Development and Physicochemical Characterization of Hydroxypropylmethylcellulose Patches Containing *Derris scandens* extract

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Pharmacy in Pharmaceutical Sciences

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Thesis Title	Development and Physicochemical Characterization of Hydroxypropylmethylcellulose Patches Containing *Derris scandens* extract
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ชื่อวิทยานิพนธ์ การพัฒนาและการตรวจลักษณะเฉพาะทางเคมีกายภาพของแผ่นแปะผิวหนังไฮดรอกซีพรอพิลเมทิลเซลลูโลสที่บรรจุสารสกัดเถาวัลย์เปรียง

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ปีการศึกษา 2561

บทคัดย่อ

การพัฒนาสูตรตำรับแผ่นแปะผิวหนังที่บรรจุสารสกัดเถาวัลย์เปรียง (DSE) โดยประเมินคุณสมบัติทางกายภาพและความสามารถในการปลดปล่อยยาออกจากแผ่นแปะผิวหนังซึ่งสามารถเตรียมแผ่นแปะโดยวิธี Solvent casting evaporation technique โดยใช้สัดส่วนที่ต่างกันของสารสกัดเถาวัลย์เปรียง (A) สัดส่วนของพอลิเมอร์ HPMC E4M:E15LV (B) และสารเพิ่มความยืดหยุ่น PEG 400 (C) โดยใช้ central composite design เพื่อพิจารณาปัจจัยที่เกี่ยวข้องกับสูตรตำรับข้างต้นที่มีผลต่อ tensile strengths ของแผ่นแปะ พบว่าค่า tensile strengths อยู่ในช่วง 151.88 ± 46.58 g/cm^2 ถึง 902.89 ± 101.05 g/cm^2 และมีความแข็งและยืดหยุ่นที่น่าพึงพอใจ สร้างสมการทางคณิตศาสตร์ที่แสดงความสัมพันธ์ระหว่างตัวแปรอิสระกับ tensile strengths และใช้เป็น optimization model เพื่อทำนายค่า tensile strength ที่ต้องการได้ ค่าที่เหมาะสมของตัวแปร A, B และ C คือ 0.6 g, 48:52 และ 40% ตามลำดับ ส่วนประกอบดังกล่าวเมื่อนำมาเตรียมแผ่นแปะจะให้ค่า tensile strengths คือ 571.28 ± 37.61 g/cm^2 ค่าดังกล่าวให้ความสัมพันธ์ที่สอดคล้องกันระหว่างค่าที่ทำนายและค่าที่ได้จริง การศึกษาการปลดปล่อยยาจากแผ่นแปะในระดับ *in vitro* drug release โดยใช้เครื่อง franz diffusion cells ในสารละลาย 2 ชนิด ได้แก่ 10% w/v ethanol in phosphate buffer saline และ 50% w/v ethanol แสดงให้เห็นว่าการปลดปล่อยยามีความเข้มข้นสูงสุดที่เวลา 6 ชั่วโมง สำหรับ 10% w/v ethanol in phosphate buffer saline และ 1 ชั่วโมง สำหรับ 50% w/v ethanol และพบว่าอัตราการปลดปล่อยยาจะลดลงเมื่อเพิ่มสัดส่วนของพอลิเมอร์ HPMC E4M:E15LV โดยลักษณะทางจลนศาสตร์ของการปลดปล่อยยาเหมาะสมเข้ากันกับโมเดลของ higuchi สำหรับแผ่นแปะที่ได้จาก optimization ดังนั้นการศึกษาการซึมผ่านผิวหนังในระดับ *in vitro* skin permeation เพิ่มเติมนั้นจะทำให้ทราบถึงศักยภาพของแผ่นแปะ DSE ที่จะเป็นรูปแบบยาเตรียมทางเลือกอื่นนอกจากการให้ทางปาก

Thesis Title Development and Physicochemical Characterization of Hydroxypropylmethylcellulose Patches Containing *Derris scandens* extract

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ABSTRACT

Formulations of topical patches containing *Derris scandens* extracts (DSE) were developed and their physicochemical properties were investigated. The patches were prepared by solvent casting evaporation technique using various proportions of DSE concentrations (A), the polymer ratios of HPMC E4M and E15LV (B), and the amount of PEG 400 (C). Central composite design was adopted to determine the effect of the above formulation factors on the tensile strengths of the patches. It was found that tensile strengths were in the range of 151.88±46.58 g/cm^2 to 902.89±101.05 g/cm^2, with suitable hardness and brittleness. The mathematical equation was used to fit the independent variables with tensile strength of the patches. The optimization model showed the prediction of the tensile strength with A, B, and C levels of 0.6 g, 48:52 and 40 %, respectively. The optimal composition resulted in suitable tensile strength of 571.28 ± 37.61 g/cm^2 and showed a good correlation between predicted and observed values. The *in vitro* drug release from the DSE

patches was performed in 10% w/ v ethanol in phosphate buffer saline and 50% w/ v ethanol. Maximum concentrations of drug released were obtained within 6 hours and 1 hour for 10 % w/ v ethanol in phosphate buffer saline and 50% w/v ethanol, respectively. The release rates were found to decrease with increasing polymer proportions of HPMC E4M and E15LV. The drug release kinetics of Higuchi models fit well to DSE release data of optimized patches. Hence, DSE patches have potential as an alternative dosage form to oral preparation and could be further developed for the study of *in vitro* skin permeation of DSE.

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Watchara Achirasena

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLUBLICATION

PAPER Achirasena W, Ketjinda W, Sakunphueak A. Formulation and physical evaluation of topical patch containing *Derris scandens. Bull. Health Sci. Technol* 2018, 16 (2): 135-146

June 5, 2018

Dear Mr. Watchara Achirasena,

I am pleased to inform that paper entitled

"FORMULATION AND PHYSICAL EVALUATION OF TOPICAL PATCH CONTAINING DERRIS SCANDENS"

of the authors named
Watchara Achirasena, Wichan ketjinda and Athip Sakunphueak
has been accepted for publication in journal "Bulletin of Health, Science and Technology (BHST)".

It will be published in journal BHST in No.2, Vol.16, 2018.

Please note that the accepted paper will be sent to the corresponding author for corrections prior to publishing in the journal.

Thank you for submitting your work to our journal.

Yours sincerely,

Thanapat Songsak, Ph.D.
Associate Editor
Bulletin of Health, Science and Technology (BHST)
Rangsit University, Thailand

INTRODUCTION

***Derris scandens* (Roxb.) Benth.**

Derris scandens (Roxb.) Benth. known in Thai as "*Tao-Wan-Priang"*, is a climbing shrub widely distributed across Southeast Asia, including Thailand, India, Malaysia and China. *D. scandens* has been used in traditional folk medicine in the form of decoction as an expectorant, diuretic, antidysentery, anti- inflammation and for treatment of muscle pain. (Mahabusarakama et al., 2004) (Wongsinkongman et al., 2013).

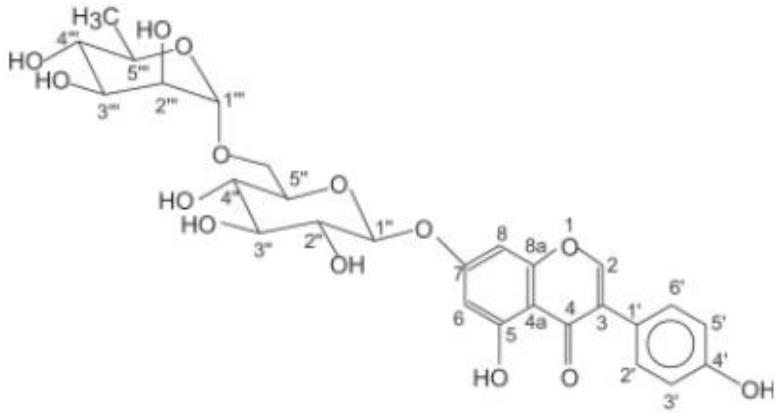


Figure 1 Structural formula of genistein-7-O- [γ-rhamnopyranosyl-(1- 6)-β-glucopyranoside] (Wongsinkongman et al., 2013)

D. scandens dried powder (used dose, 0.5–1 g immediately after meal three times per day) and 50% hydroethanolic extract (used dose, 400 mg immediately after meal two times per day) have been registered in the List of Herbal Medicinal Products in the Thailand National List of Essential Medicines for musculoskeletal pain treatment (Thailand National List of Essential Medicines, 2017). Active constituents in *D. scandens* for anti-inflammatory effect are isoflavones such as genistein-7-O- [γ-rhamnopyranosyl-(1- 6)-β-glucopyranoside] (Figure 1). *D. scandens* extract exhibits good anti-inflammatory effect by inhibition of cyclooxygenase 1 (COX-1) activity and lowering leukotriene-B4 (LTB-4) generation. It has been reported that *D. Scandens* has reduced eicosanoid synthesis via both cyclooxygenase and lipoxygenase pathways and myeloperoxide

release (Laupattarakasem et al., 2003) (Mahabusarakama et al., 2004) (Wongsinkongman et al., 2013). *D. scandens* extracts were as efficacious and safe as naproxen in relieving knee pain and improving knee functions, even though there was a trend toward a greater effect in patients receiving naproxen (Kuptniratsaikul et al., 2011).

Current research evidence on the systematic review and meta- analysis of randomized controlled trials suggests that *D. scandens* might be considered as a potential alternative for nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of musculoskeletal pain. The use of *D. scandens* in alternative medicine might be safer than NSAIDs in long-term use. Concomitant use of *D. scandens* and NSAIDs, should be avoided due to their similar adverse events (Puttarak et al., 2016). In the toxicity study of *D. Scandens,* a dose of 600 mg/body weight (75–100 times human dosage per day) was fed to Wistar rats for 6 months without any toxicity or abnormal functions of other organ systems (Chavalittumrong et al., 1999). Another study in human volunteers taking *D. scandens* 200 mg twice daily for 2 months revealed no adverse events or abnormalities in blood chemistry (Chavalittumrong et al., 2008).

Topical and Transdermal Drug products

In pharmacy, the route of administration is the path by which a drug is taken into the body. Over the last 2-3 decades, the skin has become an important route of drug delivery for topical, regional, or systemic action. The skin, however, is a physical and biochemical protective barrier. It prevents loss of water from the body and guards against entry of external toxic chemicals and pathogens into the body, thereby maintaining homeostasis or a stable, relatively constant internal environment. Stratum corneum is the outermost layer of the skin comprised of keratin-rich cells embedded in multiple lipid bilayers. It has been considered the rate- limiting structure governing percutaneous absorption of many kinds of permeants (Songkro, 2009). Drug products topically administered are classified into two general categories: local and systemic actions. Local actions include those at or on the surface of the skin or the stratum corneum and modulate the function of the epidermis and/or the dermis. Common topical products in the former category include creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, and solutions.

The most common drug products applied to the skin for systemic action are referred to as transdermal drug delivery systems (TDS) or transdermal patches (Ueda et al., 2009).

Topical patch formulation is an alternative medicine from oral therapy. Topical patches can provide a defined dose to a defined area for an extended period of time. Topical agents now available include NSAIDs, counter-irritants (e.g. capsaicin) and local anesthetics, such as the 5% lidocaine patch. Transdermally absorbed drugs, depend on systemic absorption (e.g. fentanyl, nicotine patches). The goal of topical agents is to achieve efficacy similar to oral formulations with potentially lower systemic side effects. Penetration studies confirm that topical NSAIDs reach therapeutic concentrations equivalent or greater to those seen with larger doses of oral NSAIDs (McCarberg and Argoff, 2010).

