Applied Biological Research 26(3): 538-551; (2024)
DOI: 10.48165/abr.2024.26.01.62

A COMPREHENSIVE DESIGN EXPERT SCREENING APPROACH FOR OPTIMIZING THE TRANSDERMAL DONEPEZIL DELIVERY IN ALZHEIMER'S DISEASE MANAGEMENT

Bariki Rajasekhar[1*] and Haranath Chinthaginjala[2]

[1]*Department of Pharmaceutical Sciences, JNTUA, Ananthapuramu – 515 001, Andhra Pradesh (India)*
[2]*Department of Pharmaceutics, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)-Autonomous, K. R. Palli Cross, Ananthapuramu – 515 721, Andhra Pradesh (India)*
*e-mail: *barikirajasekhar@gmail.com*

(Received 15 June, 2024; accepted 9 December, 2024)

ABSTRACT

Transdermal patches offer advantage to the absorption of systemic Donepezil (DPZ) without undergoing hepatic first-pass metabolism and yields inactive Donepezil N-oxide and other hydroxylated metabolites. They enable controlled and continuous discharge of medication into the bloodstream through intact skin over an extended period. This study was aimed to develop and characterize DPZ transdermal patches using a central composite design. The patches were prepared with PVP K30, HPMC K15, and modified chitosan by solvent-casting tactic. Design Expert software-generated central composite design was used to analyse the impact of independent variables on the response. FTIR analysis was performed to examine DPZ-excipient interactions. Donepezil transdermal patches (DTDP) formulations showed strong physicochemical properties, including appearance, thickness, uniformity of weight, folding endurance (FE), elongation at break, DPZ content, moisture content, and tensile strength. Among them, DTDP-11 showed highest DPZ release at 8, 16, and 24 h. The patches were flexible, with a breakpoint at 161-folds. Tensile strength across all batches was between 0.422 and 0.433 mg cm^{-2} h^{-1}, and moisture content was acceptable. *In vitro* testing confirmed consistent DPZ permeation across the patches, with DTDP-11 performing best, indicating that DTDP-11 improved DPZ discharge. The study concluded that using HPMC K15 (125 mg) and PVP K30 (40 mg) with modified chitosan (65.9 mg) effectively facilitate the permeation for the extended period and flexibility of DPZ transdermal patches.

Keywords: Design, donepezil, flexibility, permeation, transdermal patches

INTRODUCTION

Transdermal patches offer a unique and effective Donepezil (DPZ) delivery method as compared to the traditional dosage forms like oral tablets, injections, and topical creams. They enable DPZs to be absorbed directly into the bloodstream through skin, bypassing the gastrointestinal tract and hepatic first-pass metabolism (Zhang *et al.,* 2022). This enhances the bioavailability of DPZ and reduces side effects. Transdermal patches provide controlled and sustained DPZ release, maintaining steady plasma levels, which is particularly beneficial for DPZs requiring consistent plasma concentrations, unlike the oral tablets which can cause fluctuating DPZ levels due to variable absorption rates. This delivery method also minimizes gastrointestinal side-effects and is non-invasive, making intake easier for the patients who face difficulty in swallowing pills or who avoid injections, thereby improves compliance (Siafaka *et al.,* 2020). Additionally, adhesion issues, complexity and manufacturing cost of transdermal patches pose big challenges. Oral tablets are the most common dosage form due to

their ease of manufacture and convenience, yet they suffer extensive first-pass metabolism and gastrointestinal degradation. Injectables provide rapid and complete DPZ absorption but are invasive and often painful, requiring professional administration. Transdermal patches offer a non-invasive alternative with sustained discharge, although they are unsuitable for DPZs needing immediate effects. Topical creams used for local DPZ delivery are generally not intended for systemic effects, making them less effective at maintaining consistent plasma DPZ levels as compared to the transdermal patches.

DPZ is a centrally-acting acetylcholinesterase inhibitor primarily used to treat Alzheimer's disease (AD) by elevating cortical acetylcholine levels. AD is most prevalent type of senile dementia, impacting 6-8% of individuals over the age of 65 yr and nearly 30% of those over 85 yr (Mohamed *et al.,* 2019). By inhibiting the enzyme acetylcholinesterase, DPZ enhances cholinergic function, typically deficient in Alzheimer's patients. This action potentially improves cognitive function and slows the progression of symptoms associated with the disease, offering therapeutic benefits to individuals suffering from AD (Alzheimer's Association, 2019). Multiple experimental observations suggest that DPZ is an excellent candidate for transdermal patches designed to deliver controlled discharge formulations (Jyothika *et al.,* 2022). A matrix polymer combination of PVP K30, HPMC K15, and modified chitosan was used to effectively regulate the discharge of DPZ, demonstrating the potential of this delivery system to enhance the therapeutic management of AD by consistent and controlled discharge of medication over time (Adlimoghaddam *et al.,* 2018).

The traditional research methods often focus on studying the influence of one variable at one time due to the feasibility of manipulating variables in isolation and the ability to control and observe specific effects (Pandey and Pandey, 2021). This approach is preferred as it simplifies statistical analysis and ensures that the effects observed are attributable to the variable under study. However, in real-world systems interactions between multiple variables are frequently encountered. Examining the variables independently may overlook these interactions, potentially leading to misleading or incomplete conclusions. Therefore, a design of experiments (DOE) approach is essential for multivariate analysis, as it allows the simultaneous study of multiple variables and their interactions (Lee *et al.,* 2022). This comprehensive method enhances the accuracy and reliability of results, providing a more holistic understanding of the system under study. The present study was aimed to develop and characterize DPZ transdermal patches using a central composite design. The patches were formulated with PVP K30, HPMC K15, and modified chitosan to enhance their properties and effectiveness. The central composite design (developed with the aid of Design Expert software-11 trail) (*https://www.statease.com/software/design-expert/*) facilitated the systematic assessment of the effects of these polymers on discharge profile and mechanical properties of patches (Kusuma *et al.,* 2024). The objective was to achieve a controlled discharge formulation that could effectively deliver DPZ through skin, potentially reduce the side effects associated with oral administration, and improve the patient's compliance.

MATERIALS AND METHODS

Materials

DPZ was supplied by Intas Pharmaceuticals, Ltd., Secunderabad (India) while polymers (HPMC K15, modified chitosan, and PVP K30) were procured from SD Fine Chemicals, Mumbai, India. The cellulose acetate membrane required for transdermal patches was supplied by Chemtech International, India. All other chemicals and materials used in the study were of pharmaceutical grade.

