



Enhancing Effect of Natural Oils as Permeation Enhancer for Transdermal Delivery of Diltiazem Hydrochloride Through Wistar Rat Skin.

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ABSTRACT

The aim of the present investigation is to formulate and evaluate the biopharmaceutical behaviors of the matrix patch containing Diltiazem hydrochloride (DH) with an attempt of use of natural oils as permeation enhancers for transdermal applications. Transdermal patch prepared using 3² full factorial designs by solvent evaporation technique by incorporating propylene glycol as plasticizer and ethanol as solvent. Fourier transform infrared spectroscopy (FTIR) had employed to study drug and excipients incompatibility that showed the absence of any type of chemical interaction. Prepared patches evaluated for physico chemical parameters such as tensile strength, percent elongation, folding endurance, flatness, thickness, hardness, weight variation, percentage moisture loss and uptake, *ex-vivo* permeation study, *in-vivo* skin irritation study and stability study. The physicochemical and *ex-vivo* permeation studies indicated that Batch A2 containing HPMC K15M and psyllium in the ratio of 2:1 was better compare to all nine batches of factorial designs. Tensile strength, percent elongation and folding endurance found to be 4.48 kg/mm², 21.84±0.335 and 384±3.21 respectively, which showed good mechanical property of prepared patch. Penetration enhancing capacity of natural oils (pumpkin seed oil, jojoba oil, tea tree oil, cumin oil, and linseed oil) was determined by performing *ex-vivo* study using wistar-rat skin. A maximum steady-state skin permeation flux of 239 µg/cm²/h achieved in the batch A2 which containing 20%w/w of pumpkin seed oil. The results of highest flux revealed that compare to all essential oils pumpkin seed oil enhance the permeation of drug through the skin. The release kinetics indicates that the release pattern was diffusion controlled and it follows Higuchi and zero order kinetics. The skin irritation study performed on wistar-rats revealed that the patch was not irritating the skin after 24hours. Stability study performed according to ICH guideline and it showed that transdermal patch of DH containing natural oil was stable at accelerated conditions for six months. This research suggested that transdermal applications of DH improved patient compliance and very good alternative to oral administration of DH for the treatment of hypertension.

Keywords: Natural oil, Permeation enhancer, Hypertension, Diltiazem HCl (DH), Propylene glycol, *ex-vivo* study, wistar rat skin.

INTRODUCTION

Transdermal drug delivery offer many advantages such as it avoids hepatic first pass metabolism and maintaining constant blood levels for longer period. Therefore, dosing frequency decreases which decreases gastrointestinal irritation and improved patient compliance for long-term treatment. Easy removal of patch at any time from the target site will terminate the treatment so less chances of over or under dosing. However, transport of compounds via skin considered as a challenge, which required the passage of drug into and across the skin.¹⁻⁵

The success of a transdermal matrix patch depends on the ability of the drug to penetrate into the skin in sufficient quantities to maintain therapeutic levels. In the formulation of transdermal delivery system, many chemical enhancers are widely used that elevate the structure of stratum corneum and reduce barrier properties of skin. Literature review suggested that natural oils like essential oils and vegetable oils are most widely used as permeation enhancers because they are non-toxic, less allergic, easily available and compatible with drug and excipients. Natural oils for a longer period used in cosmetics, medicine so safe to use, also they

metabolized in the body also showed higher permeability because they containing unsaturated fatty acids which alleviating the lipid and protein structure of stratum corneum and increases penetration of drug through the skin.⁶⁻¹⁵

Selective drug candidate, Diltiazem HCl (DH) is a calcium channel blocker used in hypertension, angina pectoris and several other cardio-vascular disorders. The usual dose of drug ranges from 40-120 mg per day. The drug has short plasma half-life 3 to 4.5 hrs, so it is necessary to repeat the dose twice in the day to maintain its therapeutic level.

