ORIGINAL ARTICLE

A Design of Experiment-based Approach for the Formulation Development and Evaluation of Repaglinide Transdermal Patch Using Natural Polymers

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Abstract

Aim: The main aim of this study was to Formulation and Characterization of Repaglinide Transdermal Patch Using Natural polymers. **Materials and Methods:** The Box–Behnken design was used, which has three levels and three factors to investigate the associate impact of significant attributes on tensile strength, *In vitro* drug release, and *Ex vivo* drug permeation. Fifteen formulations were developed that differed in the polymer ratio (9:1, 8:1, and 7:1). The permeation of repaglinide through hairless goat ear skin through *Ex vivo* was found to increase with the help of permeation enhancer d-limonene. **Results:** As per the experimental data, it is concluded that the R12 is the best formulation in *ex vivo* permeation, in which we have taken 30% propylene glycol as a plasticizer, 3% d-limonene as a permeation enhancer, and this formulation shown 301.825 μg/cm^2 permeation in 24 h. **Conclusion:** After optimizing all formulations, it concluded that the ratio of polymer in 9:1, 3% of a permeation enhancer, and 30% plasticize was the optimum quantity of different excipients used in the optimized formulation. These findings suggest that transdermal repaglinide delivery may have therapeutic promise, with benefits such as reduced dose frequency, better patient compliance, non-invasive properties, enhanced bioavailability, and ease of medication discontinuation.

Key words: Box–behnken design, chitosan, gum copal, repaglinide, transdermal patch

INTRODUCTION

One of the most important ways for innovative drug delivery systems is transdermal. Transdermal drug delivery has various advantages over the conventional drug delivery methods such as oral and injection. Transdermal administration, which generally involves applying a patch to the skin containing pharmacological ingredients, is non-invasive, painless, easy, and can prevent gastrointestinal toxicity and hepatic first-pass metabolism.[1]

Repaglinide is a newer blood glucose-lowering medication from the carbamoyl methyl benzoic acid class. It promotes insulin release from pancreatic beta-cells by closing K^+ ATP channels, and it is quickly absorbed and removed from the body. Repaglinide was created to alleviate the drawbacks of existing anti-diabetic medications. Hypoglycemia, subsequent failure, and cardiovascular adverse effects are among them. Although repaglinide shares some chemical similarities with glibenclamide, a sulphonylurea-type anti-diabetic medication, it differs from other sulphonylureas in both action profile and excretion mechanism. Other sulphonylureas bind to other receptor sites than repaglinide. As a result, it is 3–5 times more effective than glibenclamide, and it does not induce insulin release in the absence of glucose, unlike glibenclamide. Repaglinide is an appealing medicine for diabetic individuals with reduced kidney function, because it is mostly eliminated through bile when taken orally or intravenously.[2]

Repaglinide is an antihyperglycemic drug that belongs to the meglitinide class of drugs. It stimulates insulin

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secretion by attaching to beta-cells in the pancreas. The medicine repaglinide is classified as a BCS Class II drug. In the upper section of the intestine, it is weakly absorbed. With a claimed bioavailability of 56%, it undergoes significant hepatic first-pass metabolism. Repaglinide plasma concentrations fall rapidly after oral administration of 2 mg repaglinide, reaching predose levels in 4–5 h. The cytochrome P450 3A4 enzyme system in the liver converts repaglinide to inactive metabolites. The biliary–fecal pathway is used to remove repaglinide and its metabolites. Approximately 90% of a single orally taken dose is excreted in feces, whereas only 8% is excreted in the urine. The maximum daily dose of repaglinide is indicated to be 16 mg. It has a log P of 3.95 and a 1-h elimination half-life.[3]

It is currently used to treat hyperglycemia in people with non-insulin-dependent diabetes mellitus. This inhibition induces cell membrane depolarization, which opens voltage-dependent calcium channels, resulting in triggering. After oral medication, gastrointestinal disturbances such as nausea, vomiting, acidosis, anorexia, and improved appetite are Repaglinide's most commonly reported side effects. Because these medications are frequently prescribed for a long period, the patient compliance is crucial. For a systemically active drug to reach a target tissue far away from the site of administration on the skin surface, it must have certain physicochemical properties that allow for drug sorption by the stratum corneum, drug penetration through various skin tissues, and drug uptake by the capillary network in the dermal papillary layer.[4]

Penetration enhancers are chemicals that make it easier to absorb penetrant through skin by temporarily lowering the impermeability of skin. These materials should ideally be pharmacologically inert, non-irritating, non-toxic, no allergenic, congenial with the drug and excipients, odorless, colorless, tasteless, and affordable and possess good solvent characteristics. The enhancer should not deplete physiological fluids, electrolytes, or other endogenous elements, and the skin should quickly regain its barrier properties after it is removed.[5]

Chemical penetration enhancers diminish diffusional resistance by reversibly destroying or modifying the physicochemical composition of the stratum corneum to promote skin permeability. Many chemical penetration enhancers irritate the skin, which is one of their drawbacks. It is unsurprising that agents that disrupt ordered lipid structures, cell membranes, and components also disrupt ordered lipid structures, cell membranes, and components. Their clinical utility has been limited due to the toxicity associated with several chemical penetration enhancers. Essential oils, terpenes, and natural polymeric enhancers, classified as Generally Regarded as Safe by the FDA, have all been investigated in recent years.[6]

MATERIALS AND METHODS

Materials

Repaglinide was purchased from Tokyo Chemical Industry Co. Ltd. Japan, chitosan was given to me as a sample as a gift from marine hydrocolloids, Kochi, Gum Copal (GC) was purchased locally, d-limonene was purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, Propylene Glycol procured from Avantor performance material India Limited, Thane, Maharashtra. The rest of the compounds were of analytical quality.

Differential scanning calorimetry analysis (DSC)

Repaglinide, Chitosan, GC, and their solid dispersion were measured using a DSC apparatus (Shimadzu Model No. DSC-60Plus) fitted with a liquid nitrogen sub-ambient accessory. The nitrogen purge gas was used at a 20 ml/min rate to operate the device. Samples (3–6 mg) were weighed in open aluminum pans and scanned at 100°C/min from 30 to 3000°C.[7]

Fourier-transform infrared spectroscopy (FT-IR) analysis

The FT-IR Spectrophotometer was used to analyze the probable chemical interactions between the medication and the polymer using FT-IR spectrum analysis (Perkin Elmer Model No. Spectrum Two Serial no. 105627 FT-IR). Under the pressure of 150 kg/cm^2, various materials were mixed with KBr and crumpled to form pellets. The FT-IR spectra of Repaglinide, Chitosan, and GC were scanned in the 4000–400 cm^{-1} range.[8]

