 ± 0.4	1.95 ± 0.3
14	300	200	1.0	210 ± 0.9	0.19 ± 0.2	4.0 ± 0.6	11.5 ± 0.2	95.5 ± 0.3	26.03 ± 0.4	24.0 ± 0.2	1.9 ± 0.3
15	200	200	0.5	207 ± 0.5	0.17 ± 1.2	3.6 ± 1.5	11.0 ± 0.6	94.4 ± 0.4	20.02 ± 0.3	21.7 ± 0.2	1.53 ± 0.8
16	300	100	0.5	208 ± 0.4	0.17 ± 0.2	4.06 ± 0.1	11.4 ± 0.42	98.6 ± 1.3	26.13 ± 2.4	24.07 ± 2.3	2.0 ± 2.5
17	100	100	0.5	195 ± 2.1	0.17 ± 1.8	2.3 ± 0.5	9.4 ± 0.4	98.2 ± 2.3	16.6 ± 1.6	16.04 ± 1.3	1.62 ± 0.4
18	200	150	1.5	209 ± 2.6	0.18 ± 0.1	3.52 ± 0.6	13 ± 3.4	98 ± 0.3	20.41 ± 0.45	22.34 ± 1.9	1.94 ± 0.3
19	200	150	0.5	208 ± 1.7	0.17 ± 1.9	3.33 ± 0.6	12.5 ± 0.32	97.4 ± 0.4	20.12 ± 0.56	22.05 ± 1.8	1.43 ± 0.4
20	200	100	1.0	208 ± 0.6	0.16 ± 0.3	3.54 ± 0.3	12 ± 1.2	97.8 ± 1.5	20.3 ± 0.4	22.1 ± 0.4	1.95 ± 0.3

films exhibit elevated elongation with tensile strength (Lin, Lee, & Lin, 1995). So, for increasing the elasticity of the film and decreasing the brittleness of the film, optimal amount of plasticizer is required (Pichyakorn et al., 2013). The percent elongation varied from 31% to 110% (Table 2). For the percent elongation, the quadratic model was observed to fit with *P* and *F* value of < 0.0500 (Linear) and 56.20, respectively which denoted that at *P* less than 0.05 was significant (Tables 4 and 5). For the chosen model, linear coefficients A, B, C and quadratic coefficients (A^2) were significant. To evaluate individual component significance and variable's strength of interaction, *P* value was used (Karacabey & Mazza, 2010). The response surface plot showed that both percentage of HPMC and DBP were the important variables which affect the percent elongation in an optimistic manner with < 0.0001 as *P* value. Plasticizers abate the cohesive forces within chains of polymer either/or disrupt matrix of polymer thus increasing chain mobility and improving matrix flexibility (Nesseem, Eid, & El-Houseny, 2011; Rujivipat & Bodmeier, 2012). ANOVA of observed values affirmed the accuracy of the model yielding linear relationship having R^2 value of 0.9133% in order to evaluate the percent elongation of film.

Quadratic equation was generated by the software is given below:

Percent elongation = −11.60832 + 0.75895 × HPMC − 0.1551 7 × EC + 23.46930 × DBP − 5.37586E−004 × HPMC × EC + 0.02 8891 × HPMC × DBP − 1.31438E−003 × $HPMC^2$ + 3.71256E −004 × EC^2 − 5.00687 × DBP^2

It is apparent from the equations, that percent elongation is positively impacted by factors A and C whereas factor C (coefficient

Table 4
Fit summary model for measured responses R1 and R2.

R2										
Source	Sum of Squares	df	Mean Square	*F* Value	*P*-value Prob > F	Remarks	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	Remarks
Mean	99687.20	1	99687.20								
Linear	6606.70	3	2202.23	35.37	< 0.0001	Suggested	7.89	0.8690	0.8444	0.7916	1584.42
2FI	69.67	3	23.22	0.33	0.8067		8.44	0.8781	0.8219	0.4621	4089.75
Quadratic	779.18	3	259.73	17.64	0.0003	Suggested	3.84	0.9806	0.9632	0.6111	2956.87
Cubic	147.26	5	29.45			Aliased	0.000	1.0000	1.0000		+
Residual	0.000	5	0.000								
Total	1.073E + 005	20	5364.50								