Calibration curve of donepezil

To create a calibration curve for donepezil, a stock solution was prepared by dissolving 10 mg DPZ in 10 mL methanol to achieve a concentration of 1,000 µg mL^{-1}. Then a series of standard solutions

were prepared with the concentrations ranging from 10 to 100 µg mL^{-1}. The absorbance of each solution was measured by using a UV-Vis spectrophotometer (Scimadzu-1900) at 271 nm wavelength, using the solvent as a blank. The absorbance was plotted against concentration to get a linear regression curve, ensuring a correlation coefficient (R^2) of ≥ 0.99 for strong linearity. This validated calibration curve was used to measure the concentration of DPZ in unknown samples. All the measurements were taken in triplicate for accuracy, following the safety protocols for chemical handling and UV-Vis spectrophotometer use (Prabhu *et al.,* 2021).

Preparation of transdermal patches

Transdermal patches containing DPZ were prepared using solvent-casting method (Suksaeree *et al.,* 2021). Three polymers, HPMC K15, PVP K30, and modified chitosan, were separately dissolved in ethanol-dichloromethane (DCM) 2:1 mixture in various proportions. The mixtures were stirred with a magnetic stirrer for 30 min, followed by the addition of propylene glycol. DPZ was then incorporated into the mixtures under continuous stirring and the resulting polymeric solutions poured into petri dishes and allowed to dry at room temperature for 6 h. After drying, 1 x 1 cm^2 patches were cut, wrapped in aluminium foil, and stored in desiccators for subsequent analysis (Ahad *et al.,* 2016).

Central composite design was used to evaluate 15 different blends using Design Expert software (version 11-trail) (*https://www.statease.com/software/design-expert/*) for the analysis of DPZ permeation (DP) at various time intervals (DP @ 8, 16, and 24 h). Significant factors influencing the regression analysis were identified through stepwise forward and backward elimination. The factors showing insignificant effects ($p > 0.05$) on dependent variables were excluded from the final model. The different formulae of polymers, evaluated in the study, uniformly had donepezil 20 mg, dichloromethane 20 mL, and propylene glycol 2 mL, besides the other variable as under:

Formulations	HPMC K15 (mg)	Modified chitosan (mg)	PVP K30 (mg)
DTDP-1	100	100	30
DTDP-2	150	100	30
DTDP-3	100	200	30
DTDP-4	150	200	30
DTDP-5	100	100	50
DTDP-6	150	100	50
DTDP-7	100	200	50
DTDP-8	150	200	50
DTDP-9	82.95	150	40
DTDP-10	167.04	150	40
DTDP-11	125	65.91	40
DTDP-12	125	234.09	40
DTDP-13	125	150	23.18
DTDP-14	125	150	56.82
DTDP-15	125	150	40

Compatibility studies

Each formulation was mixed with dry potassium bromide (KBr) powder and compressed into thin, transparent pellets. These pellets were then scanned using an FTIR spectrophotometer (Bruker IFS 125HR) in the range of 4000-400 cm^{-1}, with appropriate resolution settings, typically at 4 cm^{-1}. The recorded spectra were analysed by comparing the characteristic peaks of pure DPZ and excipients with those of physical mixtures (Magalhães *et al.,* 2021). Significant shifts, disappearance of peaks, or appearance of new peaks in the mixture's spectra indicated potential interactions. For instance, DPZ, when analysed with HPMC K15, PVP K30, and modified chitosan, should maintain its characteristic peaks without significant changes if no interactions occurred. This method provided a non-destructive way to evaluate compatibility, aiding in the formulation of stable and effective dosage forms.

Physical appearance

The colour, clarity, flexibility, and smoothness of all donepezil transdermal patch (DTDP) samples were assessed visually. The thickness of DTDP was measured using a micrometer (H.L. Scientific Industries, Ambala, India). The measurements were performed three times, and average values calculated to obtain a more accurate representation of thickness (Alhaushey and Ahmad, 2023). Visual inspection allows the qualitative assessment of DTDP samples, with colour referring to the perceived

hue, clarity to its transparency or lack of opaqueness, flexibility to the material's ability to bend or deform without breaking, and smoothness to the absence of rough or uneven surfaces. In contrast, the micrometer quantitatively showed the thickness of DTDP samples by measuring the distance between two parallel surfaces with high accuracy. The categorization of physical appearance as "good" or "excellent" for transdermal patches was based on a set of observable criteria, with emphasis on aesthetic and functional attributes. The key factors included surface uniformity, with "excellent" patches displaying a smooth, consistent surface free from bubbles, wrinkles, or particles, while "good" patches had minor, non-functional irregularities. Transparency or opacity in "excellent" patches was consistent, meeting the intended appearance precisely; whereas "good" patches showed slight variation. Further, "excellent" patches showed strong adhesive integrity without edge-lifting or peeling; while "good" patches tolerated minimal inconsistencies. Flexibility and texture distinguished these ratings, as "excellent" patches were uniformly pliable and durable, while "good" patches were slightly less flexible but still sufficient for application. Additionally, the colour consistency was critical and relevant, with "excellent" patches maintaining throughout uniform colour while "good" patches allowed minor acceptable variations. These standards ensured that the patches were both visually appealing and functionally reliable for effective application and performance.

Uniformity of weight

Each DTDP sample underwent individual weighing, and the average weight of five DTDP samples from each batch was determined. This gave a standardized assessment of DTDP sample weights within a batch (Ahad *et al.,* 2011; Jajoo *et al.,* 2023).

Folding endurance

To acquire insights into the mechanical characteristics and endurance of DTDP film, the DTDP film underwent repeated folding at the designated cutting location to evaluate its resilience to multiple pleats without any interruptions or failures. The process entailed folding the film at a precise spot and then unfolding it to its original state. This folding and unfolding sequence was reiterated many times, consistently positioning the film at the same location. The objective was to assess the maximum capacity of film to endure pleating at that specific spot without experiencing tearing or breakage (Ashfaq *et al.,* 2024). Throughout the experiment, the film underwent visual inspection after each pleating cycle to detect any damage or failure. The criteria for identifying interruptions or failures included visible tears, cracks, or structural deformations that signal the film's inability to withstand further pleats. The understanding of film's resistance to fold provides valuable knowledge for enhancing product durability and performance in practical applications.

Tensile strength

To provide support to DTDP when placed inside a patch holder, an adhesive tape was affixed at one end of DTDP. On its opposite end, a small pin was inserted into the adhesive tape to maintain its straight alignment as it expanded. Additionally, the adhesive tape was punctured in the region where the hook was inserted (Shravani *et al.*, 2021; Pandey and Gupta, 2023). To measure the tensile strength of DTDP, a pulley system was used. One end of the thread was attached to a small pin, which was then passed over a pulley. Weights were attached to the other end of thread to increase the pulling force on DTDP. As the weights were gradually added, the pointer moved along the thread, tracing its path on the baseplate of a graph paper. The breaking force of DTDP was determined by the amount of weight required to cause it to break.