Topical drug products are evaluated for various pharmacopeial and non-pharmacopeial tests to ensure their strength, efficacy, purity, and safety characteristics. These tests for topical products are listed in two categories: product quality tests (USP40 Chapter <3>) and product performance tests (USP40 Chapter <1724>). These tests are performed on drug products to provide assurance of batch-to-batch quality, reproducibility and performance. Product quality tests are performed to assess attributes such as assay, identification, content uniformity, pH, microbial limits, minimum fill, tensile strength (USP40 Chapter <881>) and are part of the compendial monograph. Product performance tests are conducted to assess drug release from the finished dosage form (United States Pharmacopeial Convention 2017). Therefore, these test methods should be employed to ensure the quality and performance of topical and transdermal drug products. Both the physical characteristics and the *in vitro* drug release are important quality attributes to consider for the development of these products.

Genistein and daidzein, phytoestrogens belonging to the isoflavone class, have been studied for topical preparation (Hwa Lee, et al. 2016). Delivering these active compounds through the skin by emulsion-based creams or similar methods was difficult because these drugs are nearly insoluble in aqueous solutions. In addition, the hydrocolloid patch containing poorly soluble drugs such as genistein and daidzein has been used as a transdermal drug delivery system (TDDS) to deliver the drugs through the skin. It was shown that the patches were helpful for the drugs to absorbed more deeply than the cream due to the occlusive effect of the patches; this was verified from the porcine skin and the confocal microscopy. The hydrocolloid patch is a better way

to deliver poorly soluble drugs through the skin than general cosmetics such as emulsion-based cream and is valuable as an effective cosmetic due to its convenient usage.

Due to the inconvenient use of *D. scandens* dried powder in conventional oral capsules with large doses, the alternative topical patch of herbal extract will be developed to improve patients' compliance and enhance local action. Therefore, the aim of the present study is to prepare an optimized topical patch containing *D. scandens* extract. The topical patches were prepared by solvent casting evaporation technique using various proportions of *D. scandens* extract, the polymer proportion of hydroxypropyl methylcellulose (HPMC) E4M and E15LV and the amount of polyethylene glycol 400 (PEG 400) as plasticizer. Hydroxypropyl methylcellulose (HPMC) was chosen as the hydrophilic polymer of the exploits of a nontoxic, nonallergic polymer having good film forming properties for the preparation of tough films (Nair et al., 2013). In the first step, the design of the experimental trial, central composite design was adopted to determine the effect of the above formulation factors on the tensile strengths of the patches. In the second step, optimization of suitable composition of variables for the topical 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 (Parhi and Suresh, 2016) (Wongpoowarak, 1992).

OBJECTIVES

1. To formulate topical patches containing *Derris scandens* extract
2. To evaluate the physicochemical properties and drug release of topical patches containing *Derris scandens* extract

MATERIALS AND METHODS

Materials

The *Derris scandens* extract was provided from Prince of Songkla Univeristy Pharmacognosy Laboratory, Thailand. Hydroxypropyl methylcellulose grade E4M and E15LV was obtained from P.C. Drug Center, Bangkok, Thailand. Polyethylene glycols 400 was obtained from P. C. Drug Center, Bangkok, Thailand. AR grade of Aluminium Chloride was obtained from Sigma®, Thailand. All organic solvents were obtained from Sigma®, Thailand.

Experimental design

The preliminary patches were selected in concentrations of 1,2,3,4 and 5 %w/w of hydroxy propyl methyl cellulose E4M:E15LV (50:50) as polymers, DSE 600 mg as a drug, and PEG 400 40 g% of dried polymers as plasticizer. These preliminary patches of 3% w/w of HPMCs were selected for topical patches preparation. The central composite design was adopted to optimize the formulation factors (i.e. DSE concentration, polymer proportion of HPMC E4M and E15LV and percentage of PEG 400) and to evaluate their effects on the response of the tensile strength of the patches. All the factors and the response variables are mentioned in Table 1. Statistical analysis for the present study was performed by Design-Expert software (Version 10, Stat-Ease Inc., Minneapolis, MN, USA). Experimental design of different formulations of topical patches is presented in Table 2.

Table 1. Variables and their levels in central composite experimental design

Variables	Levels				
	-1.68	-1	0	1	1.68
Independent factors					
A = DSE (g)	0.064	0.2	0.4	0.6	0.736
B = HPMC E4M:15LV	22:48	33.33:66.67	50:50	66.67:33.33	78:22
C = PEG400 (%)	13.18	20	30	40	46.82
Dependent response					
Tensile strength (g/cm2)			-		

Table 2. Central composite design for the preparation of different formulations

Formulations	Factors with levels		
	A = DSE (g)	B = HPMC E4M:15LV	C = PEG400 (%)
1	0.2	33.33:66.67	20
2	0.6	33.33:66.67	20
3	0.2	66.67:33.33	20
4	0.6	66.67:33.33	20
5	0.2	33.33:66.67	40
6	0.6	33.33:66.67	40
7	0.2	66.67:33.33	40
8	0.6	66.67:33.33	40
9	0.064	50:50	30
10	0.736	50:50	30
11	0.4	22:78	30
12	0.4	78:22	30
13	0.4	50:50	13.18
14	0.4	50:50	46.82
15	0.4	50:50	30
16	0.4	50:50	30

Preparation of the herbal medicine patch

The 16 formulations of topical patches (Table 2) were prepared by solvent casting evaporation technique. Initially, the 3% HPMC solution containing water and HPMC in varied proportions of E4M and E15LV was prepared. The HPMC polymers were dispersed and thoroughly hydrated in about 20-30 % of the required amount of water. The water was heated to 80 - 90 °C, and then the polymers were added by stirring. Sufficient cold water then was added to produce the required volume while continuing to stir. Required quantities of DSE and PEG 400 were added slowly to previously prepared HPMC solutions while cooling and were then mixed thoroughly. The matrix solutions were poured into a petri dish. The solvent casting was evaporated at 45 °C for 24 hours. The dried DSE films were cut into 40 cm^2 and wrapped in aluminum foil before being kept in a desiccator at 2-8 °C.

Evaluation of physicochemical properties of the patch

Appearance of patches

All the formulated DSE patches were evaluated visually for appearance in terms of surface smoothness, brittleness, flexibility and homogeneous appearance.

Thickness and weight variation

The thickness of the patch was measured three times by a Teclock® dial thickness gauge. Weight variation studies were carried out by selecting the three patches 40 cm^2 from each formulaion using analytical balance.

Swelling index

The dried patches supported by stainless steel mesh were immersed in a beaker containing 25 ml of distilled water at room temperature. At the time interval (1,3,5 and 10 minutes) the swollen samples with the pre-weighed mesh were weighed after the excess surface water was removed by blotting lightly with a filter paper. The experiment was discontinued when the patches began to disintegrate or dissolve. To quantify the swelling index, percentages were calculated as follows:

$$\text{Swelling index} = (Ws - Wd) / Wd \times 100$$

Where; Wd: the weight of the dried patch

Ws: the weight after swelling

Mechanical properties

For the measurement of mechanical properties as tensile strength (g/cm^2), % elongation at break (EB) and elastic modulus (EM), the patch specimen of specific dimension (4 × 2 cm) was fixed between two clamps of texture analyzer (TA. XT plus, Stable MicroSytems Ltd, USA). The test conditions were test speed of 10 mm/min, target load of 5 kg, holding time of 5 seconds, and trigger force of 5 g (Rabinarayan and Padilam, 2016). The mechanical properties were determined as follows:

$$\text{Tensile strength } (g/cm^2) = \text{Breaking force } (g) / \text{area of the film } (cm^2)$$

Drug content

A 1x1 cm^2 of each patch (n=3) was dissolved in 10 ml of 95 % ethanol and sonicated for 30 minutes. Then 0.5 ml of each sample solution, 1.5 ml of 95 % ethanol, 0.1 ml of 1 M. aluminium chloride and 2. 9 ml of distilled water were thoroughly mixed to obtain the completely dissolved solution. A sample blank was prepared in a similar way by replacing 1M aluminum chloride with distilled water. All prepared solutions were filtered through 0.45 μm. filter paper before measuring. The samples and the blank solution were measured by UV- VIS spectrophotometer (UV-1800, SHIMADSU) at 382 nm. Genistein standard was used as a marker for DSE to make the calibration curve (Isabela da Costa César et al., 2008).

Thermal analysis

The drug-excipient interaction was studied by a differential scanning calorimeter (DSC, Perkin Elmer, USA) with a nitrogen purge of 10 °C/min. DSC analysis of DSE, HPMC, blank patch and the optimized DSE patch were performed by heating the samples from 0 °C to 600 °C at a rate of 10 °C/min.

Scanning Electron Microscopic (SEM)

Surface morphology of the DSE patches with a proportion of HPMC E4M and E15LV at 48:52 % was examined by SEM Quanta using Maker FEI® software (Hillsboro, Oregon, USA).

***In vitro* release study**

The three suitable patch formulations from the physical properties evaluation were selected to continue the *in vitro* drug release study. The *in vitro* evaluation was carried out in Franz diffusion cells. The cellulose dialysis membranes (molecular weight cut-off 12,000 to 14,000 Da, Spectra/ Por®, Spectrum Labs, Rancho Dominquez, CA, USA) was mounted between the donor and receptor compartment of the diffusion cell. The membrane was previously soaked in receptor fluid of 10 % w/v ethanol in phosphate buffer saline (PBS) and 50% w/v ethanol for 2 hours. The

1.77 cm2 patches were placed over the membrane. The receptor compartment of the diffusion cell was filled with 12 ml of the receptor fluid. The solution in the receptor compartment was constantly and continuously stirred using a magnetic bar at 500 rpm; the temperature was maintained at 31 ± 0.5 °C. Samples were withdrawn (1 ml) at predetermined time intervals (0, 5, 15, 30, 45, 60, 120, 240, 360 and 720 minutes) and replaced with an equal volume of receptor fluid. The genistein content in these samples was determined by UV-VIS spectrophotometer at 382 nm.

Statistical analysis

In the study of physical evaluation of topical patches, polynomial models including linear, interaction, quadratic and cubic were generated for all the response variables using multiple linear regression analysis. Equations were derived and coefficients of interactions were calculated to determine the effect of each variable on the formulation characteristics. Statistical validity of the model was established on the basis of analysis of variance (ANOVA) using various statistical parameters such as p-value, coefficient estimate, determination coefficient (R^2), and adjusted determination of coefficient (Adjust R^2) by the Design Expert Software Version 10 (Stat-Ease Inc., Minneapolis, MN, USA.). In the study of drug release, the dissolution data of various formulations were fitted with the different mathematical models to determine the drug release kinetics, i.e., zero order, first order and Higuchi's model.