R1											
Source	**Sum of squares**	**df**	**Mean square**	***F* value**	***P*-value Prob > F**	**Remarks**	**Source**	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**	**Remarks**
Mean *vs* Total	1.027E + 005	1	1.027E + 005				Linear	2.71	0.9133	0.8971	0.8590	Suggested
Linear *vs* Mean	1241.13	3	413.71	56.20	<0.0001	Suggested	2FI	2.83	0.9235	0.8882	0.6904	
2FI *vs* Linear	13.82	3	4.61	0.58	0.6410		Quadratic	2.62	0.9496	0.9043	0.6017	
Quadratic *vs* 2FI	35.52	3	11.84	1.73	0.2238		Cubic	0.000	1.0000	1.0000		Aliased
Cubic *vs* Quadratic	68.45	5	13.69			Aliased						
Residual	0.000	5	0.000									
Total	1.040E + 005	20	5202.17									

Table 5
Analysis of variance table for measured responses.

Analysis of variance table (R1)						
Source	Sum of squares	df	Mean square	*F* value	*P*-value Prob > F	Significance
Model	1241.13	3	413.71	56.20	<0.0001	Significant
A-HPMC	469.47	1	469.47	63.77	<0.0001	
B-EC	739.79	1	739.79	100.49	<0.0001	
C-DBP	0.28	1	0.28	0.038	0.8487	
Residual	117.79	16	7.36			
Lack of Fit	117.79	11	10.71			
Pure error	0.000	5	0.000			
Cor total	1358.93	19				

Analysis of variance table (Partial sum of squares - Type III) (R2)						
Source	**Sum of squares**	**df**	**Mean square**	***F* value**	***P*-value Prob > F**	Significance
Model	1240.86	2	620.43	89.33	<0.0001	Significant
A-HPMC	469.19	1	469.19	67.56	<0.0001	
B-EC	741.83	1	741.83	106.81	<0.0001	
Residual	118.07	17	6.95			
Lack of fit	118.07	12	9.84			
Pure error	0.000	5	0.000			
Cor total	1358.93	19				

value 23.46) had a higher effect than factor A with a coefficient value of 0.75. In response surface plots, 3D and contour plots, the same results were viewed. With augmentation in HPMC concentration in polymer mixture, an increase in tensile strength was observed as depicted in Fig. 4A and 4B. In the study, a hydrophobic plasticizer i.e. DBP having solubility of 0.04% at 20 °C was employed (Frohoff-Hülsmann, Schmitz, & Lippold, 1999). In case of runs comprising of high concentration of HPMC, as the glass transition temperature decreases, increase in tensile strength occurs (Saettone, Perini, Rijli, Rodriguez, & Cini, 1995). For drug delivery across skin a tough and hard film is required. For the experimental run 10 (F10), the %R1 (110) was observed to be highest which was followed by experimental run 9 (F9) having %R1 90. Tensile strength is affected negatively with augment in the concentration of EC due to its hydrophobic nature (Ahmed & El-Say, 2016; Parhi & Suresh, 2016). It was concealed that tensile strength dwindles as the concentration of hydrophobic polymer i.e. EC is augmented. It can be concluded that the preparation containing higher proportion of HPMC and DBP exhibit higher tensile strength. It is also mentioned in literature that EC augments the toughness of film but not elongation which in turn is augmented by increase in the concentration of plasticizer and hydrophilic polymer to some extend (Haas, Farney, & Valle, 1952; Prajapati et al., 2011).

3.7. Effect of formulation variables on percent drug release (R2) of film

Value of *P* of regression coefficients was employed to determine significance and experimental data was evaluated by ANOVA. To illustrate the dissolution behavior of the film, the drug release is a good tool (Anand, Yu, Conner, & Davit, 2011). Poor dissolution properties indicate low drug release. The correct concentration of plasticizer and hydrophilic: hydrophobic polymer ratio result in the augmented release of drug (Jagtap, Bhujbal, & Ranpise, 2012; Kibria, Roni, & Jalil, 2008). The use of HPMC in combination with EC is largely reported in literature in various studies to enhance the drug release by various mechanisms i.e. (i) through a network of water-filled capillaries, (ii) through a hydrated swollen film or