Solubility measurement

Drug substance solubility is critical in the drug development since a drug's intended therapeutic effect cannot be accomplished if it is not sufficiently soluble in a fluid media. An adequate amount of Repaglinide was dissolved in a beaker by adding the required solvents constantly. Repaglinide was discovered to be soluble in various solvents, including ethanol, methanol, chloroform, and carbon tetrachloride. The solubility was determined in mg per ml. After determining an approximate solubility, the saturation solubility was established, and the mixture was agitated at 80 rpm for 24 h on a magnetic stirrer, filtered. The concentration of dissolved medication was evaluated spectrophotometrically at 243 nm.[9]

Drug partition coefficient

In a partition coefficient investigation, n-octanol was chosen as the oil phase and phosphate buffer (pH 7.4) as the aqueous

phase. On a mechanical shaker at 37 ± 0.5°C for 24 h, the two phases were combined in equal parts and saturated. A separating funnel was used to separate the saturated phases. Phosphate buffer pH 7.4 and n-octanol were used to make standard plots of the medication. In conical flasks, equal volumes (10 ml) of the two phases were placed in triplicate and 100 mg of drug was added to each. To ensure complete partitioning, the flasks were shaken periodically for 24 h. Centrifugation at 1500 rpm for 5 min separated the two phases, which were then tested for drug content.[10]

Statistical optimization through Box–Behnken design (BBD)

The independent and dependent variables and their low, medium, and high levels are listed in Table 1. With 3-factor and 3-level design, exploring quadratic response surfaces and constructing second-order polynomial models with Design Expert R are a breeze (11.1.0.1 Stat Ease Inc., Minneapolis, MN, USA). This statistical approach can build second-order polynomial equations and illustrate quadratic response surfaces. The response surface design is far more authentic than the full factorial design in terms of prediction variance. Quadratic equations and three-dimensional (3D) response surface plots were constructed for each response. The statistical examination of the quadratic model yielded significant *P*-values indicating goodness of fit for all responses. To validate the model, checkpoint batches were made, and the percentage relative inaccuracy was computed for each response. Table 1 shows the dependent and independent factors. This experimental design's polynomial equation (Eq. 1) is as follows:

$$Y = \beta 0 + \beta 1A + \beta 2B + \beta 3C + \beta 4AB + \beta 5AC + \beta 6BC + \beta 7A^2 + \beta 8B^2 + \beta 9C^2 \quad \text{(Eq. 1)}$$

Where Y is the measured response for each factor level combination, β0 is the constant, and A, B, and C are independent variable levels. Three independent variables such as polymer ratio (A), % d-limonene (B) and %PG (C), whereas three depended variable such Tensile strength (R1), *in vitro* % drug release (R2) and ex vivo % drug release (R3) were selected to get the optimized formulation. Analysis of variance (ANOVA) was used to examine the responses statistically. Furthermore, utilizing the desirability function, a numerical optimization process was used to determine the best formulation.[11]

Table 1: Actual and coded values of factors together with response

Factors	Levels		
	Low	Medium	High
Code values	-1	0	+1
A: Polymer ratio (Chitosan: GC)	7:1	8:1	9:1
B: d-limonene (%)	1	2	3
C: Propylene glycol (%)	20	30	40
Response			
R1:Tensile strength			
R2: *In vitro* % drug release			
R3: *Ex vivo* % drug release			

Preparation of the transdermal patch using chitosan and GC

The Matrix type transdermal patch was prepared using chitosan and GC in different proportions with the help of d-limonene as permeation enhancer and propylene glycol as a plasticizer. Total 15 formulations were developed by BBD. Chitosan solutions were prepared by dissolving the appropriate amount of chitosan in an 0.1 M acetic acid solution. The solution was homogenised and permitted to remain at room temperature for 24 h to allow the polymer to fully hydrate. In another container, GC and repaglinide dissolve in the ethanol. Now both preparation mix together, then add the appropriate amount of plasticizer and permeation enhancer, and the solution was sonicated for 5 min to get appropriate mixing of all the ingredients and eliminate air bubble. The final solution was poured into a Petri dish plate with a total area of 63.58 cm^2 allowed for air drying at room temperature for 72 h. An upturned funnel was used to evaporate the solvent over the Petri dish. The dry patch was kept in desiccators awaiting use.[12]

Physicochemical evaluation of patch

Tensile strength

Tensile strength is determined to check the mechanical strength of the patch that depends on polymers and plasticizers. The single side of the Transdermal Patch was fixed to support, and another side was tied to a pan, where weight was added gradually increased. Now note the length of the patch, where it was broken. The experiment was performed on each patch.[13]

Tensile strength was determined using the below formula (Eq.2).

$$\text{Tensile Strength} = \frac{\text{Force required to break The Patch}}{\text{Width} \times \text{Thickness}} \left(1 + \frac{\text{Length of The Patch}}{\text{Elongation before it broken}}\right) \quad \text{(Eq. 2)}$$

Thickness

The thickness of the patches was investigated at diverse locations, digital vernier Caliper (Stanley, New Delhi, India) was taken to evaluate the thickness of the patches. Average values and standard deviations of the patch be determined.[13]

Weight variation

Individually weighing ten patches at random is used to investigate weight variation. For each formulation, this determination was made, and standard deviations were determined.[14]

Folding endurance

The folding endurance of the patch was evaluated by folding a tiny section (3 cm × 4 cm) at the same point until it broke. The folding endurance rating was determined by the number of times the patch could be folded in the same region without breaking.[15]

Flatness

Each patch was sliced into two longitudinal strips, one from the left and one from the right.[16] The length of each strip was measured, and the length difference due to non-uniformity in flatness was determined using percent constriction, with 0% constriction equaling 100% flatness.[17]

%Moisture loss

The fabricated patches are weighed separately and placed at room temperature for 24 h in a desiccator containing fused calcium chloride.[18] The patches were weighed again, and the percent moisture content was estimated with the following formula (Eq. 3):

$$\% \text{ Moisture content} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Final weight}} \times 100 \quad \text{(Eq. 3)}$$

% Moisture uptake

The weighed patches are placed in a desiccator at room temperature for 24 h before being exposed to a saturated potassium chloride solution at 84% relative humidity.[19] After that, the patches were weighed, and the percent moisture uptake was estimated using the following formula (Eq. 4):

$$\% \text{ Moisture uptake} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100 \quad \text{(Eq. 4)}$$

Drug content

The patches of the particular area be cut and weighed exactly ($n = 3$). The pieces were taken into a 100-mL volumetric flask and dissolved in ethanol. The solution was filtered with the help of 0.45-µ membrane (UV-1800, SHIMADZU, Japan) before drug analysis through a UV–visible spectrophotometer at 243 nm.[20]