Moisture content

The DTDP samples were stored at room temperature in desiccators containing $CaCl_2$ to uphold a low-humidity environment and the weights of DTDP samples were assessed at predetermined intervals. The samples were initially weighed while wet and subsequently the samples were dried to remove moisture. Once the weight stabilized across consecutive weighing, the samples were deemed to be in their dry state (Dinh *et al.,* 2022). The difference between wet and dry weights gave the moisture

content in sample. Through periodic monitoring of DTDP sample moisture content, one can evaluate the desiccators' effectiveness and appraise sample stability under specific storage conditions. Such insights were vital to maintain the quality and integrity of DTDP samples across diverse applications.

Assay for donepezil content in the patches

DTDP was segmented into 1 x 1 cm^2, and immersed into a tube containing 100 mL phosphate buffer saline (PBS). The contents were stirred for 2 h by using magnetic beads. Then contents were filtrated through Whatman filter paper (pore size 0.45 µm). This filtration step segregated solid particles or insoluble components from liquid solution. The resultant filtrates were used for subsequent spectrophotometric analysis at 271 nm wavelength (Adelman and Louis, 2023). This wavelength was chosen due to its correlation with absorbance peak or characteristic absorption band pertinent to DTDP or its constituents. The process was iterated to ensure the precision and reproducibility during spectrophotometric analysis (Kılıçaslan, 2024).

In vitro diffusion study

A Franz diffusion cell system was used to assess the diffusion of DTDP through a cellophane membrane. The receptor section of Franz diffusion cell was filled with 22 mL PBS (pH 7.4) which served as receiving medium. DTDP was firmly applied onto the center of a cellophane membrane, acting as a barrier between donor and receptor compartments. Positioned within donor compartment, DTDP-loaded cellophane membrane was aligned so that it lightly contacted the surface of receptor compartment. This arrangement facilitated the diffusion of DPZ through membrane into the receptor section. To maintain consistent experimental conditions, the entire Franz diffusion cell assembly was immersed in a water bath (32°C). Regular sampling from the receptor compartment was done at every 12 h to monitor DTDP diffusion (Nakamura *et al.*, 2023). These samples were analysed to detect the presence and concentration of DPZ. Moreover, equal volumes of buffer solution were added to each receptor cell at regular intervals to uphold a constant volume and create sink conditions conducive to DPZ diffusion. The sample analysis at specified intervals helped in quantifying the DTDP diffusion across the cellophane membrane and into the receptor compartment (Buck *et al.*, 2024). The experiment explored DPZ discharge and permeation characteristics, offering insights into the factors such as DPZ solubility, concentration gradients, and diffusion kinetics.

Statistical analysis

The data was analysed using Design Expert (version 11, trial) and Microsoft Excel 2016 so as to interpret and validate the experimental findings. Descriptive statistics, specifically mean and standard deviation, were calculated. One-way analysis of variance (ANOVA) was performed to examine any significant differences among the experimental groups at $P < 0.05$.

RESULTS AND DISCUSSION

The transdermal patches containing DPZ were prepared with varying compositions (DTDP-1 to DTDP-15) as suggested by CCD from Design Expert software. Each formulation had a fixed donepezil content (20 mg), while the quantities of HPMC K15, modified chitosan, PVP K30, dichloromethane, and propylene glycol were varied. The key formulations included a range of HPMC K15 from 82.95 mg to 167.04 mg and modified chitosan from 65.91 mg to 234.09 mg. Notably, formulation DTDP-11 exhibited a balanced composition with 125 mg HPMC K15, 65.91 mg modified chitosan, and 40 mg PVP K30, indicating optimal properties for drug release.

The transdermal patches for DPZ delivery enhance the bioavailability of medicine and patient compliance of this therapeutic agent. The formulation approach revealed that balancing the components was essential to achieve optimal drug permeation and mechanical properties. The formulations with varying ratios of HPMC K15, modified chitosan, and PVP K30 were designed to

assess their influence on the release characteristics of DPZ. HPMC K15 has gel-forming properties which contribute to the sustained drug release, while modified chitosan enhances the permeation and adhesion to the skin, thus improves the overall effectiveness of transdermal system. The observed variation in drug release profiles among the formulations is attributed to the differences in polymer concentration and composition. For instance, higher amounts of modified chitosan in DTDP-12 and DTDP-14 resulted in patches that exhibited superior permeability due to chitosan's bioadhesive properties, thus allowing enhanced skin contact and improving the drug absorption. The use of dichloromethane in formulation aids in effective dissolution of polymers, thus promotes homogeneous blend for consistent drug release. The presence of propylene glycol enhances the plasticity of patches, so make them more user-friendly while maintaining sufficient mechanical strength.

FTIR spectra were used to assess the compatibility of DPZ with polymers employed (Fig. 1). The characteristic peaks of DPZ remained unchanged when combined with various excipients, indicating non-significant interactions or chemical reactions. This preservation of DPZ's structural integrity confirmed its compatibility with the selected excipients, ensuring the maintenance of DPZ's efficacy and stability. The FTIR spectra provided clear evidence of DPZ's compatibility with the excipients, essential for developing a stable and effective pharmaceutical formulation (Fig. 1). IR spectra revealed no changes in peaks and stretches when DPZ was mixed with HPMC K15, PVP K30, and modified chitosan, indicating no physical interaction between DPZ and these polymers.

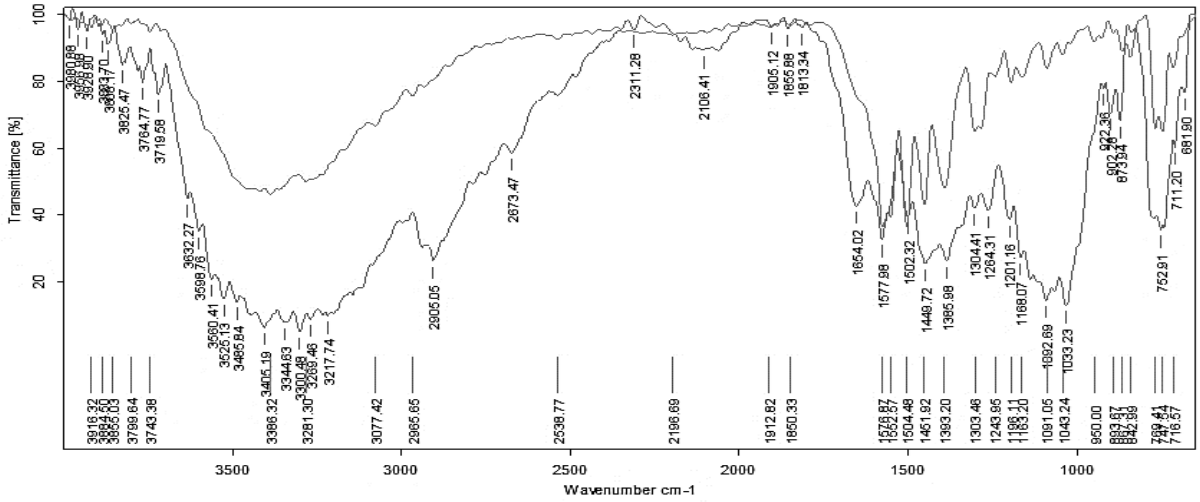

Fig. 1: FTIR spectra of DPZ and its excipients

DTDP exhibited satisfactory physical and chemical properties, including appearance, thickness, weight uniformity, folding endurance (FE), percentage elongation at break, DPZ content percentage, moisture content, and tensile strength. Specifically, DTDP-11 showed the highest DPZ discharge at 8, 16, and 24 h. In addition, they demonstrated optimal folding characteristics as they were flexible and broke after 161 folding. The tensile strength across all batches ranged from 0.422 ± 0.01 to 0.433 ± 0.02 mg cm^{-2} h^{-1}, and all batches had acceptable moisture content (Table 1).