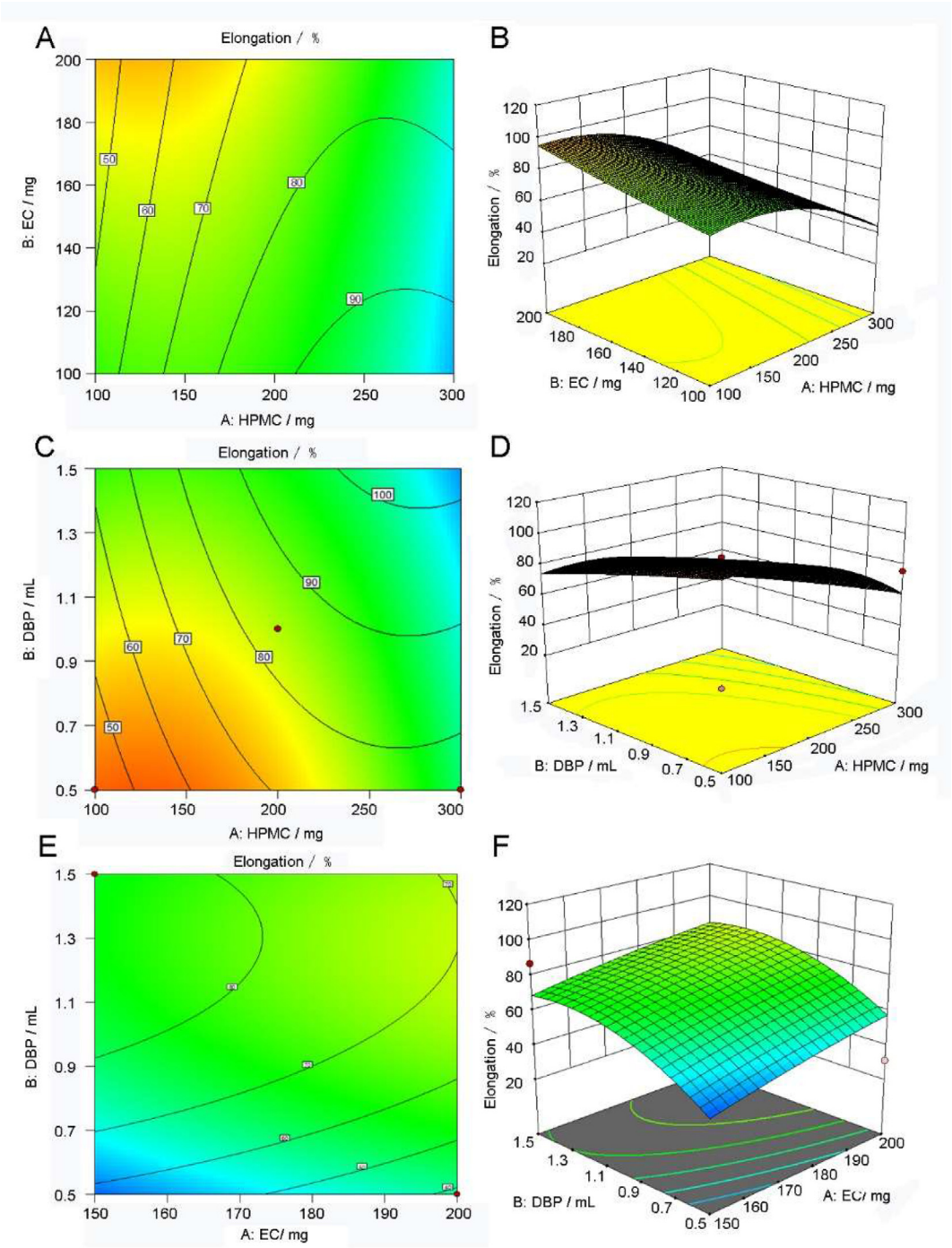

Fig. 4. Contour plots showing effect of HPMC and EC (A), DBP and HPMC (C), DBP and EC (E) on response R1 (%elongation); (B), (D), and (F) were their corresponding 3D response surface plots.