RESULTS AND DISCUSSIONS

Characterization of the DSE patches

Physical properties and mechanical parameters

All of the DSE patches were found to be flexible and brittle in appearance. The DSE patches containing HPMCs and PEG400 were not sticky and had the desired physical appearance. The physicochemical properties of DSE patches such as thickness, weight and genistein content were measured and are presented in Table 3**.**

Table 3. Physicochemical properties of DSE patches

Formulations	Thickness (mm ±s.d.)	Weight (mg ±s.d.)	Genistein content in patch (mg/40cm^2)	% drug loading (mg ±s.d.)
1	0.12 ±0.02	751.7 ±15.4	12.37 ± 0.31	76.93 ±2.51
2	0.08 ±0.01	591.6 ±1.7	34.70± 0.17	63.18 ±0.49
3	0.15 ±0.01	688.3 ±10.1	12.12± 0.24	75.37 ±1.98
4	0.10 ±0.00	610.4 ±12.0	34.91 ± 0.23	63.56 ±0.66
5	0.09 ±0.00	716.8 ±9.7	12.41 ± 0.05	77.18 ±0.41
6	0.10 ±0.01	718.3 ±19.4	34.57 ± 0.27	62.95 ±0.78
7	0.09 ±0.01	718.6 ±30.1	12.94 ± 0.16	80.47 ±1.24
8	0.09 ±0.01	711.9 ±10.9	35.07 ± 0.22	63.86 ±0.63
9	0.10 ±0.01	708.7 ±11.5	4.52 ± 0.23	66.27 ±5.08
10	0.08 ±0.01	561.0 ±31.1	42.72 ± 1.34	71.14 ±3.14
11	0.11 ±0.02	711.2 ±5.3	26.57 ± 0.04	57.73 ±0.15
12	0.17 ±0.02	562.0 ±14.4	26.50 ± 0.20	57.58 ±0.75
13	0.09 ±0.00	620.1 ±7.9	26.76 ± 0.35	58.15 ±1.31
14	0.11 ±0.01	771.2 ±30.4	27.48 ± 0.31	59.71 ±1.12
15	0.10 ±0.02	688.1 ±15.1	27.71 ± 0.54	60.21 ±1.94
16	0.10 ±0.01	718.0 ±4.3	27.86 ± 0.29	60.54 ±1.04

Variation of each property depends on the formulation variables used for experimental design. The genistein contents of all patches were found to be in the range of 4.52 ±0.23 to 42.72 ±1.34 mg/40 cm2. The % DSE loading implements the ability of drug incorporation in each formulation. The thickness of all patches varied between 0.08 ±0.01 to 0.17 ±0.02 mm. The variation of weight ranged from 561.00 ±31.15 mg to 771.20 ±30.41 mg. Mechanical parameters such as tensile strength (TS), % elongation at break (EB) and elastic modulus (EM) are shown in Table 4. These results indicate the rigidity and brittleness of the patches. Tensile strength values were found to be in the range of 151.88 ±46.58 g/cm^2 to 902.89 ±101.05 g/cm^2, as a result of the formulation factors, i.e., DSE concentration, polymer proportion and amount of plasticizer as further investigated by using response surface plots.

Table 4. Mechanical parameters of DSE patch

Formulations	Tensile strength (TS) (g/cm^2±s.d.)	Elongation at break (EB) (%±s.d.)	Elastic modulus (EM) (MPa±s.d.)
1	829.34±38.35	114.49±12.78	7.10±1.66
2	447.26±35.84	92.48±11.94	4.74±1.51
3	902.89±101.05	56.23±33.68	14.29±1.34
4	377.23±40.13	18.18±13.37	22.60±2.07
5	422.65±58.07	79.51±19.35	4.18±1.15
6	470.95±41.40	75.34±13.8	6.45±1.62
7	469.85±60.75	122.41±20.25	4.43±1.56
8	439.11±19.03	96.25±6.34	4.47±1.84
9	443.08±6.29	81.67±2.09	5.26±1.01
10	355.43±19.97	84.63±6.65	7.37±1.74
11	709.84±58.90	92.70±19.63	4.69±1.22
12	151.88±46.58	67.81±15.52	5.14±1.34
13	880.42±166.31	100.76±35.43	7.72±1.62
14	573.49±61.34	64.90±20.44	2.29±0.09
15	562.74±57.21	77.94±19.07	8.98±1.78
16	635.65±9.02	70.08±3.01	9.19±1.89

Analysis of suitable mathematical models

The essential components of statistical design, include defining output response and input variables, design of experiment, running the experiment, statistical analysis and optimization of formulation. In the current study, the combined effect of process variables such as DSE concentrations, ratios of HPMC E4M and E15LV, and PEG 400 concentrations at different levels on dependent variable (tensile strength) were studied using central composite design method. Experimental data were fitted to different polynomial models and statistical were performed to determine the adequacy of models (Table 5 and 6).

Table 5. Model Summary Statistics

Source	Std.dev.	R^2	Adjusted R^2
Linear	176.72	0.4087	0.2609
Quadratic	143.67	0.8046	0.5115
Cubic	53.11	0.9911	0.9332

Table 6. ANOVA for Response Surface Quadratic model

Model	Coefficient estimate	P-values <0.05
Model	596.24	0.1159
A-DSE	-75.90	0.0987
B-HPMC	-67.40	0.1337
C-PEG400	-93.09	0.0537
AB	-27.70	0.6052
AC	115.79	0.0629
BC	1.36	0.9795
A^2	-63.56	0.2268
B^2	-52.38	0.3096
C^2	52.30	0.3103

Effect of formulation variables on tensile strength

The quadratic model was found to be a fit for the response tensile strength with R^2 = 0.8046. All terms were selected for the model and the generated quadratic equation was as follows:

$$\text{Tensile strength } (g/cm^2) = 596.24 - 75.90A - 67.40B - 93.09C - 27.70AB + 115.79AC + 1.36BC - 63.56A^2 - 52.38B^2 + 52.30C^2$$

The equation shows that factors A and C have negative effects on the tensile strength of patches. However, the coefficient value of factor C (-93.09) is higher than that of factor A (-75.90), having a coefficient value of -75.90. The interaction factors (AC) had synergistic effect on decreasing tensile strength due to their positive coefficient value. In addition, the influences of patch formulation variables on the tensile strength can be further explained by using response surface plots based on this model as seen in figure 4. The correlation between the data from the experiments and the theoretical value can be evaluated. The values of three factors (A, B and C) were substituted in the polynomial equation to calculate the theoretical value of the response (tensile strength). The theoretical (predicted) values and observed values were mostly in agreement as shown in Table 7.

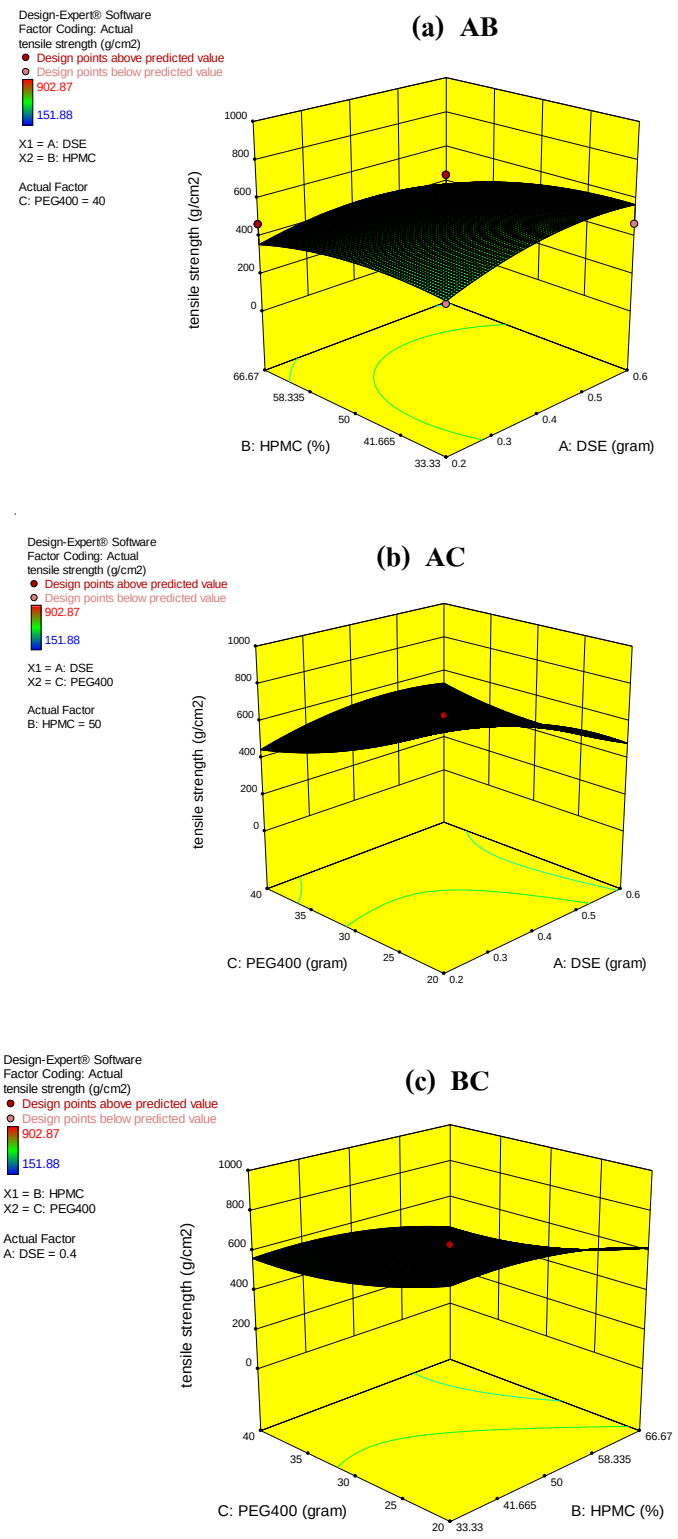


Figure 2.Effects of the contents of DSE (A), proportion of HPMC E4M and E15LV (B) and the amount of PEG 400 (C) on response shown by using response surface plot AB, AC and BC.

Table 7. Observed and predicted values of response tensile strength

Formulation	Factor 1 A = DSE (g)	Factor 2 B = HPMC E4M (%)	Factor 3 C = PEG400 (g)	Response Tensile strength (g/cm^2)	Predicted Tensile strength (g/cm^2)
1	0.2	33.33	20	829.34±38.35	858.44
2	0.6	33.33	20	447.26±35.84	530.47
3	0.2	66.67	20	902.89±101.05	779.32
4	0.6	66.67	20	377.23±40.13	337.55
5	0.2	33.33	40	422.65±58.07	437.97
6	0.6	33.33	40	470.95±41.40	573.14
7	0.2	66.67	40	469.85±60.75	361.28
8	0.6	66.67	40	439.11±19.03	385.65
9	0.064	50	30	443.08±6.29	544.37
10	0.736	50	30	355.43±19.97	289.34
11	0.4	22	30	709.84±58.90	561.63
12	0.4	78	30	151.88±46.58	335.15
13	0.4	50	13.18	880.42±166.31	900.25
14	0.4	50	46.82	573.49±61.34	587.46
15	0.4	50	30	562.74±57.21	596.24
16	0.4	50	30	635.65±9.02	596.24

Optimization of topical DSE patch formulation

Derringer's desirability function methodology was used for optimization of DSE patch formulation. Table 4 shows the mechanical properties of the DSE patches. Higher values of tensile strength (TS) are desirable for mechanical resistance. The elongation at break (EB) is a measure of the ductility of a patch which defines the patch's ability to deform before failure occurs, whereas the elastic modulus (EM) is a key indicator of the stiffness of the patch. A tough patch should have low EM, moderate TS and high EB. TS was selected as the key parameter to be optimized. Ideal patches were designed to have high DSE content by varying the polymer proportion and amount of plasticizer to obtain the moderate TS.

