Journal of Applied Pharmaceutical Research
Volume 12 Issue 3, Year of Publication 2024, Page 21 – 37
DOI: https://doi.org/10.69857/joapr.v12i3.580

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com ***ISSN: 2348 – 0335***

OPTIMIZATION AND EVALUATION OF TRANSDERMAL DELIVERY SYSTEM FOR NEBIVOLOL HYDROCHLORIDE

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Article Information
Received: 5th February 2024
Revised: 30th April 2024
Accepted: 18th May 2024
Published: 30th June 2024

Keywords
Transdermal drug delivery, Hypertension, Nebivolol hydrochloride, Central composite design

ABSTRACT
Background: Nebivolol hydrochloride, a β1-receptor antagonist known for its antihypertensive properties, boasts a plasma half-life of 10 hours and an oral bioavailability of 12%. In this study, we aimed to enhance the therapeutic effectiveness of Nebivolol hydrochloride and circumvent its extensive hepatic first-pass metabolism by developing transdermal matrix patches. **Methodology:** Utilizing Central Composite Design (CCD), nine formulations were devised, comprising Hydroxypropyl methylcellulose K15M and Eudragit S100 as independent variables, with 10% w/w triethyl citrate as the plasticizer. Key dependent variables were evaluated, including folding endurance, moisture content, tensile strength, in vitro drug release, and flux. Fourier transform infrared spectroscopy (FTIR) assessed the compatibility between the drug and polymer. **Results and discussion:** Among the formulations, FP8 demonstrated the highest drug release (85.88% over 24 hours), attributed to its elevated concentration of hydrophobic polymer. The optimized formulation was determined based on the results of dependent variables. **Conclusion:** These findings suggest that the developed matrix transdermal film holds promise as a potential candidate for sustained drug release over a 24-hour.

INTRODUCTION

The transdermal route of drug administration has emerged as a promising method for delivering medications locally and systemically. It offers several advantages over oral dosage forms, including enhanced patient adherence during long-term therapy [1], bypassing of first-pass metabolism [2], sustained drug release, maintenance of consistent plasma drug levels [3], reduction of variability between patients [4], and the ability to discontinue treatment when necessary. Moreover, transdermal delivery can mitigate gastrointestinal side effects associated with oral medications, making it a preferred option for patients with sensitive digestive systems [5]. However, the stratum corneum's structured barrier hinders drug penetration, necessitating modification to facilitate the delivery of poorly permeating drugs [6]. Chemical penetration enhancers can broaden the range of drugs suitable for transdermal delivery by altering the skin barrier properties and enhancing drug permeation. The formulation of a transdermal delivery system involves the careful selection of excipients and the design of a delivery matrix capable of enhancing drug permeation while ensuring patient

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safety and comfort [7]. The physicochemical properties of Nebivolol hydrochloride, including its molecular weight, lipophilicity, and pharmacokinetic profile, must be considered during formulation design to achieve optimal drug release and skin permeation. Additionally, selecting suitable polymers, permeation enhancers, and adhesive agents plays a pivotal role in determining the performance of the transdermal patch. Furthermore, optimizing formulation parameters, such as polymer concentration, drug loading, and patch thickness, necessitates a systematic approach involving experimental design and statistical analysis.

Nebivolol hydrochloride, a selective β1-adreno receptor blocker, demonstrates unique hemodynamic effects, such as lowering peripheral vascular resistance and exerting a neutral impact on cardiac output. Clinical trials have confirmed its safety and efficacy in reducing blood pressure through vasodilation. Despite its low oral bioavailability, attributed to extensive metabolism [8], Nebivolol hydrochloride's favorable characteristics, including low molecular weight, short half-life, moderate lipophilicity, and low dosage requirement, make it an ideal candidate for transdermal drug delivery.

By selectively blocking β1-receptors, Nebivolol hydrochloride counteracts the effects of epinephrine, lowering heart rate and blood pressure [9]. Additionally, β blockers inhibit renin release, which constricts blood vessels. At higher concentrations, Nebivolol hydrochloride may also bind β2-receptors [10]. The present study proposes a matrix-type transdermal drug delivery system for Nebivolol hydrochloride using various hydrophilic and hydrophobic polymers, including HPMC K15M and Eudragit S100. Furthermore, the study will investigate the impact of formulation variables, such as polymer concentration, on the release profile and skin permeation of Nebivolol hydrochloride. Additionally, the study will assess the physicochemical properties of the formulated transdermal patches, including their mechanical strength, adhesive properties, and stability under ambient storage conditions. Overall, the research aims to develop an optimized transdermal delivery system for Nebivolol hydrochloride (NBH), potentially improving patient compliance and therapeutic outcomes in the treatment of hypertension. Through this comprehensive investigation, the study endeavors to contribute valuable insights into the development of transdermal drug delivery systems and advance the field of pharmaceutical sciences.

MATERIAL AND METHODS

Materials

Sun Pharmaceutical Industries Ltd, Baroda, generously provided nebivolol hydrochloride. Colorcon Asia Pvt Ltd., Goa, India graciously supplied HPMC K15M. Eudragit S100 was a kind donation from Evonik Industries, Mumbai, India. Triethyl citrate was sourced from Sd Fine Chemicals Limited, Mumbai, while glycerine was procured from RFCL Limited (Rankem), New Delhi. All additional solvents, including ethanol and acetone, were of analytical grade.

METHODOLOGY

Drug polymer interaction

In the study on drug-polymer interaction, both the pure NBH and a blend of it with HPMC and Eudragit polymers were individually mixed with IR grade KBr at a ratio of 100:1. Subsequently, pellets were formed by applying 5.5 metric tons of pressure using a hydraulic press [11]. These pellets were subjected to scanning across a wave number range of 4000-400/cm using a Jasco FTIR 4100 instrument [12]. The obtained spectra for the drug and its physical mixtures with polymers were then compared.

Differential Scanning Calorimetry (DSC) is a vital tool for investigating drug-polymer interactions in transdermal systems. By subjecting drug-polymer blends to controlled temperature changes, DSC analyses heat flow between the sample and a reference material [13]. This technique helps identify changes in thermal properties, such as melting point, glass transition temperature, and crystallinity, which reflect interactions between the drug and polymer. Understanding these interactions is crucial for optimizing formulation parameters, ensuring compatibility, and enhancing the efficacy and stability of transdermal drug delivery systems.

Preparation of Transdermal Films

In this investigation, matrix-type transdermal patches were fabricated using the solvent casting method [14, 15, 16]. The polymers are dissolved in the chosen solvent using gentle heating and stirring to achieve thorough dissolution, and the solution of drug is then added to the polymer solution, resulting in a homogeneous combination. This solution is then poured onto a flat, inert surface, such as a glass or Teflon plate, forming a thin, uniform layer. The thickness of the film is regulated by altering the volume of the solution and the dimensions of the

casting surface, which is then spread evenly with a casting knife or applicator. Solvent evaporation is permitted to occur, either at room temperature or under regulated conditions in a drying oven, ensuring progressive evaporation and preventing air bubbles or inconsistencies.

After the solvent has completely evaporated, a dry, flexible film forms and is carefully removed from the casting surface. In this study, HPMC K15M was gradually introduced into hot boiled water with continuous stirring and allowed to cool at room temperature. Meanwhile, Eudragit S100 was dissolved in acetone (10 mL), and nebivolol hydrochloride drug was dissolved in ethanol (2.5 mL); both solutions were combined and added to the HPMC solution. Subsequently, triethyl citrate (10% w/w) was incorporated into the polymeric solution.