(iii) through flaws, cracks and imperfections (Andersson et al., 2013; Hjärtstam & Hjertberg, 1998; Lindstedt, Ragnarsson, & Hjärtstam, 1989; Lindstedt, Sjöberg, & Hjärtstam, 1991; Sakellariou & Rowe, 1995). DBP is also reported in literature to increase the drug release by modifying the hydrophilicity of the film (Saettone et al., 1995). Percent drug release ranged between 59.4% and 83.3% (Table 3) in patches containing DBP. Major linear coefficients affecting drug release were A and B. To evaluate individual component significance and variable's strength of interaction, *P* value was used. The *P* -value and the *F* value of the model were observed to be < 0.0001 and 89.33 respectively, that pointed the model's significance having 0.0500, as *P* value (Tables 4 and 5). The response surface plot (Fig. 5A and 5B) showed that both percentage of HPMC and DBP were the important variables which affect the percent drug release in an optimistic manner with < 0.0001 as *P* value. Plasticizers improve the flexibility of the film thereby improving the drug release. ANOVA of observed values affirmed the accuracy of the model with a R^2 value of 0.9806% yielding linear relationship. Software generated linear equation is depicted below:

Percent drug release = +81.28938 + 0.063115 × HPMC − 0.15 370 × EC + 0.29771 × DBP

It is apparent from the equations, that percent drug release is positively impacted by factors A and C. Factor C having a coefficient value of 0.29731 exerts more impact than factor A having coefficient value of 0.063 as is also revealed from response surface plots. Therefore it was concealed that with increase in concentration of HPMC (300–100 mg) and DBP (0.5–1.5 mL), drug release increases. (Fig. 5A and 5B). The percent drug release for experimental run 10 (F10) was observed to be highest (83.3%) followed by experimental run 16 (F16) i.e. 82.2%. It was revealed that the release of drug decreases as concentration of EC (hydrophobic polymer) increases. Between the observed and predicted values of responses, linear correlation plots are illustrated. The R^2 value varied between 0.6111 and 0.9632 for R2 response. As displayed in Fig. 5E and 5F with increase in concentration of EC (hydrophobic polymer), drug release decreases. Linear correlation plots are illustrated between observed and predicted values for the responses (Fig. 6A and 6B). The R^2 values between 0.6111 and 0.9632 for response R2 and between 0.8590 and 0.8971 for response R1 for linear correlation plots drawn between the predicted and observed values indicated correctness of the model (Ahmed & El-Say, 2016; Parhi & Suresh, 2016).