Bioavailability of this drug is only about 40% due to extensive hepatic metabolism. Several attempts made to resolve the disadvantages of oral administration, transdermal drug delivery is one of the best alternative.

The biological properties of Diltiazem HCl (DH) such as extensive hepatic metabolism, low therapeutic dose, its suitability for patients requiring long-term treatment and repetitive dosing make Diltiazem HCl (DH) a suitable candidate for transdermal patch also the physico-chemical properties such as, Log P value 2.8 at 20-25 °C, low molecular weight 450.98 g/mol and melting point



207-212°C indicates a good probability of Diltiazem HCl (DH) to cross the lipophilic skin barrier.¹⁶⁻¹⁹

A transdermal diffusional matrix type design selected for this investigation due to its ease of manufacturing and optimization of formulation variables carried out by 3² full factorial design using Design Expert software 9.0.2. All nine batches evaluated for physico-chemical characteristics, *ex-vivo* drug permeation study and skin irritation study. The final optimized batch subjected to *in-vivo* skin irritation study and stability study for six months.²⁰⁻²²

MATERIALS AND METHODS

Materials

Diltiazem HCl (DH) was gift sample from Centurion laboratories, Vadodara, India. Psyllium purchased from Shiv psyllium Industries, North Gujarat, India. Natural oils (Jojoba oil, Cumin oil, Linseed oil, tea tree oil and pumpkin seed oil) purchased from Hamdard Laboratories, Ghaziabad, India, Hydroxyl Propyl methylcellulose K-15M, propylene glycol and tween-80 supplied by S.D. Fine Chemicals Ltd., Mumbai, India. All remaining chemicals and solvents were reagent grade. Double distilled water used throughout the study.

Animals

Animals used in this work supplied by Zydus Research center, (Village Moraiya, Near Nova Petrochem, Ahmedabad, Gujarat) including wistar rats (180-200g, 6-8 week old). The animal experiments skin-irritation study and *ex-vivo* permeation study using wistar-rat skin performed in accordance with the guidelines given by Animal Ethical Committee, CPCSEA. Protocol No. is 984/06/2014-2-06.

Investigation of physicochemical compatibility between drug and polymers

FTIR spectroscopy used as analytical tool to find out compatibility between Diltiazem HCl, HPMC K15M, psyllium, tea tree oil and propylene glycol. FTIR spectra of pure drug and final optimized formulation carried out using KBr disc method.²³

Solubility measurement

Solubility study performed using saturation solubility method in which saturated solution of drug was prepared and transferred in a glass vial. The solution kept at 37°C for 24 hours in a mechanical shaker to achieve the equilibrium. The concentration of drug in the filtrate was determined using UV spectrophotometer at 237 nm after 24 hours.²⁴

Partition coefficient (kp)

The partition coefficient of the drug was determined by shaking equal volumes of oil and aqueous phase in a separating funnel. A drug solution of 1 mg/ml was prepared in distilled water and 50 ml of this solution taken in a separating funnel, shaken with an equal

volume of octanol for 10 minutes, and allowed to stand for 24 hours with intermittent shaking. Analysis of drug present in both the phase done by UV spectrophotometer. For finding the partition coefficient, ratio of drug present in aqueous phase and in organic phase was calculated.²⁵⁻²⁶

Preliminary studies for improvement of skin permeability using enhancers

Now a day's very less numbers of transdermal drug delivery systems are available. The less availability is due to ionic characteristic of drugs and large molecular weight of drugs, which decreases the permeability of drugs through the skin. Linseed oil, Pumpkin seed oil, Jojoba oil and tea-tree oil evaluated for permeation enhancement activity across wistar rat skin. A 5 ml solution of drug (0.1mg/ml) was prepared in pH 6.8 buffers and it incorporated with 10% v/v concentration of each permeation enhancer includes Linseed oil (LO), Pumpkin seed oil (PSO), Jojoba oil (JO), and tea-tree oil (TTO). For this, preliminary batches V1 to V5 were prepared. The permeation study performed as described in method of *ex-vivo* permeation study.