The resulting mixture was manually agitated to achieve a homogeneous viscous solution and subjected to sonication to eliminate trapped air [16]. The mixture was then cast onto a plastic petri plate and dried in a hot air oven at 40°C for 24 hours. To finalize the transdermal therapeutic system for NBH, we applied a backing membrane (3M™ Scotchpack™ 9733) and a release liner (3M™ Scotchpack™ 1022) on both sides of the film. The NBH patches were then stored in a desiccator until they were ready for use.

Design of Experiment

For this investigation, a central composite design (CCD) was employed [17]. This design assessed two factors, HPMC K15M and Eudragit S100, each at five distinct levels. All nine feasible combinations of experimental batches were executed, as detailed in Table 1. Independent variables, including the quantities of HPMC K15M (X1) and Eudragit S100 (X2), were determined, as outlined in Table 1.

Moisture uptake assesses the patch's ability to absorb and retain moisture, which is critical for keeping it intact and functioning during storage (between 20°C to 25°C and 68°F to 77°F) and use. High moisture uptake can cause swelling, mechanical property changes, and possible degradation of the active pharmaceutical ingredient, whereas low moisture might cause brittleness and poor adherence.

Tensile strength measures the mechanical durability of a patch, guaranteeing that it can survive handling and wear without tearing or losing structural integrity. Adequate tensile strength is required for the patch to stick securely to the skin and deliver consistent medication release. The percentage of drug release over time reveals the patch's efficiency and consistency in drug administration, which has a direct bearing on its therapeutic potential. Optimizing% drug release ensures that the patient receives the appropriate dosage of medication over the prescribed time period, hence preserving therapeutic drug levels and reducing side effects.

Therefore, the dependent variables selected for analysis were Moisture Uptake (Y1), Tensile Strength (Y2), and % Drug Release (Y3). To evaluate the impact of polymers on drug release and the dependent variables, contour and 3-D response surface plots were generated using Design-Expert® 13 software developed by Stat-Ease®. The specific variable values utilized in the CCD are documented in Table 1. A statistical model, incorporating interactive and polynomial terms, was applied to compute the responses.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1 X_1 + b_{22} X_2 X_2 \quad (1)$$

Where, Y is dependent, b_0 is the arithmetic mean response of all trials, and b_i (b_1, b_2, b_{12}, b_{11} and b_{22}) is the estimated coefficient for the corresponding factor X_i (X_1, X_2, $X_1 X_2$, X_{11} and X_{22}) which represents the average result of changing one factor at a time from its low to high value. Equation 1 depicts a second-order polynomial regression model used in response surface methodology to investigate the impact of independent factors on response variables. In this context, Y represents the response variable (e.g., moisture uptake, tensile strength, percent drug release), while X_1 and X_2 represent the independent variables (HPMC K15M (X_1) and Eudragit S100 (X_1)).

The equation's coefficients have precise meanings: b0 is the intercept term, which represents the anticipated value of Y when all independent variables are zero, and b_1 and b_2 are linear coefficients that quantify the effect of each independent variable on Y. The term b_{12} is the interaction coefficient, demonstrating how the combined values of X_1 and X_2 influence Y, and b_{11} and b_{22} are quadratic coefficients that account for curvature in the response surface, implying non-linear effects of the independent variable on Y. This model provides a full understanding of the interactions between inputs and responses, allowing for better optimization and prediction of outcomes.

Table 1: Central Composite Design for NBH Transdermal Delivery System

Formulation	**HPMC K15M (X_1)**		**Eudragit S100 (X_2)**		
FP1	-1		-1		
FP2	1		-1		
FP3	-1		1		
FP4	1		1		
FP5	- α		0		
FP6	α		0		
FP7	0		- α		
FP8	0		α		
FP9	0		0		
Independent factors	**- α**	**-1**	**0**	**+1**	**+ α**
X_1 (HPMC K15M)	358.58	400	500	600	641.42
X_2 (Eudragit S100)	358.58	400	500	600	641.42

* Each formulation incorporating 10% w/w Triethyl citrate as a plasticizer

Evaluation of Transdermal Films

Physical characteristics such as film thickness, weight uniformity, folding endurance, tensile strength, moisture content, moisture absorption, and drug content were evaluated.

Film Thickness

The thickness of each film was measured using a screw micrometer (Usico, India) at various points, and the average thickness was calculated [18]. The concentration of hydroxypropyl methylcellulose and Eudragit polymers primarily influences the film's thickness. A higher polymer concentration increases thickness, leading to a longer path length and slower drug release rate.

Weight Uniformity

To assess weight consistency, 10 randomly selected films were individually weighed, and the average weight was determined. To ensure uniformity, each film's weight should not significantly deviate from the average weight [14].

Measurement of Drug Content

The film was dissolved in 25 mL of methanol to determine the drug content and left on a rotary flask shaker for 24 hours [19]. Following this, the solution underwent filtration and appropriate dilution, and the drug content per film was assessed using a UV-visible spectrophotometer at a wavelength of 282.5 nm. This information is crucial for assessing the quality and consistency of the formulation, ensuring that each film delivers the intended dosage of the active pharmaceutical ingredient. Additionally, the test allows for monitoring batch-to-batch variability and verifying compliance with regulatory standards, thereby enhancing the overall quality control measures in pharmaceutical manufacturing [20].

Moisture content determination

Initially, the films were weighed (W1) and then placed in a desiccator with anhydrous calcium chloride until they reached a constant weight (W2). The final weight was recorded when there was no further change in the film's weight [21]. The moisture content was determined using the following equation:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100 \qquad (2)$$

Where W1 represents the initial weight of each strip, and W2 denotes the final weight of each strip.

Study on Moisture Uptake

The films underwent a controlled process to evaluate moisture uptake. Initially, they were positioned in a desiccator with anhydrous calcium chloride until a stable weight (W1) was achieved. Subsequently, the films were transferred to a separate desiccator containing a saturated solution of aluminum chloride ($AlCl_3$) at 25°C and 75% relative humidity until another stable weight (W2) was attained [22]. The moisture uptake capacity was determined using the formula:

$$\text{Moisture uptake (\%)} = \frac{W_2 - W_1}{W_1} \times 100 \qquad (3)$$

W1 represents the initial weight of each strip, and W2 signifies the final weight of each strip.

Flatness Assessment

Longitudinal segments were excised from the prepared medicated patches, and the lengths of each segment were meticulously measured. The measurement aimed to quantify the length variation attributed to the non-uniformity in flatness. The degree of flatness was determined by assessing the constriction of strips, where zero percent constriction corresponded to one hundred percent flatness [23]. The flatness percentage (% Constriction) was calculated using the formula:

$$\% \text{ Constriction} = \frac{L_1 - L_2}{L_1} \times 100 \qquad (4)$$

Where L1 represents the initial length of each strip, and L_2 denotes the final length of each strip.

Tensile strength

Polymeric films were clamped between corked linear iron plates to evaluate tensile strength. One end of the film remained fixed with an iron screen, while the other was connected to a movable thread passing over a pulley. Gradual weights were added to the pan attached to the hanging end of the thread. A scale with a pointer measured film elongation, and the weight required to break the film was recorded [24]. Tensile strength was then calculated using the formula:

$$\text{Tensile strength} = \frac{F}{a \times b} \times \frac{1+L}{l} \qquad (5)$$

F represents the breaking force, a is the film width, b is the film thickness, L is the film length, and l is the elongation at the breaking point.

Folding Endurance Test

Three distinct films were chosen for evaluation to assess folding endurance. The procedure involved iteratively folding each film at the same location until a break occurred. The folding endurance value was quantified as the number of times the film could be folded at the same spot without breaking. The results were derived from the average of three determinations [15].