The optimum compositions were found to be 0.6 g for DSE, 48:52% for proportion of HPMC (E4M:E15LV) and 40% for PEG 400 which predicted that the value of TS would be 550 g/cm^2 with a desirability value of 0.999. The experiment was performed to prepare the DSE patch according to the optimized composition for validation purpose. The average optimized patches (n=5) values of 571.28 ±37.61 g/cm^2 for TS and 83.5 % for EB and 6.71 MPa for EM were obtained. The result of TS was closely related with the data predicted from the above numerical optimization technique using desirability methodology. The EB value was high whereas EM value was low, indicating that the optimized patch had the required mechanical property. The result was also in accordance with a recent study which used the response surface methodology to investigate the mechanical properties of the transdermal patch of simvastatin (Parhi and Suresh 2016). It was reported that high drug concentration (2%), medium polymer ratio (50%) and high amount of plasticizer (40%) resulted in mechanical properties of high EB (85.33%) and low EM (20.39 MPa). The optimized composition was used to prepare DSE patches for the *in vitro* drug release study.

Swelling property

Swelling indexes of all patches which indicate the swelling properties are shown in Figure 5**.** The result implied that the low polymer ratios of HPMC E**4**M and E**15**LV as seen from formulations **1**,**2**,**5**,**6** had higher swelling indexes than those of the high polymer ratio patches from formulations **3**,**4**,**7**,**8.** It could be explained that the water uptake by the patches increased as the amount of HPMC E**15**LV increased because HPMC E**15**LV was more hydrophilic and easily leached from the patch, leaving behind a porous structure and thereby increasing penetration of water**.** Consequently, the portion of HPMC E**4**M in the patch imbibed more water to swell**.** In addition, DSE concentration also had an agonistic effect on swelling properties**.** The patch with a composition of high DSE concentration and high polymer proportion had a lower swelling index as seen from formulations **13-16.** As seen in formulation **9**, the patch with the lowest DSE concentrations had the highest swelling index**.**

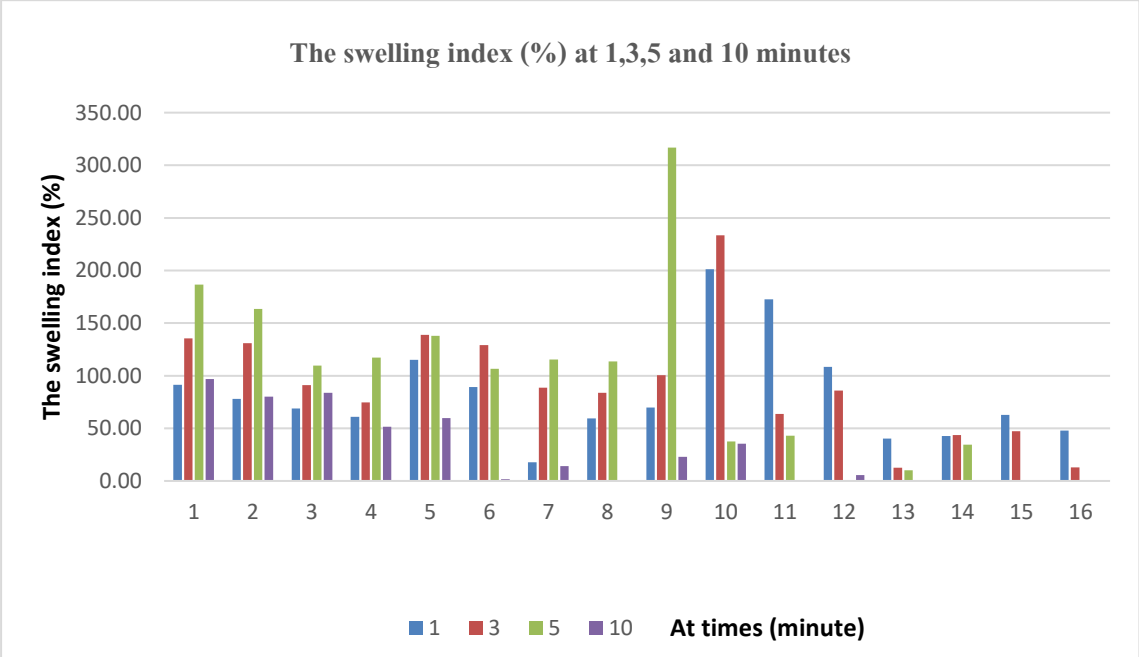


Figure 3. The swelling (%) at 1, 3, 5 and 10 minutes of formulations 1-16.

Thermal analysis

The drug-excipient interaction was studied by differential scanning calorimeter (DSC, Perkin Elmer, USA) with a nitrogen purge of 10 °C/min. DSC analysis of DSE, HPMC, blank patch and the optimized DSE patch were performed by heating the samples from 0 °C to 600 °C at a rate of 10 °C/min. The thermogram of DSE (Fig. 2) shows the endothermic peak at 117.17 °C. The polymers show broad peaks at 88.67 °C for HPMC, and 85.00 °C for blank patch. The thermogram of an optimized DSE patch shows a shifted endothermic peak at 87.50 °C. There is no similar endothermic peak of DSE in the thermogram of the DSE patch because the amount of DSE in the patch is considerably less than that in pure DSE samples. However, interaction of drug and polymer cannot be observed due to the nearly identical identity between the thermogram of DSE patch and of the blank sample. The thermogram of HPMC is gradually different from the DSE patch and blank as the result of the higher moisture content (Ford JL 1999). Thus, DSE was found to be compatible with the HPMC polymer, suggesting that HPMC could be used for the preparation of the topical patches.

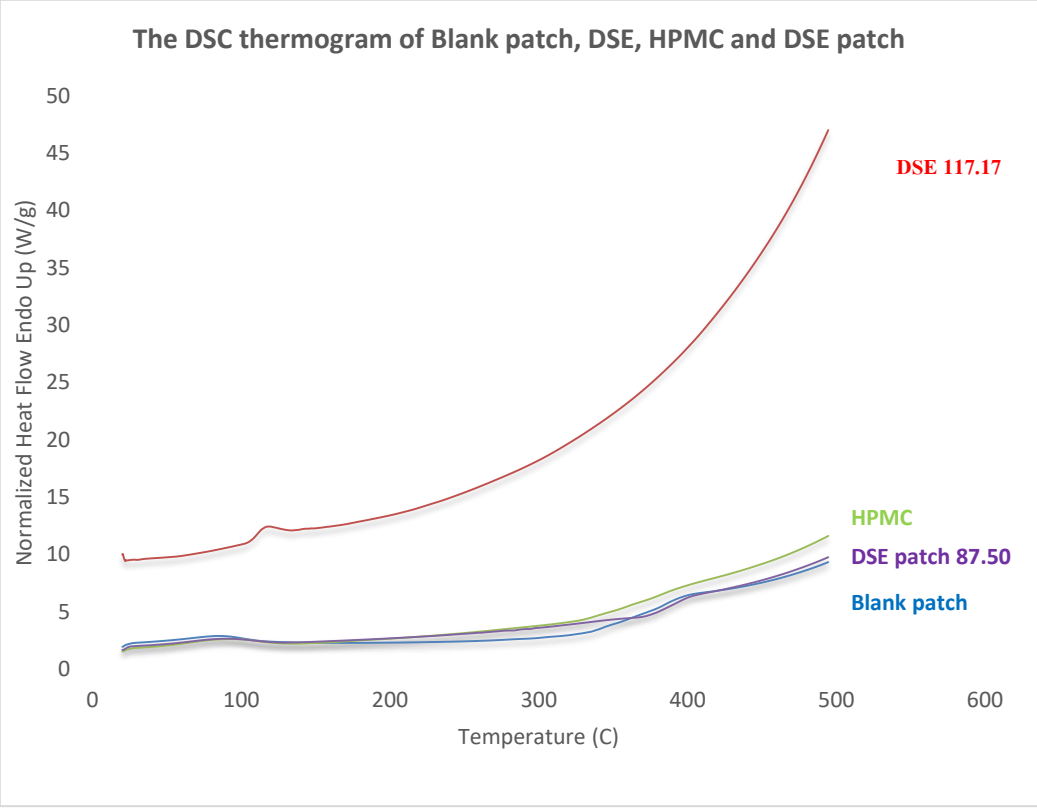
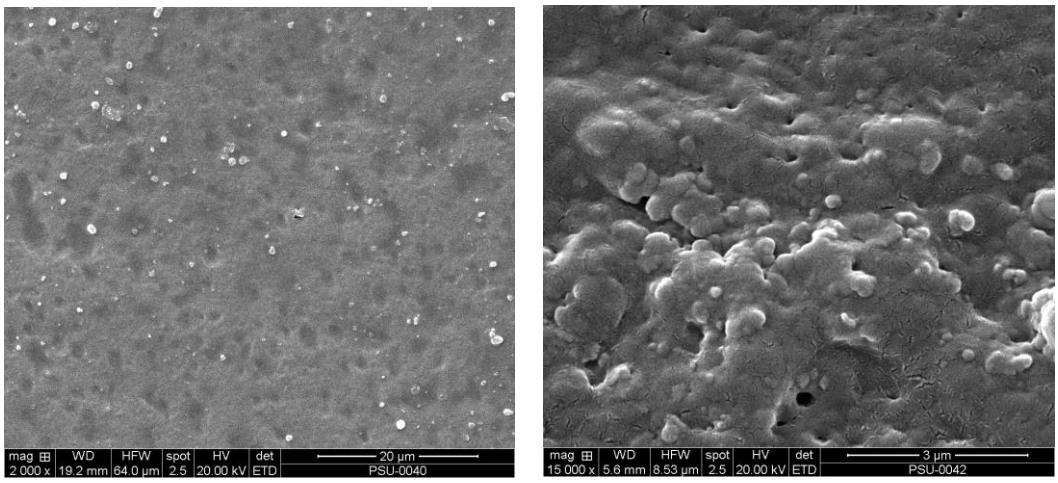


Figure 4. The DSC thermogram of blank, DSE, HPMC and DSE patch

Scanning Electron Microscopic (SEM)

The morphology of the DSE patches was prepared by solvent casting evaporation. DSE seemed to be dispersed homogeneously in a hydrophilic of HPMC as the patch obtained was thoroughly stained dark green, the color of DSE. In addition, Fig. 3 shows SEM micrographs of the patch matrices with proportions of HPMC E4M and E15LV at 48:52%. The white drug particles are dispersed thoroughly in the porous matrix of DSE patches and can be seen in SEM at 2,000X magnification. The drug particles can be seen close-up in cross-sectional SEM imaging at 16,000X magnification showing that the particles are embedded in the patch matrix consistently. The clearer SEM comparison could be made between drug patches and blank patches.