In vitro drug release studies

The *in vitro* release of Repaglinide from various matrix systems was investigated using a Franz diffusion cell with a receptor compartment capacity of 10 ml and a cellophane membrane. There are two compartments in the cell: One for the donor and one for the receptor. The donor chamber was exposed to the atmosphere, because it was open at the top. The receptor compartment was equipped with a sampling port and was encircled by a water jacket to keep the temperature at 37 ± 0.5°C. Magnetic beads were used to stir the diffusion media in the receptor compartment. Phosphate buffer 7.4 pH solution was utilized as the diffusion medium. The drug-containing patch was held in the donor compartment with the help of a backing membrane, and it was separated from the receptor compartment by a conventional membrane. Powerful grip clamps hold the donor and receptor compartments together. The water in the outer jacket from the organ bath was circulated to keep the receptor compartment containing dissolution media at 37 ± 0.5°C. The diffusion medium was agitated using a magnetic bead with a diameter of 2 mm and a length of 6 mm, which was driven by a magnetic stirrer.[21]

The solution in the receptor compartment was removed and replaced with a fresh phosphate buffer solution at each sample interval. UV spectrophotometry at 243 nm was used to decide the drug concentration for the drug content. The table shows cumulative percent drug release data *In vitro* for several polymeric patches.

Ex vivo skin permeation studies

Preparation of skin

Hairless animal skin is preferred, because it is easy to obtain from animals of a certain age or sex. The skin of a goat ear was obtained from a nearby slaughterhouse. An animal hair clipper was used to remove hairs from the goat ear's skin, subcutaneous tissue was surgically detached, and the dermis side was cleaned with isopropyl alcohol to remove any remaining clinging fat. The skin was washed with distilled water. After that, the skin was wrapped in aluminium foil and frozen at −2°C until needed. The skin was defrosted at room temperature before use. In *ex vivo* skin permeation studies on goat ear skin, Franz diffusion cells with a contact surface area of 11 cm^2 were utilized.[22]

Drug permeation study

The Franz diffusion cell was used to conduct *ex vivo* permeation tests. The epidermis was facing the donor, and the positioned among the donor and receptor compartments. 10 mL of phosphate buffer saline (pH 7.4) was used to the receptor compartment as of release media. Over the epidermis, the prepared patch was placed between the receptor and the donor compartment. The diffusion cell assembly was rotated at 150 RPM on a magnetic stirrer, and the complete assembly was kept at 32 ± 1°C. To maintain sink conditions, aliquots of 1 ml were taken at fixed time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 h) and replaced with an equal volume of fresh media. The absorption at 243 nm was determined spectrophotometrically after using Whatman filter paper to

purify the samples. Over time, the cumulative percent of drug penetrated per cm square was calculated and plotted.[23]

Model fitting

A variety of kinetic models were employed to examine the *In vitro* release data and define the release kinetics. The systems in which the drug release rate is independent of its concentration are described by the zero-order rate Eq. (5). The first-order Eq. (6) defines the release from a system with a concentration-dependent release rate. Higuchi (1963) defined drug release from the insoluble matrix as a time-dependent square root process based on Fickian diffusion. (Eq. 7).[24]

Where, k_0 is zero-order rate constant expressed in units of con centration/time and t is the time.

$$Q = K_0.t \qquad \text{(Eq. 5)}$$

$$\text{Log}\, C = \text{Log}C_0 - \frac{kt}{2.303} \qquad \text{(Eq. 6)}$$

Where, C_0 is the initial concentration of drug and k is first order constant.

$$Q = Kt_{1/2} \qquad \text{(Eq. 7)}$$

Where, K is the const. reflecting the design variables of the system.

The following plots were made:

i. Zero-order kinetic model represent, cumulative % drug release versus time
ii. First-order kinetic model represent, Log cumulative of % drug remaining versus time
iii. Higuchi model represent, Cumulative % drug release versus square root of time
iv. Korsmeyer model represent, Log cumulative % drug release versus log time.

Scanning electron microscopy (SEM)

A field emission scanning electron microscope is used to record the patch's surface morphology (Model: Quanta FEG 250). Double-sided sticky tape was used to adhere the sample on an aluminium stubby. Then, it was placed in a sputter coater (Model: LEICA EM ACE 200) for gold coating. The sample was exposed to a 10–50 mm vacuum during the gold coating process. A 5kV accelerating voltage was applied, and the image was photographed.[25]

RESULTS AND DISCUSSION

DSC

Repaglinide alone revealed a pronounced endothermic peak at 135.52°C, corresponding to its melting point in DSC analysis. In the presence of any of the polymers investigated, DSC examination of physical mixes of the drug, chitosan, and GC revealed no change in the melting point of repaglinide [Figure 1].

FT-IR analysis

Figure 2 illustrates the FTIR spectra of repaglinide and a physical combination. The NH stretching peak was

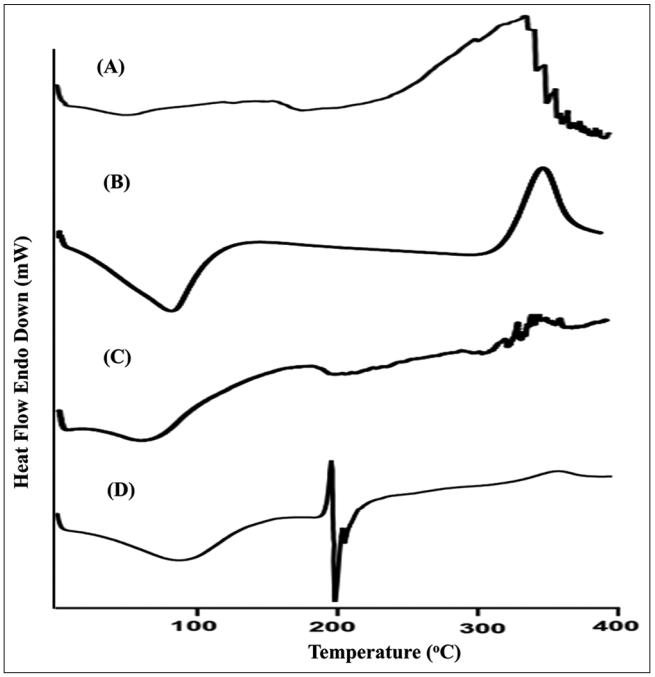

Figure 1: Differential scanning calorimetry of (A) gum copal, (B) chitosan, (C) physical mixture of drug and polymes, and (D) pure Drug (Repaglinide)