In vitro drug diffusion study

In vitro diffusion investigations were conducted using a Franz diffusion cell having a receptor compartment capacity of 20 ml [25]. An egg membrane was positioned between the donor and receptor compartments of the diffusion cell [26, 27]. The film was placed on the diffusion membrane and then covered with aluminum foil. The receptor compartment was filled with phosphate buffer pH 7.4. The phosphate buffer pH 7.4 was chosen as the receptor compartment medium in *in vitro* diffusion studies because the pH of human blood is roughly 7.4, making this buffer physiologically relevant and ensuring that the experimental circumstances closely resemble the *in vivo* environment. This relevance aids in precisely determining the drug's release and penetration characteristics from the transdermal patch into the bloodstream. Furthermore, phosphate buffers have a strong buffering capacity around pH 7.4, ensuring a steady pH throughout the experiment and reliable results despite pH changes. The entire setup was affixed onto a hot plate magnetic stirrer, ensuring continuous stirring of the solution in the receptor compartment via magnetic beads, while maintaining a constant temperature of 32±0.5°C. Samples were withdrawn at varying intervals and subjected to spectrophotometric analysis for drug content. An equal volume of phosphate buffer was added each time a sample was withdrawn to replenish the receptor phase.

Stability testing

The optimized formulations were tested for stability under accelerated conditions (40 ± 0.5˚C and 75 ± 5% relative humidity) in a programmable environmental test chamber for six months [15]. This rigorous testing environment mimicked severe storage circumstances to assess the formulations' durability and stability over time. The samples were comprehensively examined for moisture content, tensile strength, and percentage of drug release. These stability studies are crucial in assessing whether the formulations are adequate for long-term shelf life and constant therapeutic efficacy in clinical settings.

RESULTS AND DISCUSSIONS

Fourier transform infrared spectroscopy analysis

FTIR spectroscopy is crucial in pharmaceutical analysis as it allows for the identification of functional groups present in drug molecules and polymers [28]. Comparing spectra of pure substances with those of formulations helps assess the compatibility and potential interactions between drugs and excipients (Figure 1). In the context of transdermal film formulation, FTIR analysis provides valuable insights into the integrity of the formulation components, ensuring drug stability and efficacy [29]. In the analysis of NBH using FTIR spectroscopy, distinct peaks were observed at specific wavenumbers, indicating the presence of key functional groups (Figure 1 (a)). These include peaks at 3299 cm^{-1} corresponding

to the secondary amine group (-CH_2NH) and N-H stretching, 1138 cm^{-1} attributed to the presence of fluorine on the aryl ring, and C-F stretching, 1260 cm^{-1} indicating the presence of a secondary alcohol group (-CH_2OH) and C-O stretching, and 1215 cm^{-1} suggesting the presence of the aryl-O-CH_2 group and C-O stretching. A peak at 1621 cm-1 was also observed, representing the aromatic C=C stretching peak. Similarly, FTIR analysis of HPMC K15M revealed peaks at 3462 cm^{-1} corresponding to O-H stretching vibration and 2932 cm^{-1} associated with C-H stretching vibration. Meanwhile, FTIR spectra of Eudragit S100 displayed peaks at 2952 cm^{-1}, which is attributed to CH aliphatic stretching, and 1735 cm-1 is due to -C=O stretching. Moreover, the FTIR spectrum of the transdermal film formulation demonstrated peaks at 3431 cm^{-1} for the secondary amine group (-CH_2NH) and N-H stretching, 1260 cm^{-1} indicating the presence of the secondary alcohol group (-CH_2OH) and C-O stretching, 1215 cm^{-1} suggesting the presence of the aryl-O-CH_2 group and C-O stretching, and 1623 cm^{-1} representing the aromatic C=C stretching peak. The comparison of these spectra with those of the pure drug revealed closely similar peaks, indicating minimal interaction between the drug and polymers used in the formulation. This lack of significant interaction is confirmed by the transdermal film formulation spectrum, which closely resembles the addition spectrum of the pure drug and polymer, suggesting a simple physical mixture without notable chemical interactions.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is critical in formulating transdermal films by providing valuable insights into the compatibility and interaction between drug and polymer constituents. By precisely detecting changes in thermal properties such as melting points and crystallinity [30], DSC helps optimize formulation parameters to ensure the stability and efficacy of transdermal patches. Additionally, DSC analysis aids in identifying any potential issues, such as drug-polymer incompatibility or the presence of amorphous phases, guiding formulation adjustments for enhanced drug delivery performance and patient safety. Overall, DSC is a crucial analytical technique in developing transdermal film formulations, facilitating the design of effective delivery systems for various therapeutic applications. In this study, we employed DSC to analyze the thermogram of Nebivolol hydrochloride and its physical mixture. The pure Nebivolol hydrochloride exhibited a distinct sharp endothermic peak at 228°C, indicating its melting point. Interestingly, when analyzing the physical mixture of the drug with polymers, minimal alteration was observed in the melting point of Nebivolol hydrochloride, suggesting compatibility with the polymer blend under study. Furthermore, the optimized formulation of the transdermal patch displayed a broadened peak in its DSC thermogram, attributed to the amorphous nature of the compound [31]. These DSC analysis findings underscore the drug's and polymer components' compatibility, as illustrated in Figure 2.

Impact of Variables

Impact on Moisture uptake

Moisture uptake test holds significance in transdermal delivery systems as it assesses the film's ability to absorb moisture, which is crucial for maintaining stability and efficacy. Excessive moisture uptake can lead to undesirable changes in the film's properties, such as degradation or altered drug release kinetics [32]. By understanding the moisture uptake capacity (Table 2), formulation scientists can optimize the composition and design of transdermal patches to ensure optimal performance and shelf-life stability. Regarding moisture absorption, analysis via multiple linear regression unveiled that coefficient b1 displays a positive trend, while coefficient b2 exhibits a negative trend. A positive coefficient for X1 implies that elevating the concentration of X1 (HPMC K15M) correlates with heightened moisture absorption in the transdermal film [32]. Conversely, the negative coefficient for X2 suggests that augmenting the concentration of X2 (Eudragit S100) leads to decreased moisture absorption in the transdermal film. The fitted equation illustrating the relationship between folding endurance response and transformed factors is expressed as follows:

$$Y_1=2.05+0.29X_1 – 0.11X_2+0.08X_1X_2 + 0.01X_{11} – 0.01X_{22} \quad (6)$$

All batches exhibit a strong correlation coefficient of 0.99595 for moisture absorption. Elevated moisture absorption significantly jeopardizes the stability of the matrix transdermal film, with X1 being primarily responsible for increased moisture uptake. Further insight into the correlation between formulation variables (X1 and X2) and moisture absorption was gained through contour and response surface plot analysis, as depicted in Figure 3. The response surface plot illustrates that at the highest levels of X1, moisture absorption increases as X2 decreases from level -1 to level +1, and the same findings are also confirmed in the perturbation plot.