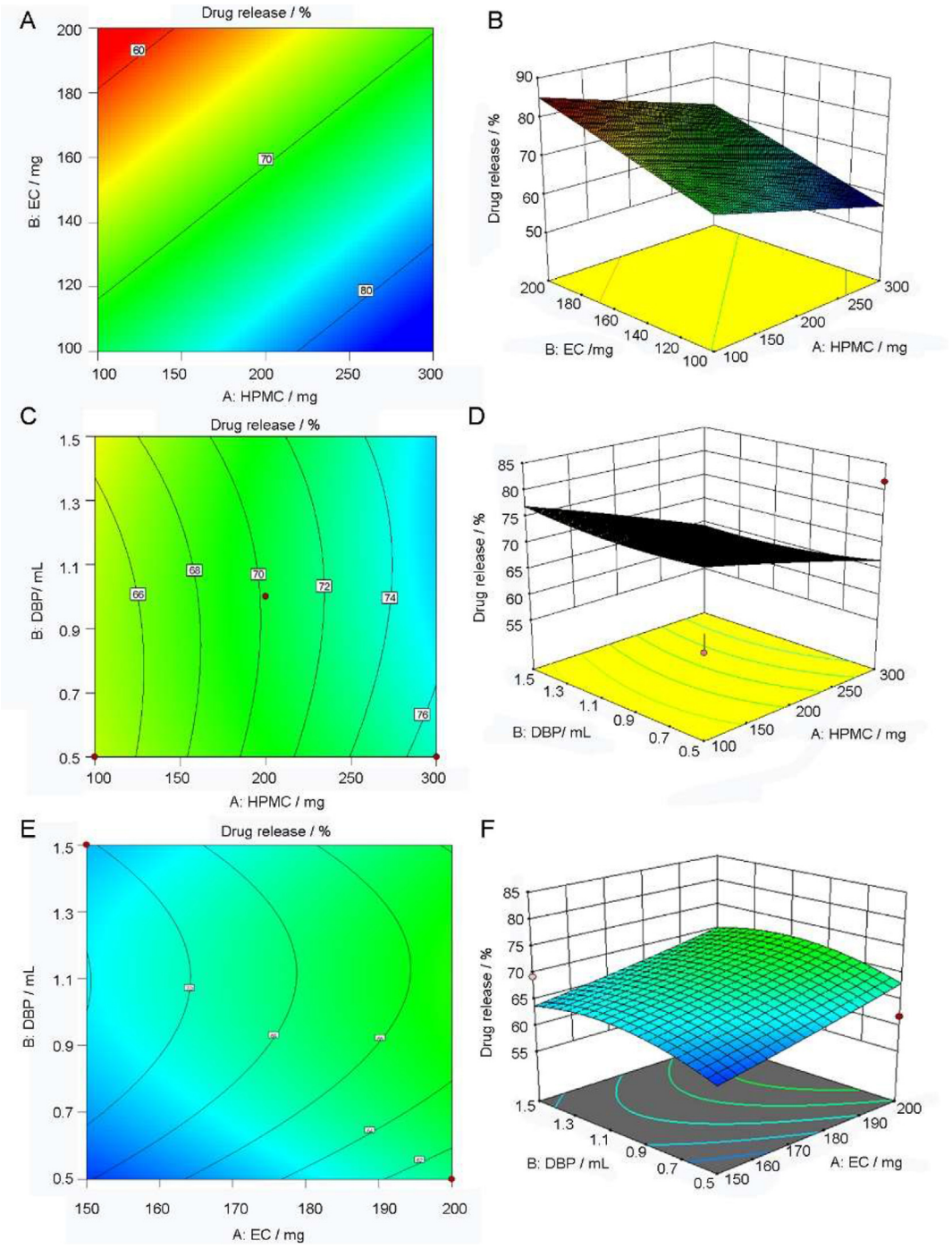

Fig. 5. Contour plots showing effect of HPMC and EC (A), DBP and HPMC (C), DBP and EC (E) on response R2 (% drug release); (B), (D), and (F) were their corresponding 3D response surface plots.

Table 6
Optimum combination of factors, predicted, observed values, residuals, and predicted error percentage for optimized formulation of curcumin loaded transdermal film.

Factors	Optimum			
HPMC percentage	242.061			
EC percentage	109.598			
DBP percentage	1.030			
Responses	Predicted	Observed	Residual	Prediction error/%
R1 (Elongation/%)	94.35	95.02	±0.67	0.71012
R2 (Drug release/%)	80.0306	81.034	±0.998	1.2470

Note: Residual = predicted value − observed value
Prediction error = (predicted value − observed value/predicted value) × 100.

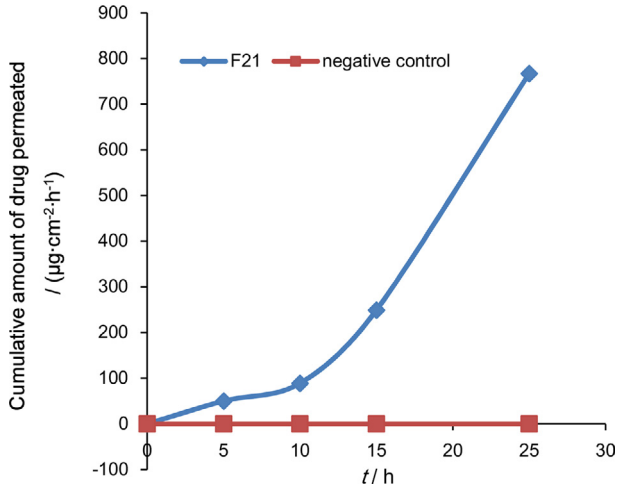

Fig. 7. Cumulative amount of drug permeated across rat skin through optimized formulation.