Ex-vivo drug permeability study using wistar rat skin

Preparation of wistar rat skin³⁴⁻³⁶

A full thickness of skin cut from dorsal site of dead rat and washed with water. The fatty tissue layer and hairs removed using scissor. The skin kept in normal saline solution in refrigerator at 4 to 5 °C, until skin used for diffusion study. Prior to use, the skin was allowed to equilibrate with room temperature. Then skin mounted between donor and receptor compartment of cell. The skin clamped in such a way that the dermal side was being in contact with receptor medium.

Method of permeation study

The receptor compartment filled with 20 ml of pH 6.8 buffer. The donor compartment contained 5 ml solution of DH in pH 6.8 buffer having concentration of 0.1 mg/ml. The temperature of diffusion medium maintained at 32 ± 2°C. This whole assembly kept on a magnetic stirrer and solution in the receiver compartment constantly and continuously stirred using magnetic bead. Samples were withdrawn (2 ml, each time) at different time interval and replaced with equal amount of pH 6.8 buffer. Sample analyzed at 237nm after suitable dilution using UV spectrophotometer. Amount of drug permeated per square centimeter at each time interval was calculated and plotted against time. The *Ex-vivo* release data subjected to various kinetic equations to find out release mechanism and order of drug release. Flux was determined directly from the slope of the curve that constructed by the steady state values of the cumulative amount of drug permeated (mg/cm²) vs time.

Permeation coefficients (cm/hr) were deduced by the dividing the flux by initial drug loading (mg/cm²). Lag time calculated from back extrapolation. Diffusion coefficient



(D/h^2) and permeability coefficient (K_p) was also calculated for the *ex-vivo* studies using below mentioned equations respectively,

$$D/h^2 = 1/6 \times T_{lag}, J_{ss} = (dq/dt) \cdot 1/A, K_p = J_{ss}/C_s.$$

The regression analysis of steady state data and release rate was calculated. The experiment was performed in triplicate and mean results were recorded.

Enhancement ratio

Enhancement ratio calculates using below mentioned equation to evaluate the effect of permeation enhancer on diffusion and permeation of drug across the skin.

Enhancement ratio = K_p of drug with penetration enhancer / K_p of drug without penetration enhancer.

Formulation development

Dose calculation

Dose for the transdermal patch calculated on the bases of targeted flux. Targeted flux i.e. amount of drug permeated per centimeter square area which required to maintain therapeutic level, targeted flux of Diltiazem HCl (DH) is **247 $\mu\text{g}/\text{cm}^2/\text{hr}$** (calculated from equation: targeted flux $J_{ss} = C_{ss} \times Cl_t \times BW/A$, where 5 $\mu\text{g}/\text{l}$ and 10 ml/min/kg are C_{ss} and Cl_t respectively for Diltiazem HCl). Oral dose of Diltiazem HCl is 20 mg or 40 mg once daily and bioavailability is 40%. Therefore, orally available dose to maintain plasma concentration is only 8 mg. To surplus the loss of drug in different layers of skin and for getting required flux here, double dose is required. Therefore, in

present study dose of DH for the preparation of transdermal patch, having 4- cm^2 was 16mg (146 mg for total 6.8cm petriplate) and it gives flux 330 $\mu\text{g}/\text{hr}/\text{cm}^2$, which is very nearer to required flux.