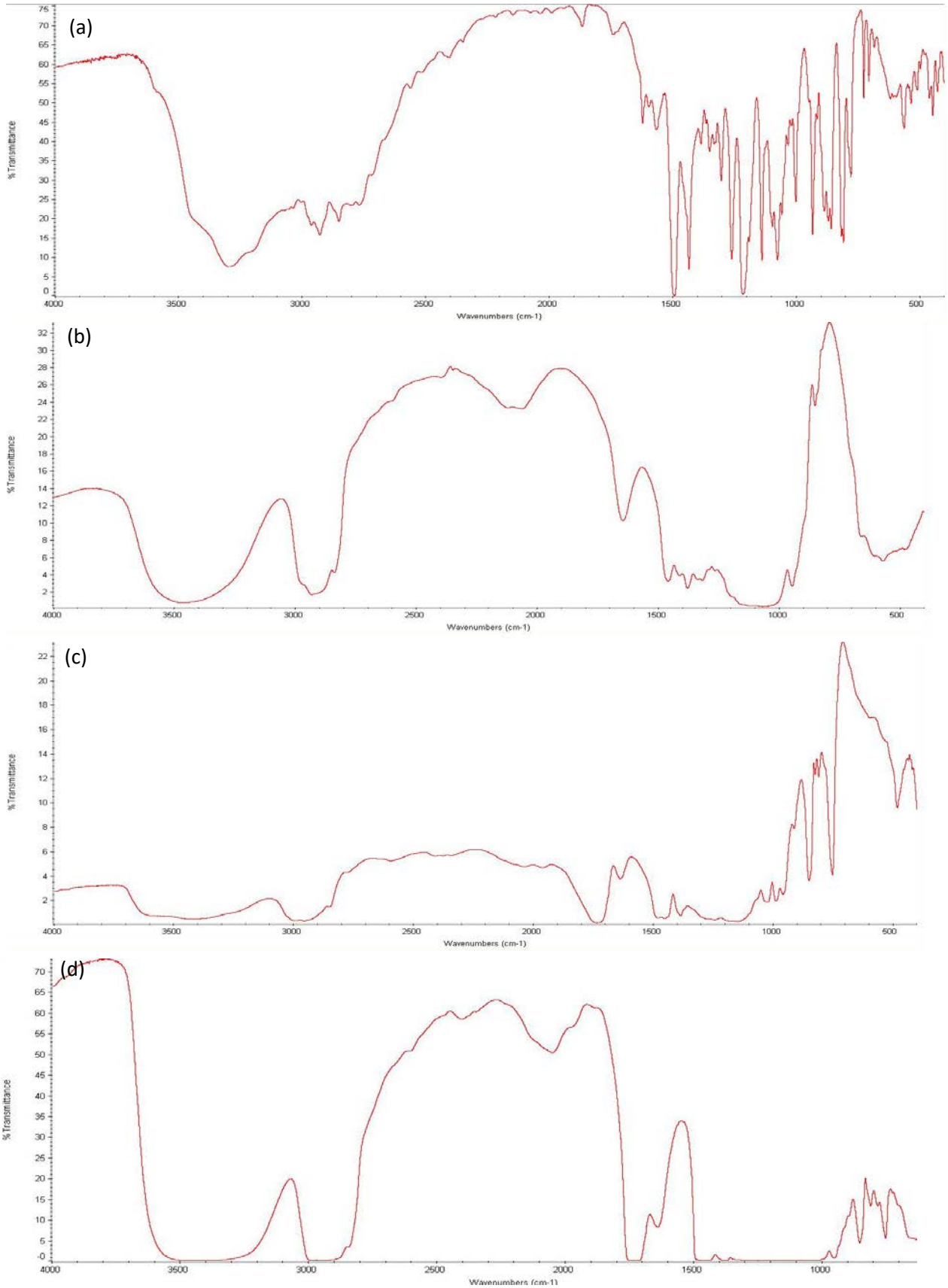

Figure 1: FTIR Spectrum of Nebivolol hydrochloride (a); HPMC K15M (b); Eudragit S100 (c); optimized transdermal formulation (d)

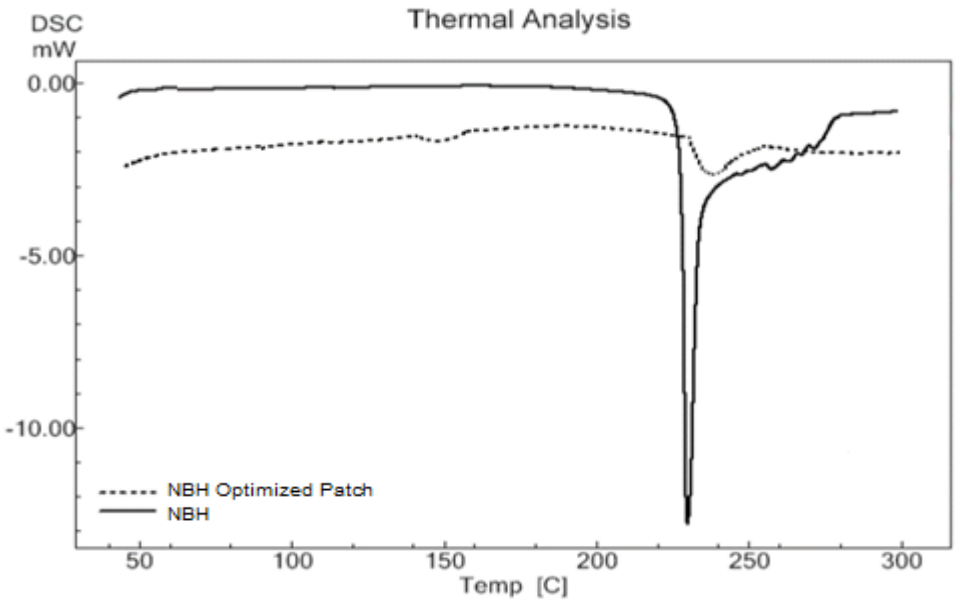

Figure 2: Differential scanning calorimetry of pure drug Nebivolol hydrochloride and transdermal patch