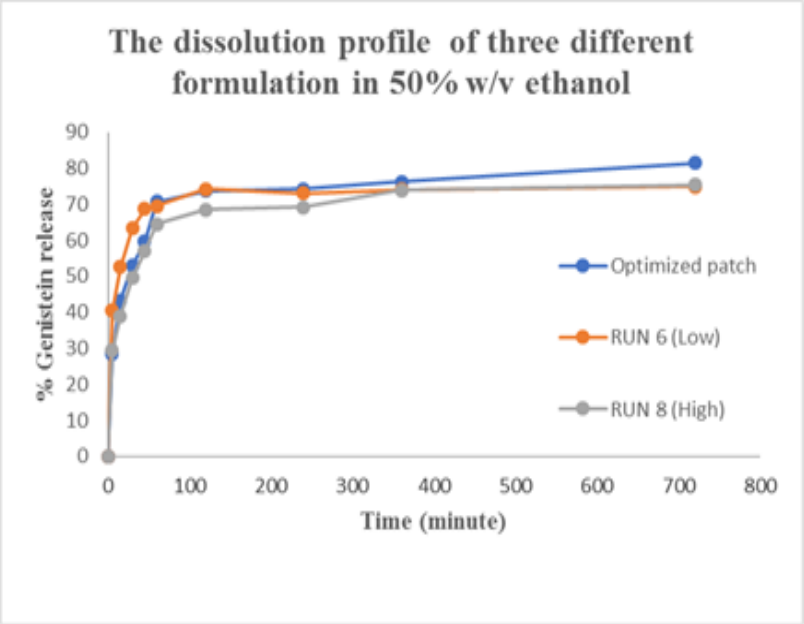
(a) **(b)**

Figure 5. The scanning electron microscopic of the DSE patch; (a) at 2,000 X and (b) cross-section at 16,000X magnification

***In vitro* drug release study**

The *in vitro* drug release of DSE patches was carried out using 10% w/v ethanol in phosphate buffer saline and 50% w/v ethanol. The dissolution profiles of three different formulations (optimized patch, formulation 6 [low polymer proportion], formulation 8 [high polymer proportion]) in different media are shown in Figure 6. The results showed that maximum concentrations of drug released were obtained at the 1st hour and 12th hour for 50% w/v ethanol and 10% w/v ethanol in phosphate buffer saline, respectively. From Figure 6a, it was found that all patches released genistein rapidly in 50% w/v ethanol medium. The percent release at the 60th minute of optimized, low and high polymer proportion patches were 70.76 %, 69.52 % and 64.44 % respectively due to the high solubility of genistein in ethanol. From Figure 6b, it was found that all patches released genistein more slowly in 10% w/v ethanol in phosphate buffer saline. The percent release at the 240th minute of optimized, low and high polymer proportion patches were 67.28, 70.28 and 62.17 % respectively. In the case of drug release performed in 50% w/v ethanol, the release of drugs from the patch is governed by the diffusion of solute which dissolved initially by the medium within the matrix phase and continuously decreases as the concentration gradient decreases. Therefore, the higher drug solubility could facilitate higher drug release from the patches. While drug release performed in 10% w/v ethanol in phosphate buffer saline, the release of drugs from the patch is governed by the dissolution of the patch which swelled initially by the imbibition of the medium leading to the lag time of the drug release for the first 45 minutes. Due to less solubility of DSE in 10% w/v ethanol in phosphate buffer saline, the drug particle was embedded in the polymer matrix and could not exit with the medium as in the case of drug release in 50% ethanol. Consequently, the drug release was minute. Until the drug release evolved into erosion of the patch matrix, it caused a burst of drug dissolved in the medium after lag time. This mechanism of drug release was in substantial agreement with the kinetic model further analyzed.

(a)



(b)

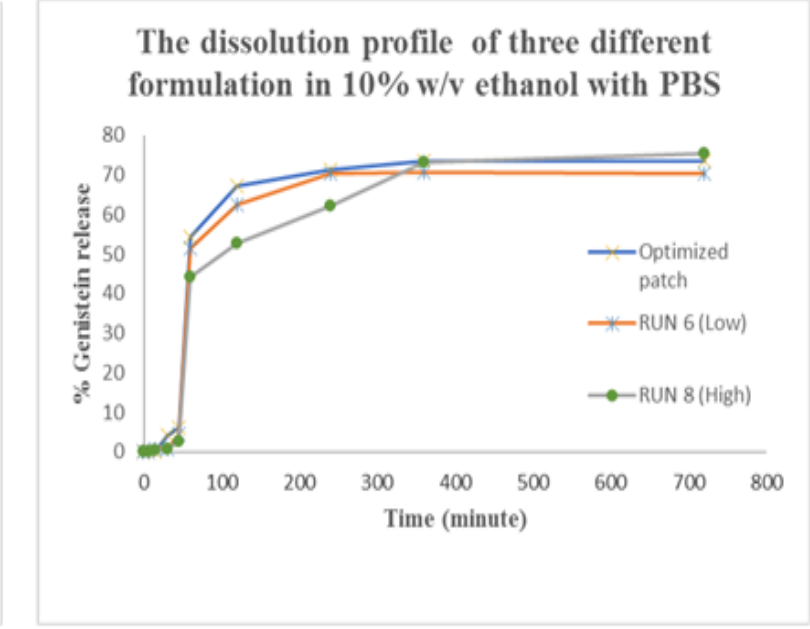


Figure 6. The *in vitro* drug release profile of 3 different patch formulations in 50% w/v ethanol (a) and 10% w/v ethanol with PBS (b) at 31 ± 0.5 °C.

Kinetic modeling of dissolution data

Drug release kinetics were analyzed by various mathematical models such as a zero-order and first-order kinetic models; Higuchi and Korsmeyer–Peppas models to ascertain the kinetics of drug release.

- Zero order kinetics; $Q_1 = Q_0 + K_0 t$

Where Q_1 is the amount of the drug dissolved in time *t*, Q_0 is the initial amount of drug in the solution (most times, Q_{50}) and *K* is the zero-order release constant.

- First order kinetics; $\ln Q_t = \ln Q_0 + K_1 t$

Where Q_t is the amount of drug released in time *t*, Q_0 is the initial amount of drug in the solution and *K* is the first order release constant.

- Higuchi model; $Q_t = K_H t_{1/2}$

Where Q_t is the amount of drug released in time *t*, K_H is release rate constants.

To explain the drug release kinetics of genistein from the topical patches, the mean dissolution curves including the fraction between 10-90% of drug release was calculated by linear regression analysis of various mathematical models. Neither one of the models could provide good coefficients of determination except Higuchi's (R^2 0.962, 0.534 and 0.899) which gave R^2 close to 1 for the test run in receptor fluid of 10% w/v ethanol in PBS as tabulated in Table 5. Hence, the release mechanism of genistein from the topical patches is governed by diffusion and dissolution processes. It might be explained that the patches with the higher proportion of hydrophilic polymer lead to higher erosion of the patches and thereby facilitate rapid drug release from the patches. According to this fact, the release rates were found to decrease with an increase in polymer proportion of HPMC E4M and E15LV. The results of the swelling index supported the drug release in the corresponding manner.

Table 8. Coefficient of determination from linear regression analyses of various mathematical models

DSE patches	Zero order $Q_t = K_0t + Q_0$	First order $LogQ_t = K_1t + LogQ_0$	Higuchi $Q_t = K_Ht^{1/2}$
Receptor fluid; 50% w/v ethanol			
Optimized	0.665	0.393	0.639
Formulation 6	0.831	0.464	0.452
Formulation 8	0.815	0.453	0.641
Receptor fluid; 10% w/v ethanol in PBS			
Optimized	0.674	0.735	0.962
Formulation 6	0.468	0.490	0.534
Formulation 8	0.774	0.822	0.899

CONCLUSIONS

The present study was undertaken to optimize a suitable combination of DSE concentration (A), the polymer proportion of HPMC E4M and E15LV (B), and the amount of PEG 400 (C) for topical and transdermal drug delivery systems using central composite design and response surface methodology. By utilizing Derringer's desirability prediction tool, the optimum compositions were found to be 0.6 g for DSE, 48:52% for proportion of HPMC (E4M:E15LV) and 40 % for PEG, resulting in the tensile strength of 571.28 g/cm^2 and high elongation at break of 83.5 %. This indicated that the patch was tough and inherited good mechanical properties. The *in vitro* drug release from the DSE patches were studied in 10% w/v ethanol in phosphate buffer saline and 50% w/v ethanol. Maximum concentrations of drug released was obtained within 12 hours and 1 hour for 10 % w/v ethanol in phosphate buffer saline and 50% w/v ethanol, respectively. The release rates were found to decrease with the increase in polymer proportion of HPMC E4M and E15LV. The drug release kinetics of Higuchi models fit well to DSE release data of optimized patches. Hence DSE patches have potential as an alternative dosage form to oral preparation and could be further developed for the study of *in vitro* skin permeation of DSE.

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APPENDICES

Appendix A **Full Paper Bulletin of Health, Science and Technology**

Appendix B **Raw data of calibration curve, dissolution test and swelling test**

Appendix A Full Paper Bulletin of Health, Science and Technology

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FORMULATION AND PHYSICAL EVALUATION OF TOPICAL PATCH CONTAINING *DERRIS SCANDENS*

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Abstract: Formulation of topical patch containing *Derris scandens* extracts (DSE) for topical and transdermal drug delivery systems were developed and their physical properties including drug release were investigated. The topical patches of DSE were prepared by solvent casting evaporation technique using various proportions of DSE concentration (A), the polymer proportion of HPMC E4M and E15LV (B) and the amount of PEG 400 (C). Central composite design was adopted to determine the effect of the above formulation factors on the tensile strengths of the patches. It was found that tensile strengths were in the range of 151.88±46.58 g/cm^2 to 902.89±101.05 g/cm^2, demonstrating the patches with suitable hardness and brittleness. The mathematical equation was used to relate independent variables with tensile strength. The optimization model predicted the desired tensile strength with A, B, and C levels of 0.6 g, 48:52 and 40 %, respectively. This optimum composition resulted in suitable tensile strength of 571.28 ± 37.61 g/cm^2 and showed a good correlation between predicted and observed value. The *in vitro* drug release from the DSE patches were studied in 10%w/v ethanol in phosphate buffer saline and 50% w/v ethanol. Maximum concentrations of drug released obtained within 12 hours and 1 hour for 10 % w/v ethanol in phosphate buffer saline and 50% w/v ethanol, respectively. The release rates were found to decrease with the increase in polymer proportion of HPMC E4M and E15LV. The drug releasee kinetics of Higuchi models fit well to DSE release data of optimized patches. Hence DSE patches have a potential as an alternative dosage form to oral preparation and could be further developed for the study of *in vitro* skin permeation of DSE.