Preparation of transdermal matrix patches

Transdermal matrix patch prepared by solvent evaporation method using 3^2 full factorial designs from Design Expert software 9.02. The polymer fixed weight ratio (X1) & concentration of pumpkin seed oil (X2) selected as two independent variables, both the variables evaluated at three different levels. Tensile strength (Y1), cumulative drug release in 1hr (Y2) & cumulative drug release in 16 hrs (Y3) selected as dependent variables. Psyllium and HPMC K15M accurately weighed and dispersed in 12ml water and kept aside for 2 hrs, in another beaker drug dissolved in 8 ml ethanol then slowly with continuous stirring, drug solution added into above polymeric solution and stirred the mixture until it become clear. Into this clear solution, 20 % (w/w of polymer dry wt) propylene glycol and 30 % (w/w of polymer dry wt) pumpkin seed oil added as a plasticizer and permeation enhancer respectively. The resulted clear solution was casted on the previously lubricated petri plate and dried at room temperature in dark condition for 24 hours. Inverted funnel placed over the petri plate for uniform evaporation of the solvent¹⁹⁻²¹. After complete drying the dried films removed and stored in desiccators for further studies. Formulation compositions of A1 to A9 represented in Table 1.

Table 1: Formulation of 3^2 Full Factorial Design Batches A1-A9

Formulation code	A1	A2	A3	A4	A5	A6	A7	A8	A9
Diltiazem-HCL	146	146	146	146	146	146	146	146	146
HPMC K15M(mg)	200	200	200	150	150	150	100	100	100
Psyllium(mg)	100	100	100	150	150	150	200	200	200
Pumpkin seed oil (10%w/w of dry polymer weight)	10	20	30	10	20	30	10	20	30
Water(ml)	12	12	12	12	12	12	12	12	12
Ethanol(ml)	8	8	8	8	8	8	8	8	8
Propylene glycol(%w/w of dry polymer wt)	20	20	20	20	20	20	20	20	20

Evaluation of transdermal patches³¹⁻³³

Physicochemical characterizations of transdermal patches

Prepared patches inspected for colour, clarity and smoothness. The thicknesses of the patches measured at five different points by using screw gauge and average thickness was determined, ten patches from each batch weighed individually and the average weight determined.

Folding endurance measured manually to check the efficiency of the plasticizer and the strength of the patch. It was determined by repeatedly folding the patch at the same place until it broken. The number of times the patch

can folded at the same place without breaking/cracking gave the value of folding endurance.

Percentage moisture absorption and loss

The patch weighed accurately and placed in the desiccators containing 100 ml of saturated solution of potassium chloride, which corresponds to 85% RH. After 3 days, the patches removed from the desiccators' and weighed. The percentage of moisture uptake calculated as the difference between final and initial weight with respect to initial weight.

For moisture loss patch weighed accurately and kept in a desiccators containing anhydrous calcium chloride, which



corresponds to 30% RH. After 3 days, the patches removed from the desiccators' and weighed. The percentage of moisture loss calculated as the difference between initial and final weight with respect to initial weight.

Tensile strength

The tensile strength of the patch was measured using tensile strength instrument locally assembled. One end of the patch fixed between the two iron screens to give support to the patch and another end connected to the paper holder in which hook inserted. A thread tied to this hook, passed over the pulley and a small pan attached to the other end to hold the weight. A small pointer attached to the thread, which travels over the scale affixed on the base plate.

To determine tensile strength, the patch pulled by means of a pulley system. Weights gradually added to the pan to increase the pulling force until the patch was broken. The weights required to break the patch considered as a tensile strength and it was calculated by given equation

$$\text{Tensile strength} = \frac{F}{a \times b} \left(1 + \frac{L}{I}\right)$$

Where, **F** is force required to break; **a**, **b**, and **L** are width, thickness and length of patch respectively and **I** is elongation of patch at break point.

Drug content uniformity of patches

This study performed to checked uniform and complete dispersion of drug in the formulation. In this test, three patches of the same batch individually dissolved in 50 ml of pH 6.8 buffer and 50 ml of ethanol. Aliquots were prepared with suitable dilution and analyzed for drug content at 237 nm against pH 6.8 buffer as blank using UV-spectrophotometer.