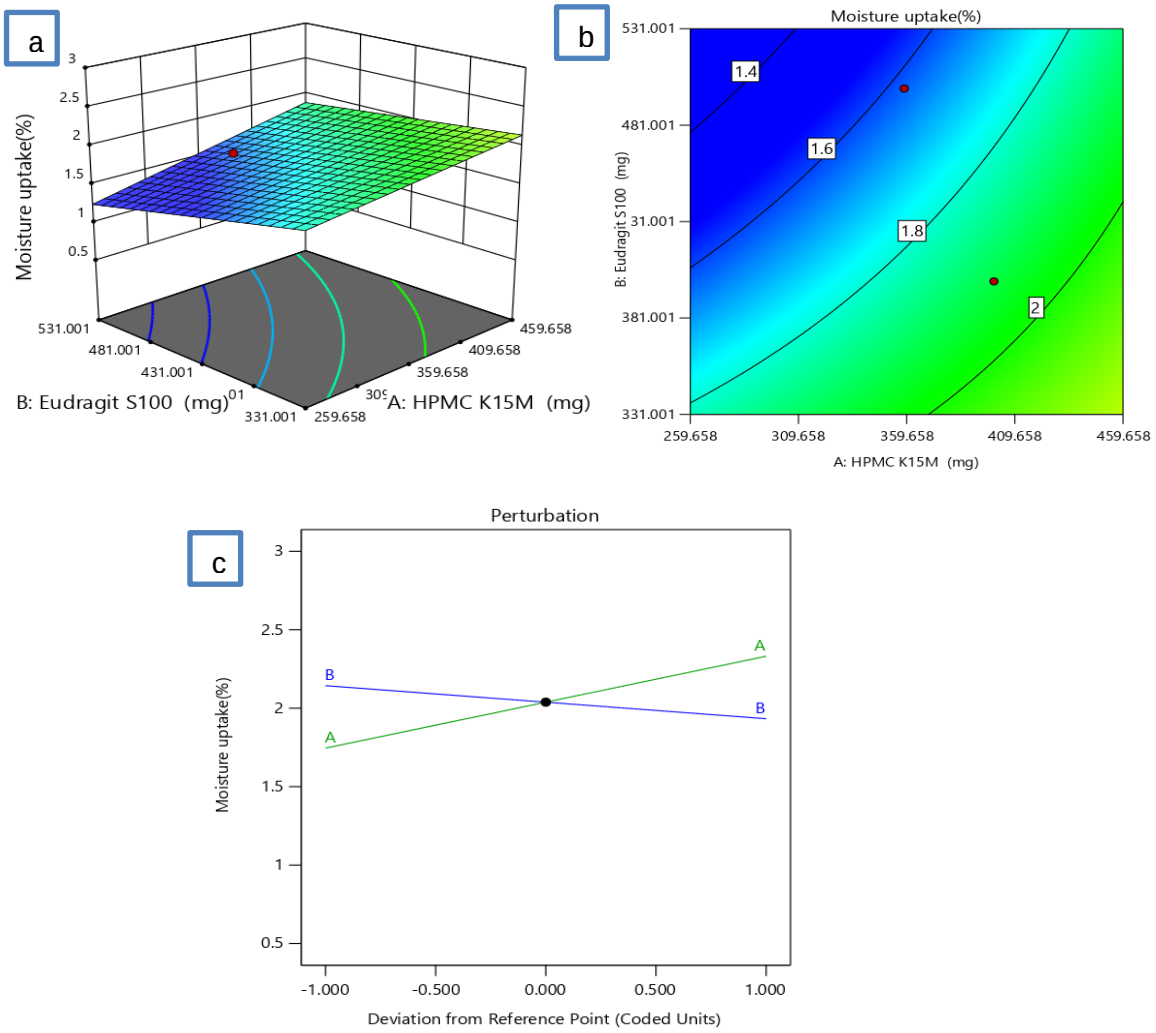

Figure 3: The response surface plot (a), contour plot (b), and perturbation plot (c) depicting impact of independent variables on moisture uptake

Tablet 2: Evaluation parameters of transdermal patch containing NBH

Batch	Thickness	Weight uniformity	Drug content	Moisture content (%)	Moisture uptake (%)	Folding Endurance	Tensile strength	Elongation	Flatness (%)	% Drug Release
F1	0.23±0.02	50.53±4.04	98.03±1.03	2.76±0.34	1.92±0.08	253±10.52	1.62±0.18	3.19±0.21	99.97±1.75	96.37±3.21
F2	0.29±0.05	53.45±3.17	97.43±0.9	3.52±0.29	2.39±0.14	152±9.01	1.13±0.19	2.16±0.14	95.91±3.16	99.79±2.07
F3	0.27±0.06	51.59±4.13	97.92±0.89	2.19±0.39	1.54±0.13	327±7.74	1.86±0.16	3.57±0.18	99.63±1.73	90.03±2.81
F4	0.37±0.03	58.54±1.11	97.97±0.83	3.44±0.34	2.31±0.24	249±10.73	1.77±0.17	3.28±0.08	95.09±1.71	92.77±1.93
F5	0.24±0.02	55.67±2.26	97.87±0.79	2.27±0.36	1.65±0.04	302±9.73	2.19±0.16	4.12±0.18	95.55±2.89	88.26±3.01
F6	0.35±0.04	56.08±1.08	96.74±0.91	3.73±0.39	2.43±0.14	230±7.87	1.43±0.15	2.63±0.22	99.36±3.62	96.93±2.13
F7	0.25±0.02	54.51±3.04	97.69±0.72	3.17±0.51	2.14±0.24	174±9.27	1.35±0.18	2.51±0.08	95.37±1.75	99.97±2.74
F8	0.34±0.03	58.56±1.12	97.16±0.91	2.75±0.34	1.87±0.19	272±8.44	2.14±0.21	3.86±0.13	95.81±3.16	85.88±3.13
F9	0.28±0.02	54.23±2.05	97.92±1.17	2.91±0.42	2.05±0.14	235±9.81	1.65±0.17	3.21±0.14	92.48±1.67	95.39±2.51

* Results are the mean of three observations ± SD

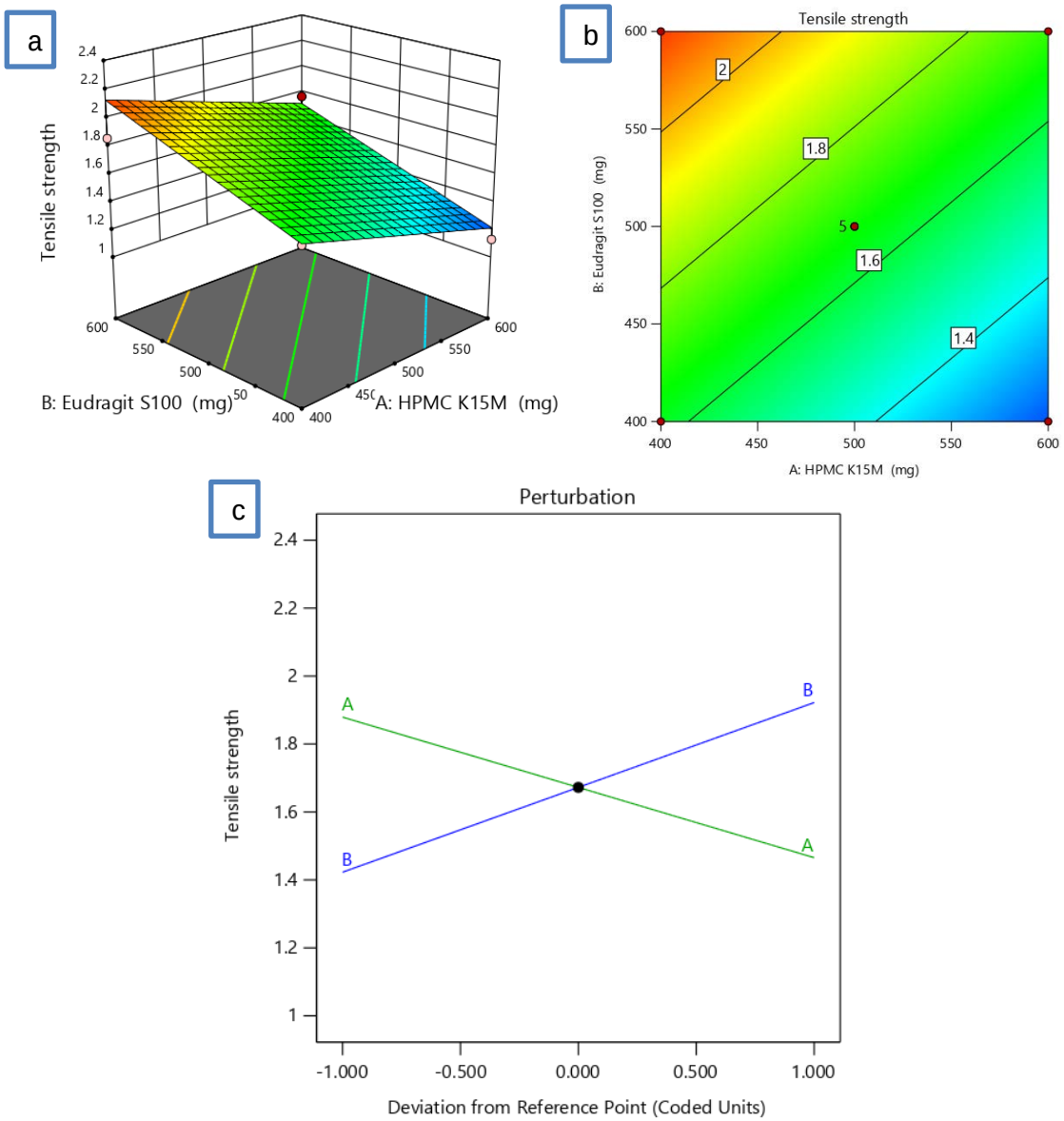

Figure 4. The response surface plot (a), contour plot (b), and perturbation plot (c) depicting impact of independent variables on tensile strength

Impact on tensile strength

A tensile strength test is crucial in transdermal delivery systems to assess the mechanical integrity and durability of polymeric films used as drug carriers [33]. Understanding tensile strength helps ensure that the film can withstand the stresses encountered during application, removal, and wear without compromising drug release kinetics or causing discomfort to the patient. Additionally, it aids in optimizing formulation parameters to achieve the desired balance between flexibility and strength for efficient drug delivery through the skin. Regarding tensile strength, multiple linear regression analysis revealed that coefficient b1 has a negative sign, while coefficient b2 is positive. The negative coefficient for X1 (HPMC K15M) suggests that decreasing its concentration increases transdermal film tensile strength [34]. Conversely, the positive coefficient for X2 (Eudragit S 100) indicates that increasing its concentration results in higher tensile strength. The equation derived from the analysis is as follows:

$$Y_2=1.70 - 0.21X_1+ 0.25X_2+0.01X_1X_2–0.23X_{11} - 0.13X_{22} \quad (7)$$

All batches exhibit a strong correlation coefficient of 0.9253, indicating a robust relationship between variables. A high tensile strength is crucial for withstanding mechanical pressure in matrix transdermal films, with X2 playing a significant role in achieving this. We utilized contour and response surface plots to explore further the relationship between formulation variables (X1 and X2) and tensile strength. Figures 4 depict the effects of X1 and X2 on tensile strength. The plots illustrate that at the highest levels of X2, tensile strength increases when X1 is decreased from -1 to +1 levels.