The physicochemical characteristics of DTDP patches suggested that they were well-suited for transdermal drug delivery applications. The uniform appearance, consistent thickness, and weight across the batches reflected a controlled formulation process was essential for reliable drug delivery. The high folding endurance value indicated a flexible yet durable patch, which can withstand the physical stresses associated with wear without breaking prematurely. The range of tensile strength values suggested sufficient mechanical stability, which is crucial for maintaining patch integrity during application (Yongping *et al.*, 2018).

The superior drug release profile of DTDP-11 at multiple time points suggested that this formulation achieved a desirable controlled-release mechanism. This sustained release could support continuous therapeutic effects, reducing the need for frequent reapplication. The moisture content of

Table 1: Physicochemical of different formulations of DTDP

Formulations	Physical appearance	Thickness (mm)	Uniformity of weight (mg)	Folding endurance	Moisture content (%)	Tensile strength (mg cm^{-2} h^{-1})	Assay (%)
DTDP-1	Good	39.29 ± 0.22	704.60 ± 2.53	135 ± 2	3.3 ± 0.03	0.428 ± 0.02	93.39 ± 2.36
DTDP-2	Good	38.72 ± 0.35	706.51 ± 5.15	160 ± 5	3.2 ± 0.06	0.426 ± 0.01	94.53 ± 5.67
DTDP-3	Good	39.17 ± 2.02	718.44 ± 4.95	137 ± 3	3.3 ± 0.06	0.427 ± 0.01	94.32 ± 2.52
DTDP-4	Good	38.89 ± 1.25	711.73 ± 2.57	151 ± 2	3.5 ± 0.01	0.429 ± 0.01	96.62 ± 3.48
DTDP-5	Good	39.54 ± 1.11	709.28 ± 1.97	134 ± 5	3.6 ± 0.04	0.431 ± 0.01	97.51 ± 2.58
DTDP-6	Good	36.22 ± 0.89	702.71 ± 3.53	155 ± 4	3.4 ± 0.05	0.429 ± 0.02	96.84 ± 2.08
DTDP-7	Good	37.14 ± 0.62	702.28 ± 3.67	155 ± 2	3.1 ± 0.06	0.427 ± 0.02	96.62 ± 3.59
DTDP-8	Good	36.07 ± 0.13	718.46 ± 6.32	154 ± 2	3.2 ± 0.03	0.428 ± 0.02	94.58 ± 4.27
DTDP-9	Excellent	39.42 ± 0.80	709.21 ± 4.80	153 ± 4	3.3 ± 0.02	0.422 ± 0.01	97.21 ± 3.01
DTDP-10	Excellent	37.42 ± 0.67	705.77 ± 2.34	152 ± 4	3.3 ± 0.06	0.425 ± 0.01	95.85 ± 4.88
DTDP-11	Excellent	38.52 ± 0.50	707.59 ± 1.25	161 ± 2	3.2 ± 0.04	0.433 ± 0.02	98.23 ± 5.02
DTDP-12	Excellent	37.71 ± 0.75	707.28 ± 3.40	157 ± 2	3.1 ± 0.01	0.429 ± 0.01	96.29 ± 4.67
DTDP-13	Good	36.66 ± 0.46	704.54 ± 2.75	158 ± 3	3.2 ± 0.04	0.427 ± 0.02	94.33 ± 3.95
DTDP-14	Good	38.84 ± 0.95	708.29 ± 1.62	154 ± 3	3.1 ± 0.06	0.426 ± 0.03	92.52 ± 2.84
DTDP-15	Excellent	38.51 ± 0.82	706.48 ± 3.95	152 ± 5	3.2 ± 0.05	0.423 ± 0.02	97.74 ± 4.58

The values are mean ± SD; n = 3

patches also met the stability requirements, thereby minimize the risk of patch drying or degradation, which could impact drug release and absorption. Overall results showed that DTDP patches, especially DTDP-11, possess the necessary qualities for effective transdermal drug delivery, combining structural robustness with prolonged and steady drug permeation.

In vitro DPZ permeation

I*n vitro* DPZ permeation was conducted to assess the controlled discharge capabilities of various patches, notably DTDP-11 formulation which comprised of HPMC (125 mg), PVP K30 (40 mg), and modified chitosan (16.91 mg), exhibited superior DPZ discharge as compared to the other formulations. This suggested that the specific combination of ingredients (as in DTDP-11) contributed to its enhanced performance in facilitating the DPZ discharge. The amount of drug permeated was assessed from the calibration curve (Fig. 2). Results revealed consistent DPZ permeation across all the patches (Fig. 3). The *in vitro* permeation study showed the effectiveness of DTDP patches in delivering DPZ in a controlled manner. The consistent permeation across the formulations underscored the reliability of patch matrix in maintaining the drug release rates. Notably, the superior discharge performance of DTDP-11 is attributed to its specific composition. HPMC provides a stable, hydrophilic matrix, while PVP K30 enhances patch adhesion and drug dispersion. Modified