3.8. Optimization

Physicochemical properties of drug i.e. morphology and structure of polymer matrix govern the release of drug through transdermal patches (Langer & Peppas, 2006). With augment in the proportion of HPMC, drug release increases owing to high water absorbing property of polymer due to increased porosity and pore diameter of polymer matrix leading to rapid diffusion of drug (Hollenbeck, Swarbrick, & Boylan, 2013). Channels are formed for the diffusion of drug from the film due to higher rate of swelling of HPMC on contact with dissolution medium (Khan, Stedul, & Kurjaković, 2000). DBP also displays good solubilizing property and is miscible with the solvent mixture. It boosts diffusion of curcumin by down turning skin resistance. As depicted in literature, if the drug solubilizes in matrix of polymer permeation of drug is augmented owing to increased thermodynamic activity in base (El-Say, Ahmed, Aljaeid & Zidan, 2015; El-Say, Ahmed, Badr-Eldin et al., 2015; Elshafeey, Hamza, Amin, & Zia, 2012). After experimental variable analysis, an optimized preparation of curcumin with required elongation and drug release was inferred to contain 242.06% HPMC, 109.59% EC and 1.03 DBP (Table 6). Table 6 showed the observed, predicted values, prediction errors and residuals. Predicted values of responses for optimized formulation ensured preferred mechanical and physicochemical properties. Insignificant prediction error percentage (<6%) was observed between observed and predicted values for optimized transdermal film and residuals. It can be concluded that for the formulation of transdermal patch of curcumin with desirable properties, optimization technique is highly reproducible and reliable (Ahmed & El-Say, 2016; Parhi & Suresh, 2016).

3.9. Ex vivo drug permeation studies using rat skin

The *ex-vivo* study performed using rat skin revealed that cumulative drug release from an optimized film containing DBP showed maximum flux i.e. (30.68 ± 18 μg/cm^2/h) as permeation enhancers (Fig. 7). Initially during the first 5 h, the percent cumulative drug release was nearly same but with time the release increased more up to 25 h. In case of negative control patch, no release was observed since curcumin was not incorporated in the patch.

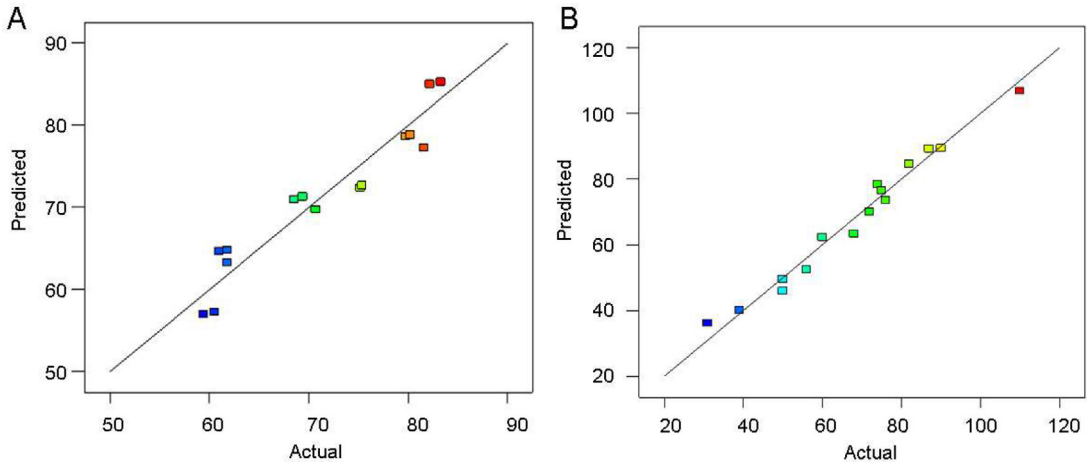

Fig. 6. Linear correlation plots between actual and predicted values of % elongation (R2) (A) and between actual and predicted values of % drug release (R1) (B).

4. Conclusion

The Box–Behnken design was an efficient tool in optimizing the three variables used to prepare curcumin TDDS through studying their effect on the quality attributes and dissolution behavior of the prepared curcumin-loaded transdermal films. The optimized formulation showed good physicochemical and mechanical properties as well as enhanced dissolution characteristics. The *in vitro* and *ex vivo* study reveals that the prepared formulation has potential to be used as effective alternative to the current drugs available in the market. More research and optimization and clinical studies of herbal formulations must be done to get better products in future. Though many formulations of curcumin are reported in literature to have potential therapeutic effects but lack of proper clinical studies is hindering the pathway of this pragmatic drug molecule to come into existence as better therapeutic option.

Conflict of Intrest

There is no conflict of intrest.

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