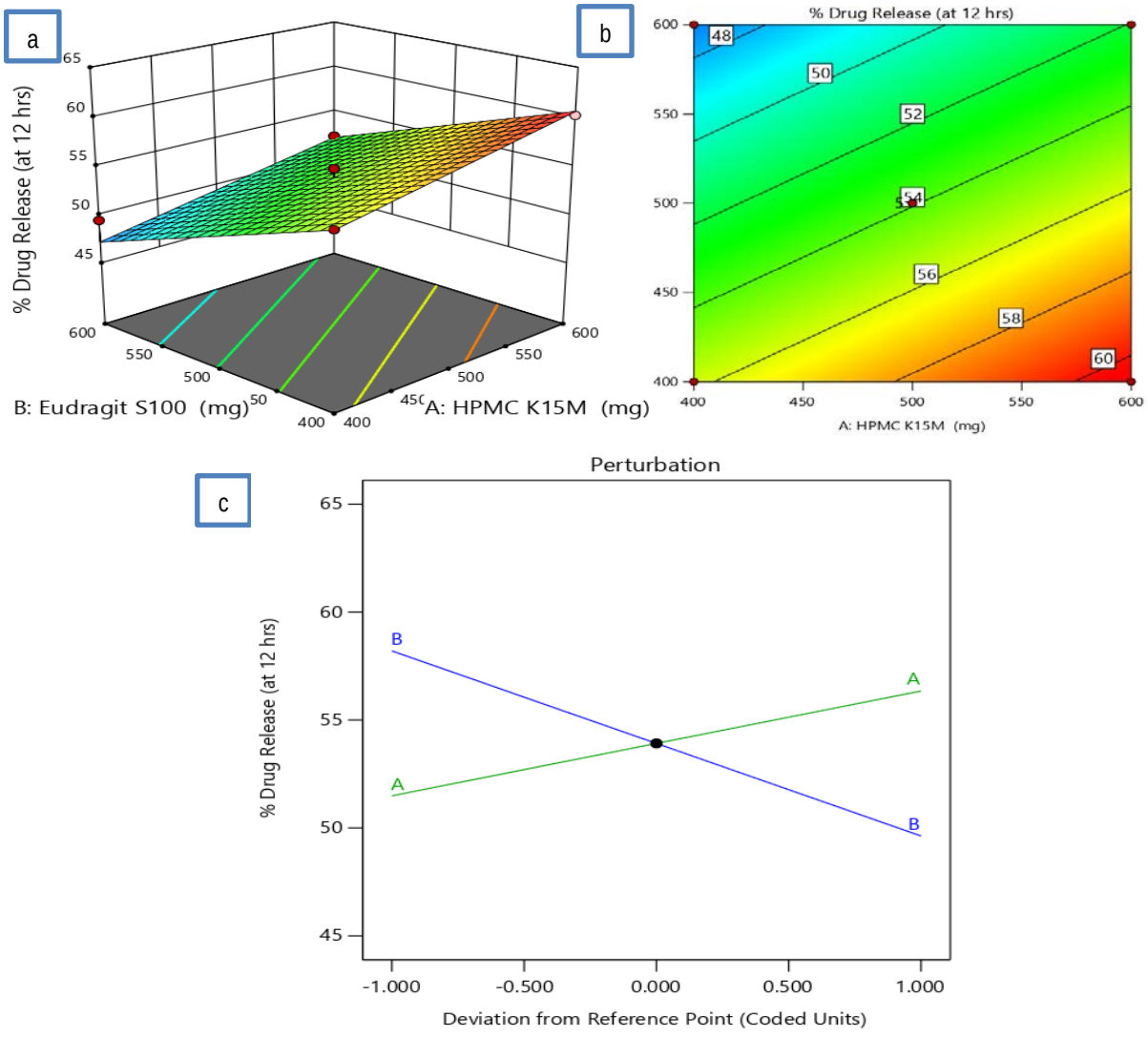

Figure 5: The response surface plot (a), contour plot (b), and perturbation plot (c) depicting impact of independent variables on drug release.

Impact on % Drug Release

The examination of variables impacting the percentage of drug release revealed intriguing insights. Multiple linear regression analysis showed that the coefficient b1 exhibited a positive inclination, while b2 displayed a negative orientation. This implies that as the concentration of X1 (HPMC K15M) escalates, there is a concurrent elevation in drug release from the transdermal film [13]. Conversely, an increase in the concentration of X2 (Eudragit S 100) leads to a decrease in drug release from the transdermal film. The formulated equation depicting the moisture uptake response of the transformed factors is presented as follows:

$Y_3=54.05 + 2.43X_1 - 4.29X_2 – 0.42X_1X_2+1.63X_{11} - 1.23X_{22}$ (8)

The drug release across all batches demonstrates a commendable correlation coefficient of 0.9165. A controlled drug release profile is essential for maintaining sustained release from the matrix transdermal film. Further elucidation of the relationship between formulation variables (X1 and X2) and drug release was achieved through contour and response surface plots. These plots, depicted in Figure 5, illustrate the effects of X1 and X2 on drug release. It was discerned from the response surface plot that at the highest levels of X1, there was an augmentation in drug release. However, with an increase in X2 from the -1 level to the +1 level, drug release exhibited a decline.

Upon assessing the transdermal patch, we focused on moisture uptake, tensile strength, and drug release as dependent variables. Notably, the coefficient b1 for moisture uptake proved significant ($P<0.05$), with its positive value indicating an increase in moisture uptake as X1 values rose. Conversely, the significant negative coefficient b2 suggested a decrease in moisture uptake with higher X2 values. Furthermore, a significant interaction between both independent variables was observed, denoted by a P-value of <0.05 for the b12 coefficient. The remaining coefficients were deemed insignificant ($P\geq0.05$) [30].

Regarding tensile strength, the significant negative coefficient b1 ($P<0.05$) indicated an increase in strength with decreasing X1 values. However, the other coefficients were not statistically significant ($P\geq0.05$). In the drug release study, the significant positive coefficient b1 ($P<0.05$) suggested an increase in release with higher X1 values. Conversely, the significant negative coefficient b2 indicated a decrease in release with higher X2 values. Similar to the previous analyses, the other coefficients were not statistically significant ($P\geq0.05$). These results are summarized in Table 3.