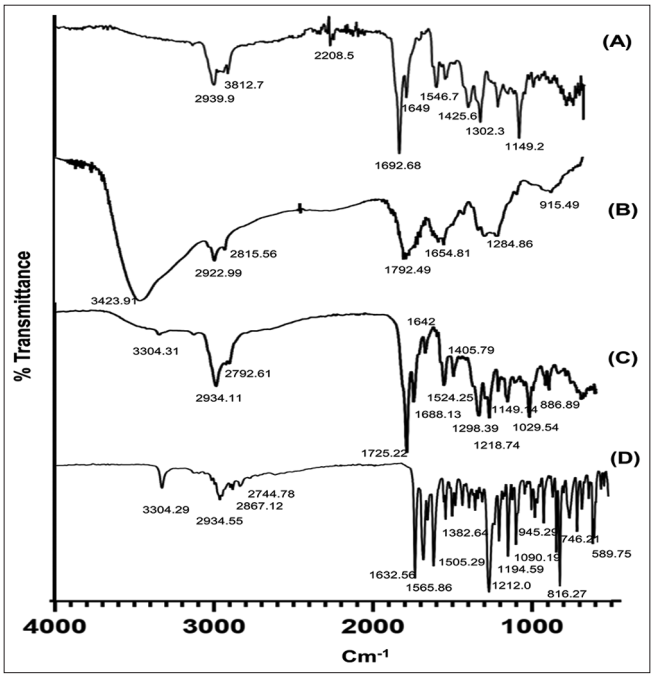

Figure 2: Fourier-transform infrared of (A) gum copal, (B) chitosan, (C) physical mixture of Drug and Polymes, and (D) pure drug (repaglinide)

3304.29 cm^{-1}, the CH stretching peak was 2934.5 cm^{-1}, and the C = O stretching peak was 1685.32 cm^{-1} in the FTIR of pure repaglinide. C–O stretching in the phenyl alkyl ether structure is linked to the bands at 1040.26 cm^{-1} and 1212 cm^{-1}. Aromatic C = C and N–H bending cause the bands at 1566.86 cm^{-1} and 1632.56 cm^{-1}, respectively, while C–H banding causes the band at 1382.64 cm^{-1}.

In the FTIR spectrum of the Physical combination, there were some broadening and reduction in the intensity of the drug's characteristic peaks. Some of the peaks were displaced with very little modification in the wavenumber. These findings indicate that repaglinide and excipients had no interaction.

Solubility measurement

Repaglinide is very soluble in methanol, ethanol, chloroform, and acetone. Hence, based on experimental analysis, ethanol was selected as a solvent, and it was 74 ± 1.6 mg/ml.

Drug partition coefficient

The partition coefficient of repaglinide in n-Octanol: water, n-Octanol: phosphate buffer saline (pH 5.5), and n-Octanol: phosphate buffer saline (pH 7.4) was 2.723 ± 0.126, 2.451 ± 0.081, and 2.187 ± 0.119, respectively. This shows that the drug is highly lipophilic and suitable for developing a transdermal drug delivery system.

Statistical optimization through BBD

ANOVA for tensile strength response

Response 1 – tensile strength

The model has a significant F-value of 14.65, indicating that it is significant. Due to noise, an F-value of this magnitude has a 0.43% probability of occurring. $P < 0.0500$ is considered significant in model terms. In this circumstance, significant model terms include B, C, AB, A^2, and C^2. The model terms are meaningless if the value is >0.1000. Model reduction might help you enhance your model if it has a lot of nominal model terms (not including those required to support hierarchy). The F-value of the Lack of Fit is 2.57, which means that it is not statistically significant when compared to the pure error. A significant Lack of Fit F-value has a 29.27% chance of occuring due to noise. We want the model to fit since a minor lack of fit is good [Figure 3].

The final data analysis equation to obtain the coding factor may be seen in the equation (Eq. 8).

$$R1(Tensile\ Strength) = 0.8017 - 0.008 \times A + 0.1466 \times B + 0.2014 \times C + 0.1978 \times AB - 0.0467 \times AC - 0.1030 \times BC + 0.1369 \times A^2 - 0.0838 \times B^2 + 0.1087 \times C^2 \quad \text{(Eq. 8)}$$

ANOVA for In vitro drug release response

Response 2 – In vitro drug release

The F-value of 61.76 infers that the model is statistically significant. Due to noise, an F-value of this magnitude has a 0.01% probability of occurring. $P < 0.0500$ is considered significant in model terms. In this circumstance, the model terms A, B, B^2, and C^2 are significant. The model terms are meaningless if the value is greater than 0.1000. Model reduction might help you enhance your model if it has a lot of nominal model terms (not including those required to support hierarchy). The Lack of Fit has an F-value of 0.36, indicating that it is not statistically significant when compared to the pure error. There's a 79.02% likelihood that a big Lack of Fit F-value is caused by noise. We want the model to fit. Thus, a minor lack of fit is good [Figure 4].

The final equation of data analysis to achieve the coding factor is seen in the equation (Eq. 9).

$$R2(In\ vitro\ drug\ release) = 71.65 + 7.41 \times A + 11.46 \times B - 0.4731 \times C + 1.68 \times AB + 0.07365 \times AC - 1.05 \times BC + 1.03 \times A^2 - 3.91B^2 + 2.5C^2 \quad \text{(Eq. 9)}$$

ANOVA for Ex vivo drug permeation response

Response 3 – Ex vivo drug permeation

The statistical significance of the model is indicated by the Model F-value of 53.50. Due to noise, an F-value of this magnitude has a 0.02% probability of occurring. $P < 0.0500$ is considered significant in model terms. In this circumstance, the model terms A, B, B^2, and C^2 are significant. The model terms are meaningless if the value is >0.1000. Model reduction might help you enhance your model if it has a lot of nominal model terms (not including those required to support hierarchy). The Lack of Fit has an F-value of 0.06, indicating that it is not significant when compared to the pure error. There is a 97.70% likelihood that a big Lack of Fit F-value is caused by noise. We want the model to fit. Thus, a minor lack of fit is good [Figure 5].

The final equation of data analysis to obtain the coding factor can be seen in the equation (Eq. 10).

$$R3(Ex\ Vivo\ drug\ permeation) = 245.05 + 28.16 \times A + 40.30 \times B - 4.05 \times C + 1.47 \times AB + 4.78 \times AC - 6.99 \times BC + 1.52 \times A^2 - 16.15 \times B^2 + 10.72 \times C^2 \quad \text{(Eq. 10)}$$

Physicochemical evaluation of patch

The physical parameter such as effect of polymeric system, tensile strength, thickness, weight variation, folding