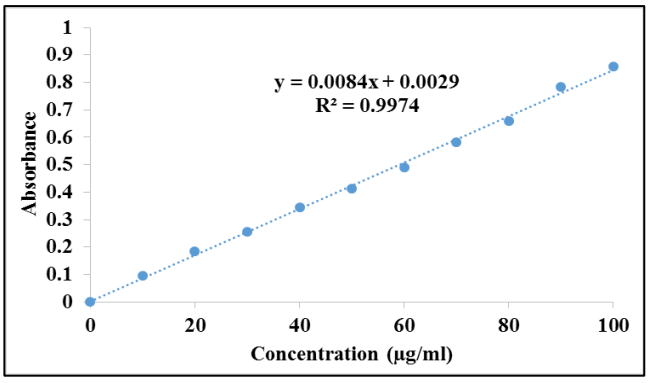

Fig. 2: Calibration curve of Donepezil showing linearity

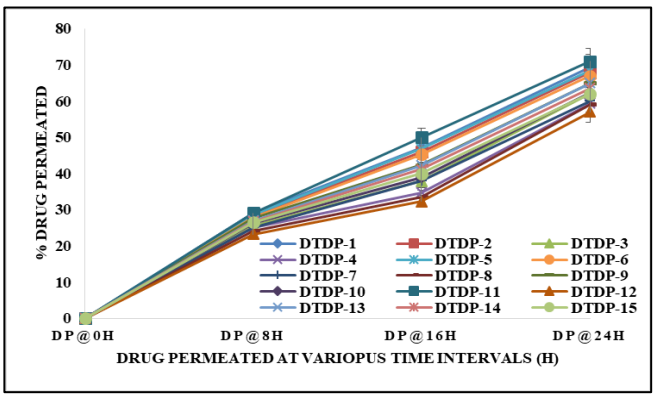

Fig. 3: *In vitro* drug DPZ permeation from the patches

chitosan, known for its muco-adhesive and permeation-enhancing properties, may have further facilitated the DPZ diffusion across the membrane, contributing to the observed sustained release. The synergistic effect of these ingredients in DTDP-11 optimize DPZ permeation, thus suggesting it to be a promising formulation for achieving controlled drug delivery in transdermal applications.

Model fit summary

Analysis of responses (DP @ 8 h, DP @ 16 h, and DP @ 24 h) using Design Expert software yielded the fit summary (Table 2) and ANOVA details (Table 3). It revealed that reducing the number of terms in model can be advantageous if it contains many insignificant terms, provided the hierarchy is maintained. The final equation for the responses was as follows:

$DP@8h = +26.49 - 0.356A - 1.74B - 0.182C - 0.0337AB - 0.044AC - 0.068BC + 0.1675A^2 - 0.117B^2 + 0.115C^2$

$DP@16h = +40.01 - 1.17A - 5.18B - 0.294C - 0.611AB - 0.224AC + 0.004BC + 0.213A^2 + 0.386B^2 + 0.572C^2$

$DP@24h = +61.99 - 0.807A - 4.06B - 0.491C - 0.107AB + 0.262AC - 0.0725BC + 0.601A^2 + 0.721B^2 + 0.825C^2$

(Where A: HPMC K15; B: Modified chitosan; C: PVP K30)

The results of DP analysis highlight the effectiveness of quadratic model in capturing the effects of formulation factors on drug permeation over time. By removing insignificant terms while retaining the model's hierarchy, the final equations simplify the interpretation of each component's role in permeation at different time points, aiding in targeted formulation optimization. The interaction and quadratic terms revealed the complex relationships among formulation components, where interactions like AB and AC (representing various combinations of factors) affect permeation differently across time intervals. Specifically, the presence of quadratic terms (A^2, B^2, and C^2) suggests non-linear responses to changes in each component, indicating that optimal performance may be achieved within a particular range of each factor (Somadasan *et al.*, 2024).

Table 2: Model fit summary and analysis of drug permeation efficiency in prepared transdermal patches across 8, 16, and 24 h intervals

Sources	Sequential p-value	Adjusted R^2	Predicted R^2
Response 1: DP@8h			
Linear	< 0.0001	0.9716	0.9578
2FI	0.9084	0.9633	0.9474
Quadratic	0.0291	0.9890	0.9633
Cubic	0.2000	0.9990	0.9781
Response 2: DP@16h			
Linear	< 0.0001	0.9802	0.9721
2FI	0.0771	0.9879	0.9840
Quadratic	0.0215	0.9968	0.9896
Cubic	0.1754	0.9998	0.9951
Response 3: DP@24h			
Linear	< 0.0001	0.9697	0.9661
2FI	0.7845	0.9633	0.9492
Quadratic	0.0074	0.9937	0.9774
Cubic	0.1870	0.9995	0.9891

The consistency between observed and predicted data confirmed the model's reliability, making it a valuable tool for formulating transdermal patches with precise release profiles at 8, 16, and 24 h. This approach provides insight into how specific the amounts of ingredients can be adjusted to achieve sustained and controlled drug release, and enhance the therapeutic effectiveness of transdermal delivery systems.

The model F-values of 140.46, 483.15, and 246.60 indicated significant models, with only 0.01% chance of these values occur due to noise. P-values < 0 .05 marked significant model terms: A, B, and C in the first model; A, B, C, AB, B^2, and C^2 in the second; and A, B, C, A^2, B^2, and C^2 in the third. Values > 0.10 denoted insignificant terms, suggesting that model reduction may enhance the models if many insignificant terms are present.

The high F-values across all three models underscore the statistical significance and reliability of models in predicting the permeation responses at different time intervals. This significant predictive ability, coupled with minimal noise interference, confirms the robustness of model structure. Notably, the presence of interaction terms (e.g., AB) and quadratic terms (e.g., B^2 and C^2) in 16 h and 24 h models suggests complex relationships among the factors that influence drug permeation. For instance, the interaction effects observed in 16 h model indicate that combinations of formulation components

have a greater impact on permeation at this stage, while quadratic terms in 24 h model suggest non-linear responses, where optimal performance may require fine-tuning of individual component levels. Model reduction by eliminating non-significant terms may improve model simplicity without sacrificing its predictive power, thereby making it more efficient for practical applications in transdermal patch optimization (Khan *et al.*, 2020).

The ANOVA analysis of drug permeation rates at 8, 16, and 24 h for transdermal patch formulations revealed high F-values, indicating the significance of quadratic models at each time interval. For drug permeation at 8 h (DP@8h), the model yielded an F-value of 140.46 and a p-value of < 0.0001, highlighting a very strong significance in the model's predictive ability. Similarly, at 24 h mark (DP@24h) an exceptionally high F-value of 246.6 and a p-value of < 0.0001 suggested strong reliability of model in predicting permeation outcomes. For 16 h interval (DP@16h), the model showed an F-value of 24.28 with a p-value of 0.0124, indicating moderate significance but still affirming the model's capacity to describe permeation trends effectively (Table 3). ANOVA results emphasize the quadratic model's strength in explaining the drug permeation dynamics at different time intervals. The high F-values for DP@8h and DP@24h, coupled with p-values < 0.0001, indicate that these models are robust, and accurately reflect the underlying processes influencing the drug release at these stages. These strong correlations suggest that the formulation components and their interactions are well-accounted for in the model, especially at 8 and 24 h. The moderate F-value of 24.28 at 16 h mark, while significant (p = 0.0124) indicates that the model may capture slightly less variance in the drug release process at this stage compared to the other intervals. This could be due to changes in drug permeation rate over time, which might reflect shifts in the formulation's structural or compositional influences (Padmaja and Rani, 2024). Overall, the ANOVA findings validated the quadratic model as a highly effective tool for predicting drug permeation, especially at early and late stages, providing insights for further optimization of transdermal patch formulations.