The moisture uptake, tensile strength, and drug release exhibited a high correlation with their respective R^2 values of 0.9969, 0.9253, and 0.9165, showcasing the robust relationship between dependent and independent variables. To streamline the models, insignificant terms with $P\geq0.05$ were omitted, while those with $P<0.05$ were deemed statistically significant and retained. Table 3 displays the coefficients for both full and reduced models across response variables. In the analysis of moisture uptake, ANOVA revealed that the coefficients b11 and b22 had P values greater than 0.05, leading to their exclusion from the full model. Statistical results are presented in Table 4, where coefficients b1, b2, and b12 were deemed significant at $P <0.05$ and retained in the reduced model. As shown in Table 3, model testing involved assessing whether these coefficients provide significant predictive information regarding moisture uptake. The critical F value for $\alpha = 0.05$ (DF = 2,7) was determined to be 4.74, with the calculated value (F = 2.99) falling below this threshold. Therefore, it can be concluded that terms b11 and b22 do not significantly contribute to moisture uptake prediction and can be excluded from the full model in favor of the reduced model.

Upon conducting ANOVA for tensile strength, it was observed that the significance levels of coefficients b12, b11, and b22 exceeded a P value of >0.05. Consequently, these coefficients were excluded from the full model, resulting in a reduced model. The statistical analysis results are detailed in Table 4. Notably, coefficients b1 and b2 exhibited significance at $P <0.05$, thus meriting retention in the reduced model. The reduced model underwent testing to assess the significance of coefficients b11, b22, and b12 in predicting tensile strength. The outcomes of this testing, illustrated in Table 4, revealed that the calculated F value (2.462) fell below the critical value of F (4.35) for $\alpha = 0.05$ (DF = 3,7) [35]. Consequently, it can be deduced that terms b11, b22, and b12 do not significantly contribute to predicting tensile strength and can thus be excluded from the full model to derive the reduced model.

ANOVA was conducted for the drug release study to assess the significance levels of coefficients b12, b11, and b22. Those with greater P values were excluded from the full model, resulting in a reduced model. The statistical analysis results are detailed in Table 4. Notably, coefficient b2 remained significant at $P < 0.05$

and was retained in the reduced model. Subsequently, the reduced model underwent testing to determine the contribution of coefficients b12, b11, and b22 to drug release prediction. The testing results, presented in Table 3, revealed that the calculated F value (0.850) was lower than the critical value (4.35) for α = 0.05, indicating that terms b12, b11, and b22 do not significantly contribute to drug release prediction and can be omitted from the full model.

Table 3: Regression analysis of Central Composite design batches of NBH transdermal patches

Coefficients	*Moisture Uptake (Y_1)*		*Tensile strength (Y_2)*		*% Drug Release (Y_3)*	
	FM [a]	*RM* [b]	*FM*	*RM*	*FM*	*RM*
b_0	2.05	2.04	1.70	1.67	54.05	53.92
b_1	0.29	0.29	-0.21	-0.21	2.43	2.43
b_2	-0.11	-0.11	0.25	0.25	-4.29	-4.29
b_{12}[d, e]	0.08	0.08	0.10	-	-0.42	-
b_{11}[c, d, e]	0.01	-	-0.23	-	1.63	-
b_{22}[c, d, e]	-0.01	-	0.13	-	-1.23	-

[a] FM, Full model; [b] RM, Reduced model; [c] Non significant (P>0.05) coefficients for Y_1; [d] Non significant (P>0.05) coefficients for Y_2; [e] Non significant (P>0.05) coefficients for Y_3

Table 4: Calculation for testing the model in portions for NBH transdermal patches.

Model	***df*** [c]	***SS*** [d]	***MS*** [e]	***R^2***
Moisture Uptake (Y_1)				
Regression				
FM[a]	5	0.8001197	0.1600239	0.99596
RM[b]	3	0.7973433	0.2657811	0.9925
Residual				Fcal = 2.990
FM	7	0.0032496	0.0004642	Fcritical = 4.74
RM	9	0.0060259	0.0006695	df = (2, 7)
Tensile strength (Y_2)				
Regression				
FM	5	0.9192206	0.1838441	0.9253
RM	2	0.8409112	0.4204556	0.84647
Residual				Fcal = 2.462
FM	7	0.0742102	0.0106015	Fcritical = 4.35
RM	10	0.1525195	0.015252	df = (3, 7)
% Drug Release (Y_3)				
Regression				
FM	5	200.75396	40.150793	0.91652
RM	2	194.09147	97.045735	0.8861
Residual				Fcal = 0.850
FM	7	18.285866	2.6122666	F critical = 4.35
RM	10	24.948361	2.4948361	df = (3, 7)

[a] FM, Full model; [b] RM, Reduced model; [c] df, Degree of freedom; [d] SS, Sum of squares; [e] MS, Mean of squares

Optimization of Transdermal patch

Following the application of the ANOVA model, the optimization of the transdermal patch was conducted using an overlay plot. The overlay plot displayed the optimized batch transdermal patch's predicted values (Figure 6). Subsequently, upon evaluating the optimization transdermal patch, the

experimental values were obtained (Table 5). The comparison between the predicted and experimental values revealed that the % bias did not exceed 8 % [36]. This outcome demonstrates the effectiveness of the applied model in accurately predicting the investigation outcomes.

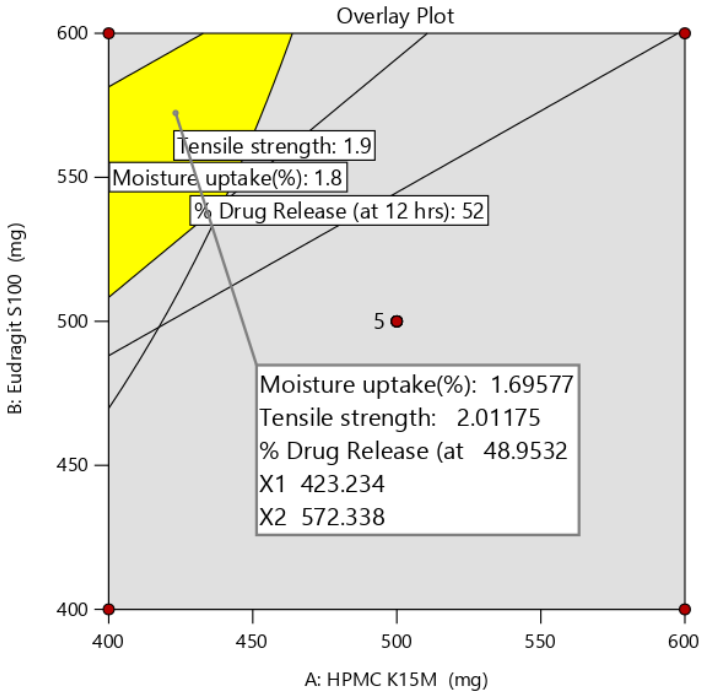

Figure 6: Overlay plot for optimization of NBH transdermal system

Table 5: Results of optimized batch of NBH transdermal patches

Response	*Predicted Value*	*Experimental Value*	*% Relative Error*
Moisture Uptake (%)	1.69	1.73 ± 0.14	-2.31%
Tensile strength	2.01	2.14 ± 0.35	-6.07%
% Drug Release	48.95	47.12 ± 2.47	3.88%

*****Results are the mean of triplicate observations ± SD

Evaluation of transdermal patch

Each trial within the central composite design underwent analysis for folding endurance, tensile strength, moisture absorption, percentage elongation, thickness, weight consistency, drug content, moisture levels, and flatness. Detailed outcomes are presented in Table 2.

Folding endurance

The folding endurance test holds paramount significance in developing transdermal delivery systems. The mechanical robustness of the film, measured through its ability to endure repeated folding without breaking, indicates its flexibility and resilience [37]. In transdermal patches, which are subjected to various degrees of manipulation during application and wear, a high folding endurance ensures the structural integrity of the delivery system. This, in turn, contributes to the reliability of the patch in maintaining a consistent drug release profile over the intended duration, thus enhancing the overall efficacy of transdermal drug delivery. The films exhibited diverse folding endurance values, ranging between 152 and 327. This parameter gauges the film's resilience against rupture. The evaluation of folding endurance was conducted manually, revealing that the films possess robust integrity, sustaining general skin folding without breaking. Refer to Table 2 for a presentation of the folding endurance results.