Ex-vivo skin permeation studies of transdermal patches³⁷⁻⁴¹

Ex-vivo skin permeation study for the prepared transdermal matrix patch was performed same as the method given for the drug permeability study using wistar rat skin but instead of drug solution, prepared diffusional matrix patch was attached on the wistar rat skin.

In-vivo skin irritation study⁴²

The study performed on wistar rats to determine irritancy after single application of prepared transdermal patch. Accurately cut 2×2 cm² size patch applied on the clean hairless backside skin of rat and removed after 24 hours. The exposed skin graded for formation of edema and erythema and intensity of irritation. The rats were divided into 2 groups of 3 rats in each group (n=6), one group as control and another group as test (prepared matrix patch).

Prior permission taken from the animal ethical committee for this study.

Stability study⁴³⁻⁴⁷

The final optimized batch A2 subjected to stability study to evaluate any physicochemical change in the performance and appearance when exposed to accelerated conditions of environment during storage, handling, transport and use. The patch sealed in the aluminum foil and kept in stability chamber at 40°C and 75% RH for a period of six months. The patch sample with an area of 2×2 cm² cut and it analyzed for physicochemical parameters at particular time intervals.

RESULTS AND DISCUSSION

Evaluation of preformulation study

FTIR study carried out to find out any incompatibility between drug & polymers used with analytical method of drug estimation. IR spectra of pure drug Diltiazem HCl showed sharp peak at 3342.14 cm⁻¹, 2937.30 cm⁻¹, 1744.27cm⁻¹ & 1680.01cm⁻¹ that confirmed the presence of N-H, C-H, C=C and C=O stretching, respectively. All the major peaks remain present in the IR spectra of drug with polymers; it indicates there was no interaction. Results of solubility study, melting point and partition coefficient indicates that the observed values exactly similar with original values. FTIR spectra shown in Figure 1 (a and b).

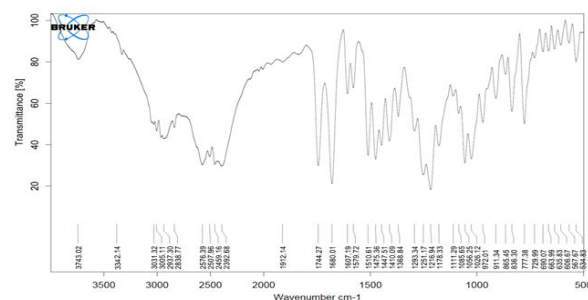


Figure 1(a): Infrared Spectra of Diltiazem Hydrochloride

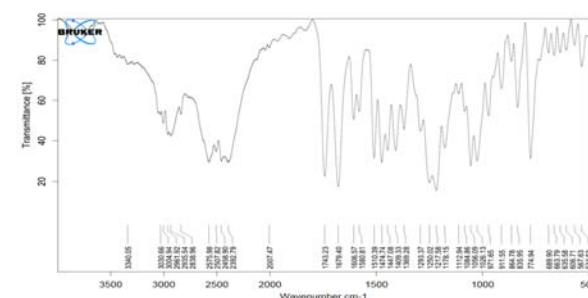


Figure 1(b): Infrared Spectra of Final Formulation - A2

Ex-vivo permeation data of pure drug with permeation enhancers using wistar rat skin

All calculated results of flux, permeability coefficient, diffusion coefficient and enhancement ratio given in Table 2.

The results of flux value, permeability coefficient, diffusion coefficient and enhancement ratio suggested that the enhancement ability of pumpkin seed oil was highest compared to linseed oil, jojoba oil and tea-tree oil.