Table 3: ANOVA analysis using a quadratic model to evaluate drug permeation rates in transdermal patches at 8, 16, and 24 h intervals

Response	F-value	p-value
DP@8h	140.46	< 0.0001
DP@16h	24.28	0.0124
DP@24h	246.60	< 0.0001

The residual vs. predicted plots (Fig. 4A, 3C, and 3E) demonstrated a strong correlation between observed and predicted values for drug permeation at 8, 16, and 24 h, indicating that the model accurately describes the permeation data. The residuals were distributed randomly around zero line, with no clear patterns or trend, supporting the model's reliability and lack of bias. Cook's distance plots (Fig. 4B, 3D, and 3F) showed no points of excessive influence, confirming that no individual data points disproportionately affected the model outcomes. This implied that the effects of HPMC K15, modified chitosan, and PVP K30 on drug permeation were consistently accounted for across all time intervals without any outlier influence.

The residual vs predicted plots suggest that the quadratic model provided a good fit for drug permeation data at 8, 16, and 24 h, with minimal discrepancies between observed and predicted values. This alignment indicated that the combination and concentration of HPMC K15, modified chitosan, and PVP K30 are well captured by the model, making it robust for predicting drug permeation behaviour over time. Additionally, Cook's distance analysis highlights that no single data point unduly impacted the model, enhancing confidence in the observed effects of formulation components (Kriplani *et al.*, 2021). This uniformity across the plots underscores that the chosen concentrations of HPMC K15, modified chitosan, and PVP K30 contribute consistently to drug permeation, affirming their roles in modulating the release rates stably and predictably. These results reinforce the validity of model and suggest its suitability for further optimization of transdermal patch formulations.

The 2D (Fig. 5A, 4C, and 4E) and 3D response surface plots (Fig. 5B, 4D, and 4F) illustrate the effects of HPMC K15, modified chitosan, and PVP K30 on drug permeation at 8, 16, and 24 h. The 2D plots revealed clear gradients revealing that as the concentrations of these components vary the drug permeation rates change significantly. Increasing the HPMC K15 and PVP K30 concentrations

generally correlates with enhanced drug permeation at all-time intervals, while modified chitosan showed a more complex effect depending on its concentration and the interactions with other components. The 3D plots provide further insight, visually emphasizing the interactive effects of these excipients on drug permeation. For instance, at higher concentrations of HPMC K15 and PVP K30,

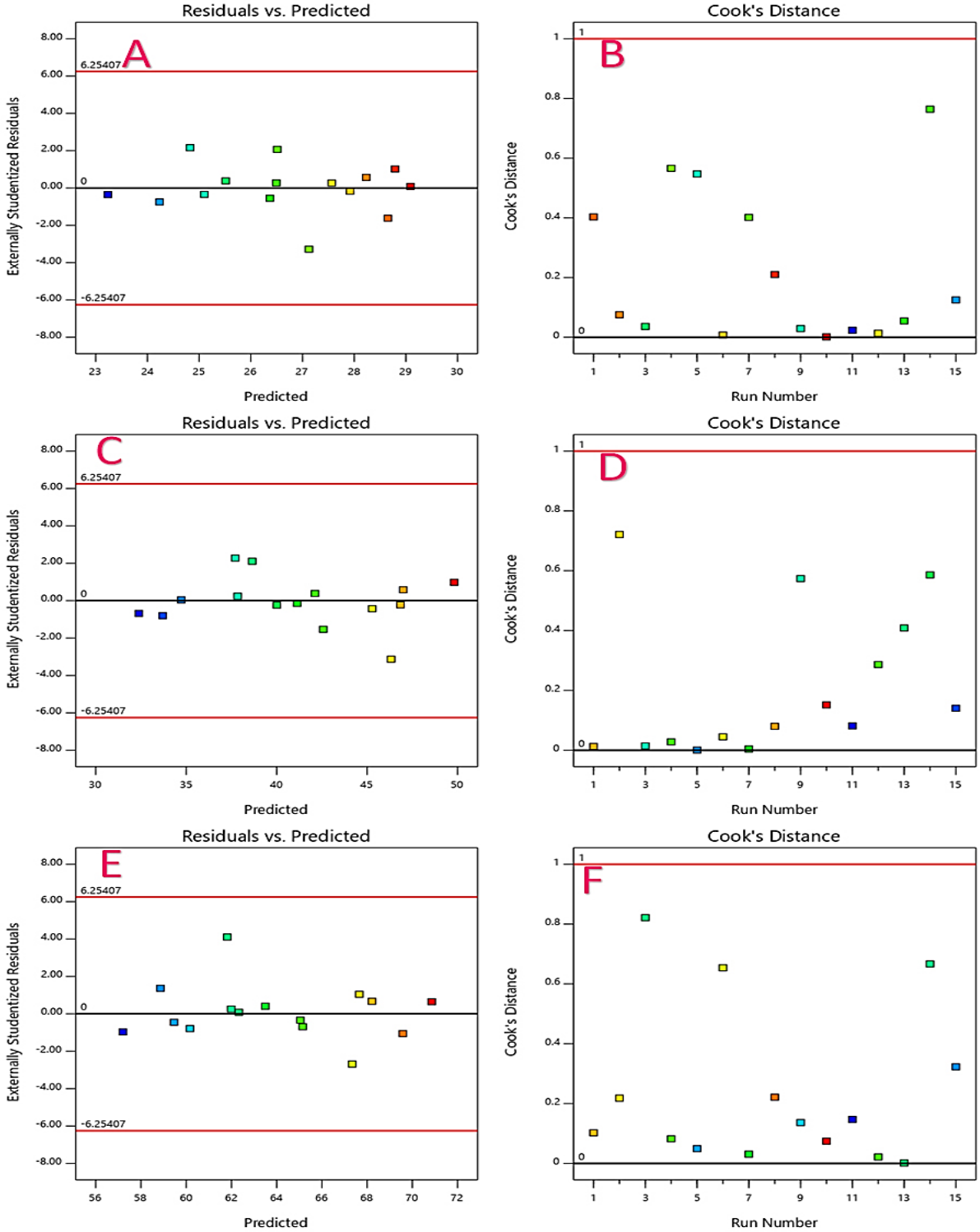

Fig. 4: Residual vs. predicted (A, C & E), Cook's distance (B, D & F) showing the effect of inputs on the response