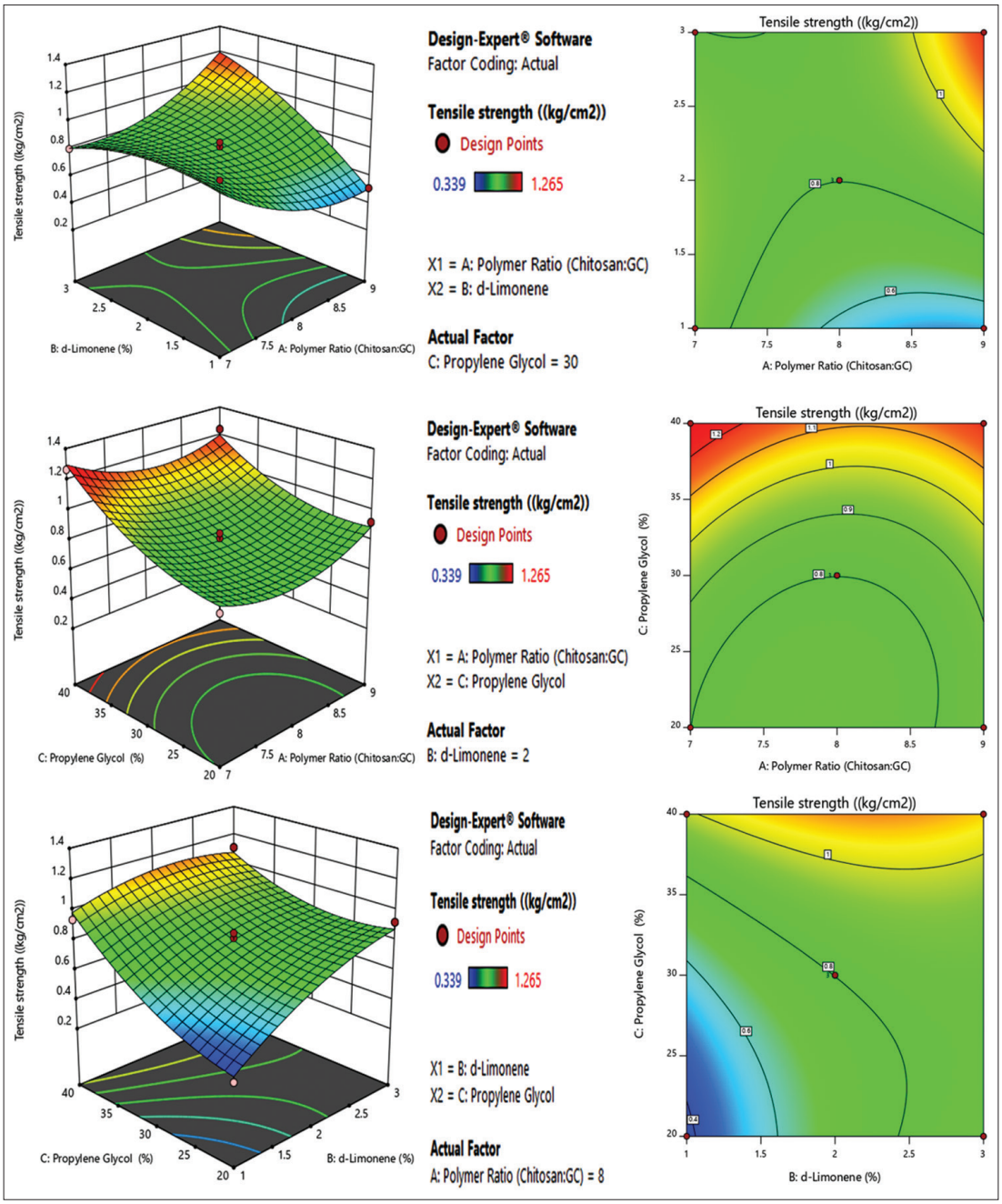

Figure 3: Surface plot response showing effect of α polymer (Chitosan: Gum Copal [GC]) ratio (a) and Limonene (%w/w (b); β polymer (Chitosan: GC) ratio (a) and concentration of propylene glycol (%w/w) (c); γ Limonene (%w/w) (b) and concentration of propylene glycol (%w/w) (c) on R1 response (tensile strength

endurance, flatness, %Moisture Loss, %Moisture Uptake, and Drug content was determined [Table 2].

Effect of polymeric system

Chitosan is hydrophilic and GC is a hydrophobic polymer in nature and is regarded as a non-toxic, non-irritating, and non-allergic material. It has good patch-forming properties that enable the preparation of mechanically strong structures. GCs benefits have been combined with other (hydrophilic) polymers. This provides a drug release interface that will allow the appropriate dose to be achieved, which would otherwise be impossible due to the display of an extremely sluggish and prolonged release profile. Improved polymer-medium interaction can produce a more uniform diffusion layer. Within 24 h, 88.33% of the medication was released constantly, according to the *In vitro* release profile. This release would have been significantly slower without GC, resulting in a lower concentration of medication available to achieve therapeutic action.[26]

The presence of chitosan in the formulation, on the other hand, facilitates moisture uptake, which has other good