Table 2: Permeability Coefficient, Flux and Enhancement Ratio of Diltiazem Hydrochloride Pure Drug with Permeation Enhancers

Batch. No	Name	Transdermal flux ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Permeability coefficient (cm/hr)	Enhancement ratio
V1	DH+PSO	208.87 \pm 0.035	0.0097 \pm 0.062	3.23 \pm 0.03
V2	DH+LO	83.39 \pm 0.032	0.0041 \pm 0.057	1.4 \pm 0.06
V3	DH+JO	79.89 \pm 0.045	0.0039 \pm 0.057	1.3 \pm 0.06
V4	DH+TTO	73.67 \pm 0.021	0.0036 \pm 0.047	1.2 \pm 0.02
V5	DH	55.93 \pm 0.010	0.0030 \pm 0.042	1.0 \pm 0.03

Physicochemical evaluations of transdermal patches (Batches A1 to A9)

Transparent, flat, flexible and uniform transdermal diffusional matrix patch obtained using mixture of natural polymer psyllium and synthetic polymer HPMC K15M. Propylene glycol 20%w/w of dry weight of polymer added as plasticizer for a good flexibility and elasticity.

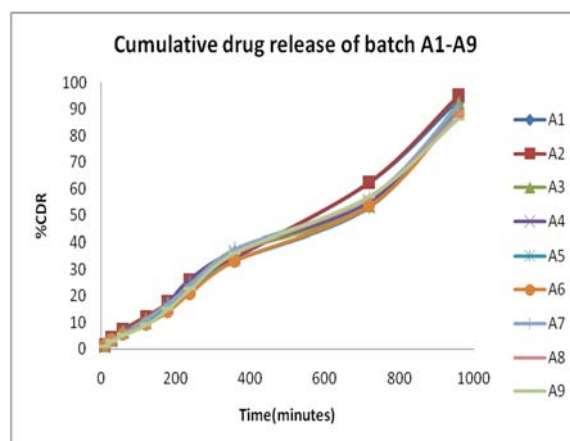
From the literature review it found that natural oils as a permeation enhancer gave good elasticity to the patch. The average weight of batches A1 to A9 range between 462 \pm 1.732 to 574 \pm 2.31mg, which indicates that all the solid excipients uniformly dispersed into the liquid and all batches were relatively in similar weights.

The thickness of the patches measured by micrometer screw gauge, results found in between 0.84 \pm 0.04 to 0.89 \pm 0.13 mm. The results revealed that solution was uniformly casted on a previously lubricated petriplate and solvent uniformly evaporated from the petriplate.

The drug content of the entire batches lie between 96 to 98 %, these results revealed that method select for the preparation of matrix patch was suitable and reproducible. The results of flatness study showed that all the batches have same length before & after cuts. Therefore, nearer to 100% flatness obtained and it indicates that all patch had a smooth surface. Tensile strength found in between 4.03 \pm 0.126 to 6.48 \pm 0.129gm/cm², which revealed that the patch had sufficient mechanical strength to withstand during handling, transportation and administration. Same way results of folding endurance study revealed that the patch would not break & maintain their integrity with general skin folding applied. The moisture uptake in the humid condition found to vary from 1.48 \pm 0.08 to 1.89 \pm 0.06 percentage. The low moisture uptake also preserves the patches from microbial attack and avoids bulkiness of the patches. The moisture loss in the dry condition found to vary from 1.68 \pm 0.07 to 1.85 \pm 0.04 percentage.

The variation in the percentage moisture loss depends on varied concentration of hydrophilic polymers in formulations. This indicates that little moisture content would make the patches more stable and prevents drying. These above all values revealed that the patches found to be stable and elastic.

Ex-vivo permeation data of transdermal matrix patches

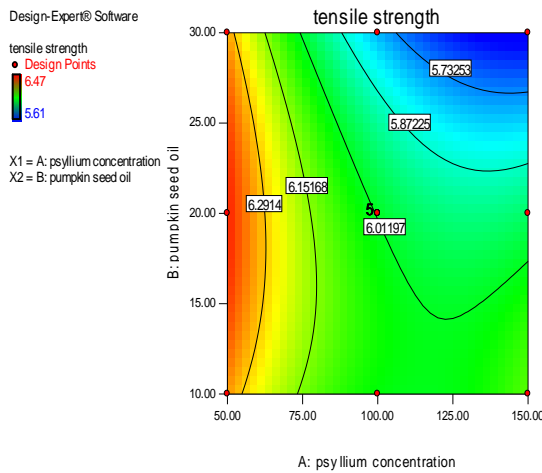
**Figure 2:** Cumulative Drug release profile of A1-A9