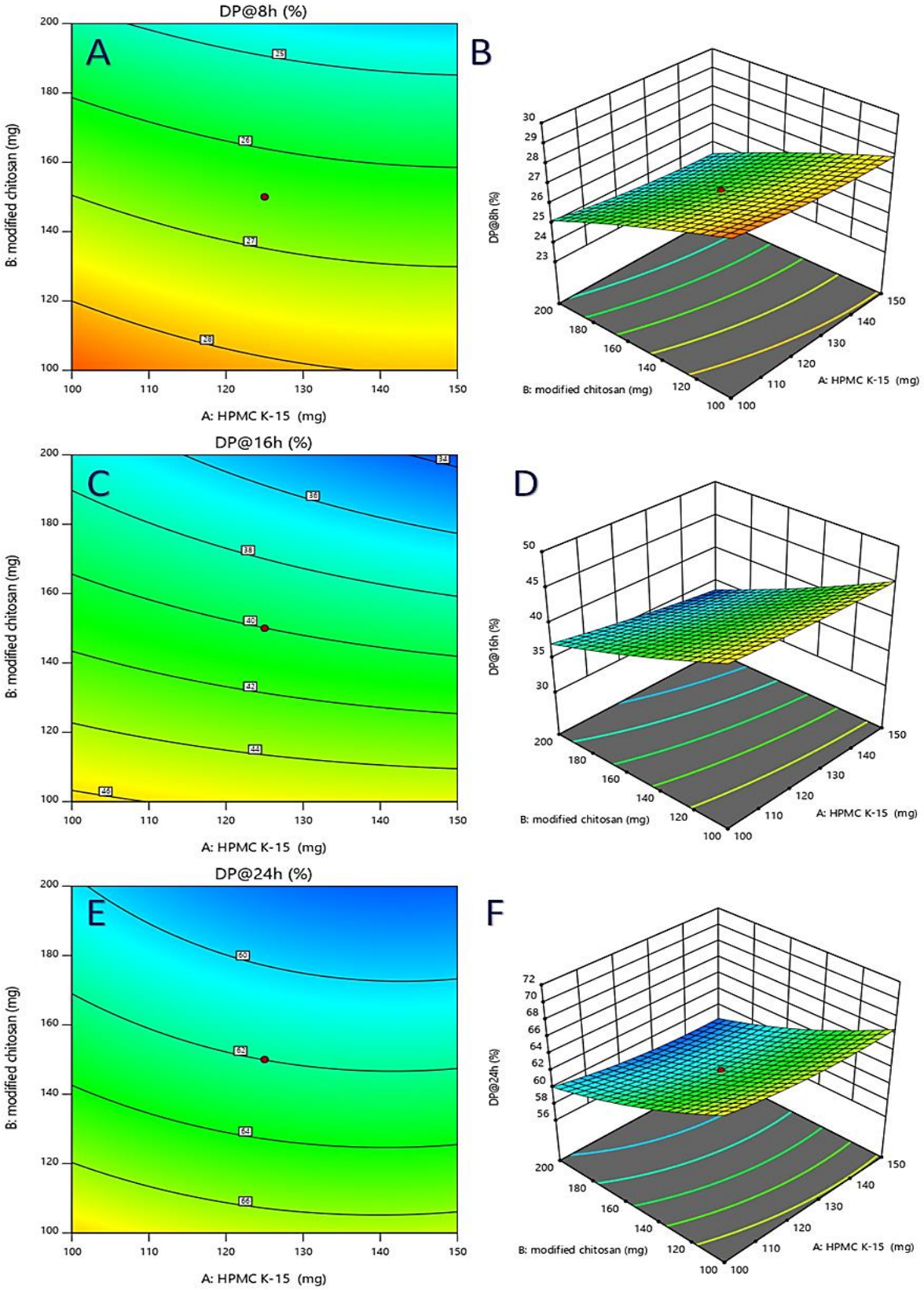

Fig. 5: Response surface plots 2D (A, C & E) and 3D (B, D & F) indicate the effect of inputs on the responses

drug permeation at 8 and 24 h appears maximized, while the effect of modified chitosan is more pronounced at intermediate levels.

The response surface plots highlight the interactive influence of HPMC K15, modified chitosan, and PVP K30 on the drug permeation rates across time. The gradients in 2D plots indicate that each component significantly contributes to permeation, with varying effects over different time intervals. HPMC K15 and PVP K30, known for enhancing drug matrix structure and stability, appear to promote controlled permeation effectively. The role of modified chitosan, which fluctuates based on its concentration and interaction with HPMC and PVP, suggests that its mucoadhesive and permeation-enhancing properties are dose-dependent, potentially modulating release rates differently across time intervals (Amarachinta *et al.*, 2021).

The 3D plots allow for comprehensive visualization of these effects, showing the synergy between HPMC K15 and PVP K30, particularly at higher concentrations, which results in maximized drug release at 8 and 24 h. This implies that adjusting these excipients in tandem can be strategically utilized to control the drug permeation rate for specific therapeutic needs. Overall, the response surface analysis underscores the importance of carefully balancing these formulation components to achieve desired permeation profiles, providing a valuable guide for optimizing transdermal patch formulations (Shiyan *et al.*, 2021).

Conclusion: The transdermal DPZ delivery system mentioned combines HPMC K15, PVP K30, and modified chitosan which demonstrated the potential for controlled discharge of DPZ (presumably a DPZ) from matrix-type transdermal patches. HPMC K15 and PVP K30 are commonly used excipients in pharmaceutical formulations. HPMC K15 is a cellulose derivative that acts as a matrix former and can control DPZ discharge rates. PVP K30 and modified chitosan enhanced DPZ solubility and bioavailability. By incorporating these polymers, the transdermal DPZ delivery system aims to achieve controlled discharge of DPZ through matrix-type transdermal patches. Matrix-type transdermal patches release the DPZ from a polymer matrix in direct contact with the skin. The combination of HPMC K15, PVP K30, and modified chitosan in the transdermal DPZ delivery system offers the potential for an extended discharge of DPZ through the skin over a desired period.

Funding: This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement: This study did not involve any animal subjects.

REFERENCES

Adelman, M. and Louis, L. 2023. A novel formulation: Donepezil patch. *The Senior Care Pharmacist*, **38**(7): 300-304.

Adlimoghaddam, A., Neuendorff, M., Roy, B. and Albensi, B.C. 2018. A review of clinical treatment considerations of donepezil in severe Alzheimer's disease. *CNS Neuroscience and Therapeutics*, **24**(10): 876-88.

Ahad, H.A., Ishaq, B.M., Shaik, M. and Bandagisa, F. 2016. Designing and characterizing of tramadol hydrochloride transdermal patches prepared with *Ficus carica* fruit mucilage and povidone. *Pakistan Journal of Pharmaceutical Sciences,* **29**(3): 945-951.

Ahad, H.A., Kumar, C.S., Anuradha, C. and Reddy, K.K. 2011. A novel mucilage from *Ficus glomerata* fruits for transdermal patches: Taking indomethacin as a model drug. *Iranian Journal of Pharmaceutical Sciences,* **7**(1): 25-36.

Alhaushey, L. and Ahmad, R.D. 2023. Formulation and evaluation of celecoxib transdermal patches. *Research Journal of Pharmacy and Technology*, **16**(4): 1574-1580.

Alzheimer's Association. 2019. Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, **15**(3): 321-387.