Keywords: *Derris scandens* extract, topical patches, topical drug delivery system

บทคัดย่อ: การพัฒนาสูตรตำรับแผ่นแปะผิวหนังซึ่งบรรจุสารสกัดเถาวัลย์เปรียง (DSE) โดยประเมินคุณสมบัติทางกายภาพและความสามารถในการปลดปล่อยยาออกจากแผ่นแปะผิวหนังซึ่งสามารถเตรียมแผ่นแปะโดยวิธี solvent casting evaporation technique โดยใช้สัดส่วนที่ต่างกันของเถาวัลย์เปรียง (A) สัดส่วนของพอลิเมอร์ HPMC E4M:E15LV (B) และสารเพิ่มความยืดหยุ่น PEG400 (C) ใช้ central composite design เพื่อพิจารณาหาผลของปัจจัยที่เกี่ยวข้องกับสูตรตำรับข้างต้นที่มีผลต่อ tensile strength ของแผ่นแปะ จากการทดลองพบว่าค่า tensile strength อยู่ในช่วง 151.88 ± 46.58 g/cm^2 ถึง 902.89 ± 101.05 g/cm^2 โดยให้ความแข็งแรงและยืดหยุ่นเป็นที่น่าพอใจ สร้างสมการทางคณิตศาสตร์ที่แสดงความสัมพันธ์ระหว่างตัวแปรอิสระกับ tensile strength ใช้ optimization model ที่สร้างขึ้นทำนายค่า tensile strength ที่ต้องการได้ จากค่าของ A, B และ C ที่ 0.6 g, 48:52 และ 40% ซึ่งส่วนประกอบดังกล่าวจะให้ผลเป็นที่น่าพอใจคือแสดงค่า tensile strength: 571.28 ± 37.61 g/cm^2 และให้ความสัมพันธ์ที่สอดคล้องกันระหว่างค่าที่ทำนายและค่าที่ได้จริง การศึกษาการปลดปล่อยยาในระดับ *in vitro* drug release โดยใช้เครื่อง Franz diffusion cell: ในสารละลาย 2 ชนิด ได้แก่ 10% w/v ethanol in phosphate buffer saline และ 50% w/v ethanol แสดงให้เห็นว่ามีลักษณะของการปลดปล่อยยาที่ความเข้มข้นสูงสุดที่ 12 ชั่วโมง สำหรับ 10% w/v ethanol in phosphate buffer saline และ 1 ชั่วโมง สำหรับ 50% w/v ethanol พบว่าอัตราการปลดปล่อยยาจะลดลงเมื่อเพิ่มสัดส่วนของพอลิเมอร์ HPMC E4M ต่อ E15LV โดยลักษณะทางจลนศาสตร์ของการปลดปล่อยยาเข้ากันได้ดีกับโมเดลของ Higuchi สำหรับแผ่นแปะที่ได้จาก optimization ดังนั้นการศึกษาการซึมผ่านผิวหนังในระดับ *in vitro* skin permeation เพิ่มเติมนั้นจะทำให้ทราบถึงศักยภาพของแผ่นแปะ DSE ที่จะเป็นรูปแบบยาเตรียมทางเลือกอื่นนอกจากการให้ทางปาก

คำสำคัญ: สารสกัดเถาวัลย์เปรียง แผ่นแปะผิวหนัง ระบบนำส่งยาผ่านทางผิวหนัง

INTRODUCTION

Derris scandens (Roxb.) Benth. (Fabaceae) or Thao-Wan-Priang (Thai-name) is known as herbal medicine in the South-East Asia and has been traditionally used as a diuretic, antitussive, expectorant, anti-dysentery and muscle pain treatment. It is also claimed as cancer prevention, and health promotion herb in cardiovascular patients and postmenopausal women (Sriwanthana and Chavalittumrong, 2001; Kuptniratsaikul *et al*., 2011). Active ingredients of *Derris scandens* for anti-inflammatory effect are genistein glycoside derivatives (isoflavones). Current evidence suggested that *Derris scandens* might be considered as a potential alternative for nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of musculoskeletal pain. The use of *Derris scandens* as alternative medicine might be safer than NSAIDs in long-term use (Puttarak *et al*., 2016).

The term of topical drug products referring to all formulations applied to the skin except transdermal delivery systems (TDS) or transdermal patches that will be addressed separately (Pharmacopeial Forum, 2009). Topical patch formulation is the alternative medicine apart from oral therapy. In contrast to conventional topical formulations, such as creams, gels and sprays, topical patch provides a defined dose to a defined area for an extended period time. In addition, application of the patch is devoid of the messiness or staining of the skin that may occur while applying creams or gels. Sun Hwa Lee et al. (2016) studied the hydrocolloid patch including genistein and daidzein to deliver them through the skin. It was confirmed that the patches are helpful for the drugs to be absorb much and more deeply than the cream due to the occlusive effect of patch. In this study, the topical patch consisted of *Derris scandens* extracts (DSE) was developed as alternative drug delivery. Aim of this study is to investigate the drug released and evaluate the physical properties of the formulation of topical patches.

MATERIALS AND METHODS

Materials

Derris scandens extract (PSU pharmacognosy laboratory), Genistein standard (Sigma®, Thailand), Hydroxypropyl methylcellulose grade E4M and E15LV (P.C. Drug Center, Bangkok, Thailand), Polyethylene glycols 400 (P.C. Drug Center, Bangkok, Thailand). 95% Ethanol, AR grade (Lab-Scan®, Bangkok, Thailand), Aluminium Chloride AR grade (Sigma®, Thailand)

Methods

Experimental design

The central composite design was adopted to optimize the formulation factors, i.e. DSE concentration, polymer proportion of HPMC E4M and E15LV and percentage of PEG 400 and to evaluate their effects on a response of the tensile strength of the patches. All the factors and the response variables are mentioned in Table 1.

Table 1. Variables and their levels in central composite experimental design

Variables	Levels				
	-1.68	-1	0	1	1.68
Independent factors					
A = DSE (g)	0.064	0.2	0.4	0.6	0.736
B = HPMC E4M:15LV (%)	22	33.33	50	66.67	78
C = PEG400 (%)	13.18	20	30	40	46.82
Dependent response					
Tensile strength (g/cm^2)			-		

Statistical analysis for the present study was performed by Design-Expert software (Version 10, Stat-Ease Inc., Minneapolis, MN, USA). Experimental design of different runs (formulations) of topical patch is presented in Table 2.

Table 2. Central composite design for the preparation of different formulations

Runs	Factors with levels		
	DSE (g)	HPMC E4M (%)	PEG400 (g)
1	0.2	33.33	20
2	0.6	33.33	20
3	0.2	66.67	20
4	0.6	66.67	20
5	0.2	33.33	40
6	0.6	33.33	40
7	0.2	66.67	40
8	0.6	66.67	40
9	0.064	50	30
10	0.736	50	30
11	0.4	22	30
12	0.4	78	30
13	0.4	50	13.18
14	0.4	50	46.82
15	0.4	50	30
16	0.4	50	30

Preparation of the herbal medicine patch

The 16 formulations of topical patches (Table 2) were prepared by solvent casting evaporation technique. Initially, the 3% HPMC solution containing water and HPMC in varied proportions of E4M and E15LV were prepared. The HPMC polymers were dispersed and thoroughly hydrated in about 20-30 % of the required amount of water. The water was heated to 80 -90 °C, and then the polymers were added with stirring action. Sufficient cold water then was added to produce the required volume while continuing to stir. Required quantities of DSE and PEG 400 were added slowly to previously prepared HPMC solutions while cooling followed by mixing them thoroughly. The matrix solutions were poured on to a petri dish. The solvent casting was evaporated at 45 °C for 24 hours. The dried DSE films were cut into 40 cm^2 and wrapped in aluminum foil before being kept in a desiccator 2-8 °C (Rabinarayan and Padilam, 2016).

Evaluation of physicochemical properties of the patch

1) Thickness and weight variation

The thickness of patch was measured three times by Teclock® dial thickness gauge. Weight variation studies were carried out with selected the three patches)40 cm^2 (from each run using analytical balance (Rabinarayan and Padilam, 2016).

2) pH measurement

The pH of patches solution (n=3) were measured by pH meter (Professional Benchtop pH meter BP3001 Trans Instruments., Singapore).

3) Swelling index

The dried patches supported by stainless steel mesh were immersed in a beaker containing 25 ml distilled water at room temperature. At the 2^{nd} minute time interval the swollen samples with the pre-weighed mesh were weighed after removal of excess surface water by blotting lightly with a filter paper. The experiment was discontinued when the patches began to disintegrate or dissolve. To quantify the swelling index, percentage was calculated as follows:

$$\text{Swelling index} = \frac{(W_s - W_d)\times 100}{W_d} \quad (1)$$

Where; W_d: the weight of the dried patch
W_s: the weight after swelling

4) Mechanical properties

For the measurement of mechanical properties as tensile strength (g/cm^2), % elongation at break (EB) and elastic modulus (EM), the patch specimen of specific dimension (4 × 2 cm) was fixed between two clamps of texture analyzer (TA. XT plus, Stable MicroSytems Ltd, USA). The test condition were ; test speed of 10 mm/min, target load of 5 kg, holding time of 5 seconds, and trigger force of 5 g (Rabinarayan and Padilam, 2016). The mechanical properties were determined as follows:

Tensile strength (g/cm^2) = Breaking force (g) / area of the film (cm^2) (2)

5) Drug content

A 1x1 cm^2 of each patch (n=3) was dissolved in 10 ml of 95 % ethanol and sonicated for 30 minutes. Then 0.5 ml of each sample solution, 1.5 ml of 95 % ethanol, 0.1 ml of 1 M aluminium chloride and 2.9 ml of distilled water were thoroughly mixed to obtain the completely dissolved solution. Sample blank was prepared in similar way by replacing 1M aluminium chloride with distilled water. All prepared solution was filtered through 0.45 µm. filter paper before measuring. The samples and blank solution were measured by UV-VIS spectrophotometer (UV-1800, SHIMADSU) at 382 nm. Genistein standard as marker for DSE was used to make the calibration curve (Isabela da Costa César *et al*., 2008).

6) In vitro release study

The three suitable patch formulations from the physical properties evaluation were selected to continue the *in vitro* drug release study. The *in vitro* evaluation was carried out in Franz diffusion cell. The cellulose dialysis membranes (molecular weight cut-off 12,000 to 14,000 Da, Spectra/ Por®, Spectrum Labs, Rancho Dominquez, CA, USA) was mounted between the donor and receptor compartment of the diffusion cell. The membrane was previously soaked for 2 hours in receptor fluid of 10 % w/v ethanol in phosphate buffer saline

(PBS) and 50% w/v ethanol. The 1.77 cm^2 patches were placed over the membrane. The receptor compartment of the diffusion cell was filled with 12 ml of the receptor fluid. The solution in the receptor compartment was constantly and continuously stirred using magnetic bar at 500 rpm; the temperature was maintained at 31 ± 0.5 °C. Samples were withdrawn (1 ml) at predetermined time intervals (0, 5, 15, 30, 45, 60, 120, 240, 360 and 720 minutes) and replaced with an equal volume of receptor fluid. The genistein content in these samples was determined by UV-VIS spectrophotometer at 382 nm.

7) Statistical analysis

In the study of physical evaluation of topical patch, polynomial models including linear, interaction, quadratic and cubic were generated for all the response variables using multiple linear regression analysis. Equations were derived and coefficients of interactions were calculated to determine the effect of each variable on the formulation characteristics. Statistical validity of the model was established on basis of analysis of variance (ANOVA) using various statistical parameters such as p-value, coefficient estimate, determination coefficient (R^2), adjusted determination of coefficient (Adjust R^2) by the Design Expert Software Version 10 (Stat-Ease Inc., Minneapolis, MN, USA).

In the study of drug release, the dissolution data of various formulations were fitted with the different mathematical models to determine the drug release kinetics, i.e., zero order, first order and Higuchi's model.