Ex-vivo permeation studies are prediction of an in-vivo performance of a drug. Permeation studies carried out with 2 \times 2 cm² patch having 16 mg of drug and various concentrations permeation enhancers using wistar-rat skin. The results of ex-vivo permeation study of batches A1 to A9 showed that flux was significantly enhance with the addition of permeation enhancer than the formulation containing no permeation enhancer. This enhancement depends on the mechanism of permeation of drug through the skin. This results shows that as amount of oil as permeation enhancer increase they enhance permeation of drug. This may be occurring due to the presence of unsaturated fatty acids in the oils. This result of permeation studies were found to be different when different oils were use but among all oils pumpkin seed oil showed maximum enhancement so, it was selected as one independent factor for the formulation of 3² full factorial designs, batches A1 to A9. The results of these nine batches showed that all three concentrations of pumpkin seed oil enhance the permeation of Diltiazem HCl through excised wistar rat skin than the control batch V5 prepare without permeation enhancer, but the formulation batch A2 containing 20%w/w (dry weight of polymers) of pumpkin seed oil exhibited the highest % cumulative amount of drug permeate % in 16 hrs. The results revealed that enhancement ability of 20%w/w of pumpkin seed oil exhibited highest flux 239.09 \pm 0.14 $\mu\text{g}/\text{cm}^2/\text{hr}$ and 89.93% drug release in 16 hrs because higher content of fatty acids of pumpkinseed oil. This acids increase the permeation through a mechanism involving elevating stratum corneum structure by modifying lipids and proteins cell. Cumulative drug release plot of *ex-vivo* studies represented in Figure 2. Data of *ex vivo* release was fitted into different kinetic equations to explain the release kinetics of Diltiazem HCL.

The cumulative amount of drug released from the patches, when plotted against time, the release profiles of drug seemed to follow zero order and drug release mechanism was diffusion controlled so it followed Higuchi model. The correlation coefficient of R² values for batch A2 was r² = 0.9976 for zero order and r² = 0.9733 for

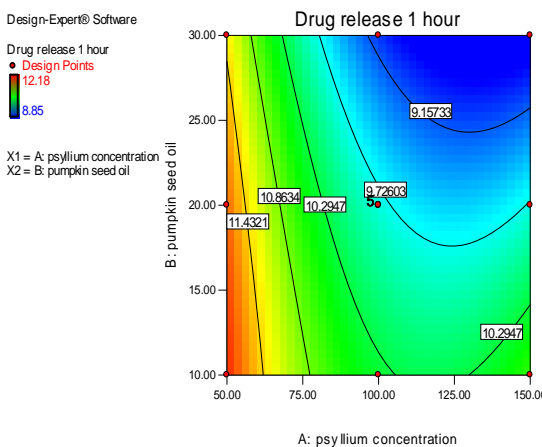
higuchi model. Response surface plots such as 3D & contour plots of dependent variables indicates that as concentration of pumpkin seed oil increases drug release increases in 1hr and in 16 hrs.