Amarachinta, P.R., Sharma, G., Samed, N., Chettupalli, A.K., Alle, M. and Kim, J.C. 2021. Central composite design for the development of carvedilol-loaded transdermal ethosomal hydrogel for extended and enhanced anti-hypertensive effect. *Journal of Nanobiotechnology*, **19** (1): 1-5.

Ashfaq, A., Riaz, T., Waqar, M.A., Zaman, M. and Majeed, I. 2024. A comprehensive review on transdermal patches as an efficient approach for the delivery of drug. *Polymer-Plastics Technology and Materials*, **63**(8): 1045-1069.

Buck, A., Rezaei, K., Quazi, A., Goldmeier, G., Silverglate, B. and Grossberg, G.T. 2024. The donepezil transdermal system for the treatment of patients with mild, moderate, or severe Alzheimer's disease: A critical review. *Expert Review of Neurotherapeutics*, **24**(6): 607-614.

Dinh, L., Lee, S., Abuzar, S.M., Park, H. and Hwang, S.J. 2022. Formulation, preparation, characterization, and evaluation of dicarboxylic ionic liquid donepezil transdermal patches. *Pharmaceutics*, **14**(1): 205-220.

Jajoo, V.S., Shrirame, D.S., Sawale, A.V. and Atram, S.C. 2023. Formulation and evaluation of transdermal patch for the treatment of migraine. *Journal of Drug Delivery and Therapeutics*, **13**(5): 47-52.

Jyothika, L.S.K., Ahad, H.A., Haranath, C., Kousar, S., Pal Gowd, H.D. and Sadiya, S.H. 2022. Types of transdermal drug delivery systems: A literature report of the past decade. *Research Journal of Pharmaceutical Dosage Form and Technology*, **14**(2): 157-162.

Khan, D., Qindeel, M., Ahmed, N., Khan, A.U., Khan, S. and Rehman, A.U. 2020. Development of novel pH-sensitive nanoparticle-based transdermal patch for management of rheumatoid arthritis. *Nanomedicine*, **15**(6): 603-624.

Kılıçaslan, D.J.C.S.J. 2024. Donepezil-squaric acid hybrid: Synthesis, characterization and investigation of anticholinesterase inhibitory, DNA binding and antioxidant properties. **45**(2): 216-226.

Kriplani, P., Guarve, K. and Baghel, U.S. 2021. Formulation optimization and characterization of transdermal film of curcumin by response surface methodology. *Chinese Herbal Medicines*, **13**(2): 274-285.

Kusuma, K., Subhash, P., Ahad, H.A., Poojari, S.J. and Abhishek, J. 2024. Deciphering the puzzle: A thorough examination of microsphere optimization through factorial design methodology, unveiling novel strategies and promising directions. *Naturalista Campano*, **28**(1): 3143-3155.

Lee, B., Mahtab, M., Neo, T., Farooqi, I. and Khursheed, A.J.J. 2022. A comprehensive review of design of experiment (DOE) for water and wastewater treatment application - Key concepts, methodology and contextualized application. *Journal of Water Process Engineering*, **47**(1): 102673. [*https://doi.org/10.1016/j.jwpe.2022.102673]*.

Magalhães, S., Goodfellow, B.J. and Nunes, A.J.A.S.R. 2021. FTIR spectroscopy in biomedical research: How to get the most out of its potential. *Applied Spectroscopy Reviews*, **56**(8): 869-907.

Mohamed, F.A., Khashaba, P.Y., Shahin, R.Y. and El-Wekil, M.M. 2019. Tunable ternary nanocomposite prepared by electrodeposition for biosensing of centrally acting reversible acetyl cholinesterase inhibitor donepezil hydrochloride in real samples. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **567**(1): 76-85.

Nakamura, Y., Kim, R., Nishiyama, K., Kikuchi, T., Ishikawa, I. and Aoki, H. 2023. Efficacy and safety of a transdermal donepezil patch in patients with mild-to-moderate Alzheimer's disease: A 24-week, randomized, multicenter, double-blind, parallel group, non-inferiority study. *Geriatrics & Gerontology International*, **23**(4): 275-281.

Padmaja, B. and Rani, S.S. 2024. Development of rosuvastatin calcium nano-carrier patches by central composite design. *GSC Biological and Pharmaceutical Sciences*, **27**(3): 123-133.

Pandey, A. and Gupta, S. 2023. Evaluation of formulated transdermal patches. *Journal of Population Therapeutics and Clinical Pharmacology,* **30**(16): 793-798.

Pandey, P. and Pandey, M.M. 2021. *Research Methodology Tools and Techniques*. Bridge Center, Buzau, Romania.

Prabhu, K., Prabhu, P., Bhat, P. and Patil, S. 2021. Development and validation of a UV spectrophotometric method for the estimation of donepezil hydrochloride in bulk and pharmaceutical formulations. *Asian Journal of Pharmaceutical and Clinical Research,* **14**(1): 61-66.

Shravani, Y., Ahad, H.A., Haranath, C., Gari Poojitha, B., Rahamathulla, S. and Rupasree, A. 2021. Past decade work done on cubosomes using factorial design: A fast track information for researchers. *International Journal of Life Sciences and Pharmaceutical Research,* **11**(1): 124-135.

Shiyan, S., Marketama, M.M. and Pratiwi, G. 2021. Optimization transdermal patch of polymer combination of chitosan and HPMC-loaded ibuprofen using factorial designs. *Pharmaciana,* **11**(3): 406-415.

Siafaka, P.I., Bülbül, E.Ö., Mutlu, G., Okur, M.E., Karantas, I.D., Okur, N.Ü.J.C. and Targets, N.D.D. 2020. Transdermal drug delivery systems and their potential in Alzheimer's disease management. *Journal of Current Drug Delivery*, **19**(5): 360-373.

Somadasan, S., Subramaniyan, G., Athisayaraj, M.S. and Sukumaran, S.K. 2024. Central composite design: An optimization tool for developing pharmaceutical formulations. *Journal of Young Pharmacists*, **16**(3): 400-409.

Suksaeree, J., Maneewattanapinyo, P., Panrat, K., Pichayakorn, W., Monton, C.J.J.O.P. 2021. Solvent-cast polymeric films from pectin and Eudragit® NE 30D for transdermal drug delivery systems. *Journal of Polymers and the Environment*, **29**(1): 3174-3184.

Yongping, Z.H., Jian, X.U. and Weijie, X.I. 2028. Optimization of the matrix formulation of new indomethacin hydrophilic gel patches based on central composite design-response surface methodology and its molding process inspection. *Herald of Medicine*, **37**(5): 593-599.

Zhang, X., Lian, S., Zhang, Y. and Zhao, Q. 2022. Efficacy and safety of donepezil for mild cognitive impairment: A systematic review and meta-analysis. *Clinical Neurology and Neurosurgery*, **213**(1): 107134. [*https://doi.org/10.1016/j.clineuro.2022.107134*].