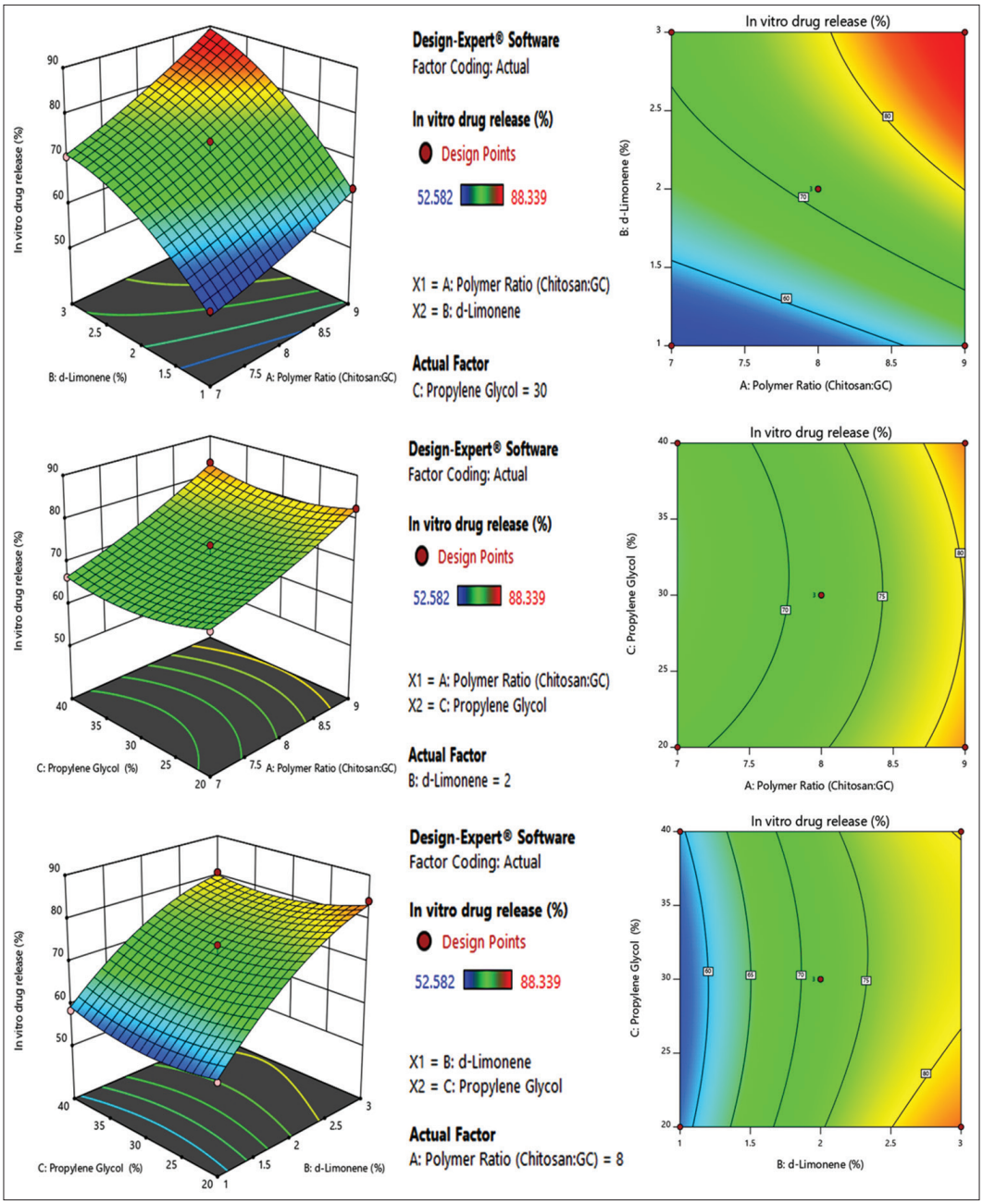

Figure 4: Surface plot response showing effect of α polymer (Chitosan: Gum Copal [GC]) ratio (a) and Limonene (%w/w (b); β polymer (Chitosan: GC) ratio (a) and concentration of propylene glycol (%w/w) (c); γ Limonene (%w/w) (b) and concentration of propylene glycol (%w/w) (c) on R2 response (*In vitro* drug release)

impacts on drug crystallinity architecture, favoring the amorphous state. Moisture uptake in formulated dosage forms aids in medication dispersion throughout the system, increasing drug solubility overall. The inclusion of chitosan is a crucial aspect of favorable thermodynamic activity for Repaglinide delivery from the patch system in this regard.[27]

Tensile strength

Tensile strength of the transdermal patches is a very important parameter since they are expected to be sufficiently flexible and to have a mechanical strength on skin for an long time. Tensile strength results showed that strength of patch was in a range from 0.217 to 0.935 kg/mm^2. The folding endurance determines the capacity of patch to hold out damage. The results showed that all batches of patches would not be damaged and would keep their veracity when used with ordinary skin folding.

Thickness

The thickness of the patches varied from 129 ± 0.006 mm to 180 ± 0.016 mm. A low standard deviation in patch thickness

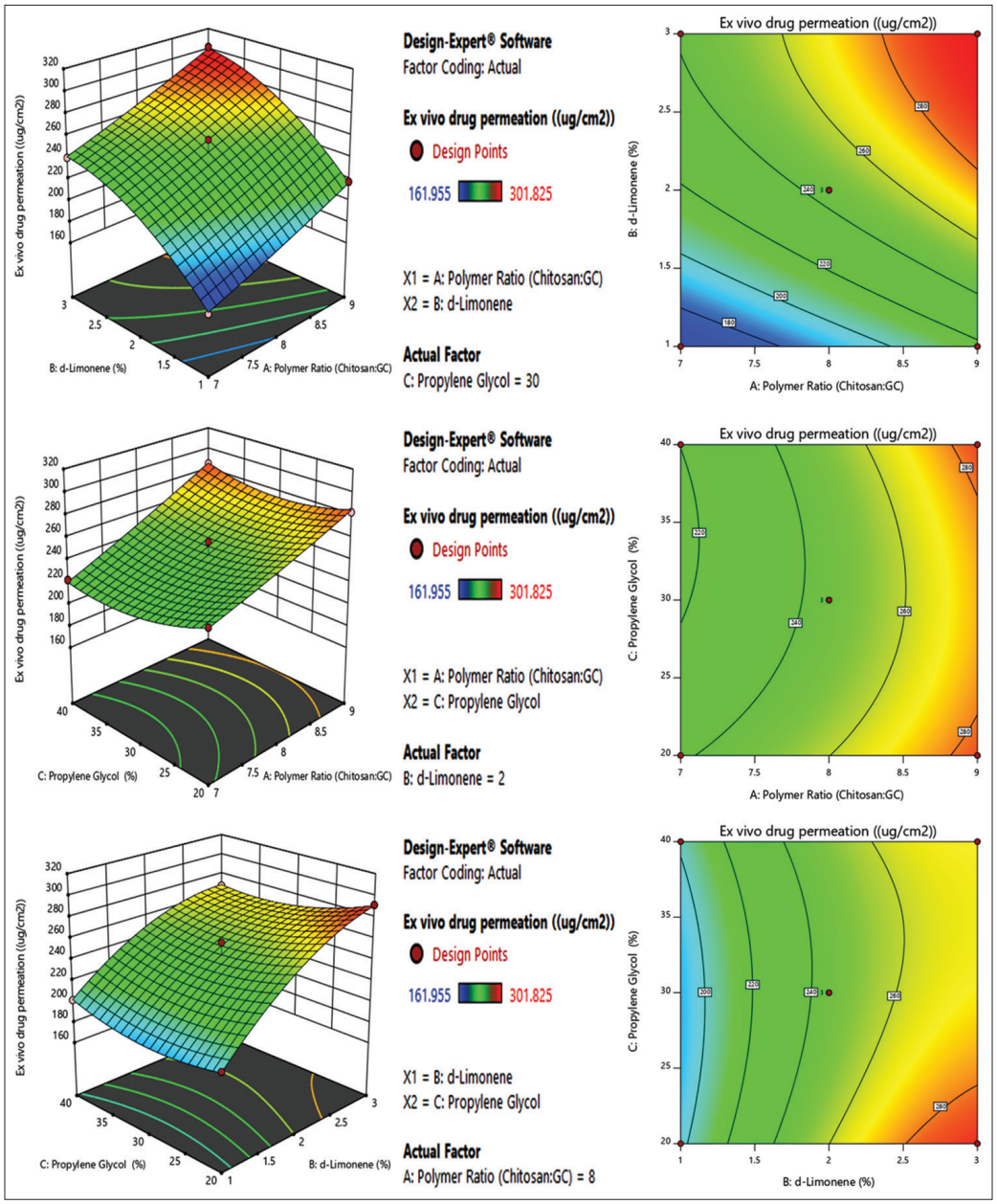

Figure 5: Surface plot response showing effect of α polymer (Chitosan: Gum Copal [GC]) ratio (a) and limonene (%w/w (b); β polymer (Chitosan: GC) ratio (a) and concentration of propylene glycol (%w/w) (c); γ Limonene (%w/w) (b) and concentration of propylene glycol (%w/w) (c) on R3 response (*Ex vivo* drug permeation)

measurements ensured the homogeneity of the patches made by the solvent casting approach.