RESULTS AND DISCUSSION

Evaluations of physicochemical properties and mechanical parameter of DSE patches

The physicochemical properties of DSE patches such as, thickness variation, weight variation, pH and genistein content were measured and presented in Table 3. The genistein content of all patch were found to be in the range of 4.52 ±0.23 to 42.72 ±1.34 mg/40 cm^2. The thickness of all patches was varied between 0.085 ±0.008 to 0.177 ±0.027 mm. The variation of weight ranged from 561.0 ±31.15 mg to 771.2 ±30.41 mg. The pH of solution ranged from 8.48 ±0.03 to 8.86 ±0.01. Mechanical parameters such as tensile strength (TS), % elongation at break (EB) and elastic modulus (EM) are shown in Table 4. which indicated the hardness and brittleness of prepared patches. Tensile strength values were found to be in the range of 151.88 ±46.58 g/cm^2 to 902.89 ±101.05 g/cm^2, as a resulting of the formulation factors, i.e., DSE concentration, polymer proportion and amount of plasticizer as further discussed by using response surface plots.

Table 3. Results of physicochemical properties of DSE patches

Runs	Thickness (mm)	Weight (mg)	pH	Amount of genistein in patch (mg)
1	0.121 ±0.024	751.7 ±15.4	8.86±0.01	12.37 ± 0.31
2	0.085 ±0.008	591.6 ±1.7	8.85±0.01	34.70± 0.17
3	0.151 ±0.014	688.3 ±10.1	8.55±0.04	12.12± 0.24
4	0.102 ±0.006	610.4 ±12.0	8.86±0.01	34.91 ± 0.23
5	0.099 ±0.006	716.8 ±9.7	8.50±0.01	12.41 ± 0.05
6	0104 ±0.009	718.3 ±19.4	8.50±0.01	34.57 ± 0.27
7	0.094 ±0.005	718.6 ±30.1	8.59±0.02	12.94 ± 0.16
8	0.098 ±0.010	711.9 ±10.9	8.55±0.01	35.07 ± 0.22
9	0.103 ±0.015	708.7 ±11.5	8.47±0.03	4.52 ± 0.23
10	0.086 ±0.011	561.0 ±31.1	8.54±0.03	42.72 ± 1.34
11	0.113 ±0.029	711.2 ±5.3	8.53±0.03	26.57 ± 0.04
12	0.177 ±0.027	562.0 ±14.4	8.51±0.04	26.50 ± 0.20
13	0.091 ±0.005	620.1 ±7.9	8.52±0.04	26.76 ± 0.35
14	0.114 ±0.015	771.2 ±30.4	8.55±0.04	27.48 ± 0.31
15	0.101 ±0.020	688.1 ±15.1	8.59±0.04	27.71 ± 0.54
16	0.105 ±0.011	718.0 ±4.3	8.48±0.03	27.86 ± 0.29

Table 4. Results of mechanical parameters of DSE patch

Runs	Tensile strength (g/cm^2) (TS)	% Elongation at break (EB)	Elastic modulus (MPa) (EM)
1	829.34±38.35	114.49	7.10
2	447.26±35.84	92.48	4.74
3	902.89±101.05	56.23	14.29
4	377.23±40.13	18.18	22.60
5	422.65±58.07	79.51	4.18
6	470.95±41.40	75.34	6.45
7	469.85±60.75	122.41	4.43
8	439.11±19.03	96.25	4.47
9	443.08±6.29	81.67	5.26
10	355.43±19.97	84.63	7.37
11	709.84±58.90	92.70	4.69
12	151.88±46.58	67.81	5.14
13	880.42±166.31	100.76	7.72
14	573.49±61.34	64.90	2.29
15	562.74±57.21	77.94	8.98
16	635.65±9.02	70.08	9.19

Swelling indexes of all patches which indicate the swelling properties are shown in Figure 1. The result implied that the low polymer proportion of HPMC E4M and E15LV as seen from runs 1,2,5,6 had higher swelling index than those of the high polymer ratio patches from runs 3,4,7,8. It could be explained that the water uptake by the patches increased as amount of HPMC E15LV increased because HPMC E15LV was more hydrophilic and easily leached from the patch and left behind a porous structure and thereby increased penetration of water. Consequently, the portion of HPMC E4M in the patch imbibed more water to swell. In addition, DSE concentration had also agonist effect on swelling property. The patches with composition of high DSE concentration and high polymer proportion had lower swelling

index as seen from runs 13, 14, 15 and 16. The patch with the lowest DSE concentration as seen in run 9 had the highest swelling index.

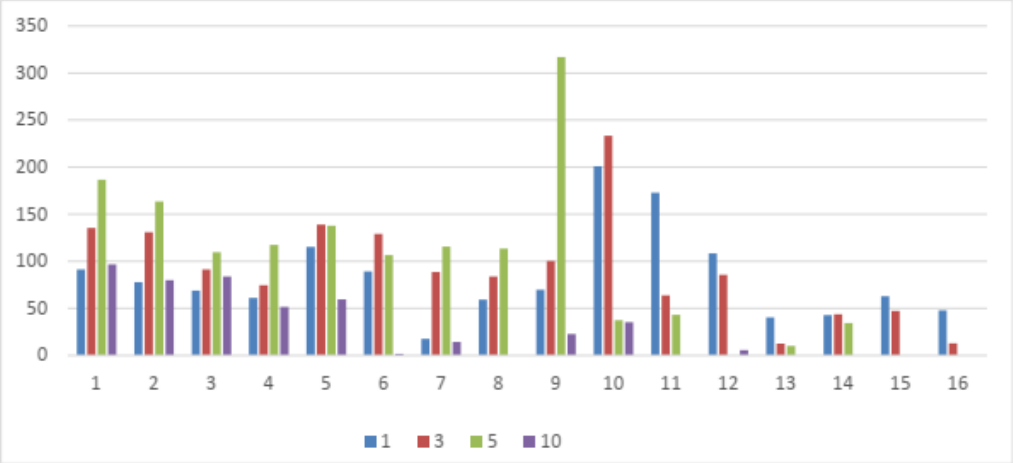


Figure 1. The swelling (%) at 1st, 3rd, 5th and 10th minutes of runs 1-16.

Analysis of suitable mathematical model

In a statistical design, the essential components include defining output response and input variables, design of experiment, running experiment, statistical analysis and optimization of formulation (suitable combination of independent variables). In the current study, the combined effect of process variables such as DSE concentration, proportion of HPMC E4M and E15LV and PEG 400 concentration at different levels on dependent variable (tensile strength) were studied using central composite design method. Experimental data were fitted to different polynomial models including linear, quadratic and cubic models and statistical tests such as sequential model sum of squares and model summary were performed to determine the adequacy of models (Table 5 and 6).

Table 5. Model Summary Statistics

Source	Std.dev.	R^2	Adjusted R^2
Linear	176.72	0.4087	0.2609
Quadratic	143.67	0.8046*	0.5115
Cubic	53.11	0.9911	0.9332

Table 6. ANOVA for Response Surface Quadratic model

Model	Coefficient estimate	P value
Model	596.24	0.1159
A-DSE	-75.90	0.0987
B-HPMC	-67.40	0.1337
C-PEG400	-93.09	0.0537
AB	-27.70	0.6052
AC	115.79	0.0629
BC	1.36	0.9795
A^2	-63.56	0.2268
B^2	-52.38	0.3096
C^2	52.30	0.3103

Effect of formulation variables on tensile strength of DSE patch

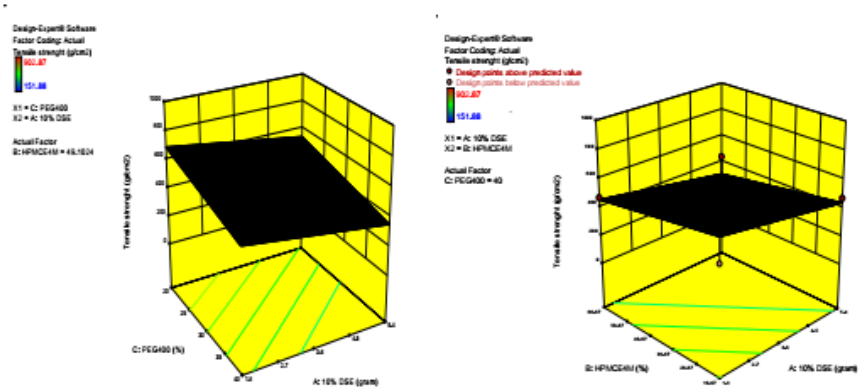
The experimental data was evaluated by ANOVA and the significance was measured by the corresponding p-value of the regression coefficients as shown in Table 5. Quadratic model was found to be fitted for the response tensile strength with $R^2 = 0.8046$. All terms were selected for model and generated quadratic equation is given to:

$$\text{Tensile strength (g/cm}^2\text{)} = 596.24 - 75.90A - 67.40B - 93.09C - 27.70AB + 115.79AC + 1.36BC - 63.56A^2 - 52.38B^2 + 52.30C^2 \quad (3)$$

The equation shows that factors A and C have negative effects on the tensile strength of patches. However, the coefficient value of -93.09 for factor C indicates its higher influence on the patch's tensile strength than factor A, having coefficient value of -75.90. The interaction factor (AC) has synergistic effect on decreasing tensile strength due to their positive coefficient value. In addition, the influences of patch formulation variables on the tensile strength can be further explained by using response surface plots based on this model. The values of three factors (A, B and C) were substituted in the polynomial equation to calculate the theoretical value of the response (tensile strength). The theoretical (predicted) values and observed values were mostly in agreement as shown in Table 7.

Table 7. Observed and predicted values of response tensile strength

Runs	Factor 1 A = DSE (g)	Factor 2 B = HPMC E4M (%)	Factor 3 C = PEG400 (g)	Response Tensile strength (g/cm^2)	Predicted Tensile strength (g/cm^2)
1	0.2	33.33	20	829.34±38.35	858.44
2	0.6	33.33	20	447.26±35.84	530.47
3	0.2	66.67	20	902.89±101.05	779.32
4	0.6	66.67	20	377.23±40.13	337.55
5	0.2	33.33	40	422.65±58.07	437.97
6	0.6	33.33	40	470.95±41.40	573.14
7	0.2	66.67	40	469.85±60.75	361.28
8	0.6	66.67	40	439.11±19.03	385.65
9	0.064	50	30	443.08±6.29	544.37
10	0.736	50	30	355.43±19.97	289.34
11	0.4	22	30	709.84±58.90	561.63
12	0.4	78	30	151.88±46.58	335.15
13	0.4	50	13.18	880.42±166.31	900.25
14	0.4	50	46.82	573.49±61.34	587.46
15	0.4	50	30	562.74±57.21	596.24
16	0.4	50	30	635.65±9.02	596.24



(a) (b)

Figure. 2 Response surface plots showing the effects on the tensile strength of three formulation factors (a) between DSE concentration (A) and amount of plasticizer (C); (b) between DSE concentration (A) and polymer proportion (B)

The 3D response surface plots were shown in Figure 2. The effect of DSE concentration (A) and amount of plasticizer (C) at mid-point of factor B on the response tensile strength was exhibited in Figure 2a. Both factor A and C adversely affected the tensile strength. The results were found that the increase in DSE concentration and amount of plasticizer resulted in a substantial decrease in tensile strength. The effect of DSE concentration (A) and polymer ratio (B) at mid-point of factor C on the response was also shown in Figure 2b and implied that both factor A and B had negative effect on tensile strength. It could be suggested that the DSE concentration, amount of plasticizer and polymer proportion had the optimal levels to use in the patch formulation.