Tensile strength

The strength of the films varied between 1.13 and 2.19 kg/cm^2, indicating their tensile strength and susceptibility to cracking. However, no cracking was observed in the prepared transdermal films, possibly due to the inclusion of 10% triethyl citrate as a plasticizer. Tensile strength data are presented in Table 2. Higher amounts of HPMC K15M in the film correlated with increased tensile strength. An increase in Eudragit S100 content in the polymer blend also led to a corresponding increase in tensile strength [38].

Elongation (%)

Assessing the elongation percentage of transdermal films is crucial in formulation development, as it directly impacts their mechanical properties and overall performance [39]. Films with adequate elasticity can conform to the skin's contours, ensuring better adhesion and comfort for the user. Additionally, elasticity is essential for preventing film brittleness, which can compromise drug delivery efficacy and patient compliance. By evaluating elongation percentage, formulators can optimize formulations by selecting suitable plasticizers and polymers to achieve the desired flexibility and durability, thus enhancing the efficacy and acceptability of transdermal drug delivery systems. The films' elongation percentage ranged from 2.16% to 4.12%, indicating varying degrees of elasticity and susceptibility to brittleness. This critical characteristic was assessed to determine the films' flexibility and durability, particularly in transdermal applications. Adding the plasticizer triethyl citrate significantly enhanced the films' elasticity, as evidenced by the results presented in Table 2. Moreover, incorporating Eudragit led to

increased elongation in the transdermal formulations, highlighting its role in enhancing film flexibility and performance.

Thickness

Determining film thickness plays a critical role in transdermal film formulation as it directly influences drug delivery kinetics, patient comfort, and product performance [40]. Optimal film thickness ensures proper adherence to the skin, facilitating efficient drug absorption while minimizing discomfort for the user. Additionally, uniform film thickness is essential for maintaining dosage consistency and ensuring reproducibility across batches, thus enhancing product efficacy and safety. By assessing film thickness, formulation scientists can fine-tune manufacturing processes to achieve desired therapeutic outcomes and improve patient compliance. Variations in film thickness were observed due to varying concentrations of polymers, ranging from 0.23 to 0.37 mm. Table 2 provides the corresponding values for all formulations. The consistently low standard deviation suggests physical uniformity across the films, while the minimal coefficient of variation further supports this uniformity. The films' thin nature makes them aesthetically pleasing and meets acceptability standards. This underscores the suitability, reproducibility, and consistency of the film preparation process, ensuring minimal variability in the final product.

Weight uniformity

The film weights ranged from 50.53 to 58.56 mg across formulations, as detailed in Table 2. The consistently low standard deviation values suggest a uniform dispersion of polymers throughout the films, ensuring consistent quality and performance.

Drug content

Ensuring content uniformity in transdermal film formulations is crucial for maintaining consistent drug delivery. Spectrophotometric analysis at a specific wavelength allows for accurate quantification of drug content, aiding in assessing the quality and uniformity of the formulation. A uniform drug distribution throughout the film ensures reliable and predictable release kinetics, enhancing therapeutic efficacy and patient safety [41]. Additionally, it enables regulatory compliance by ensuring that each formulation unit delivers the intended dose, contributing to the overall success and acceptance of transdermal drug delivery systems. The drug concentration in all formulations was assessed using spectrophotometry at 282.5 nm, ranging from 96.74% to 98.03%. Minimal standard deviation implied a homogeneous dispersion of the drug within the films. Results detailing content uniformity are provided in Table 2.

Moisture content

Moisture content analysis is crucial in developing transdermal delivery systems as it assesses the presence of water within the film or patch. Excessive moisture content can lead to instability, degradation of active ingredients, and compromised performance of the transdermal delivery systems [42]. By accurately measuring moisture content, developers can ensure transdermal formulations' quality, stability, and efficacy, ultimately enhancing patient safety and treatment outcomes. The results of the conducted experiments in Table 2 demonstrate a slight variation in moisture content across different formulations. Notably, an increase in the hydrophilic polymer HPMC K15M led to increased moisture content within the matrix transdermal films. Despite these fluctuations, the overall moisture content of the prepared transdermal films remained low. This attribute is significant as it contributes to the stability of the formulations, preventing excessive drying and reducing the risk of brittleness during ambient storage. By monitoring moisture levels, formulation developers can optimize product stability and enhance the shelf-life of transdermal films, ensuring their efficacy and integrity over time.

Flatness

In transdermal film formulation, flatness studies play a crucial role in assessing the quality and performance of the product. By evaluating the uniformity of weight distribution and polymer consistency, these tests provide insights into the film's structural integrity and its ability to maintain a smooth surface over time. This is particularly significant as any irregularities or inconsistencies in the film's surface could affect its efficacy and patient comfort during application, highlighting the importance of meticulous quality control measures in transdermal drug delivery systems. Flatness assessments were conducted to evaluate this quality. Table 2 displays the flatness study results indicating minimal disparity in strip lengths pre- and post-cutting, suggesting uniform polymer distribution across the transdermal films. Nearly 100% flatness was observed in all formulated films, indicating minimal contraction, and ensuring a smooth, uniform surface upon skin application.

Stability testing

The stability investigations were carried out using an optimized formulation of Nebivolol hydrochloride film. The effects of temperature and humidity on the physicochemical parameters of formulations held for six months were also investigated. Table 6 shows the results for all physicochemical characteristics, such as moisture content (%), tensile strength (kg/cm^2), and percentage of drug release after the stability period. The data of the improved formulation after the stability time was found to be almost identical to those of the film before the stability period and rather stable, as demonstrated by stability studies done by ICH criteria. As a result, the stability research shows that the formulation is quite stable at accelerated settings [15].

Table 6: Stability studies of optimized NBH film

Parameters	Storage Periods		
	At initial time	3 months	6 months
Moisture Content (%)	1.73 ± 0.14	1.69 ± 0.15	1.74 ± 0.64
Tensile strength (kg/cm^2)	2.14 ± 0.35	2.05 ± 0.67	2.34 ± 0.37
% Drug Release	47.12 ± 2.47	46.73 ± 3.04	47.78 ± 2.21

* All results are the mean of three observations ± SD

CONCLUSION

Medicated transdermal films of Nebivolol hydrochloride can be prepared from blends of HPMC K15M and Eudragit S100 showed good mechanical performance. When high mechanical performance is required, a higher amount of Eudragit S100 in the blends has to be used. An in vitro drug release profile indicates that the drug release is sustained by increasing the amount of Eudragit S100 in the blends. Moreover, a general conclusion that can be drawn is that the selection of a particular blend formulation can vary the diffusion of the drug significantly.

It may also be concluded that adding plasticizer to the HPMC K15M/ Eudragit S100 systems could be a promising approach for altering drug diffusion. HPMC K15M/ Eudragit S100 polymer blends could potentially formulate TDDS as they have a good film forming property and mechanical strength. However, these systems' pharmacodynamic and pharmacokinetic evaluation in animals and human volunteers is necessary to confirm these findings.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

P.V. Shelke conceptualized and designed the research and experimental work, including formulation development and optimization of the transdermal delivery. Punit R. Rachh contributed significantly to the supervised project and provided critical insights into the methodology and data interpretation. S.D. Mankar played a key role in data collection, analysis, and interpretation, ensuring the accuracy and reliability of the experimental results. Prasad L. Gorde contributed to the manuscript, including drafting and revising the content for intellectual clarity and scientific accuracy. All authors have reviewed and approved the final version of the manuscript, demonstrating their collective effort and expertise in advancing the research on transdermal delivery systems for Nebivolol Hydrochloride.

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