Weight variation

The weight variation ranged between 114.33 ± 8.993 and 125.66 ± 8.082 mg, indicating that all formulations had the same weight and the standard deviation values indicating patch uniformity.

Folding endurance

Folding endurance scores ranged from 168 ± 2.16 to 200 ± 3.08. The goal of folding endurance was to determine the patch's ability to survive breakdown as it was applied to the skin's surface. A minimal value of <150 for folding endurance indicates that the patch will maintain its integrity and form during application.

Flatness

The percent flatness investigation revealed that all formulations (F1–F15) had the same strip length before and after cutting, showing that all formulations were 100% flat. As a result, no constriction occurred, and all patches had a smooth, flat surface that could be maintained when applied to the skin.

Table 2: Physicochemical properties of repaglinide transdermal patch

S. No.	Formulation code	Thickness (mm)	Folding Endurance	% Moisture Loss	% Moisture Uptake	%Drug Content	Wt. Variation (mg)	Flatness (%)
1	R01	0.172±0.013	188.33±6.40	2.206±0.225	2.236±0.397	94.58±2.122	120.66±4.784	100
2	R02	0.180±0.016	185.33±3.30	2.166±0.57	1.969±0.269	95.966±0.612	121.33±3.681	100
3	R03	0.140±0.010	200.66±3.08	1.916±0.118	2.083±0.278	96.77±0.420	117.66±13.012	100
4	R04	0.146±0.008	198.0±8.28	2.373±0.424	2.05±0.084	96.443±0.507	119.66±6.599	100
5	R05	0.156±0.006	167.66±6.23	1.873±0.114	1.923±0.258	97.186±0.263	125.0±6.482	100
6	R06	0.165±0.009	205.33±6.54	1.926±0.181	1.763±0.147	96.85±0.753	115.66±4.109	100
7	R07	0.166±0.019	185.66±3.68	1.996±0.126	1.916±0.061	95.403±0.588	121.66±4.109	100
8	R08	0.144±0.010	178±2.16	1.996±0.245	2.01±0.192	95.179±0.451	118.33±8.993	100
9	R09	0.134±0.005	181.33±5.79	2.143±0.166	1.939±0.261	95.746±0.510	114.33±10.498	100
10	R10	0.129±0.006	170.33±1.70	1.753±0.143	2.35±0.037	96.673±0.552	120.0±6.164	100
11	R11	0.166±0.006	183.33±2.05	1.77±0.086	1.993±0.108	95.25±0.711	118.66±5.436	100
12	R12	0.165±0.016	188.0±2.44	2.019±0.209	1.863±0.187	96.046±0.218	121.66±9.104	100
13	R13	0.158±0.012	166.33±1.70	2.163±0.401	1.829±0.141	95.913±0.192	120.66±4.109	100
14	R14	0.155±0.101	165.66±2.86	2.133±0.134	1.98±0.206	95.653±0.700	125.66±8.082	100
15	R15	0.139±0.009	190.33±2.49	2.026±0.073	1.990±0.314	96.126±0.83	120.33±4.921	100

Values are expressed as mean±S.D., *n*=3

% Moisture loss

The percent moisture loss study of the different formulations is shown in Table 2. The result indicated that if the concentration of Chitosan increases, the percent moisture loss also increases.

% Moisture uptake

The weight difference compared to the starting weight after exposing the prepared patches to 84% relative humidity was used to compute the percentage moisture uptake (saturated solution of aluminum chloride). The results of moisture uptake trials for various formulations. The produced formulation had a low moisture uptake, which could help protect the formulations from microbial contamination and reduce largeness. With increasing concentrations of the hydrophilic polymer, the percentage moisture uptake was likewise shown to raise. Significant changes in characteristics such as increased porosity, increased pore diameter, and lower crushing strength for matrix patches containing hydrophilic polymers are caused by moisture uptake from the atmosphere.

Drug content

The range of drug content in the patches was from 94.580 ± 2.122 to 97.186 ± 0.263 %, which indicated that the method used to formulate transdermal patches in this study could produce patches with consistent drug content and minimal patch variability. It is shown good uniformity of percentage drug content among the patches.

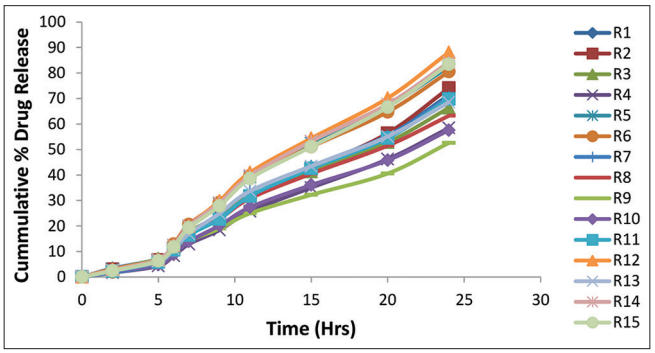

Figure 6: *In vitro* % drug release from F1 to F15 formulations

In vitro drug release studies

The Franz diffusion cell test apparatus was used as a dissolution apparatus for different formulations through phosphate buffer, with 7.4 pH, as a dissolution media at 32 ± 0.5°C. The release study of all formulations was done.

The cumulative *In vitro* drug release results from transdermal patches are shown in Figure 6. The factors such as polymer ratio and %PG have non-linear effects on the response in vitro % drug release as shown in Figure 6. It was observed that the formulation R12 exhibited the highest cumulative amount of Repaglinide released. For the subsequent formulations, that is, R1 to R11 and R13 to R15, the release of Repaglinide was found to be progressively slower. It was shown that as the concentration of hydrophilic polymer (Chitosan) was increased in the formulation, the dissolution rate of the formulation also increased gradually. Transdermal patch R12 has the highest drug release, 88.339 ± 2.191% at the

end of 24 h, while R9 has a minimum drug release of 52.582 ± 1.484% among all 15 preparations. It was shown that the release of Repaglinide decreased as the ratio of chitosan declined and the ratio of GC increased. This phenomenon was done due to the hydrophilic nature of chitosan and the hydrophobic nature of GC polymer.

Hence, the use of permeation enhancer (d-limonene) also affects the drug release profile of the transdermal patch, and it also plays an important role in the release profile of repaglinide. d-limonene (Cyclic terpene) is harmless for humankind due to naturally occurring compounds.