Regression analysis of the optimization of formulation



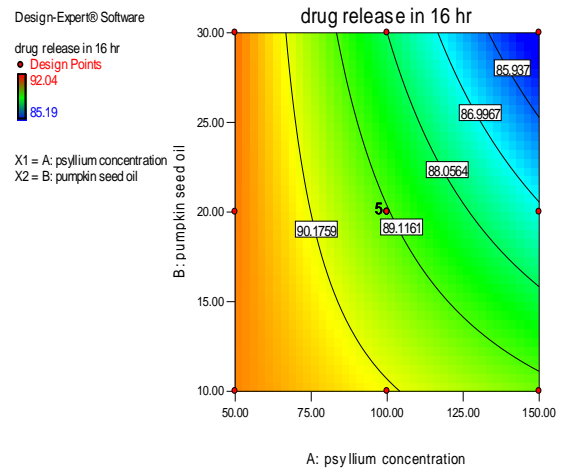
Tensile strength: (Y1) = +5.98 - 0.13X1 - 0.24X2 - 0.12X1X2 - 0.085X1² + 0.20X2²

Figure 3: (a) counter plot of effect of psyllium and pumpkin seed oil on tensile strength



Percentage Drug release in 1 hr: (Y2) = +9.75 - 0.65X1 - 1.01X2 - 0.33X1X2 - 0.065X1² + 0.99X2²

Figure 4: a) counter plot of effect of psyllium and pumpkin seed oil on percentage drug release in 1 hr



Percentage Drug release: (Y3) = +88.75 - 1.10X1 - 2.03X2 - 1.15X1X2 - 0.47X1² + 1.03X2²

Figure 5: a) counter plot of effect of psyllium and pumpkin seed oil on percentage drug release in 16 hr

Based on the values of dependent variables polynomial equations generated, listed with contour surface plots shown in Figure 3, 4, and 5. An obtained plot indicates that both the formulation variables X1 and X2 played an important role in a controlled release of DH from the transdermal matrix patches. Obtained results revealed that the selected model was significant and drug release from the matrix patch in a controlled manner for period of 16 hrs.

In-vivo Skin irritation studies

Results of skin irritation study revealed that neither placebo patch nor prepared patch A2 containing Diltiazem HCL (DH) showed negligible erythema or edema on rat skin throughout the period of 16 hrs indicates that patches were compatible with the skin.

Stability studies

The optimized batch A2 exposed for stability studies as per ICH guidelines. The patches were wrapped in aluminium foil and stored at 40±2°C and 75± 5% RH for 6 months gave satisfactory results compared to accelerated condition. The sample withdrawn at 0, 15, 30, 90 and 180 days time interval. The samples analyzed for the drug content, folding endurance and drug releasing study, results shown in Table 3.

Table 3: Results of Stability data for A2 batch

Stability condition	Sampling time	Folding endurance	Drug content uniformity (%)	Visual appearance
Room Storage (40±2°C and 75±5% RH)	Initial (0 th day)	301±1.52	98.78±0.65	Clear homogeneous appearance
	After 15 days	298±1.06	98.34±0.21	Clear homogeneous appearance
	After 30 days	304±2.64	98.45±0.65	Clear homogeneous appearance
	After 90 days	307±2.98	98.77±0.65	Clear homogeneous appearance
	After 180 days	306±3.65	98.11±0.12	Clear homogeneous appearance

CONCLUSION

In the present study the transdermal patch of Diltiazem HCl (DH) prepared by solvent evaporation method showed acceptable physico-chemical and biological properties such as folding endurance, tensile strength, thickness, % elongation, % moisture loss, % moisture uptake, drug content, *in-vivo* skin irritation study and satisfactory *ex-vivo* controlled drug release after 16 hours. The prepared patches were transparent with smooth surface without having any drug-excipients interactions. The regression analysis of the results leads to equation that describes adequately the influence of the selected variables concentration of pumpkin seed oil on the responses under study. The higher drug release, folding endurance and the well observed mechanical properties of film could be helpful for the treatment of hypertension with improved patient compliance and drug absorption properties.

Moreover, the use of natural oils for pharmaceutical applications is attractive because they are economical, biocompatible, readily available, non-toxic, capable of chemical modifications and potentially biodegradable. Hence, by fabricating transdermal matrix patch of Diltiazem Hydrochloride one of the first line antihypertensive drugs it would be possible to control the release of drug, decrease-dosing frequency and improve patient compliance in the treatment of hypertension.

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