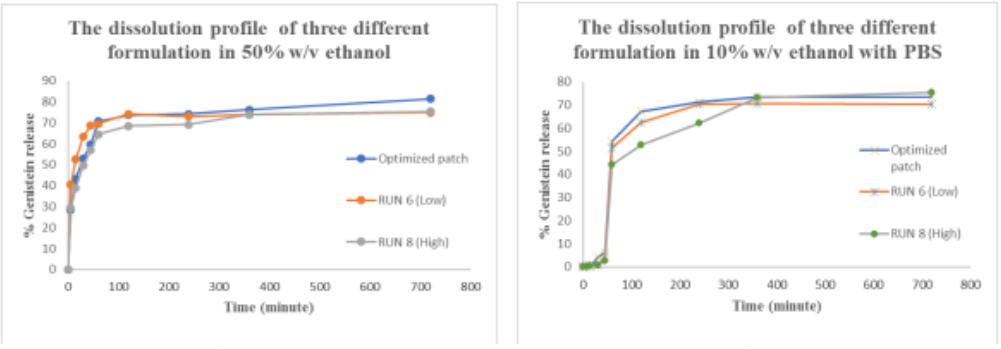
Optimization of DSE patch formulation

Derringer's desirability function methodology was used for optimization of DSE patch formulation. Table 4 shows the mechanical properties of the DSE patch. Higher values of tensile strength (TS) are desirable for mechanical resistance. The elongation at break (EB) is a measure of the ductility of a patch which defines the ability of a patch to deform before failure occurs whereas the elastic modulus (EM) is a key indicator of the stiffness of the patch. The tough patch should have low EM, moderate TS and high EB. The TS was selected as the key parameter to be optimized. The desirability was designed to have high DSE content and varied the polymer proportion and amount of plasticizer to obtain the moderate TS. The optimum compositions were found to be 0.6 g for DSE, 48:52 % for proportion of HPMC (E4M:E15LV) and 40 % for PEG 400 which predicted that value of TS would be 550 g/cm^2 with desirability value of 0.999. The experiment was performed to prepare the DSE patch as according to the optimized composition for validation purpose. The average optimized patches (n=5) values of 571.28 ±37.61 g/cm^2 for TS and 83.5 % for EB and 6.71 MPa for EM were obtained. The result of TS was closely related with the data predicted from the above numerical optimization technique using desirability methodology and EB value was high whereas EM value was low, indicating that the optimized patch had the required mechanical property. The result was also in accordance with the study of Rabinarayan and Padilam (2016) who use the response surface methodology to investigate the mechanical properties of the transdermal patch of simvastatin. It was reported that high drug

concentration (2%), medium polymer ratio (50%) and high amount of plasticizer (40%) resulted in mechanical properties of high EB (85.33%) and low EM (20.39 MPa). The optimized composition was used to prepare DSE patch for the *in vitro* drug release study.

In vitro drug release study

The *in vitro* drug release of DSE patches were carried out using 10% w/v ethanol in phosphate buffer saline and 50% w/v ethanol. The dissolution profiles of three different formulations, i.e., optimized patch, run 6 (low polymer proportion), run 8 (high polymer proportion) in different media are shown in Figure 3. The results showed that maximum concentrations of drug released were obtained at the 1st hour and 12th hour for 50% w/v ethanol and 10% w/v ethanol in phosphate buffer saline, respectively. From Figure 3a, it was found that all patches released genistein rapidly in 50% w/v ethanol medium. The percent release at the 60th minute of optimized, low and high polymer proportion patch were 70.76 %, 69.52 % and 64.44 % respectively due to the high solubility of genistein in this medium. From Figure 3b, it was found that all patches released genistein more slowly in 10% w/v ethanol in phosphate buffer saline. The percent release at the 240th minute of optimized, low and high polymer proportion patch were 67.28, 70.28 and 62.17 % respectively. In both cases, the release of drugs from the patch is governed by the diffusion of solute which dissolved initially by the medium within the matrix phase and continuously decreasing when the concentration gradient is smaller. Therefore, the higher drug solubility could facilitate higher drug release from the patches. To explain the drug release kinetics of genistein from the topical patchs, the mean dissolution curves included the fraction between 10-90% of drug release was calculated by linear regression analysis of various mathematical models but neither one of the models could provide good coefficients of determination except Higuchi's (R^2 0.962, 0.534 and 0.899) which gave R^2 close to 1 as tabulated in Table 8 for the test run in receptor fluid of 10% w/v ethanol in PBS. Hence, the release mechanism of genistein from the topical patches is governed by diffusion process. The R^2 of the DSE patch with low polymer proportion is lower than that with high polymer proportion. It might be explained that the patches composed of the more proportion of hydrophilic polymer, leading to high erosion of the patches and thereby facilitating rapid drug release from the patches by dissolution. According to this fact, the release rates were found to decrease with increase in polymer proportion of HPMC E4M and E15LV. The results of the swelling index supported the drug release in the corresponding manner.



(a) (b)

Figure 3. The *in vitro* drug release profile of 3 different patch formulations in 50% w/v ethanol (a) and 10% w/v ethanol with PBS (b)

Table 8. Coefficient of determination from linear regression analyses of various mathematical models

DSE patches	Zero order $Q_t = K_0t + Q_0$	First order $LogQ_t = K_1t + LogQ_0$	Higuchi $Q_t = K_Ht^{1/2}$
Receptor fluid; 50% w/v ethanol			
Optimized	0.665	0.393	0.639
Run 6	0.831	0.464	0.452
Run 8	0.815	0.453	0.641
Receptor fluid; 10% w/v ethanol in PBS			
Optimized	0.674	0.735	0.962
Run 6	0.468	0.490	0.534
Run 8	0.774	0.822	0.899

CONCLUSION

Formulation of *Derris scandens* extracts (DSE) for topical patches were developed and evaluated for the physical properties and drug release. The optimum compositions were found to be 0.6 g for DSE, 48:52% for proportion of HPMC (E4M:E15LV) and 40 % for PEG, resulting in the tensile strength of 571.28 g/cm^2 and high elongation at break of 83.5 %. It indicated that the patch was tough and inherited a good mechanical property. The release rates were found to decrease with increase in polymer proportion of HPMC E4M and E15LV. The drug release kinetics of Higuchi models (R^2 0.962, 0.534 and 0.899) fit to DSE release data better than the others. However, the optimized patch has the potential as an alternative to oral preparation and could be developed further for the study of *in vitro* skin permeation of DSE.

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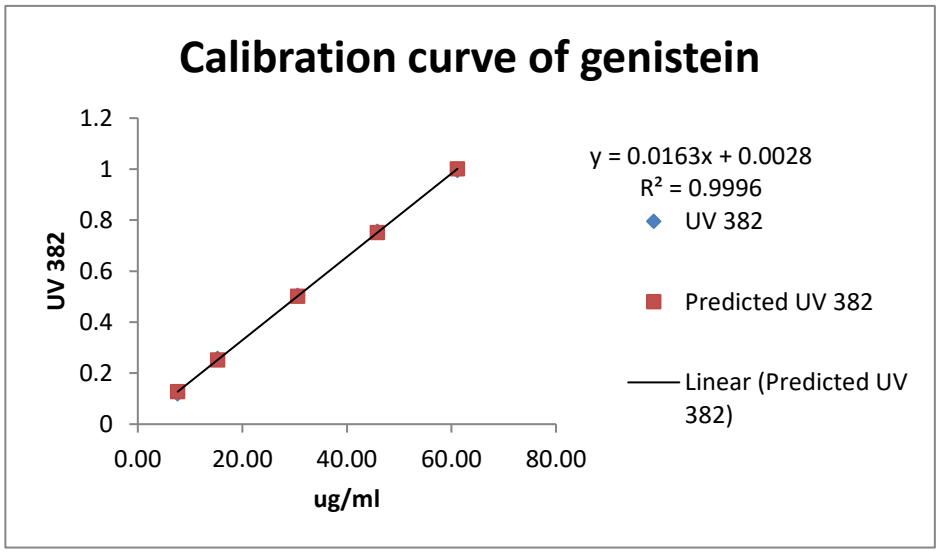
Appendix B Raw data

The calibration of genistein standard

Samples genistein standard	Concentration (µg/ml)	Absorbance @ 382 nm
1	7.6428	0.119
2	15.2856	0.258
3	30.5712	0.506
4	45.8568	0.757
5	61.1424	0.995



Raw data of dissolution test

Time (minute)	Dissolution test of HPMC patches containing *Derris scandens* extract (µg/ml) in 10% w/v ethanol in phosphate buffer saline		
	Optimized patch	**Run 6**	**Run 8**
0	0.00	0.00	0.00
5	0.01	0.01	0.15
15	0.05	0.26	0.01
30	1.40	0.26	0.20
45	2.16	1.55	0.93
60	15.25±0.26	17.89±0.21	15.35±0.55
120	18.93±0.24	21.79±0.11	18.36±0.91
240	23.43±0.36	24.47±0.15	21.65±0.62
360	24.86±0.15	24.59±0.45	25.45±0.45
720	25.64±0.45	24.51±0.36	26.25±0.87

Raw data of swelling test

Runs	Factor 1 A = DSE (g)	Factor 2 B = HPMC E4M (%)	Factor 3 C = PEG400 (g)	% Swelling index at times (minute)			
				1	3	5	10
1	0.2	33.33	20	91.37±15.23	135.33±22.32	**186.67±30.22**	96.84±14.65
2	0.6	33.33	20	77.83±10.11	130.95±25.65	**163.46±24.95**	80.09±15.33
3	0.2	66.67	20	68.81±12.28	91.19±14.88	**109.56±20.33**	83.78±14.77
4	0.6	66.67	20	61.01±13.54	74.57±10.69	**117.25±21.36**	51.43±11.14
5	0.2	33.33	40	115.24±18.22	**138.96±22.95**	137.89±22.54	59.69±12.56
6	0.6	33.33	40	89.33±15.49	**128.97±20.11**	106.67±15.98	1.69±0.56
7	0.2	66.67	40	17.65±4.95	88.57±15.12	**115.51±16.54**	14.21±2.44
8	0.6	66.67	40	59.39±12.56	83.91±14.78	**113.63±11.25**	0.00
9	0.064	50	30	69.63±14.46	100.41±17.96	**316.92±57.99**	22.80±6.54
10	0.736	50	30	201.05±49.65	**233.30±55.41**	37.39±9.65	35.35±10.22
11	0.4	22	30	**172.71±36.54**	63.80±13.33	43.00±10.23	0.00
12	0.4	78	30	**108.52±20.56**	85.78±14.69	0.00	5.62±1.19
13	0.4	50	13.18	**40.29±10.66**	12.57±4.44	10.25±3.66	0.00
14	0.4	50	46.82	**42.79±11.59**	43.75±10.24	34.48±9.87	0.00
15	0.4	50	30	**62.80±12.34**	47.25±10.11	0.00	0.00
16	0.4	50	30	**47.75±9.79**	12.75±3.65	0.00	0.00

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