Ex vivo skin permeation studies

Animal skin (Goat ear skin) models have been successfully considered alternatives for human skin. Accordingly, this skin is regarded as a successful *ex vivo* model for research into various drug penetration mechanisms. Figure 7 showed the cumulative amount of repaglinide permeated through the unit area of goatskin from formulae R1 to R15 compared. Some amount of drug was detected in the receptor compartment after the ½-h, indicating the appropriate drug release and its permeation through the skin. All tested preparations showed that the amounts of repaglinide released within the first ½-h were relatively low. Still, the amount of repaglinide released was highest for R12 (301.825 ± 1.050 µg/cm^2) after 24 h with a flux of 12.09 ± 0.181 compared with those of F9 (161.955 ± 1.040 µg/cm^2) with a flux of 6.015 ± 143. The enhancement ratio of R12 compared with the transdermal patch of repaglinide without using penetration enhancer was 2.39 fold batter.

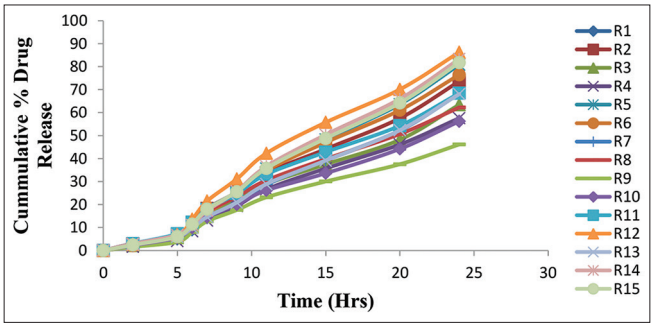

Figure 7: *Ex vivo* % drug release from F1 to F15 formulations

Model fitting

In vitro drug release was fitted into kinetic models to determine the release kinetics of Repaglinide from the transdermal patch. The zero-order, first-order, Higuchi kinetics, and Korsmeyer-Peppas models were kinetic models [Table 3]. All formulations followed the zero-order release, as evidenced by the uppermost correlation coefficient (R^2 = 0.982–0.998). The log mt/mT versus Log time curve (Korsmeyer- Peppas) slop (1.404>*n*>723) suggested a non-Fickian transport function (1.404 *n*.723).

SEM analysis

The optimized transdermal Patch (R12) surface morphology before and after the *Ex vivo* permeation study was scanned using a field emission scanning electron microscope (Quanta FEG 250). The SEM images have shown the homogeneous dispersion of repaglinide in the polymer matrix. Uniform

Table 3: Comparative table of different release kinetics models

Formulation Code	Zero-order kinetics (R^2)	First-order kinetics (R^2)	Higuchi-order kinetics (R^2)	Korsmeyer - peppas kinetics	
				(R^2)	*n*
R1	0.992	0.99	0.975	0.945	0.753
R2	0.990	0.989	0.976	0.935	0.750
R3	0.995	0.983	0.969	0.975	0.644
R4	0.994	0.989	0.953	0.950	0.807
R5	0.986	0.98	0.976	0.954	0.664
R6	0.987	0.978	0.983	0.894	0.960
R7	0.990	0.988	0.975	0.972	0.926
R8	0.993	0.991	0.972	0.928	0.935
R9	0.998	0.997	0.957	0.993	0.723
R10	0.992	0.990	0.963	0.800	1.404
R11	0.993	0.989	0.976	0.923	0.976
R12	0.982	0.974	0.981	0.902	0.749
R13	0.994	0.989	0.972	0.949	0.845
R14	0.984	0.980	0.982	0.925	0.859
R15	0.991	0.974	0.977	0.924	0.857

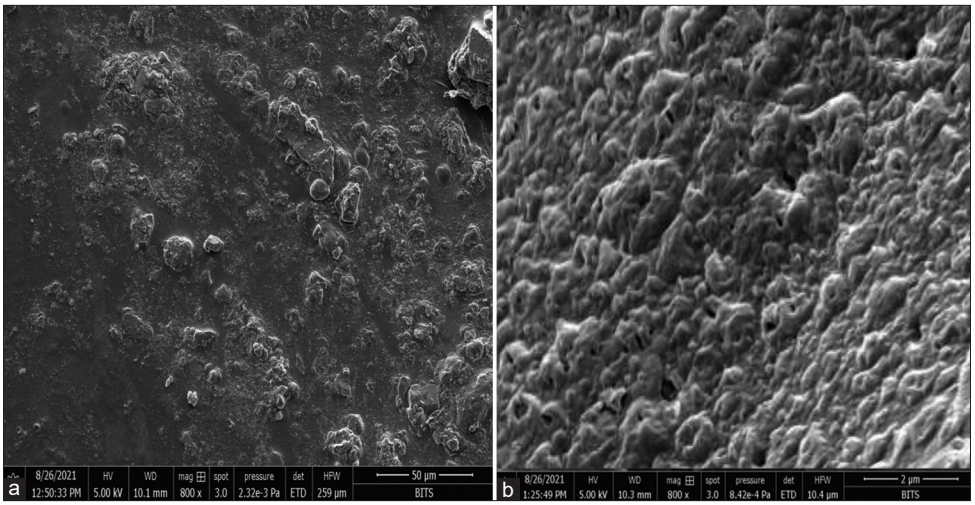

Figure 8: Scanning electron microscopy images (a) Before permeation at ×800, (b) after permeation at ×800

distribution of the drug in the transdermal patch is one of the important characteristics, and that also ensures the uniform sustained release of drug substances from the patches.

Figure 8 is an SEM image of the patch before and after the medicine has been released. According to the image, the patch has a very rough surface and many pores that allow the medicine to diffuse from the polymer matrix. Therefore, we can say that combining both polymers (chitosan and GC) provides a satisfactory sustained release from the transdermal patch of repaglinide.

CONCLUSION

Transdermal patches are very useful for those drugs that undergo hepatic first-pass metabolism and have a short half-life of less than 1h. The transdermal patch of repaglinide has been successfully formulated as a once-daily formulation by solvent evaporation technique. We have taken 15 formulations from R1 to R15 using different chitosan and GC ratios as hydrophilic and hydrophobic natural polymers. The prepared patch's tensile strength, thickness uniformity, weight variation, folding endurance, flatness, percent moisture loss, percent moisture uptake, and drug content uniformity were all evaluated, indicating that the method used for transdermal patch formulation was repeatable and ensured excellent quality and uniformity in patch characteristics with minimal variability. Further, *In vitro* and *Ex vivo* drug release studies for all the formulations showed that drug release among these formulations, R12 is the best formulation, because it shows maximum release in a sustained manner in 24 h. The role of d-limonene as permeation enhancer also enhances very effectiveness of optimizing formulations. After optimizing all formulations, it concluded that the ratio of polymer in 9:1, 3% of a permeation enhancer, and 30% plasticizer was the optimum quantity of different excipients used in the optimized formulation. These findings suggest that transdermal repaglinide delivery may have therapeutic promise, with benefits such as reduced dose frequency, better patient compliance, non-invasive properties, enhanced bioavailability, and ease of medication discontinuation. The benefits of repaglinide should be amplified even more, because it must be administered long-term.

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